Regulation of Egg Antigen-Induced In Vitro Proliferative Response by Splenic Suppressor T Cells in Murine *Schistosoma japonicum* Infection

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Beginning about 5 weeks after infection, C57BL/6J mice infected with *Schistosoma japonicum* developed granulomas around parasite eggs trapped in the liver. These granulomas attained peak size about 9 weeks after infection and then spontaneously regressed. This regression was also induced by the injection of serum immunoglobulin G1 but not lymphoid cells from chronically infected mice, but it was conceivable that lymphoid cells from mice infected for 10 weeks could also induce regression. We investigated the possibility of cellular suppression of egg antigen-induced immune responses by coculturing spleen cells from 5- to 6-week-infected mice with spleen cells from mice infected for 10 weeks or longer. Mitomycin C-resistant Thy 1.2$^+$, Lyt 2.2$^+$ splenic T cells from mice infected for 10 to 25 weeks consistently suppressed the egg antigen-stimulated proliferation of spleen cells from 5- to 6-week-infected mice. Suppression was dependent upon specific antigen and optimal concentrations of egg antigen and T suppressor cells. Once induced, the suppressor cells were nonspecific. Cultured T cells from uninfected mice also occasionally suppressed the acute spleen cell proliferative response, but these cells were mitomycin C sensitive. These in vitro observations suggest that granulomatous inflammation in vivo may also be down regulated by suppressor T cells and that these cells may also be implicated in the nonspecific depression of cellular and humoral responses to antigens observed during the course of this infection.

In schistosomiasis japonica, granulomatous inflammation occurs around the parasite eggs trapped in the liver. This lesion results in obstruction of the portal blood flow, elevation of portal pressure, and esophageal varices, the major morbid sequelae of both human and murine infections. In C57BL/6J mice infected with *Schistosoma japonicum* there is spontaneous down regulation, termed modulation, of both the granulomatous inflammation and the portal hypertension about 8 to 10 weeks after infection, resulting in their survival for at least 30 weeks (7, 23). Temporally correlated with this spontaneous modulation of granulomatous inflammation and portal hypertension are parallel decreases in both immediate and delayed hypersensitivity reactions to soluble egg antigen (SEA) and in SEA-induced proliferation and immunoglobulin synthesis by the spleen cells (SC) of these animals (11). It was also shown that the adoptive transfer of immunoglobulin G1 (IgG1) prepared from the sera of mice infected for 30 weeks to acutely infected recipients reduces granulomatous inflammation in vivo (23) and proliferative responses in vitro (12). In addition there was nonspecific depression of T-cell (concanavalin A) and B-cell (lipopolysaccharide) mitogenic responses (11) and of cellular and humoral immunity to injected myoglobin (10) in infected mice. Recently, human T cells of the Leu 2a$^+$3a$^-$ phenotype were shown to block in vitro human T-cell proliferation induced by *S. japonicum* adult worm antigen, suggesting that T-suppressor cells play a role in the human disease (21). These human T-suppressor cells were mitomycin C resistant.

In a previous study (23) the adoptive transfer of serum IgG1 but not lymphoid cells from mice infected for 30 weeks to acutely infected recipients, reduced their granulomatous inflammation. However, it was possible that lymphoid cells from mice infected for 10 weeks, when spontaneous modulation begins, would also modulate this inflammation. In the present study this possibility of cellular regulation of SEA-induced immune responses was investigated with the SEA-induced SC proliferative response as target. This system was used because a strong temporal correlation between the humoral (IgG1-mediated) regulation of this in vitro SEA-evoked response (12) and of both granulomatous inflammation and portal hypertension in vivo had been observed (23). SC were tested throughout the course of this infection for their capacity to reduce this proliferative reaction, with particular emphasis on cells taken from infected animals at the time of their maximum modulation of the disease, i.e., 10 to 20 weeks. Inasmuch as a number of studies (2, 9, 14, 20, 24) showed that culture of normal murine SC generated suppressor cells for humoral and cellular immune responses, normal SC or T cells were used as controls.

