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Cloning of TH0- and TH2-Type Helper Lymphocytes from Liver Granulomas of *Schistosoma mansoni*-Infected Mice

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The pathological manifestations of schistosomiasis mansoni are primarily induced by circumovum hepatic granuloma formation and fibrosis. The growth and modulation of the granulomas are regulated by T lymphocytes and their products. In the present study, we isolated T-lymphocyte clones from lesions at the acute and chronic stages of murine infection. All of the T-cell clones were characterized by immunofluorescence as $CD4^+CD8^-$ helper cells. Three T-cell clones derived from vigorous granulomas produced interleukin 2 (IL-2), IL-4, and gamma interferon (IFN-γ) and were therefore classified as TH0-type T lymphocytes. Of the three clones derived from immunomodulated lesions, two produced IL-2 or IL-4 and/or IFN-γ (TH0 type) and the other one did not produce IFN-γ but did produce IL-4 and was therefore characterized as a TH2-type helper clone. The clones were further characterized by their responsiveness to soluble egg antigen fractions. The acute infection-derived clones responded to the lower-molecular-mass (60- to 66-kDa and 25- to 30-kDa) fractions, whereas the immunomodulated-granuloma-derived clones responded to the 60- to 66-kDa and higher-molecular-mass (70- to 90-, 93- to 125-, and >200-kDa) fractions. Upon adoptive transfer into naive mice, both the TH0- and TH2-type clones were capable of inducing granuloma formation of similar magnitude around antigen-coated beads and/or freshly isolated parasite eggs. The present study revealed the presence of TH0-type precursor helper cells within the liver granulomas. The findings underscore the complexity of the granulomatous response at the T-cell level and demonstrate that both TH0- and TH2-type granuloma lymphocytes play a role in parasite egg-induced granuloma formation.

Schistosomiasis mansoni caused by *Schistosoma mansoni* is a helminth-induced disease characterized by hepatic and intestinal granuloma formation around deposited parasite eggs and tissue fibrosis (2). In mice, the egg-induced immune response is divided into two distinct stages. The deposited eggs secrete soluble egg antigens (SEA) (4) into the surrounding tissue and evoke a T-cell-mediated granulomatous response which peaks at the acute stage (8 to 10 weeks of infection) and is accompanied by peak lymphokine production. Subsequently, the inflammatory response wanes, and diminished lymphokine production, granuloma formation, and cumulative fibrosis lead to the chronic stage (16 to 20 weeks of infection) of the disease (2, 3, 12). This diminished, yet pathologic, response continues for the remainder of the infection.

The characterization of the T-lymphocyte responses that control the evolution of the granuloma has been of great interest. Previous studies established that T helper $Lyt1^+$ $L3T4^+$ ($CD4^+$) splenic or granuloma-derived lymphocytes are capable of inducing the granulomatous response (8, 9, 20, 24). The formation of the early, preacute-stage (6 weeks of infection) granuloma is accompanied primarily by an intragranulomatous TH1-type gamma interferon (IFN-γ) response (18). By 8 weeks of the acute infection, granuloma formation is influenced by both interleukin 2 (IL-2) and IL-4 lymphokines produced by splenic or granuloma lymphocytes (5, 6, 15, 16, 19, 20, 29). Until recently, investigators have relied on lymphokine production profiles of unseparated or isolated populations to define the T-cell subsets involved in the granulomatous response. A recent study has demonstrated that SEA-specific spleen-derived TH1-type clones were able to evoke a granulomatous response when transferred into naive mice (8, 9). However, TH2-type (IL-4- and/or IL-5-producing) clones have not, as yet, been shown by adoptive transfer to induce a granulomatous response.

In the present study, we have isolated and expanded SEA-specific T-lymphocyte clones from a population of liver granuloma lymphocytes. Characterization of clones by their lymphokine production profiles demonstrated that the majority of the clones belonged to the TH0 subset of cells. Upon adoptive transfer into naive mice, both the TH0- and TH2-type clones produced an anamnestic circumovum granulomatous response. In addition, little difference in the intensity of granuloma formation between clones isolated from acute or chronic infection-stage granulomas was observed.

