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Deficiency of Interleukin-2 Production upon Addition of Soluble Egg Antigen to Cultures of Isolated Hepatic Granulomas or Hepatic Granuloma Cells from Mice Infected with *Schistosoma japonicum*

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***Schistosoma japonicum*-infected C57BL/6 mice show similar dynamics of hepatic granulomatous inflammation and delayed hypersensitivity elicited by soluble egg antigens (SEA) which reach peak levels at 9 weeks of infection and then spontaneously regress. In an attempt to link the level of interleukin 2 (IL-2) production to the spontaneous regression of hepatic granulomatous inflammation, the study determined the dynamics of IL-2 production by SEA-challenged isolated hepatic granulomas (HG) and cells isolated enzymatically from the HG. The production of IL-2 by SEA-stimulated HG or HG cells reached its peak when these preparations from 9-week-infected mice were stimulated and fell thereafter. Some possible mechanisms that might explain the IL-2 deficiency were examined. This deficiency is not due to the in vitro binding of IL-2 by the HG cells of infected mice and is, therefore, due rather to underproduction of IL-2. The deficiency was also not explained by reduced numbers of antigen-presenting cells (macrophages or B cells) or of L3T4$^+$ T lymphocytes. In vitro SEA-induced IL-2 production by HG cells from acutely infected mice was suppressed consistently by Lyt-2$^+$ T cells from the spleens and in the majority of our experiments by Lyt-2$^+$ T cells from the HG of mice infected for 10 weeks. These findings are consistent with the main features of our working hypothesis, but it remains to be proven that in vivo deficiency of lymphokine(s) such as IL-2 is responsible for the spontaneous decrease in granulomatous inflammation and that this lymphokine deficiency is a result of suppression.**

C57BL/6 mice infected with *Schistosoma japonicum* develop hepatic granulomatous inflammation (HGI) (14) and delayed hypersensitivity (DH) (2) as cellular immune responses to soluble egg antigens (SEA) released by parasite eggs trapped in the hepatic sinusoids. The resultant obstruction to the portal circulation causes a rise in portal pressure. Between 9 and 15 weeks of infection, there is a dramatic spontaneous regression in hepatic granuloma (HG) size (13), portal pressure (13), and DH to SEA (2). Adoptive transfer experiments implicate Lyt-2$^+$ T cells in the initial modulation of HGI (14) at 10 weeks. In vitro experiments also point to a role for suppressor cells in the reduced SEA-induced proliferative response which occurs at 7 to 8 weeks of infection (23).

In searching for a common denominator for these events, it was proposed (21) that the spontaneous regression of HGI was due to (i) the down regulation of the T_H1 subset of helper cells, which upon antigenic challenge produce lymphokines such as interleukin-2 (IL-2) (12), and (ii) the down regulation of this subset by suppressor T cells in the HG (23). In the initial test of this hypothesis, it was found that the in vitro production of IL-2 by SEA-challenged spleen cells (SC) reached its peak when cells from 7-week-infected mice were cultured and that production fell at about the same time as the HGI and DH responses (22). There was evidence that this IL-2 deficiency was caused by underproduction rather than hyperutilization of IL-2 and not by a deficiency of antigen-presenting cells (APC) or of L3T4$^+$ cells, which are required for cellular immune responses (22). There was also evidence that the deficiency might be caused by suppression of IL-2 production by Lyt-2$^+$ T cells from the spleens of 10-week-infected mice (22).

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Although the deficiency in SEA-induced IL-2 production by SC was temporally almost coincident with the drop in SEA-evoked HGI and DH, the relevance of this deficiency of in vitro IL-2 production to in vivo HGI remains to be established. Therefore, the present study examined IL-2 production upon SEA challenge and culture of both intact HGs and isolated HG cells as a function of the duration of infection. A deficiency in SEA-induced IL-2 production was observed in these cultures, and the dynamics of this deficiency corresponded temporally to the spontaneous modulation of HGI and DH in vivo. This IL-2 deficiency was not due to the in vitro binding of IL-2 by HGs or isolated HG cells, nor was the deficiency due to reduced numbers of APC or of L3T4$^+$ T lymphocytes or to suppression of IL-2 production by macrophages (Mϕ). SEA-stimulated IL-2 production by HG cells from acutely infected mice was inhibited by the coculture of Lyt-2$^+$ T cells isolated from the spleens or HGs of 10-week-infected mice.

MATERIALS AND METHODS

Mice. C57BL/6 mice were obtained from the Jackson Laboratory, Bar Harbor, Maine, and infected at Lowell University, Lowell, Mass., with 25 cercariae of a Philippine strain of *S. japonicum* (16) (Public Health Service supply contract AI 052590 from the National Institute of Allergy and Infectious Diseases). These mice consistently develop an infection in which DH (1) and HGI (13) reach a peak at 9 weeks and then spontaneously diminish to basal levels by 15 weeks.