The data obtained in this study show that there is a population of Thy 1.2$^+$, Lyt 2.2$^+$, mitomycin C-resistant T cells in the spleens of mice infected for 10 to 25 weeks which inhibits the in vitro SEA-induced proliferative response of SC from acutely infected mice.

MATERIALS AND METHODS

Mice. Female C57BL/6J mice obtained from Jackson Laboratories, Bar Harbor, Maine, were infected at Lowell University, Lowell, Mass., with 25 cercariae of a Philippine strain of *S. japonicum* (26). Mice infected with this intensity will survive for at least 30 weeks.

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Antigens. Eggs were obtained from the livers of CF1 mice infected with 50 cercariae of the same strain of *S. japonicum*. SEA was prepared from homogenized, ultracentrifuged eggs (1), dialyzed extensively against phosphate-buffered saline (PBS), sterilized by passage through Millex filters (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.) and assayed for total protein by the method of Lowry et al. (18). The possibility that the observed responses were due to contamination of the SEA with lipopolysaccharide or other mitogenic substances was ruled out by the observation that the addition of the concentrations of SEA employed in the proliferative assay did not stimulate [^{3}H]thymidine ([^{3}H]Tdr) uptake or antibody synthesis by the SC of uninfected mice. Nor did the injection of SEA induce immediate or delayed footpad swelling in normal mice. Similar negative responses were obtained in vitro and in vivo when chicken ovalbumin, 5× crystallized (Miles Laboratories, Kankakee, Ill) was substituted for SEA. Sperm-whale myoglobin (Mb) was obtained from Sigma Chemical Co., St. Louis, Mo.

Cell preparations. Single-cell preparations of SC were prepared from C57BL/6J mice infected for different lengths of time with *S. japonicum*. Infection was determined by the presence of hepatomegaly and splenomegaly in chronically infected mice. Infection was verified by the presence of adult worms in the mesenteric vasculature and the microscopic demonstration of eggs in liver sections. The single-cell suspensions were washed twice and then suspended in complete RPMI 1640 medium (GIBCO, Grand Island, N.Y.) supplemented with 10% fetal calf serum (FCS) (M.A. Bioproducts, Walkersville, Md.), 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid), 100 U of penicillin per ml, 50 μg of streptomycin per ml, and 5×10^{-5} M 2-mercaptoethanol. T cells were prepared on nylon wool columns (15) and found to contain 4 to 5% surface immunoglobulin-positive (SIg$^+$) cells by immunofluorescent assay, 3 to 5% macrophages by the nonspecific esterase stain (16), and about 5 to 10% granulocytes by Giemsa stain. More homogeneous T-cell populations were prepared by a modification of the panning method on anti-mouse immunoglobulin-coated plates (19). These preparations contained fewer than 1% SIg$^+$ cells and about 3% macrophages. Affinity-purified rabbit antibody to mouse Fab (15 ml at 1 μg/ml) in Tris-hydrochloride (pH 9.6) was added to a plastic dish (15 by 100 mm) which was incubated at 4°C overnight. The plate was then washed 3× with 8 ml of cold PBS. Five ml of PBS–5% FCS was added, and the plates were held at 4°C for 30 min. The solution was removed, and 5 ml of cells (10^7/ml) in L-15 medium–5% FCS was added. The plate was incubated for 90 min at 4°C with a gentle swirl at 45 min. The nonadherent (T-enriched) cells were collected gently and saved. Seven ml of L-15–FCS was added to the plates which were gently swirled, and the cells were again removed gently and saved. The plates were washed 2× with PBS–1% FCS, which was discarded. To obtain the adherent cells, 10 ml of PBS–1% FCS was vigorously squirted on the plate, the cells were removed, and the procedure was repeated. The T cells prepared from 10-week-infected mice by either method incorporated increased amounts of [^{3}H]Tdr upon addition of concanavalin A, but not upon addition of lipopolysaccharide, and did not synthesize any detectable immunoglobulin upon addition of SEA, in contrast to what was found when lipopolysaccharide and SEA were added to unfractionated SC from mice infected for 10 weeks (11).