MATERIALS AND METHODS

Mice and infection. Female CBA/J (*H-2^k^*) mice (purchased from Jackson Laboratories, Bar Harbor, Maine) were used throughout the study. The mice were maintained with standard laboratory care. Six- to 8-week-old mice were infected subcutaneously with 25 cercariae of the Puerto Rican strain of *S. mansoni*.

Preparation of antigen. Eggs were isolated from the livers of 200 cercaria-infected mice at 8 weeks of infection. SEA was prepared from homogenized eggs as previously described (4).

Preparation of SEA fractions. SEA fractions were separated on a sodium dodecyl sulfate (SDS)–12% polyacrylamide gel (100 μg per lane) under nonreducing conditions as previously described (17). After separation, the gel was stained (0.1% amido black in 20% methanol, 10% acetic acid), and bands were identified. The lanes were cut into nine regions, comprising the <21-, 25- to 30-, 30- to 38-, 40- to 46-, 50- to 56-, 60- to

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66-, 70- to 90-, 93- to 125-, and >200-kDa molecular mass fractions. The proteins were electroeluted from gel slices with Tris-glycine buffer (25 mM Tris, 192 mM glycine [pH 8.1]), applying 8 to 10 mA per gel fraction at 4°C for 3 to 4 h (Bio-Rad, Richmond, Calif.). The effluents of a particular fraction were pooled from several consecutive elutions and dialyzed overnight in phosphate-buffered saline (pH 7.2) or RPMI 1640 with dialysis tubing with a 12- to 14-kDa exclusion. The dialyzed fractions were sterilized with a 0.22-μm-pore-size syringe filter, and the protein concentration was determined with a Bio-Rad protein assay kit. The protein concentration of each fraction was adjusted to 6 to 8 μg/ml. A blank elution consisting of polyacrylamide gel devoid of proteins was also processed and was used as a control fraction. The individual fractions were separated by SDS-polyacrylamide gel electrophoresis on a 12% gel and were silver stained (Quick-Silver; Amersham) to ascertain that only the amido black-stained proteins were present in each fraction.

Isolation and cloning of granuloma T lymphocytes. Granuloma T cells were isolated from collagenase enzyme-dispersed liver granulomas as previously described (24). The single-granuloma-cell suspension was pelleted, resuspended, and passed through both gauze and glass wool filters to remove clumped cells. For removal of macrophages, the washed granuloma cells were plated at a concentration of 5×10^6 cells per ml onto plastic tissue culture-grade petri dishes for 60 to 90 min. By Wright's stain, the nonadherent fraction of granuloma cells contained 3 to 5% macrophages, 12 to 15% lymphocytes, and 65 to 75% eosinophils.

The granuloma cell suspension was cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 10^{-5} M 2-mercaptoethanol, 2 mM sodium pyruvate, 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, 2 mM L-glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml (Sigma) with SEA (10 μg/ml) for 4 days in 75-mm^2 tissue culture flasks to allow expansion of the SEA-specific T lymphocytes. The activated blast cells were then isolated by Ficoll-Hypaque separation, counted, combined with irradiated (1,600 rads) splenic feeder cells (3×10^6/ml), and cloned in the same culture medium by limiting dilution at 1, 10, and 100 cells per well in 96-well round-bottom tissue culture plates in the presence of IL-2 (100 U/ml) and SEA (10 μg/ml). The first attempt to obtain clones from the 1-cell-per-well dilution was not successful. We initially cloned cells from the 10-cell-per-well dilution. After 6 to 10 days, positive wells were identified by recognition of activated T-lymphocyte blasts, which grew at the periphery of the cell button. The frequency of positive wells was between 8 and 30%. Positive wells were transferred into new plates with fresh irradiated feeder cells and restimulated with SEA and IL-2 for expansion on an 11-day cycle. When clones were expanded to sufficient numbers, they were tested for SEA-specific proliferation without added IL-2. Highly specific clones were then recloned at the 1-cell-per-well dilution. In the secondary cloning, the number of positive wells was 4 to 15 per 96-well plate. According to the calculations of the Poisson distribution, when the number of positive wells per plate is below 37%, the cells are considered to arise from a single cell (23). The twice-cloned T lymphocytes were then expanded and characterized by proliferation, lymphokine production, SEA-fraction responses, and granulomagenic activity.

