INFECTION AND IMMUNITY, May 1986, p. 513–518
0019-9567/86/050513-06$02.00/0

Vol. 52, No. 2

Mechanisms of In Vivo Modulation of Granulomatous Inflammation in Murine Schistosomiasis Japonicum

G. RICHARD OLDS* AND ABRAM B. STAVITSKY

Division of Geographic Medicine, Departments of Medicine and of Molecular Biology and Microbiology, Case Western Reserve University and University Hospitals, Cleveland, Ohio 44106

Received 11 November 1985/Accepted 31 January 1986

In schistosomiasis japonicum, the major pathologic lesion is the granulomatous inflammation that occurs around parasite eggs trapped in the liver. The size of these granulomas and their major sequela, a rise in portal pressure, both peak between 8 and 10 weeks after infection and then spontaneously decrease. We have shown that the adoptive transfer of the serum, but not lymphoid cells, of 30-week-infected mice caused decreases in both the size of the hepatic granulomas and the portal pressure of acutely infected recipient mice. The present study examines the role of both humoral (serum) and cellular immune mechanisms of modulation throughout the course of murine infection. Pools of serum (0.3 ml), spleen cells (5×10^7), or splenic T cells (2×10^7) from mice infected for 10, 20, and 30 weeks were adoptively transferred into mice at 4 and 5 weeks of infection. One week later (6 weeks postinfection), the portal pressure and size of hepatic granulomas in all recipient mice were determined. The 10-week-infected mouse serum occasionally lowered these values, but serum from 20- and 30-week-infected animals was consistently suppressive. The active component of 30-week-infected mouse serum coeluted with immunoglobulin G1. In contrast, 10-week-infected spleen cells or T cells consistently lowered portal pressure and granulomatous inflammation, but 20- and 30-week spleen cells did not. The phenotype of these suppressive T cells was Lyt-1^-2^+. These in vivo observations confirm earlier in vitro studies on cellular and humoral immune modulation of egg antigen-induced spleen cell blastogenesis. The current study demonstrates that both cellular and humoral regulation of granulomatous inflammation occur in murine schistosomiasis japonicum but with different kinetics: cellular mechanisms are maximal early (10 weeks) while humoral mechanisms predominate late during the chronic stage of infection (20 and 30 weeks).

During infection of C57BL/6 mice with *Schistosoma japonicum*, granulomas form around parasite eggs trapped in the hepatic sinusoids. This results in obstruction of portal blood flow and in bleeding esophageal varices. As the disease progresses from acute (6 through 15 weeks) to chronic (20 and 30 weeks), granulomatous inflammation, portal pressure, and several immune responses to soluble egg antigens (SEA) decrease dramatically (9, 10, 19). This process begins soon after the onset of egg deposition (3 to 4 weeks), with a diminution in SEA-induced responses occurring as early as 7 weeks of infection (9). This spontaneous regression of pathology and of immune responses to SEA has been termed modulation and appears to be mediated in part by serum factors (10, 19). Thus, the adoptive transfer of serum from 30-week-infected mice to acutely infected recipient animals reduced granulomatous inflammation and portal pressure (19). In contrast, the adoptive transfer of spleen cells (SC) from 30-week-infected mice had no effect. In vitro, the immunoglobulin G1 (IgG1) fraction of serum from chronically infected mice suppressed SEA-induced blastogenesis of SC from acutely infected mice (10). In vitro, SC blastogenesis is inhibited by both anti-SEA and anti-idiotypic IgG1 antibodies, which increase in titer and change in specificity during infection (17). The most dramatic diminution in granulomatous inflammation and portal pressure, however, occurs between 9 and 15 weeks of infection (19). Serum or its IgG1 fraction taken from 10- to 15-week-infected mice was significantly less effective in suppressing SEA-induced blastogenesis in vitro than 20- or 30-week serum pools (17). Other regulatory mechanisms were therefore investigated during the time of maximal downregulation. We have recently shown that Lyt-1^-2^+ suppressor T cells from the spleens of 10-week-infected mice suppress SEA-induced blastogenesis of SC from 5- or 6-week-infected mice in vitro (20).