Lyt 1.2$^+$ T cells were lysed with a 1/400 monoclonal antibody (NEI-017; New England Nuclear Corp., Boston, Mass.) and Cedarlane Low-Tox rabbit complement incu-

bated for 60 min at 37°C. The Lyt 2.2$^+$ cells were similarly deleted with a monoclonal antibody (TIB 150 hybridoma obtained from the American Type Culture Collection, Rockville, Md.) and complement. This hybridoma was originally produced by Gottlieb et al. (13). In some experiments the desired populations were prepared by panning with monoclonal rat IgG2a antibodies to Lyt 1$^+$ and 2$^+$ produced by hybridomas obtained from the American Type Culture Collection (TIB 104 and 105, respectively). These antibodies were affinity purified and screened for cytotoxicity before use. These hybridomas were originally produced by Ledbetter and Herzenberg (17). The protocol was essentially the same as for the RAM immunoglobulin plates with the following modifications. The plates were coated with 50 μg of affinity-purified rabbit antibody to rat IgG2a in 50 μM Tris-hydrochloride, (pH 9.6). The T cells were suspended at 10^7/ml in L-15 medium (no FCS), and anti-Lyt 1.2 or anti-Lyt 2.2 was added at a final concentration of 1 μg/10^6 cells and incubated on ice for 30 min, washed 1×, suspended at 10^7 in L-15–5% FCS. Five ml was added to each plate, and panning was allowed to proceed as for removal of SIg$^+$ cells. The purity was assayed by fluorescence.

In a number of experiments the SC from uninfected or chronically infected mice were treated with mitomycin C before they were cocultured with SC from acutely infected animals. 1×10^7 to 6×10^7 SC per ml were treated with 25 μg of mitomycin C for 20 min at 37°C (protected from light). The cells were then washed three times in excess balanced salt solution–5% FCS before coculture.

Proliferative assay. 2×10^5 SC were cultured in 0.2 ml of medium in a 96-well (flat-bottom) microtiter plate (Costar, Cambridge, Mass.). Various numbers of SC or T cells from uninfected or infected mice were then added to each well in the absence of SEA and with 2.5, 5, or 10 μg of SEA per ml, and the plates were then cultured at 37°C for 72 h. [^{3}H]Tdr (1 μCi per well, 5 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) were added to each well for the final 6 to 18 h of culture. The cells were then harvested (microtiter harvester; Otto Hiller Co., Madison, Wis.), and the samples were subjected to liquid scintillation counting (11).

Assay for inhibition of acute proliferation by chronic cell preparations. The usual target system for inhibition of proliferation consisted of SC prepared from mice infected for 5 to 6 weeks plus various concentrations of SEA. SC or T cell preparations (2×10^2, 2×10^3, or 2×10^4) from mice infected for from 10 to 26 weeks were added to each well, and the plates were cultured in a humidified 7% CO_2 incubator at 37°C for 72 h. A similar number of SC or T cells from uninfected mice was used as controls. In some experiments putative suppressor-cell populations were treated with mitomycin C before incubation with the target populations to eliminate any participation of these cells in the proliferative response. [^{3}H]Tdr (1 μCi per well) was added for the last 6 to 18 h of incubation. The results of the individual experiments are expressed as counts per minute ± standard error of the mean. Differences between groups were determined by Student's *t* test.