Preparation of granulomas and granuloma cells. The livers containing HGs were homogenized for 20 s at low speed in a commercial Waring blender. The granulomas were collected and washed free of cells and parenchyma over a 50-mesh steel sieve. The granulomas were pelleted in a 50-ml plastic centrifuge tube and then suspended in 4 volumes of a sterile

medium containing RPMI 1640, 5% fetal calf serum (Hyclone Laboratories, Logan, Utah), 2,000 U of type II collagenase per ml and 20 μg of DNase I per ml (both from Sigma Chemical Co., St. Louis, Mo.), penicillin, and streptomycin. The cells were then suspended in complete medium (RPMI 1640, 10% fetal calf serum, glutamine, sodium pyruvate, arginine, folic acid, 2-mercaptoethanol, HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] buffer, penicillin, and streptomycin). After this procedure, about 90% of the cells were viable as judged by the fluorescein diacetate method (18). To determine whether this digestion regimen alters the surface of lymphocytes, one sample of SC from infected mice was digested and another sample was retained as a control. After digestion, the enzyme-treated cell suspension was indistinguishable from the untreated sample with respect to total cell number and by flow cytometry with regard to the percentages of Thy-1^+, L3T4^+, Lyt-1^+, Lyt-2^+, and immunoglobulin-positive lymphocytes.

Introduction of IL-2 production. Fifty isolated HGs or 2.5 $\times 10^6$ isolated HG cells per ml were cultured in the complete RPMI 1640 medium with 1 or 5 μg of SEA for 24 h, at which time the supernatant was collected for IL-2 assay. Twenty-four hours was found to be the time of peak IL-2 production when either isolated HGs or HG cells from acutely infected mice were challenged with antigen. At least 1 μg of SEA was required for consistent induction of IL-2 production.

Production of rat and mouse factors. SC were prepared from 250-g Sprague-Dawley rats or C57BL/6 mice. A crude IL-2 preparation was made from concanavalin A (ConA)-stimulated rat or mouse SC by adding 1 μg of ConA to 10^6 cells in complete RPMI 1640 medium for 48 h. The crude rat factor was used for the routine maintenance of CTLL-20 cells and as a positive control in the IL-2 assay. ConA-stimulated mouse SC provided a rich source of mouse IL-2, which was used in the IL-2 uptake experiments.

IL-2 assay. IL-2 activity in SC supernatants was assayed in triplicate by the capacity of these fluids to promote the proliferation of CTLL-20 (3). CTLL-20 is an IL-2-dependent, cloned murine cytotoxic T-cell line. This line was propagated in Dulbecco modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) fortified with 2% fetal calf serum (Hyclone), glutamine, sodium pyruvate, arginine, folic acid, 2-mercaptoethanol, 50% rat factor, penicillin, and streptomycin. For the assay, CTLL cells (10^4) were incubated with 100 μl of serial doubling dilutions of the samples in flat-bottom wells for 18 h and then pulsed for 6 h with 0.5 μCi of [^{3}H]thymidine per well (Dupont, NEN Research Products, Boston, Mass.). The cells were then harvested, and the samples were subjected to liquid scintillation counting. In each set of assays, standard human IL-2 (Jurkat) obtained from the Biological Response Modifiers Program, Biological Resources Branch, National Cancer Institute Frederick Cancer Research Center, Frederick, Md., was assayed at levels of 1, 5, 10, and 50 U/ml to permit probit analysis (6) of the data and the determination of the number of units of IL-2 generated. Each assay was done in triplicate.

Preparation of T-cell subsets. Lyt-1.2^+ T cells were lysed with a 1/400 dilution of monoclonal antibody (NEI-017; New England Nuclear Corp., Boston, Mass.) and Cedarlane Low-Tox rabbit complement incubated for 60 min at 37°C. The Lyt-2.2^+ cells were similarly depleted with a monoclonal antibody (TIB 150 hybridoma obtained from American Type Culture Collection, Rockville, Md.) and complement. This hybridoma was originally produced by Gottlieb et al. (4). In some experiments, the desired populations were prepared by panning with monoclonal rat immunoglobulin G2a (IgG2a) antibodies to Lyt-1^+ and Lyt-2^+ cells produced by hybridomas obtained from American Type Culture Collection (TIB 104 and 105, respectively), as previously described (23). These antibodies were affinity purified and screened for cytotoxicity before use. These hybridomas were originally produced by Ledbetter and Herzenberg (10). Briefly, the plates were coated with 50 μg of affinity-purified rabbit antibody to rat IgG2a. T cells prepared on nylon columns were suspended in L-15 medium (no fetal calf serum), anti-Lyt-1.2 or anti-Lyt-2.2 (1 μg/10^6 cells) was added, and the mixture was incubated on ice. The cells were then washed and suspended in L-15–5% fetal calf serum. Upon analysis by flow cytometry, these preparations contained about 95% Lyt-1^+ and 96% Lyt-2^+ cells, whether prepared by antibody-complement lysis or by panning.