Inasmuch as previous in vitro studies suggested that both cellular and humoral mechanisms could affect cellular responses to SEA, the present study examined the in vivo role of both humoral (serum) and cellular mechanisms throughout the course of a murine infection with *S. japonicum*. Adoptive transfer of cells, serum, or both was performed on acutely infected animals prior to the onset of any immunomodulatory events. We found that SC, specifically Lyt-1^-2^+ suppressor T cells, modulated disease early in infection (10 weeks), while humoral mechanisms, specifically IgG1 antibody, were more prominent late in the infection (20 to 30 weeks).

MATERIALS AND METHODS

Mice and their infection. C57BL/6 female mice weighing 18 to 22 g (Jackson Laboratories, Bar Harbor, Maine) were injected intraperitoneally with 25 cercariae of a Philippine strain of *S. japonicum* as Lowell University, Lowell, Mass. More than 90% of the mice with this degree of infection survive for 30 weeks.

Adoptive transfer of serum and SC. Uninfected mice and animals infected for 10, 20, or 30 weeks with *S. japonicum* were used as donors of cells and serum. Serum was prepared from blood obtained by retroorbital bleeding. In some experiments serum was chromatographed on a protein A-Sepharose column (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated at pH 8.0 (10). IgM, IgA, and nonimmunoglobulin proteins were eluted with 0.1 M sodium cit-

* Corresponding author.

TABLE 1. Effect of adoptive transfer of serum or SC from 10-week-infected mice on *S. japonicum* infection in acutely infected recipients[a]

Transfer procedure	No. of mice	Adult worms (pairs/mouse)	Body wt (g)	Spleen wt (g)	Liver wt (mg)	Hematocrit	Portal pressure (cm/H_2O molecule)	Granuloma area (10^3 μm^2)
No transfer								
Uninfected mice	3		20.3 ± 1.3	77 ± 9	1,533 ± 73	43 ± 2	5.7 ± 0.3	
6-wk-infected, untreated mice	3	5.1 ± 0.7	21.7 ± 1.9	200 ± 9	2,187 ± 103	36 ± 1	14.7 ± 0.3	92 ± 5
Transfer								
Serum type								
Normal	8	4.1 ± 0.6	21.3 ± 0.5	240 ± 14	2,134 ± 491	38 ± 1	14.0 ± 0.3	85 ± 3
10-wk infected	8	4.8 ± 0.5	21.6 ± 0.8	211 ± 9	2,174 ± 53	40 ± 1	12.4 ± 0.3	79 ± 3
SC type								
Normal	8	4.5 ± 0.7	21.6 ± 0.5	231 ± 15	2,186 ± 15	39 ± 1	12.8 ± 0.5	72 ± 2[b]
10-wk infected	8	4.5 ± 0.5	21.0 ± 0.4	194 ± 6	2,161 ± 44	40 ± 1	10.1 ± 0.5[c]	50 ± 3[c]
Serum + SC type								
Normal	8	4.6 ± 0.4	21.3 ± 0.5	208 ± 6	2,160 ± 53	39 ± 1	13.0 ± 0.6	70 ± 3[b]
10-wk infected	8	5.0 ± 0.7	21.4 ± 0.7	196 ± 6	2,174 ± 68	40 ± 1	8.9 ± 0.7[d]	52 ± 2[d]

[a] Animals were given 0.3 ml of serum, 5×10^7 SC, or both at 4 or 5 weeks of *S. japonicum* infection (25 cercariae). All animals were sacrificed at week 6. Data are means ± SEM for each group.
[b] Significantly lower than untreated animals ($P < 0.05$).
[c] Significantly lower than controls receiving an equivalent number of SC from untreated mice ($P < 0.025$).
[d] Significantly lower than controls receiving an equivalent number of SC and serum from untreated mice ($P < 0.01$).

rate, pH 8.0. Subsequently, IgG1, IgG2a, and IgG2b/IgG3 were eluted with 0.1 M citrate buffers at pH 6, 4.5, and 3.5, respectively. Fractions were pooled, concentrated, and checked for purity by microdiffusion against anti-mouse IgM, IgA, IgG1, IgG2a, IgG2b, and IgG3 antisera (Southern Biotech Association, Birmingham, Ala.) (10). Protein content was determined by the method of Lowry et al. (15).