Specificity of suppression. To determine whether suppression of SC proliferation was specific, the effects of coculture of T cells from chronically infected mice on proliferation induced by T- or B-cell mitogens or by a noncross-reactive antigen (Mb) was determined. To obtain SEA- and Mb-primed SC C57BL/6J mice were injected with 100 μg of SEA and Mb in complete Freund adjuvant (GIBCO, Detroit, Mich.) subcutaneously in the nape of the neck. SC were removed 7 days later. Antigen-primed SC (2×10^5) were

TABLE 1. Specific antigen, cell concentration, and antigen concentration dependence of induction of suppression of the acute spleen cell proliferation by splenic cells from chronically infected mice

Expt no.	SEA (μg/ml)	[³H]Tdr incorporation (10³ cpm) by[a]:			
		5- or 6-wk SC (2 × 10⁵)	2 × 10⁵ 5- or 6-wk SC +		
			10-wk T cells (2 × 10³)	19-wk T cells (2 × 10³)	25-wk SC (2 × 10⁴)
1	0	3 ± 0.4[b]			2 ± 0.1
	2.5	9 ± 1			<u>2</u> ± 0.4
	5.0	12 ± 1			<u>2</u> ± 0.3
2	0	3 ± 0.5	4 ± 0.8	5 ± 1	
	2.5	23	<u>10</u> ± 1	<u>8</u> ± 1	
3	0	4 ± 0.3	<u>2</u> ± 0.3		
	2.5	14 ± 2	<u>7</u> ± 1		
	5.0	18 ± 3	<u>3</u> ± 0.4		

[a] [³H]Tdr present during 66 to 72 h of culture.

[b] Mean of quadruplicate determinations ± standard error of the mean. The underlined values are significant at least at the 0.05 level by Student's *t* test compared to antigen-stimulated cells without added 10-week, 19-week, or 25-week cells.

then cultured with 100 μg of Mb per ml and/or 100 μg of SEA per ml for 120 h. At 18 h before the end of the culture, 1 mCi of [³H]Tdr was added.

The T-cell mitogenic response was generated by adding 1 μg of concanavalin A (Pharmacia, Uppsala, Sweden) to 5 × 10⁶ normal SC in 1 ml of RPMI 1640–10% fetal serum. The B-cell mitogenic reaction was generated by the addition of 5 μg of lipopolysaccharide per ml (*Escherichia coli* type 1 lipopolysaccharide; Sigma). At the end of 24 h [³H]Tdr (2.5 μCi) was added, and the cells were collected for scintillation counting after another 24 h of incubation.

The assays for inhibition of mitogen- and Mb-induced proliferation were performed exactly as those for inhibition of the SEA-induced proliferative response by the addition of 5 × 10⁴ or 5 × 10⁵ cells from chronically infected mice to 2 × 10⁵ Mb-primed or normal SC. (In some experiments the SC from the chronically infected mice were treated with mitomycin C before coculture.)

RESULTS

Determination of optimal conditions for antigenic stimulation and regulation of the acute splenocyte proliferative response to SEA. Maximal proliferation was observed when 2 × 10⁵ SC or T cells from mice infected for 5 or 6 weeks were challenged with 2.5 or 5.0 μg of SEA per ml and then incubated for 3 days. Equivalent proliferation was obtained with T cells prepared on nylon wool columns or by single or double panning on anti-immunoglobulin-coated plastic plates. Inasmuch as spontaneous regression of granulomatous inflammation (23) and delayed hypersensitivity (11) to SEA first occur in mice infected for 10 weeks, the initial experiments employed 10-week SC as the source of potential regulatory cells. In these experiments the coculture of 2 × 10⁵ target SC (5- or 6-week-infected mice) with 2 × 10³ or 2 × 10⁴ SC (from 10-week-infected mice) in the presence of 2.5 or 5 μg of SEA per ml resulted in significant reduction of proliferation.