Removal of Mϕ by adherence. Mϕ were removed from HG preparations by adherence to the bottom of 2.5-ml wells in plastic plates for 2 h at 37°C in the RPMI 1640 medium in 5% CO_2–95% air and a humidified atmosphere. The number of remaining Mϕ in the nonadherent cell population was determined by phagocytosis of fluorescein-labeled microspheres.

Analysis of cell populations by flow cytometry. The following reagents were utilized for flow cytometry of cell populations from the spleens of uninfected mice and mice infected for different lengths of time. For direct analysis, phycoerythrin-labeled rat IgG2b antibody to L3T4 (GK 1.5 clone) and fluorescein-labeled rat IgG2b antibody to Thy-1.2 (30-H12 clone) were obtained from Becton Dickinson Labware, Mountainview, Calif. The percentages of Lyt-1^+ and Lyt-2^+ cells were determined by treating the cells with monoclonal antibodies (TIB 104 and 105, respectively) and then with fluorescein-conjugated antibody to rat IgG (H^+L^+) (Caltag Laboratories, South San Francisco, Calif.). For analysis of B cells, a fluorescein-conjugated affinity-purified goat antibody to mouse IgG and IgM (Kierkegaard and Perry, Gaithersburg, Md.) was used. That this fluoresceinated antibody to mouse IgG did not react with Mϕ bearing cytophilic immunoglobulin was shown by its failure to stain peritoneal Mϕ from infected mice or Mϕ isolated from the spleens or granulomas of infected animals by adherence to plastic. Erythrocytes were lysed with NH_4Cl-Tris before analysis. Gating for lymphocytes utilized thymocytes as controls. Fluorescence was measured on an ORTHO II2 flow cytometer interfaced to the ORTHO 2151 data acquisition system. Dead cells were gated out after treatment of the preparations with propidium iodide. A total of 20,000 cells were analyzed.

Early detection of infection. Although all of the mice were originally infected with cercariae, an occasional mouse was found not to be infected; neither adult worms nor granulomas could be found by 6 to 10 weeks after infection. Therefore, to distinguish infected from noninfected mice during the early weeks before granulomas, adult worms, or antibodies to SEA were detectable, advantage was taken of our previous observation of the early development of immediate hypersensitivity and DH responses to SEA (2). Fifty micrograms of SEA in 0.05 ml of phosphate-buffered saline was injected into the left hind footpads, and 50 μg of bovine serum albumin was injected into the right hind footpads of infected mice. The immediate hypersensitivity and DH responses were measured 1 and 24 h later, respectively, by measuring the thickness of each hind footpad with vernier calipers. The DH response typically consisted almost exclusively of a mononuclear cell infiltrate. Net footpad swelling was the difference in thickness between the pad that received the SEA and that which was injected with albumin. Significant differences in both immediate hypersensitivity

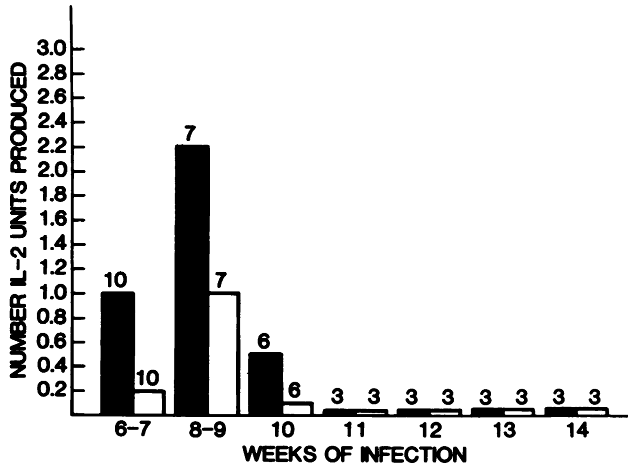

FIG. 1. Dynamics of induction of IL-2 production upon addition of SEA to isolated HGs (■) or isolated HG cells (□) from C57BL/6 mice infected with 25 cercariae of *S. japonicum* for different lengths of time. SEA (1 μg/ml) was added to 50 isolated HGs or 5×10^6 isolated HG cells for 24 h. IL-2 assays were performed in triplicate, and the values shown are the means of the highest values at each time point. The numbers at the top of each column indicate the number of experiments done at each time point; usually livers from two infected mice were utilized to prepare HGs and then to isolate cells from the HGs. Standard errors of the mean are not indicated because this value was always less than 10% of the mean value. Upon application of the Student *t* test, the following conclusions were reached. There were significant increases in the production of IL-2 from 6 to 7 and 8 to 9 weeks of infection and significant decreases in this production from 8 to 9 and 10 to 14 weeks of infection.

and DH were observed as early as 3 to 4 weeks after infection.

RESULTS

Background IL-2 activity. Before the dynamics of the SEA-induced IL-2 activity in HGs and granuloma cell cultures from infected mice were determined, a large number of IL-2 assays were performed on supernatants of cultures of 50 granulomas and 5×10^6 granuloma cells from mice infected for 6 to 20 weeks. In all instances, these supernatants contained less than 1 U of IL-2.