Proliferation assay. Cloned cells were suspended at a concentration of 3×10^4 to 5×10^4 cells in 100 μl of RPMI 1640 medium supplemented with 10% fetal calf serum, 10^{-5} M 2-mercaptoethanol, 2 mM sodium pyruvate, 20 mM HEPES buffer, 2 mM L-glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml (Sigma). Clones were supplemented with 5×10^5 1,600-rad-treated normal splenic feeder cells suspended in 50 μl of complete medium. One microgram of SEA in 50 μl of complete medium was then added. Clones were incubated in 200-μl volumes in 96-well microtiter plates (Corning Glass Works, Corning, N.Y.) at 37°C in 5% CO_2 for 4 days. The cells were then pulsed with 1 μCi of [^{3}H]thymidine (ICN Radiochemicals, Irvine, Calif.) for 12 h and harvested onto glass wool filters. The dried filters were immersed in 1 ml of scintillation cocktail and measured for radioactivity in a liquid scintillation counter (Beckman Instruments, Irvine, Calif.). Significant proliferation was defined as an increase in counts twofold over the response of the blank control.

Preparation of SEA-coated beads. Antigen-coated beads were prepared by coupling crude SEA to cyanogen bromide-activated Sepharose 4B beads (Pharmacia, Piscataway, N.J.) sieved to obtain a diameter between 40 and 80 μm. The SEA protein was coupled in 0.1 M $NaHCO_3$–0.5 M NaCl (pH 8.3) buffer. A ratio of 0.5 mg of SEA protein per 1-ml pellet of swollen beads was coupled at 4°C overnight. Unreacted sites were blocked by addition of 1 M ethanolamine-HCl (pH 8.0) for 2 h at 4°C.

Measurement of lung granulomas elicited by T-lymphocyte clones. Two thousand SEA-coated beads or freshly isolated eggs mixed with 10^6 cells of an individual T-lymphocyte clone were injected in 0.5 ml of medium into the tail vein of naive mice. After 2 days, animals were sacrificed, and their lungs were inflated and preserved with 10% phosphate-buffered formalin (Fisher, Springfield, N.J.), sectioned, and stained with hematoxylin and eosin. The area of the granulomas was measured by means of computerized image analysis with computer analysis software (Microcomp; Southern Micro Instruments, Atlanta, Ga.) and was expressed in square micrometers. Only those lesions around beads with diameters ranging between 40 and 75 μm were measured.

Lymphokine production. Unfractionated SEA (10 μg/ml) or SEA fractions (4 to 6 μg/ml) were incubated with 2×10^4 cloned T lymphocytes in 0.2 ml of RPMI 1640 containing 5% fetal calf serum, 10^{-5} M 2-mercaptoethanol, 2 mM sodium pyruvate, 20 mM HEPES buffer, 2 mM L-glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml (Sigma). Normal spleen cells irradiated with 1,600 rads were added at a concentration of 1×10^5 to 5×10^5 cells to serve for antigen presentation. On the basis of previous kinetic studies, supernatants were collected after 24 h for IL-2 and IL-4 and after 48 h for IFN-γ assays.