Spleens were removed, and single-cell suspensions were made in phosphate-buffered saline. T cells were prepared by incubating 10^8 SC on a nylon wool column for 30 min at 37°C (13). The eluted cells were 95% Thy-1^-2^+, as assayed by complement lysis with anti-Thy-1.2 antibody and complement (Cedarlane, Hornby, Ontario), and contained fewer than 5% macrophages and B cells, as assessed by phagocytosis of latex particles and immunofluorescence. In some experiments spleen T cells were treated with alloantiserum to Lyt-1.2 or -2.2 determinants and complement (alloantiserum and Low Tox-M rabbit complement, Cedarlane) (11). Purity was again tested by immunofluorescence.

Recipient mice were infected with 25 cercariae of *S. japonicum*. Four weeks later they were injected intravenously with 0.3 ml of serum, 0.5 mg of purified immunoglobulin, 5×10^7 SC, 2×10^7 T cells, or a combination of these. Treatment with cells or serum was repeated 1 week later (5 weeks of infection). At 6 weeks of infection a laparotomy was performed, and portal pressures were determined. Mice were then sacrificed for the measurement of a variety of parameters (16).

Determination of portal pressure, granuloma size, and other pathologic and parasitologic parameters. The mice were anesthetized intraperitoneally with sodium pentobarbital, and a laparotomy was performed. Portal pressure was measured with a pressure manometer following cannulation of the portal vein. The liver and spleen were removed and weighed, and sections were processed for histologic examination (16). The diameter of granulomas around single freshly deposited eggs was measured in liver sections with a Vickers image-splitting eyepiece (16). At least 20 granulomas per liver were measured, and the mean of means for each group was determined for each experiment. Significance was evaluated by Student's *t* test between groups. For repeated experiments a paired *t* test was used.

RESULTS

Adoptive transfer of SC and serum from 10-week-infected mice. Adoptive transfer was performed at 4 and 5 weeks of infection, and the animals were assayed at 6 weeks since this time point was before the onset of spontaneous immunomodulation (9, 19). Eight weeks of infection has been traditionally used in adoptive transfer experiments with *S. mansoni*, but modulation in *S. japonicum* occurs earlier after patent infection. Therefore, 6 weeks has been used as the standard time of assay in murine *S. japonicum* infections (19). At 6 weeks of infection, untreated mice already had significant enlargement of the liver and spleen (60% and 43% increase, respectively), lower hematocrits (18% decrease), and higher portal pressures (160% increase) than uninfected controls (Table 1).

In the initial experiment, the adoptive transfer of serum from either normal mice or 10-week-infected animals had no effect on any of these parameters or on the size of granulomatous inflammation around freshly deposited ova observed in pathologic sections of the liver (Table 1). The adoptive transfer of 5×10^7 SC from uninfected mice did not significantly reduce portal pressure but did significantly reduce the area of granulomas from 92 (±5) $\times 10^3$ to 72 (±2) $\times 10^3$ μm^2 ($P < 0.05$). This suppressive effect of normal SC was seen in two of four in vivo experiments. A similar suppressive effect has been seen in in vitro experiments in which coculture of SC from normal animals significantly reduced SEA-stimulated thymidine incorporation by SC taken from acutely (5-week) infected mice (20). Therefore, the effects of adoptive transfer of infected SC were always compared with those for normal SC. The adoptive transfer of

5×10^7 SC from 10-week-infected mice resulted in a significant decrease in both portal pressure (from 12.8 to 10.1 cm per molecule of H_2O) and granulomatous inflammation (from 72×10^3 to 50×10^3 μm^2) (both $P < 0.01$) compared with the response in animals receiving an equivalent number of normal SC. No statistically suppressive effect was observed when 2×10^7 SC were transferred (data not shown). Larger quantities (more than 5×10^7) were not attempted since these transfers were associated with significant mortality. A suppressive effect was seen when both 5×10^7 SC and infected mouse serum were adoptively transferred into the same animal. The adoptive transfer of cells or serum or both had no effect on body weight, spleen and liver weight, or hematocrit. More importantly, there was no effect on the number of adult worms recovered from each animal.