Dynamics of induction of IL-2 activity upon addition of SEA to HGs and HG cells from mice infected for different lengths of time. Egg laying begins during week 4 of infection. HGs are first seen during week 6. The HGs attain peak size during week 9 of infection and are then spontaneously down modulated during the next 6 weeks (13). Assayable IL-2 activity was found upon addition of SEA to isolated HGs and HG cells from mice infected for 6 to 7 weeks. Therefore, the experiments on SEA-induced IL-2 activity employed intact HGs and isolated HG cells from mice infected for 6 to 7, 8 to 9, 10, 11, 12, 13, and 14 weeks of infection.

Figure 1 presents the results of these experiments. The activity rose significantly to a peak ($P < 0.05$ by the Student *t* test) by 8 to 9 weeks of infection, dropped significantly by 10 weeks of infection, and remained low for the remainder of the time frame which was examined. Significantly more IL-2 activity was generated by the HGs than by the HG cells at all of the time points up to 10 weeks.

It was conceivable that the lowered IL-2 activity by 10 weeks was due to altered kinetics of IL-2 production or

TABLE 1. Failure of supernatants from SEA-challenged HGs or HG cells from 10-week-infected mice to inhibit the IL-2 assay[a]

Amt of IL-2[b] (U) added	Amt of IL-2 (U) assayed in presence of: Control	HG-SEA supernatant	HG cell-SEA supernatant
1	0.5	0.5	0.5
10	4.5	5.0	4.5
50	24.0	25.0	24.0

[a] 100 μl of control supernatant (medium alone), HG plus 1 μg of SEA, or HG cells plus 1 μg of SEA was added to the 100 μl of IL-2 containing the designated number of units of IL-2. Therefore, the IL-2 concentration was diluted 1:2.

[b] Jurkat IL-2.

altered antigen concentration requirements for maximal induction. However, increasing the SEA concentration to 25 μg/ml and lengthening the time of incubation of HGs or HG cells with antigen to 48 and 72 h did not increase the amount of generated IL-2 activity (data not shown).

It was also possible that the supernatants generated by the culture of HGs or HG cells from mice infected for 10 weeks or longer reduced the response of the CTLL-20 assay cells to IL-2. This possibility was addressed by adding 100 μl of supernatants from SEA-challenged HGs or HG cells from 10-week-infected mice to the assay of cells in the presence and absence of 1, 10, and 50 U of IL-2 (Jurkat). The assay results were identical in the presence of these supernatants (Table 1).

Uptake of IL-2 by HGs and HG cells from mice infected for different lengths of time. The deficiency in IL-2 production shown in Fig. 1 might be caused by hyperutilization of IL-2, including greater uptake of IL-2 by HGs or HG cells of mice infected for 10^+ weeks, or by underproduction of IL-2 or both. Therefore, the uptake of IL-2 was examined under the same conditions under which the deficiency in IL-2 production was found. Compared with that of the control CTLL cells, little or no uptake of IL-2 could be demonstrated with HG cells or intact HGs from mice infected for different lengths of time (Table 2).

Flow cytometric analysis and enumeration of cell populations and phagocytic cells in livers and granulomas of mice infected for different lengths of time. Attention was turned next to the consideration of possible mechanisms of underproduction of IL-2 by HGs or HG cells of infected mice. One

TABLE 2. In vitro uptake of IL-2 by HGs and by isolated HG cells from mice infected for different lengths of time with *S. japonicum*[a]

Expt	No. of cells	Amt of mouse IL-2[b]	Amt of IL-2 (U) in supernatant after incubation with[c]: CTLL	Intact HGs 8–9 wk	Intact HGs 11 wk	HG cells 6–7 wk	HG cells 8–9 wk	HG cells 10 wk	HG cells 11 wk
1	1×10^7	20	1	15	16	16	17	17	18
2	2×10^6	5	ND	ND	ND	4.5	4	4.5	4.5

[a] In each experiment, the cells from one mouse were used at each time interval.

[b] Mouse IL-2 was the ConA-induced supernatant from spleen cells of this species.

[c] CTLL is an IL-2-dependent cell line (see Materials and Methods). The standard error of the mean was less than 10% of the indicated values for all determinations.

ND, Not done.

TABLE 3. Cell populations in livers of uninfected mice and in HGs of mice infected with *S. japonicum*[a]

Wk of infection[b]	Total cells (no. of mice)	% of each cell population[c] (no. of mice)					
		Thy-1.2	L3T4+	Lyt-1.2+	Lyt-2.2+	Ig+	Phagocytic
0	2.1 (3)	4.5 (3)	0 (3)	2.7 (3)	0 (3)	0 (3)	7.5 (3)
4–5	3.2 (3)	12.7 (3)	6 (3)	10 (3)	0 (3)	0 (3)	17 (4)
6–7	4.0 (4)	51 (3)	35 (4)	14 (4)	11.8 (4)	1.5 (4)	24 (4)
8–9	4.2 (4)	43 (4)	24 (3)	18.6 (3)	11 (3)	32 (3)	25 (3)
10	3.7 (3)	43 (3)	20 (3)	21 (3)	15 (3)	28 (3)	24.5 (4)
11	2.3 (3)	50 (3)	21.6 (3)	21 (3)	12 (3)	15 (3)	29 (4)
14	1.8 (3)	54 (3)	24 (4)	19.8 (4)	4.5 (3)	13 (4)	20 (4)