Lymphokine assays. Culture supernatants were assayed for IL-2 with the CTLL-20 cell line (provided by Frank Fitch, University of Chicago, Chicago, Ill.). The supernatants were serially diluted in CTLL medium and plated in triplicate in 96-well round-bottom plates. CTLL cells, maintained in Dulbecco's modified Eagle's medium (GIBCO, Gaithersburg, Md.), supplemented as described above with 5% concanavalin A-stimulated Lewis rat spleen supernatant, were cultured in a volume of 200 μl per well. After 18 to 20 h of culture and 4 to 6 h of pulse with 1 μCi of [^{3}H]thymidine, incorporated radioactivity was measured. IL-2 (gracious gift from Cetus Corp., Emeryville, Calif.), used as a standard (510 pM [20 U/ml]), was serially diluted. Use of anti-IL-2 monoclonal antibody from the S4B6 hybridoma (donated by Timothy Mosmann, DNAX Corp., Palo Alto, Calif.) confirmed specificity, because the CTLL response to all of the supernatants was abrogated in the presence of the antibody.

The CT4S IL-4-dependent cell line (donated by William Paul, National Institutes of Health, Bethesda, Md.), maintained in recombinant IL-4 (generous gift from Immunex

TABLE 1. SEA-specific proliferative responses of granuloma T-lymphocyte clones of mice with acute or chronic infection[a]

Clone	Granuloma source (wk)	Proliferation index (exptl vs control results)
RF 10.3-2	8	6–19
RF 10.3-12	8	6–23
RL 10.12-8	8	4.5
RE 10.35-7	20	4–16
RE 10.35-23	20	3–15
RG 10.1-9	20	2.5–22

[a] The mean background counts per minute ± standard error of unstimulated acute infection clones was 862 ± 15 (range, 339 to 1,660), and that of chronic infection clones was 958 ± 11 (range, 545 to 1,265). The surface marker identified on all clones was $CD4^+CD8^-$.

TABLE 2. Lymphokine profiles of granuloma T-lymphocyte clones from mice with acute or chronic infection

Clone	Infection source (wk)	Lymphokine production[a]		
		IL-2	IL-4	IFN-γ
RF 10.3-2	8	2.15	1,094	152
RF 10.3-23	8	1.26	1,015	138
RL 10.12-8	8	0.72	295	593
RE 10.35-7	20	1.06	699	138
RE 10.35-23	20	2.62	1,105	
RG 10.1-9	20		291	

[a] Lymphokine production was measured in units per milliliter for IL-2 and IL-4 and picograms per milliliter for IFN-γ.

Corp., Seattle, Wash.) was used for the IL-4 determinations in the supernatants. The serially diluted supernatants were compared against a standard curve by using recombinant IL-4 serially diluted from 50 pM (100 U/ml). The supernatants were incubated with 5×10^3 CT4S cells in a volume of 200 μl in 96-well plates for 24 h and pulsed with [^{3}H]thymidine for an additional 24 h to assess proliferation. Use of anti-IL-4 monoclonal antibody produced by 11B11 hybridoma (provided by William Paul) confirmed that 100% of the proliferation of the CT4S was due to IL-4 in the supernatants.

Culture supernatants at 48 h were assayed for IFN-γ by use of an enzyme-linked immunosorbent assay kit (Genzyme Corp., Boston, Mass.) which utilizes a hamster anti-mouse IFN-γ epitope-specific monoclonal capture antibody and a goat anti-mouse IFN-γ polyclonal antibody with multiple epitope specificity. A standard curve was generated by serial dilutions of mouse recombinant IFN-γ. The assay had a detection limit of 10 ng/ml.

Statistical analysis. The unpaired Student's *t* test was used to examine the granuloma elicitation data. Significant granuloma formation was determined by *P* values of <0.05.

RESULTS

Granuloma T-lymphocyte source and specificity. The SEA-specific responsiveness of the twice-cloned T cells was confirmed by proliferation assays without added IL-2. Table 1 presents data from the proliferation index (experimental results/control results) of the individual T-lymphocyte clones. During cloning with the increase in specific stimulation cycles, the antigen responsiveness of each clone increased; thus, a range of stimulation indices are presented by the assays. The T-lymphocyte clones listed in Table 1, each derived from acutely or chronically infected mice, were all identified by immunofluorescence as $CD4^+CD8^-$ T lymphocytes.