These adoptive transfer experiments were repeated four times with different groups of 10-week-infected mice as donors for cells and serum. In addition, 2×10^7 nylon wool-purified T cells were also used. This was done since in vitro-purified T cell populations from normal animals had less effect than normal unfractionated SC on SEA-induced blastogenic responses (20). In the subsequent three experiments, no significant in vivo suppressive effect was observed with the adoptive transfer of 2×10^7 normal T cells. Some pools of 10-week-infected mouse serum suppressed both granuloma area and portal pressure (Table 2), but in general this suppression was less than that observed following adoptive transfer of 10-week-infected SC or T cells (31% suppression for four experiments with serum versus 45% suppression with SC and 56% suppression with T cells). For the same serum pool, equivalent effects were observed whether 0.3 ml or as much as 1 ml of serum was passively transferred. In these experiments portal pressure was reduced from an average of 12.3 ± 1.3 (mean of means ± SEM for four experiments) to 10.3 ± 1.4 cm of H_2O for SC ($P < 0.01$) and to 7.5 ± 0.6 cm of H_2O for T cells ($P < 0.001$). Granuloma area was reduced from an average of 62 (±9) $\times 10^3$ μm^2 for animals receiving SC from uninfected mice to 49 (±11) $\times 10^3$ μm^2 ($P < 0.025$) for animals receiving 10-week-infected SC. A more dramatic decrease was seen with 2×10^7 nylon wool-purified T cells, which reduced granuloma area from 63 (±16) $\times 10^3$ (normal T cells) or 68 (±8) $\times 10^3$ (no treatment) to 26 (±5) $\times 10^3$ μm^2 (57% suppression; $P < 0.001$). The adoptive transfer of both serum and SC to the same group of animals occasionally had additive effects on granulomatous inflammation (Table 3), but more commonly serum plus cells had no greater effect than either agent alone.

Dynamics of these two modulatory activities. Since these results were in contrast to those previously obtained with 30-week serum and lymphoid cells, the dynamics of serum and cellular modulating mechanisms were examined during the course of 30 weeks of infection. In two experiments, the modulatory activities of serum and SC from mice infected for 10, 20, and 30 weeks were directly compared (Table 3). The serum of 10-week-infected mice was minimally suppressive. Serum pools from animals infected for 20 and 30 weeks were consistently more suppressive of both portal pressure and granuloma area.

In contrast, 10-week SC were more inhibitory for both parameters than 20- and 30-week SC. The adoptive transfer of SC from the second two time points had no effect on either portal pressure of granuloma area. In an additional experiment, 2×10^7 T cells from 30-week-infected mice also had no effect on either parameter (data not shown).

Phenotype of suppressive T cells. To determine the phenotype of the suppressive T cell population of 10-week-infected SC, deletion experiments were performed. Lyt-1^+ or Lyt-2^+ cells were removed from SC or purified T cell populations by treatment with specific alloantisera and complement (11). The lysis of Lyt-2.2-positive lymphocytes abrogated the suppressive effect of 10-week SC for both portal pressure and granuloma area (Table 4). Removal of Lyt-1.2-positive cells had no effect. A similar result was obtained with nylon wool-purified T cells from which Lyt-1.2 and Lyt-2.2 cells were removed (data not shown).

Fractionation of 30-week mouse serum. To determine the active serum component for humoral-mediated modulation, highly suppressive 30-week mouse serum was fractionated on a protein A-Sepharose column. This was done because in vitro the IgG1 fraction of this serum contained all of the SEA-specific immunosuppressive activity (10). Purified IgG1 (1 mg) from 30-week-serum (0.5 mg/week for 2 weeks), but not IgG1 from the serum of uninfected mice, reduced the elevation in portal pressure by 50% and granuloma area by 40% compared with the response in acutely infected animals who received 1 mg of IgG1 purified from normal mouse serum (Table 4).