[a] The standard error of the mean was less than 10% of the indicated values for all determinations.
[b] At week 0, liver cells were prepared by collagenase digestion of livers from uninfected mice. Mice were infected by the criterion of positive immediate and delayed footpad reactions to locally injected SEA (see Materials and Methods). At weeks 4 to 5, liver cells were isolated by collagenase digestion. At later time points, cells were prepared by collagenase digestion of isolated HGs.
[c] Phagocytic cells were determined by the phagocytosis of fluorescein-labeled microspheres. The other cell populations were assayed by flow cytometry. Ig+, Immunoglobulin-positive cells.

possibility was a numerical deficiency of one or more of the cell populations required for this response. The deficiency might be due to the actual destruction of cells by autoantibodies (7) or to the emigration of cells from the HGs. The cell populations required for the induction of cellular immune responses in general and granulomatous inflammation in particular include APC, such as Mϕ and B cells expressing surface class II major histocompatibility complex molecules, and L3T4+ T cells, which actually produce IL-2 and appear to be required for the expression of granulomatous inflammation (11). There was interest in the total numbers of T (Thy-1+) and B (immunoglobulin-positive) cells as well as in the relative numbers of Lyt-1.1+ and Lyt-2.2+ T cells because of our demonstration that Lyt-2+ T cells could suppress SEA-induced SC proliferation (2) and IL-2 production (22) in vitro and SEA-elicited granulomatous inflammation in vivo (14). Finally, the total numbers of HG cells were determined.

Table 3 summarizes the data obtained in these experiments. By 4 to 5 weeks of infection, when egg laying begins, there is an increase in Thy-1+, L3T4+, and Lyt-2+ T cells in the liver as well as an increase in the number of phagocytic cells. These cell populations increased further by 6 to 7 weeks, when the cell contents of the granulomas per se were examined for the first time. By this time, a few immunoglobulin-positive cells and many Lyt-2+ cells were also demonstrable. However, there were no significant differences in the percentages of L3T4+, Lyt-1+, and Lyt-2+ T cells and in the percentages of immunoglobulin-positive cells between the granulomas examined at 8 to 9 weeks (when peak IL-2 production was observed) and those examined subsequently when IL-2 production was depressed. An exception was the dramatic drop in Lyt-2+ cells at 14 weeks.

Role of Mϕ in the deficiency of SEA-induced IL-2. The flow cytometric studies indicated that the livers and HGs of infected mice did not have reduced numbers of B lymphocytes or Thy-1+, L3T4+, Lyt-1.2+, and Lyt-2.2+ lymphocytes. However, there was an increased number of phagocytic cells, predominantly Mϕ. In view of reports that Mϕ or Mϕ products such as prostaglandin E2 (25) could suppress antigen-induced cell proliferation or IL-2 production, this possibility was studied with respect to schistosomiasis japonica.

Table 4 shows the typical results in three of the six experiments which were done. Even when the Mϕ were reduced to 6% of the total cell numbers, there was no effect on IL-2 production.

Suppression of SEA-induced granuloma cell IL-2 production by Lyt-2+ T cells from spleens and granulomas of chronically infected mice. We previously obtained evidence for the participation of suppressor T cells in regulation of cellular immune responses to SEA both in vitro and in vivo in schistosomiasis japonica. Lyt-2+ splenic T cells from chronically infected mice inhibited the SEA-induced proliferation response of SC from acutely infected animals (23). It was then observed that the adoptive transfer of Lyt-2+ splenic T cells from 10-week-infected mice into acutely infected animals resulted in significant diminution of granulomatous inflammation in the recipients (14). In the most recent study, Lyt-2+ splenic T cells from 10-week-infected mice inhibited SEA-induced IL-2 production by SC from acutely infected animals (22).

Twelve experiments have been done to determine whether IL-2 production was suppressed by Lyt-2+ T cells from the HGs of 10-week-infected mice. In eight of them, significant suppression was observed. Table 5 shows the results of one of the experiments in which significant suppression occurred. IL-2 production was suppressed when 6-week-infected HG cells were cocultured with Lyt-2+ T cells isolated from HGs or from SC of 10-week-infected animals but not when the 6-week-infected HG cells were cocultured with unfractionated T cells from HGs of 10-week-infected animals or with Lyt-2+ T cells from the spleens of uninfected mice. The remaining experiments showed very similar results.