Lymphokine profiles of the T-lymphocyte clones. To determine the phenotype of the isolated clones, T-lymphocyte clones were stimulated with SEA, and IL-2, IL-4, and IFN-γ production in the supernatants was assayed. The data in Table 2 demonstrate that two different subsets of TH clones were isolated. Three clones, RF 10.3-2, RF 10.3-23, and RL 10.12-8, isolated from acutely infected mice were all TH0-type T lymphocytes because they produced IFN-γ, IL-2, and IL-4 lymphokines. Among the chronic infection-derived clones, the RE 10.35-7 clone produced all three lymphokines, while RE10.35-23 produced IL-2 and IL-4; therefore, both were designated as having a TH0 phenotype. In contrast, RG 10.1-9 produced IL-4 but no IFN-γ, indicating a TH2-type phenotype (Table 2).

Identification of cloned T-cell responsiveness to SEA fraction. In order to identify the range of SEA protein specificity, four TH0-type and one TH2 lymphocyte clones (Table 2) were stimulated with the various isolated SEA fractions as previously described (17, 18). Each clone was incubated with the individual SEA fractions, and after 24 h, the supernatants were collected and assayed for IL-4 production as a measure of their responsiveness. As indicated in Fig. 1, the TH0-type lymphocyte clones from the acutely infected mice produced substantial amounts of IL-4 in response to the 25- to 30- and 60- to 66-kDa fractions. In contrast, the TH0 cell clones from the chronically infected mice responded to both the mid- and upper-molecular-mass fractions (60 to 66, 70 to 90, 93 to 125, and >200 kDa). The single TH2 clone (RG10.1-9) derived from granulomas of chronically infected mice also responded to the mid- to high-molecular-mass fractions, with the following amounts of IL-4 produced: >200 kDa, 13.6 U; 93 to 125 kDa, 4.7 U; 70 to 90 kDa, 8.9 U; and 60 to 66 kDa, 8.6 U. These results correspond to those from our previous studies that demonstrated a shift in the polyclonal recognition of SEA proteins in both proliferation assays (17) and granuloma formation (18) as the infection proceeded from the acute stage to the chronic stage.

Elicitation of in vivo granuloma formation by T-lymphocyte clones. We next determined whether the T-lymphocyte clones isolated from acutely and chronically infected mice could induce a granulomatous response. Five clones were used in adoptive transfer experiments in which groups of recipients were injected intravenously with a mixture of 10^6 T cells and 2,000 freshly isolated worm eggs or SEA-coated beads. As a control, similar numbers of feeder layer cells cultured in the same manner were injected with beads or eggs. In order to determine whether the reaction around the injected eggs was due to the response of the transferred clone and not to the developing primary circumovum granuloma, tissue responses were measured at 2 days postembolization. Table 3 displays the granuloma formation data from two TH0-type clones from acutely infected mice and one each of the TH0- and TH2-type clones from chronically infected mice. All four clones induced significant ($P < 0.005$ to 0.001) granuloma formation around the embolized SEA-coated beads. We next followed a similar protocol with embolized eggs and used four of the six T-lymphocyte clones. The results in Table 4 demonstrate that the TH0-type clones from both the acutely and chronically infected mice as well as the single TH2 clone from the chronically infected mice all induced significant ($P < 0.001$) circumovum granulomatous responses.

DISCUSSION

The aim of the present study was to clone T cells from vigorous and immunomodulated granulomas and characterize