Suppression of the acute splenocyte proliferative response by spleen cells from chronically infected mice is dependent upon specific antigen, antigen concentration, and cell concentration. Table 1 presents the data from four experiments which typify the range of results obtained in 40 experiments. The results of experiment 1 were typical (35 of 40 experiments); the addition of SC or T cells from chronically infected mice markedly suppressed the proliferation of SC

from acutely infected animals in the presence of SEA. Occasionally (experiment 3), significant suppression was seen in the absence of added SEA as well as in the presence of this antigen. (The addition of chronic T cells in the presence of a noncross-reacting antigen, ovalbumin, did not result in suppression of the acute SC proliferative response [data not shown]). The extent of suppression was cell concentration dependent. Thus in experiment 2, 2 × 10³ 19-week T cells were significantly more suppressive than 2 × 10⁴ 19-week T cells (data not shown). Finally, the extent of suppression was antigen concentration dependent as shown by the significantly greater inhibition observed in experiment 3 with 5 μg compared to 2.5 μg of SEA per ml.

Mitomycin C sensitivity of suppression of acute proliferation by normal splenic T cells and mitomycin C insensitivity of suppression by chronic T cells. There have been a number of reports that the culture of normal murine T cells generated suppressor cells for a variety of cellular and humoral immune responses (2, 9, 14, 20, 24). Therefore, splenocytes from normal mice were also cocultured with SC from acutely infected animals. In 12 of 22 experiments (55%) coculture of normal unfractionated SC and in 5 of 11 experiments (45%) coculture of normal T cells resulted in significant inhibition of the acute proliferative response. Mitomycin C was employed to attempt to distinguish this type of suppression from that observed upon coculture of SC from chronically infected animals and to reduce the background [³H]Tdr incorporation sometimes observed when the chronic cells were added (e.g., experiment 2, Table 1). Suppression of the proliferative response by normal T cells was completely abolished when they were treated with mitomycin C before their addition to the target cells (Table 2). In contrast, suppression by the chronic 19-week T cells was unaffected by such treatment.

Phenotype of the cell population which suppresses the acute proliferative response. Table 3 presents the results of a typical experiment which determined the phenotype of the suppressor cells in the spleens of chronically infected mice. The phenotype of the suppressor cells was Thy 1.2⁺ and Lyt 2.2⁺, since removal of cells with these markers, but not deletion of Lyt 1.2⁺ cells, resulted in abrogation of suppressive activity.

Specificity of inhibition of the proliferative response by cocultured T cells from spleens of chronically infected mice. Mice chronically infected with *S. japonicum* showed signif-

TABLE 2. Mitomycin C sensitivity of suppression of acute splenocyte proliferation by normal splenic T cells and mitomycin insensitivity of this suppression by splenic T cells from chronically infected mice

Expt no.	SEA (μg/ml)	5-wk SC (2 × 10⁵)	[³H]Tdr incorporation (10³ cpm) by[a]:			
			2 × 10⁵ 5-wk SC + 2 × 10⁴:			
			Mitomycin C-treated normal T[b]	Untreated-19 wk T	Mitomycin C-treated 19-wk T[b]	Untreated normal T
4	0	2 ± 0.2	2 ± 0.1	3 ± 0.2	4 ± 0.1	2.7 ± 0.1
	2.5	10 ± 0.1	9 ± 0.1	2 ± 0.1	4 ± 0.1	6 ± 0.3
	5.0	13 ± 0.2	14 ± 0.2	2.5 ± 0.1	4 ± 0.1	9.4 ± 0.3

[a] [³H]Tdr present during 66 to 72 h of incubation.
[b] 1 × 10⁷ 6 × 10⁷ cells per ml were treated with 25 μg of mitomycin C for 20 min at 37°C protected from light. The cells were then washed 3× in excess balanced salt solution–5% FCS before culture.

icantly diminished SC proliferative responses to T- (concanavalin A) and B- (lipopolysaccharide) cell mitogens (11) as well as significantly decreased cellular and humoral immune responses to injected Mb (10). On the other hand, in this study, it was shown that induction of splenic suppressor cells was antigen specific. It was, therefore, of interest to determine whether the splenic cells of chronically infected mice would suppress the proliferative response to these T- and B-cell mitogens as well as the proliferation induced by a noncross-reactive antigen, Mb. We utilized SC from normal mice primed both with SEA and Mb and then stimulated to proliferate by the addition of specific antigens. SEA-stimulated (line 6) T cells from chronically infected mice inhibited both the SEA- and Mb-induced proliferative responses (Table 4). Inhibition of the Mb-induced response was also unaffected by treatment of the chronic T cells with mitomycin C before their addition to the Mb-primed SC. In a number of experiments the T cells from chronically infected mice did not inhibit the proliferative response of splenic T cells to concanavalin A or of unfractionated SC to lipopolysaccharide (data not shown).