TABLE 4. Effects of removal of Mϕ on SEA-induced production of IL-2 by HG cells from mice infected with *S. japonicum*[a]

Expt	Amt of IL-2 (U) produced upon incubation of: HG cells[b] + SEA (μg)			HG cells depleted of Mϕ[c] + SEA (μg)			% Mϕ depletion	% Mϕ
	0	1	5	0	1	5		
1	0.5	0.5	0.4	0.6	0.5	0.6	50	12
2	0.8	0.8	0.7	0.8	0.9	0.8	20	20
3	0.5	0.5	0.5	0.5	0.4	0.5	70	6

[a] All cells were from 12-week-infected mice. The standard error of the mean was less than 10% of the indicated values for all determinations.
[b] 5×10^6 HG cells plus the indicated SEA concentrations.
[c] 5×10^6 HG cells after depletion of Mϕ as assayed by phagocytosis of fluorescein-conjugated microspheres. Values were not significantly different from control values ($P > 0.1$).

TABLE 5. Suppression of SEA-induced IL-2 production by Lyt-2^+ T cells from spleens and HGs of 10-week-infected mice[a]

Amt of SEA (μg)	Amt of IL-2 (U) produced upon culture of HG cells[b] plus:									
	Nothing	Nothing[c]	10-wk HG	10-wk HGT	10-wk Lyt-1^+ HGT	10-wk Lyt-2^+ HGT	10-wk Lyt-1^+ SCT	10-wk Lyt-2^+ SCT	Normal SC Lyt-1^+ T	Normal SC Lyt-2^+ T
0	0	0	0	0	0	0	0	0	0	0
1	1.4	0[d]	1.0[e]	1.0[e]	1.2[e]	0.4[d]	1.2[e]	0[d]	1.2[e]	1.2[e]

[a] Two livers from 6- and 10-week-infected mice, two spleens from 10-week-infected animals, and two spleens from uninfected mice were utilized to prepare cell populations.

[b] 2.5×10^6 cells of each population were cultured. Except where indicated, all HG cells were from 6-week-infected mice. HG, Cells prepared from HGs by collagenase digestion (see Materials and Methods); HGT, T cells prepared from HG cells on nylon columns; SCT, T cells prepared from spleens. The Lyt-1^+ and Lyt-2^+ T cells were prepared from HG cells or infected or normal spleen cells by panning (see Materials and Methods).

[c] In this control, 10-week-infected HG cells were used.

[d] Significantly different ($P < 0.05$) from 6-week-infected HG cells by the Student t test.

[e] Not significantly different ($P > 0.1$) from 6-week-infected HG cells by the Student t test.

DISCUSSION

Consideration of the dynamics of SEA-induced IL-2 production by HGs and HG cells from *S. japonicum*-infected mice was a logical next step in our studies of regulation of granulomatous inflammation in schistosomiasis japonica. Our previous study (22) showed reduced IL-2 activity upon culture of SC from 7-week-infected mice. This IL-2 deficiency was not due to excessive binding of IL-2, numerical deficiencies of APC or IL-2 producing cells, or suppression by Mϕ or Mϕ factors (22). Thus, this IL-2 deficiency was caused by underproduction. This deficiency could be reproduced by the coculture of SC from acutely infected animals and Lyt-2^+ T cells from the spleens of 10-week-infected mice. Thus, this deficiency coincided temporally with the down regulation of granulomatous inflammation. The aim of the present study was to extend these findings with SC to the HG, the arena in which this type of inflammation actually occurs.

Examination of the percentages of potential APC and of L3T4$^+$ T cells did not reveal a numerical deficiency; indeed, there were more than twice as many Mϕ in the HGs of chronically infected animals as in acutely infected animals (Table 3). However, there are no markers to distinguish the L3T4$^+$ T_H1 subset, which produces IL-2 and presumably mediates cell-mediated immune responses such as HGI, from the L3T4$^+$ T_H2 subset, which produces IL-4 and enhances IgE and IgG1 antibody production (12). Therefore, it has not been determined whether there are alterations in the relative or absolute numbers of these two different T-cell subsets in the course of this disease. The fact that both immediate hypersensitivity (2) and IgG1 antibody formation (9) to SEA occur in schistosomiasis japonica indicates that at least the T_H2 subset is functional. It has also not been determined whether in the course of this disease a reduced frequency of SEA-reactive IL-2-producing cells supervenes. The possibility of functional defects rather than numerical deficiencies in one or another cell population remains. Finally, the induction of a nonresponsive state in IL-2-producing T helper cells as found for helper T-lymphocyte clones stimulated with carbodiimide-treated APC (5) is also possible.

The data in Table 5 indicate that in vitro underproduction of IL-2 by HG cells can be caused by suppression of Lyt-2^+ HG T cells. It is not known why this type of suppression was not observed in all experiments. The Lyt-2^+ splenic T cells consistently inhibited IL-2 production by HG cells, so the latter population is susceptible to suppression. The Lyt-2^+ HG T-cell preparations were essentially indistinguishable in these 12 experiments on the basis of all of their properties which were examined, i.e., viability and cell surface markers of various T- and B-cell populations. However, the most likely explanation of the inconsistency in suppression is that our digestion regimen does not consistently yield uniformly biologically active cells. Therefore, additional criteria of cell function and alternative methods or modifications of present methods for cell preparation from HGs are being developed.