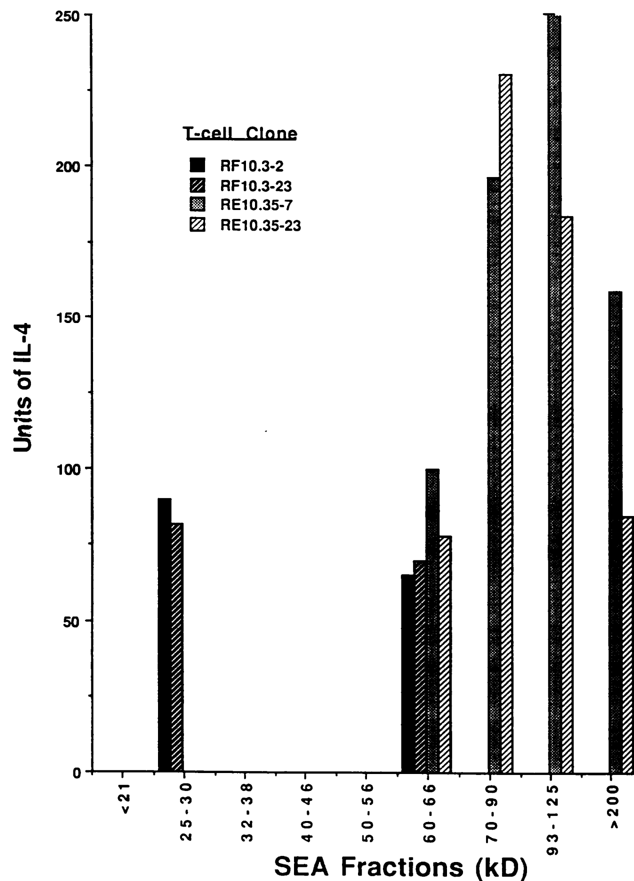

FIG. 1. T-lymphocyte clone production of IL-4 in response to SEA fractions. Cloned lymphocytes from acutely or chronically infected mice were cultured for 24 h with 10^5 irradiated feeder cells at a concentration of 2×10^4/ml in the presence of the SEA fractions. Culture supernatants were assayed for IL-4 with the CT4S IL-4-dependent cell line. Data illustrate a representative experiment; the responses of two clones, each derived from liver granulomas of acutely or chronically infected mice, are shown. Repeat experiments demonstrated similar results.

their subset identity and function. The results showed that, regardless of their source, the cloned cells were all $CD4^+$ T cells that proliferated well in response to the SEA stimulus. Five of six clones produced IL-2, IL-4, and/or IFN-γ. According to current concepts, the subset identity of cloned T cells is established by the pattern of cytokine production. Thus, TH1 clones produce IL-2 and IFN-γ but not IL-4, whereas TH2-type clones produce IL-4 without IFN-γ (21). The existence of short-term clones that exhibit cytokine production character-

TABLE 3. Granuloma induction around SEA-coated Sepharose 4B beads by adoptively transferred T-lymphocyte clones

T-cell clone (wk)	No. of mice/no. of lesions	Mean granuloma area (10^3 μm^2) ± SE	*P*
Feeder cells	6/180	5.37 ± 0.20	
RF 10.3-2 (8)	6/180	6.65 ± 0.22	<0.005
RL 10.12-8 (8)	4/122	7.77 ± 0.33	<0.001
RE 10.35-23 (20)	5/161	8.43 ± 0.46	<0.001
RG 10.1-9 (20)	6/177	6.70 ± 0.26	<0.005

TABLE 4. Granuloma induction around *S. mansoni* eggs by adoptively transferred T-lymphocyte clones[a]

T-cell clone (wk)	No. of mice/no. of lesions	Mean granuloma area (10^3 μm^2) ± SE
Feeder cells	5/100	3.90 ± 0.18
RF 10.3-2 (8)	6/180	9.44 ± 0.41
RF 10.3-12 (8)	4/95	6.64 ± 0.40
RE 10.35-23 (20)	6/180	9.03 ± 0.37
RG 10.1-9 (20)	4/80	7.71 ± 0.49

[a] *P* = 0.001 throughout.