TABLE 2. Suppression of portal pressure and granuloma area in acutely infected mice following adoptive transfer of cells or serum from 10-week-infected animals[a]

Transfer procedure and expt no.	% Suppression[b] of portal pressure	P[c]	% Suppression[d] of granuloma area	P[c]
Serum				
1	19	NS	7	NS
2	12	<0.05	33	<0.05
3	53	<0.001	12	NS
4	24	<0.05	30	<0.05
Total	31[d]	<0.01	21	<0.025
SC				
1	38	<0.025	31	<0.025
2	66	<0.001	29	<0.025
3	32	<0.05	13	NS
4	43	<0.01	32	<0.01
Total	45[d]	<0.01	27	<0.025
T cells				
1	ND[e]		ND	
2	43	<0.01	53	<0.01
3	65	<0.001	57	<0.01
4	60	<0.001	61	<0.001
Total	56	<0.001	57	<0.001
Serum + SC				
1	56	<0.01	26	<0.025
2	67	<0.001	47	<0.01
3	75	<0.001	35	<0.025
4	44	<0.01	29	<0.05
Total	61	<0.001	34	<0.01

[a] C57BL/6 mice were infected with 25 *S. japonicum* cerariae and 4 or 5 weeks later given either 0.3 ml of serum, 5×10^7 unfractionated SC, or 2×10^7 spleen T cells from mice infected from 10 weeks. Control animals received serum or cells from uninfected mice. All animals were sacrificed at 6 weeks. Portal pressure was measured, and hepatic granuloma area around single recently deposited ova was determined on pathologic sections.

[b] Suppression of portal pressure was calculated by the following formula: [(control − normal) − (experimental − normal)]/(control − normal), × 100.

[c] Determined for individual experiments by the Student *t* test and for totals by the paired *t* test. NS, Not significant. All values determined versus control.

[d] Suppression of granuloma area was calculated by the following formula: (control − experimental)/control, × 100.

[e] ND, Not determined.

TABLE 3. Suppression of portal pressure and granuloma area by serum or cells from mice infected for various periods with *S. japonicum*[a]

Transfer material and length of donor infection (wk)	% Suppression[b]			
	Portal pressure	P[c]	Granuloma area	P[c]
Serum				
10	22	<0.05	19	NS
20	65	<0.001	52	<0.001
30	44	<0.01	56	<0.001
SC				
10	37	<0.02	23	<0.05
20	14	NS	6	NS
30	8	NS	8	NS

[a] C57BL/6 mice were infected with 25 *S. japonicum* cercariae and adoptively transferred with 0.3 ml of serum or 5×10^7 SC at 4 and 5 weeks of infection. Donor animals for serum and SC had been infected for 10, 20, or 30 weeks with *S. japonicum*. Controls were given serum or SC from uninfected animals. Recipient animals were sacrificed at 6 weeks, and portal pressure and hepatic granuloma area were determined.

[b] Suppression of portal pressure or granuloma area compared with controls. Data are means of two experiments with six to eight animals per group.

[c] Determined versus animals receiving normal SC or serum by paired *t* test. NS, Not significant.

DISCUSSION

The pathogenesis of the hepatic egg-induced granuloma and its modulation have been studied extensively in murine schistosomiasis mansoni (5–7). The formation of an egg granuloma is a delayed-type hypersensitivity (DTH) response to SEA released by the trapped ova. Granulomatous inflammation is much larger than the egg that evokes the response, and thus the immunopathology of this infection reflects largely the host response. This process is mediated by Lyt-1^+2^- helper T cells, which migrate into the egg granuloma (6). This process is modulated by both Lyt-1^-2^+ cyclophosphamide-sensitive Ia^+ splenic T suppressor cells and Lyt-1^+2^- suppressor inducer cells which develop during chronic infection (4–7). These cells have been shown to reduce the size of granulomatous inflammation after their adoptive transfer into acutely infected recipient mice. In vitro, these cells have been shown to suppress SEA-induced blastogenic response and lymphokine production by SC taken from acutely infected animals (6).

The granuloma in murine *S. japonicum* infection is also a DTH response. Thus, Cheever et al., using T cell-deficient mice, have shown that the induction of granuloma formation in *S. japonicum* infection is T cell dependent (4). We have previously shown that granuloma formation around parasite eggs in the lung model of *S. japonicum* infection is also T cell dependent (18).