The finding of suppression of SEA-induced IL-2 production by Lyt-2^+ T cells isolated from HGs is in keeping with our previous findings of suppression of the in vitro SEA-induced proliferative response (23), the in vitro SEA-evoked IL-2 production by SC (22), and the suppression of HGI in vivo (14). These observations are also in keeping with reports of suppression of SEA-stimulated Mϕ inhibitory factor production by splenic suppressor cells in schistosomiasis mansoni (19), of inhibition of antigen-induced IL-2 production in granuloma-bearing mice (8), and of inhibition of mitogen-induced IL-2 production in mice infected with *Trypanosoma brucei* (20).

In our previous study (22), SEA-induced IL-2 production by 6-week-infected SC was inhibited by unfractionated T cells prepared from the spleens of 10-week-infected mice. This observation was reminiscent of our demonstration of the inhibition of the SEA-induced proliferative response of SC from acutely infected mice upon coculture with unfractionated T cells from the spleens of 10-week-infected animals (23). However, SEA-evoked IL-2 production by HG cells from 6-week-infected mice was not inhibited upon coculture with unfractionated HG T cells from 10-week-infected animals (Table 5). This discrepancy is not understood. It was demonstrated (Table 3) that the spleen and HG contained T cells of both the helper (Lyt-1^+) and suppressor (Lyt-2^+) phenotypes. However, in both systems the Lyt-1^+ T cells from the 10-week-infected mice failed to enhance IL-2 production by the SEA-challenged HG cells from 6-week-infected animals. Several non-mutually exclusive explanations may be offered for this observation. (i) The Lyt-1^+ and Lyt-2^+ preparations usually contained about 4 to 5% contamination with Lyt-2^+ and Lyt-1^+ cells, respectively. Therefore, enough Lyt-2^+ cells may have been present in the Lyt-1^+ preparation to suppress the response. (ii) The frequency of SEA-reactive Lyt-1^+ IL-2-producing T cells may have been reduced significantly in the 10-week cellular preparations. (iii) The SEA-reactive Lyt-1^+ IL-2-producing T cells may have been inactivated or tolerized by endogenous or exogenous antigen or both. These possibilities are being examined.

Table 3 presents information on the dynamics of appearance of different cell populations in the liver (before HG formation) and in the HG, presumably as a result of granulomatous inflammation in response to SEA. An increase in the number of Lyt-2^+ T cells was not observed in HGs between 6 and 10 weeks of infection, but it must be recog-

nized that these numbers do not reflect the numbers of SEA-reactive T-cell populations, which conceivably may be different at different times of infection. The frequency of these populations will be determined.

A deficiency in an eosinophile chemotactic lymphokine in the course of schistosomiasis japonica was shown previously in this (2) and another (15) laboratory. Recently, BALB/c mice infected with *S. japonicum* showed deficient ConA-induced IL-2 production by their SC (26). IL-1 production was normal. Neither suppressor Mϕ nor prostaglandin E2 caused this deficiency. The response to exogenous IL-2 was markedly suppressed by 4 weeks of infection. Thus, not only is IL-2 deficient during schistosomiasis, but a number of other cytokines involved in the cascade (1) which presumably are needed for the induction of granulomatous inflammation are also present in low concentrations. The deficiency in IL-2 has also been observed in other infectious and granulomatous diseases (17, 20, 24).

The temporal coincidence of the drop in SEA-induced IL-2 production by isolated HGs and HG cells (Fig. 1) and the spontaneous modulation of SEA-induced HGI (13) and DH (2) after 9 weeks of infection is striking. This evidence supports our working hypothesis (21) that lymphokine(s) plays an important role(s) in the pathogenesis and regulation of HGI and that regulation of lymphokine production, especially, by the T_H1 subset regulates HGI. The similarities in the numbers of cells of different types in the spleen and HG at different times after infection (22; this study) are striking and suggest that cells from the splenic microenvironment home into the liver and are responsible initially for the pathogenesis of the HG and subsequently for the regulation of HGI. Experiments are in progress in an effort to obtain additional evidence for the roles of these cells and mechanisms in the pathogenesis and regulation of HGI in schistosomiasis.