DISCUSSION

The spleens of mice infected with *S. japonicum* contain about 80% lymphocytes and about 20% other cells, including macrophages and granulocytes. The spleens of mice infected for from 10 to 25 weeks contain mitomycin C-resistant Thy 1.2⁺, Lyt 2.2⁺ T cells which markedly suppress the in vitro proliferation of SC from acutely infected animals induced by SEA, but not by the noncross-reactive antigen, OV suppression was dependent upon optimal SEA concentrations and on optimal numbers of suppressor T cells. The suppressor cells are T cells by several criteria, including nonadherence to nylon wool and to anti-mouse immunoglobulin-coated

plates and their possession of the appropriate Thy and Lyt markers. Suppression was not affected by pretreatment of the suppressor cell populations with mitomycin C. Suppression by both unfractionated splenocytes and the cells from infected mice was nonspecific; these cells also inhibited the proliferation of Mb-primed SC stimulated by Mb.

Our data are consistent with several features of the suppressor cell cascade which have been described for the NP (4-hydroxy-3 nitrophenyl acetyl) system (8). In the NP system there is a cascade of cellular events resulting in the induction of Lyt 2⁺ Ts3 suppressor cells which mediate nonspecific suppression of contact sensitivity to NP. Presumably the Lyt 2⁺ cells in the *S. japonicum* system correspond to the Ts3 cells in that they are specifically induced by SEA but are then nonspecific in their effects upon the Mb-induced proliferation of Mb-primed SC (Table 4). However, our data do not exclude the possibility that in addition to Ts3 lymphocyte-mediated suppression there is a Ts1 lymphocyte-macrophage pathway for nonspecific suppression (27). Upon activation, Ts3 release a soluble factor, TsF3, which after binding antigen, mediates nonspecific suppression of contact sensitivity. It is reasonable to presume that the Lyt 2⁺ cell in the *S. japonicum* system corresponds to the Ts3 cell in that it is specifically induced by SEA but is nonspecific in its effects upon the Mb-induced proliferation of Mb-primed SC (Table 4). In this pathway Ts1 cells elaborate a soluble suppressor factor which arms macrophages. The armed macrophages are then stimulated to produce a nonspecific soluble suppressor factor.

The suppressor cells generated upon culture of normal

TABLE 3. The T cell in the spleens of chronically infected mice which suppresses the acute proliferative response is Thy 1.2⁺ and Lyt 2.2⁺

SEA (μg/ml)	6-wk SC (2 × 10⁵)	[³H]Tdr incorporation (10³ cpm) by[a]:			
		2 × 10⁵ 6-wk SC + 2 × 10⁴ 12-wk			
		T cells[b]	T-Thy 1.2[c]	T-Lyt 1.2[d]	T-Lyt 2.2[d]
0	7 ± 1	9 ± 1	9 ± 2	9 ± 1	8.5 ± 1
2.5	21 ± 3	11 ± 4	21 ± 4	11 ± 3	23 ± 2

[a] The cultures contained [³H]Tdr during 66 to 72 h of incubation.
[b] Prepared by double panning on plastic plates.
[c] Thy 1.2 cells were lysed by antibody and complement.
[d] The Lyt 1.2 and Lyt 2.2 cells were removed by the panning procedure.