istic of both the TH1 and TH2 subsets of cells has also been described. This TH0 subset of cells is considered to be a precursor T cell from which the TH1 and TH2 phenotype cells can differentiate (1, 14, 26, 28). Indeed, schistosome eggs injected into the footpads of mice evoked a polyclonal TH0-type immune response in the lymph nodes in 3 days (28). On the basis of the pattern of cytokine production, the majority of clones isolated from the liver granulomas belong to the TH0-type lymphocytes, and a single clone derived from down-modulated granulomas was of the TH2 phenotype. We excluded several clones from the study because of low proliferative ability and poor viability. Thus, possibly, a selection was made for the stronger, faster-growing, predominantly TH0-type cells. Whether the slowly growing presumably TH1- or TH2-type cells possess similar or different granulomagenic and pathogenic potential is still to be examined. These results establish that precursor TH0-type lymphocytes are present in both the vigorous and immunologically downmodulated granulomas. The presence of TH0 cells that can produce IL-2 and IL-4 confirms our previous observation that showed both IL-2 and IL-4 production by lymphocytes of the granulomatous lesions (18, 24). Use of the sensitive ELISPOT assay (11), which identifies and quantitates single unstimulated cells that produce cytokines, may be beneficial for such analysis of T-cell subsets.

It is noteworthy that, irrespective of the derivation (acute chronic infection granuloma) or subset identity of the clones (TH0, TH2), as few as 10^6 cells adoptively transferred granulomatous responses of similar magnitude around live eggs or antigen-coated beads. This capacity is attributed to the secretion of IL-2 and/or IL-4 lymphokines that previously had been shown to be involved in the granulomatous response (5, 6, 19, 20, 29). In previous studies, lymphocytes of the modulated granulomas were shown to transfer diminished granulomatous responses that could be augmented after the deletion of the $CD8^+$ T-cell subset (24). Presently, the granulomagenic capacity of $CD4^+$ T cells cloned from modulated lesions was confirmed. We presume that the granulomagenic capacity of the $CD4^+$ subset within the modulated granulomas is subject to the action of macrophages (13, 26) and modulatory cytokines such as IL-10 (22, 25) that curtail proliferation, lymphokine production, and the intensity of the inflammatory response. A recent study indicated that TH1-type clones obtained from the lymph nodes of SEA-adjuvant-sensitized mice adoptively transferred the granulomatous response (8, 9). The present observations indicate that the TH0- and TH2-type T cells, as suggested previously (15, 16), can also carry out similar inflammatory functions. Thus, the repertoire of T helper subsets that possess granulomagenic capacity is widened. Whether the different subsets can contribute to the pathogenesis of the inflammation to the same degree is still to be elucidated. The presence, within the granulomas, of a less-differentiated TH0 cell subset that produces two granu-

loma promoter lymphokines and, upon the receipt of lymphokine-mediated (1, 27) or other signals, can develop into TH1 or TH2 cell lineages underscores the functional versatility of such cells.

It is of interest that TH0 clones derived from the vigorous granulomas recognized the lower-molecular-mass (60- to 66- and 25- to 30-kDa) fractions within SEA. Under reducing conditions, the 60- to 66-kDa protein broke into 25- to 30-kDa fragments (data not shown), suggesting that the T-cell clones recognized the same subunit epitopes on the whole molecule. This observation is similar to that of a recent study that showed granulomagenic TH1-type clones responding to two molecules with molecular masses of 64 to 68 and 38 to 42 kDa. The authors of that study also suggested that the 38- to 42-kDa molecule is a subunit of the larger 64- to 68-kDa moiety (9). In this study, the clones of the modulated lesions recognized the mid- to upper range of molecular mass fractions. This shift in antigenic recognition has been previously described with regard to the polyclonal responses of T cells of the modulated granulomas (17, 18). Its significance needs further elucidation. We presume that the response of the chronic infection granuloma-derived clones to multiple higher-molecular-mass (60- to 200-kDa) fractions is attributable either to the formation of macromolecular aggregates of smaller-molecular-mass (60- to 66-kDa) antigens or signifies the presence of repeated or cross-reactive epitopes on the antigens. The antigen responsiveness of the clones to a variety of fractions underscores the wide granuloma-inducer capability of SEA.

In summary, we cloned $CD4^+$ and TH0- and TH2-subset lymphocytes from both the vigorous and downmodulated liver granulomas. Our results demonstrate that both subsets induce granulomas of similar magnitude. At the clonal level, differences in antigenic recognition among the T cells were observed. These data provide further insight into the complexity of the granulomatous response manifested at the T-helper lymphocyte level.

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