By week 4 of *S. japonicum* infection, large hepatic granulomas can be found, and SC from these animals spontaneously secrete the lymphokine ESP, proliferate vigorously, and secrete immunoglobulin after stimulation with SEA (9, 10, 19). Both immediate and DTH reactions can be elicited by footpad injections of SEA (9). However, by week 7 the production of ESP, SEA-stimulated proliferative response of SC, and immediate and DTH responses to SEA begin to decline, so that by 10 to 15 weeks of infection these responses can no longer be elicited (9, 10). Interestingly, humoral immune responses such as total serum immunoglobulin and anti-SEA-specific immunoglobulin titers do not modulate and continue to rise throughout infection (14). Antibody production to SEA has been shown in cultures of SC from mice infected for 4 to 22 weeks (9). Granulomatous inflammation and portal hypertension peak at between 8 and 10 weeks and then decline so markedly that by 30 weeks granuloma areas are less than 10% of their maximal value and portal pressures have returned to almost normal levels (19). Using anti-mu-treated mice, Cheever et al. have shown that this modulatory process is partly B cell dependent, in that B cell-deficient mice have a similar granulomatous response at 8 weeks (peak inflammation and pathology) as untreated controls but significantly larger granulomas at 15 weeks, after the onset of immunomodulatory mechanisms (3).

The initial adoptive transfer experiments used serum and lymphoid cells from 30-week-infected mice (19). In those experiments serum, but not lymphoid cells, suppressed both granulomatous inflammation and portal hypertension in acutely infected recipients (19). Corresponding suppression of SEA-induced SC blastogenesis and immunoglobulin synthesis by 30-week serum was also noted (10). The active suppressive component in vitro coeluted with IgG1 on a protein A-Sepharose column (10). The mechanism of serum-mediated suppression was studied in vitro and appears to involve both anti-SEA IgG1 and anti-idiotypic IgG1 antibodies (17), both of which increase during chronic infection. The in vivo relevance of these in vitro observations is unknown. In the present investigation the active component of serum from 30-week-infected mice in vivo also coeluted with IgG1. To date insufficient amounts of naturally occurring anti-SEA IgG1 or anti-idiotypic IgG1 have been available for in vivo testing.

Both in vivo (this investigation) and in vitro (17) 10-week serum pools were significantly less suppressive of granulomatous inflammation and SEA-induced blastogenesis than serum from animals infected for 20 or 30 weeks. Since the most dramatic changes in portal pressure, granulomatous inflammation, and in vitro SEA-induced responses occurred between weeks 10 and 20, the present study emphasized this time interval and examined other suppressive mechanisms.

TABLE 4. Suppressive cell population in 10-week-infected mice and suppressive serum component from 30-week-infected mice[a]

Transfer material[b]	No. of mice	Portal pressure (cm/H_2O molecule)[c]	Granuloma area (10^3 μm^2)[d]
Normal SC	6	13.5 ± 0.7	60 ± 5
Normal SC (−Lyt-2.0)	6	13.8 ± 0.8	67 ± 6
Normal SC (−Lyt-1.2)	5	13.6 ± 0.8	53 ± 4
10-wk SC	6	8.9 ± 0.5[e]	29 ± 3[f]
10-wk SC (−Lyt-2.2)	6	12.3 ± 0.7	49 ± 4
10-wk SC (−Lyt-1.2)	6	9.3 ± 0.5[e]	31 ± 2[f]
Normal IgG1	8	14.6 ± 0.4	75 ± 5
30-wk IgG1	8	9.3 ± 0.5[e]	41 ± 4[f]

[a] Animals were given 5×10^7 SC or 0.5 mg of purified IgG1 at 4 or 5 weeks of *S. japonicum* infection (25 cercariae). All animals were sacrificed at week 6, and portal pressure and granuloma area were measured.

[b] −Lyt-2.2 and −Lyt-1.2 indicate that Lyt-2.2 or Lyt-1.2 cells, respectively, were lysed by monoclonal antibody and complement.

[c] Mean ± SEM.

[d] Mean of means ± SEM.

[e] Significantly lower than controls ($P < 0.02$).