TABLE 4. Lack of specificity of suppression of splenocyte proliferative responses upon coculture of T cells from chronically infected mice with SEA- and Mb-primed T cells

Antigen (μg/ml)	[³H]Tdr incorporation (10³ cpm) by[a]:	
	SEA-/Mb-primed T cells[b] (2 × 10⁵)	2 × 10⁵ SEA-/Mb-primed normal T + 22-wk T cells (2 × 10³)
SEA, Mb		
0	4 ± 0.1	3 ± 0.2
2.5	20 ± 0.3	6 ± 0.2
5.0	18 ± 0.1	7 ± 0.4
20	18 ± 0.2	17 ± 0.3
100	17 ± 0.4	19 ± 0.4
5.0, 20		8 ± 1

[a] [³H]Tdr was present in the cultures during 96 to 120 h of incubation.
[b] See Materials and Methods for method of priming these cells.

splenic SC or T cells were also Thy 1.2^+ and Lyt 1.2^+, but in contrast to those generated from the spleens of infected mice, they were mitomycin C sensitive. Thus, these cells are also T cells, in contrast to a report that suppressor cells from normal spleens are macrophages (28). However, the mitomycin C experiments show a fundamental difference in the suppressor cells generated from these two sources; presumably the normal suppressor cells depend upon mitosis for their generation, whereas the cells from infected mice do not. The lack of a requirement of the latter for mitosis is also consistent with the presence of fully induced Ts3 suppressor cells in the spleens of infected animals. These observations are reminiscent of previous observations (20) that suppression in in vitro-induced cell-mediated cytotoxicity upon culture of normal T cells was abrogated by mitomycin C, whereas suppression by alloantigen-primed suppressor cells was resistant to this treatment.

The finding in an occasional experiment that suppression occurred in the absence of added SEA does not preclude a role for SEA in suppression since we recently showed that SEA persists in the spleens of infected mice for at least 5 months after infection (29); therefore, SC preparations from chronically infected mice would be expected to contain some endogenous SEA, presumably in association with dendritic cells (30).

The nonspecificity of suppression in vitro is in keeping with our previous observations of reduced humoral and cellular immunity to injected Mb in *S. japonicum*-infected mice (10). However, the responses of SC to T- and B-cell mitogens were also depressed in the course of this infection (11), but these responses were not depressed by T cells from chronically infected mice. Thus, the mechanism(s) of depression of these mitogenic responses in the course of this infection remain to be explained.

The observation that suppressor cells for the acute proliferative response are induced in cultures of normal T cells does not exclude a role for splenic T-suppressor cells in regulation of immune responses in vivo in schistosomiasis japonica. Indeed, we have found that the adoptive transfer of Lyt 2^+ splenic T cells from the spleens of 10-week-infected mice to acutely infected mice reduces granulomatous inflammation and the level of portal hypertension in the recipients (G. R. Olds and A. B. Stavitsky, submitted for publication).

Granulomatous inflammation in both schistosomiasis japonica (23; A. W. Cheever, J. E. Byram, and F. von Lichtenberg, Parasite Immunol., in press) and schistosomiasis mansoni (31) is largely cell-mediated. In both diseases this inflammation is spontaneously down regulated by Lyt 2^+ T cells (3; Olds and Stavitsky, submitted). Suppressor T cells are induced in vitro upon addition of SEA to splenic T cells from hosts infected with either parasite (4; this study). The major difference noted thus far is the demonstration of humoral (IgG1-mediated) inhibition of granulomatous inflammation and of in vitro SEA-evoked SC blastogenesis (12) in schistosomiasis japonica (22; D. G. Colley; personal communication), but not in schistosomiasis mansoni.

Our studies suggest a complex picture of immune regulation of the in vitro proliferative response of SC to SEA, depending upon the presence of optimal concentrations of SEA and optimal numbers of T-suppressor cells either derived from the culture of normal splenic T cells or apparently preformed in the spleens of infected mice. Immune regulation in vivo presumably is even more complex. The availability of purified egg antigens, monoclonal antibodies to these antigens, and more homogeneous T- and B-cell subpopulations better characterized functionally and anti-

genically, should permit more incisive and reproducible studies of these regulatory mechanisms.

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