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LITERATURE CITED

1. **Farrar, W. L., M. C. Burchenall-Sparks, and H. B. Young.** 1986. Interleukin-2 induction of interferon-γ mRNA synthesis. J. Immunol. **137:**3836–3849.
2. **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.
3. **Gillis, S., M. M. Ferm, W. Ou, and K. A. Smith.** 1978. T cell growth factor: parameters of production and a quantitative microassay for activity. J. Immunol. **120:**2027–2032.
4. **Gottlieb, P. D., A. Atshak-Rothstein, K. Auditore-Hargraves, D. B. Berkoben, B. A. August, E. M. Rosche, and J. D. Benedetto.** 1980. Construction and properties of new Lyt-congenic stains and anti-Lyt 2.2 and anti-Lyt 3.1 monoclonal antibodies. Immunogenetics **10:**545–555.
5. **Jenkins, M. K., D. M. Pardoll, J. Mizuguchi, T. M. Chused, and R. H. Schwartz.** 1987. Molecular events in the induction of a non-responsive state in interleukin-2-producing T-lymphocyte clones. Proc. Natl. Acad. Sci. USA **84:**5409–5413.
6. **Jordan, G. W.** 1972. Basis for the probit analysis of an interferon plaque reduction assay. J. Gen. Virol. **14:**49–61.
7. **Kawabata, M., Y. Hosaka, M. Kumada, N. Matsui, and Y. Kobayakawa.** 1981. Thymocytotoxic autoantibodies found in mice infected with *Schistosoma japonicum*. Infect. Immun. **32:**438–442.
8. **Kobayashi, K., C. Allred, and T. Yoshia.** 1985. Mechanisms of suppressed cell-mediated immunity and impaired antigen-induced interleukin-2 production in granuloma-bearing mice. J. Immunol. **135:**2996–3003.
9. **Kresina, T. F., and G. R. Olds.** 1986. Concomitant cellular and humoral expression of a regulatory cross-reactive idiotype in acute *Schistosoma japonicum* infection. Infect. Immun. **53:**90–94.
10. **Ledbetter, J. A., and L. A. Herzenberg.** 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol. Rev. **47:**63–90.
11. **Mathew, R. C., and D. L. Boros.** 1986. Anti-L3T4 antibody treatment suppresses hepatic granuloma formation and abrogates antigen-induced interleukin-2 production in *Schistosoma mansoni* infection. Infect. Immun. **54:**820–826.
12. **Mossman, T. R., and R. L. Coffman.** 1987. Two types of mouse helper T-cell clone—implications for immune regulation. Immunol. Today **8:**223–227.
13. **Olds, G. R., R. Olveda, J. W. Tracy, and A. A. F. Mahmoud.** 1982. Adoptive transfer of granuloma formation and hepatosplenic disease in murine *schistosomiasis japonica*. J. Immunol. **128:**1391–1393.
14. **Olds, G. R., and A. B. Stavitsky.** 1986. Mechanisms of in vivo modulation of granulomatous inflammation in murine schistosomiasis japonicum. Infect. Immun. **52:**513–518.
15. **Owashi, M., H. Maruyama, and Y. Nawa.** 1986. Eosinophil chemotactic lymphokine produced by egg-associated granulomas in murine schistosomiasis japonicum. Infect. Immun. **54:**723–727.
16. **Peters, P. A., and K. S. Warren.** 1969. A rapid method of infecting mice and other laboratory animals with *Schistosoma mansoni*: subcutaneous injection. J. Parasitol. **42:**564.
17. **Reiner, N. E., and J. H. Finke.** 1983. Interleukin-2 deficiency in murine *Leishmaniasis donovani* and its relationship to depressed spleen cell responses to phytohemagglutinin. J. Immunol. **131:**1487–1491.
18. **Rotman, B., and B. W. Papermaster.** 1966. The membrane properties of living mammalian cells as studied by enzymatic hydrolysis of fluorogenic esters. Proc. Natl. Acad. Sci. USA **55:**134–141.
19. **Shensue, S. W., D. L. Boros, and C. S. David.** 1980. Regulation of granulomatous inflammation in murine *schistosomiasis*. *In vitro* characterization of T lymphocyte subsets involved in the production and suppression of migration inhibition factor. J. Exp. Med. **151:**1398–1412.
20. **Silighem, M., R. Hamers, and P. de Baetselier.** 1986. Active suppression of interleukin-2 secretion in mice infected with *Trypanosoma brucei* AnTat 1.1. Parasite Immunol. **8:**641–649.
21. **Stavitsky, A. B.** 1987. Immune regulation in *schistosomiasis japonica*. Immunol. Today **8:**228–232.
22. **Stavitsky, A. B., and W. W. Harold.** 1988. Deficiency of interleukin-2 activity upon addition of soluble egg antigen to cultures of spleen cells from mice infected with *Schistosoma japonicum*. Infect. Immun. **56:**1778–1784.
23. **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.
24. **Tarleton, R. L., and R. E. Kuhn.** 1984. Restoration of *in vitro* immune responses of spleen cells from mice infected with *Trypanosoma cruzi* by supernatants containing interleukin-2. J. Immunol. **133:**1570–1575.
25. **Walker, C., F. Kristensen, F. Bettens, and A. L. De Weck.** 1983. Lymphokine regulation of activated (G1) lymphocytes. I. Prostaglandin E-2-induced inhibition of interleukin 2 production. J. Immunol. **130:**1770–1773.
26. **Yamashita, T., T. Watanabe, and F. Sendo.** 1987. Studies on the immunological disturbance in murine *schistosomiasis japonica* from the viewpoint of the interleukin cascade reaction. Immunology **62:**215–221.