[f] Significantly lower than controls ($P < 0.01$).

We have shown that splenic T cells from 10-week-infected mice will suppress SEA-induced blastogenesis in vitro (20). In the present investigation the adoptive transfer of SC from 10-week-infected mice modulated granulomatous inflammation and portal pressure in vivo. The responsible SC appeared to be T cells on the basis of their failure to be removed on nylon wool columns and their lysis with anti-Thy antibody and complement. It appeared that the phenotype was Lyt-1^-2^+ since pretreatment with Lyt-2.2 but not Lyt-1.2 alloantiserum and complement removed suppressive activity. The phenotype of the cells which were immunosuppressive in vivo, therefore, is the same as that of the cells which suppress in vitro SEA-induced blastogenesis of 5-week-infected SC (20). As has been observed in other antigen-induced systems (8, 12), both in vitro and in vivo, some suppression was seen with normal SC. This problem was circumvented in the in vitro experiments by purifying T cells and mitomycin C treatment (20). In vivo, 2×10^7 normal T cells were not suppressive and thus served as a control. As the disease progressed (20 to 30 weeks of infection), suppressive SC or T cells could no longer be demonstrated in the spleen by adoptive transfer of 4×10^7 SC or 2×10^7 splenic T cells. It is not known whether the failure of these SC to inhibit granulomatous inflammation is due to an absolute loss of a population of Lyt-2^+ suppressor cells from the spleen, to a relative loss of these cells combined with a rise in the number of T helper cells, to interference with suppression by the rising level of antibody to SEA or anti-idiotype, or to a combination of these mechanisms.

The role of IgG1 antibody to SEA as well as anti-idiotypic antibody in modulating granulomatous inflammation is supported not only by the adoptive transfer experiments and in vitro experiments but by the inverse relationship between the decline in DTH to SEA (manifested by reduced granulomatous inflammation, footpad swelling after infection of SEA, and lymphokine production by granulomas) and the rise in serum IgG1 antibody to SEA and anti-idiotypic antibody. Thus, there is split unresponsiveness in this infection; DTH to SEA is impaired but antibody production is not. This split unresponsiveness is reminiscent of the findings in some experimental models. Thus, DTH to tuberculin but not the production of circulating antibody was inhibited in guinea pigs injected with tuberculin protein before immunization with *Mycobacterium bovis* BCG (2). In another study with guinea pigs, development of DTH but not of antibody production was inhibited by prior injection of the antigen (1). Thus far there has been no generally accepted explanation of this split unresponsiveness.

The data presented strongly suggest that both cellular and humoral regulation of granulomatous inflammation and its sequela, elevated portal pressure, occurs in C57BL/6 mice infected with *S. japonicum*. Cellular suppression is mediated by Lyt-1^-2^+ T cells, occurs early in infection, and then wanes. Humoral suppression is mediated by an IgG1 antibody, presumably anti-SEA and anti-idiotype, and can be demonstrated to some extent throughout infection but is most prominent late in infection (20 to 30 weeks), when the titer of both anti-SEA and anti-idiotypic antibodies is high.

ACKNOWLEDGMENTS

G.R.O. is the recipient of a Young Investigator Award from the National Institute of Allergy and Infectious Diseases. This research was supported by Public Health Service grants AI 15351, AI-18523, and AI-21080 from the National Institutes of Health.

We thank Earlene Moss for the preparation of pathologic sections, Pierre Peters for his expert technical assistance, and Wendy Fox for typing the manuscript.

LITERATURE CITED

1. **Asherson, G. L., and S. H. Stone.** 1965. Selective and specific inhibition of 24 hour skin reactions in the guinea pig. I. Immune deviation: description of the phenomenon and the effect of splenectomy. Immunology **9**:205–209.
2. **Boyden, S. V.** 1957. The effect of previous injections of tuberculoprotein on the development of tuberculin sensitivity following BCG vaccination in guinea pigs. Br. J. Exp. Pathol. **38**:611–616.
3. **Cheever, A. W., J. E. Byram, S. Hieny, F. von Lichtenberg, M. N. Lunde, and A. Sher.** 1985. Immunopathology of *Schistosoma japonicum* and *S. mansoni* infection in B cell depleted mice. Pazrasite Immunol. **7**:399–413.
4. **Cheever, A. W., J. E. Byram, and F. von Lichtenberg.** 1985. Immunopathology of *Schistosoma japonicum* infection in athymic mice. Parasite Immunol. **7**:387–398.
5. **Chensue, S. W., and D. L. Boros.** 1979. Modulation of granulomatous hypersensitivity. I. Characterization of T lymphocytes involved in the adoptive suppression of granuloma formation in *Schistosoma mansoni*-infected mice. J. Immunol. **123**:1409–1410.
6. **Chensue, S. W., S. R. Wellhausen, and D. L. Boros.** 1981. Modulation of granulomatous hypersensitivity. II. Participation of $Ly1^+$ and $Ly2^+$ lymphocytes in the suppression of granuloma formation and lymphokine production in *Schistosoma mansoni* infected mice. J. Immunol. **127**:363–367.
7. **Cooley, D. G., F. A. Lewis, and C. W. Todd.** 1979. Adoptive suppression of granuloma formation by T lymphocytes and by lymphoid cells sensitive to cyclophosphamide. Cell. Immunol. **46**:192–200.
8. **Ferguson, R. M., S. M. Anderson, and R. L. Simmons.** 1977. In vitro generation of specific and non-specific suppression of cell-mediated cytotoxicity. Transplant. Proc. **9**:919–921.
9. **Garb, K. S., A. B. Stavitsky, and A. A. F. Mahmoud.** 1981. Dynamics of antigen and mitogen induced responses in murine schistosomiasis japonica: in vitro comparison between hepatic granulomas and splenic cells. J. Immunol. **127**:115–120.
10. **Garb, K. S., A. B. Stavitsky, G. R. Olds, J. W. Tracy, and A. A. F. Mahmoud.** 1982. Immune regulation in murine schistosomiasis japonica. Inhibition of in vitro antigen- and mitogen-induced cellular responses by splenocyte culture supernates and by purified fractions from serum of chronically infected mice. J. Immunol. **129**:2752–2758.
11. **Henry, C.** 1980. Specific antisera and complement, p. 211–212. *In* B. B. Mishell and S. M. Shiigi (ed.), Selected methods in cellular immunology. W.H. Freeman & Co., San Francisco.
12. **Janeway, C. A., Jr., S. O. Sharrow, and E. Simpson.** 1975. T cell populations with different functions. Nature (London) **253**:544–546.
13. **Julius, M. H., E. Simpson, and L. A. Herzenberg.** 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur. J. Immunol. **3**:645–649.
14. **Little, J. V., C. E. Carter, and D. G. Colley.** 1982. Serologic responses to *Schistosoma japonicum*: evaluation of total and parasite-specific immunoglobulins during the course of murine infection. J. Parasitol. **68**:519–527.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
16. **Mahmoud, A. A. F., M. A. Mandel, K. S. Warren, and L. T. Webster, Jr.** 1975. Niridazole. II. A potent long-acting suppressant of cellular hypersensitivity. J. Immunol. **114**:279–281.
17. **Olds, G. R., and T. F. Kresina.** 1985. Network interactions in *Schistosoma japonicum* infection: identification and characterization of a serologically distinct immunoregulatory auto-anti idiotypic antibody population. J. Clin. Invest. **76**:2338–2347.
18. **Olds, G. R., and A. A. F. Mahmoud.** 1981. Kinetics and

mechanisms of pulmonary granuloma formation around *Schistosoma japonicum* eggs injected into mice. Cell. Immunol. **60**:251–260.

19. **Olds, G. R., R. Olveda, J. W. Tracy, and A. A. F. Mahmoud.** 1982. Adoptive transfer of modulation of granuloma formation and hepatosplenic disease in murine schistosomiasis japonica by serum from chronically infected animals. J. Immunol. **128:** 1393–1393.
20. **Stavitsky, A. B., G. R. Olds, and L. B. Peterson.** 1985. Regulation of egg antigen-induced in vitro proliferative response by splenic suppressor T cells in murine *Schistosoma japonicum* infection. Infect. Immun. **49**:635–640.