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Deficiency of Interleukin-2 Activity upon Addition of Soluble Egg Antigen to Cultures of Spleen Cells from Mice Infected with *Schistosoma japonicum*

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***Schistosoma japonicum*-infected C57BL/6 mice show similar dynamics of hepatic granulomatous inflammation (HGI) and delayed hypersensitivity (DH) elicited by soluble egg antigens (SEA) which reach peak levels at 9 weeks of infection and then spontaneously regress. The in vitro SEA-induced proliferation of spleen cells (SC) from infected animals attained its high point and then declined when SC from 5-week-infected mice were used. The present study determined the dynamics of interleukin-2 (IL-2) production by SEA-challenged SC from infected mice in an attempt to link the level of IL-2 production to the spontaneous regression of the aforementioned T-cell-mediated immune responses. The production of IL-2 by SEA-stimulated SC reached its peak when cells from 7-week-infected mice were challenged at least 2 weeks after the peak of the proliferative response, but declined at about the same time as the HGI and DH responses. Therefore, the decline in IL-2 activity cannot alone explain the diminished proliferative response but could account for the reduction in HGI and DH in vivo. 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 SC of infected mice and is, therefore, likely to be due to underproduction of IL-2. Nor is the deficiency explained by reduced numbers of antigen-presenting cells (macrophages and B cells) or of L3T4$^+$ T lymphocytes or by suppression of IL-2 production by macrophages or macrophage products such as prostaglandins. However, suppression of IL-2 production was observed consistently upon coculture of SC from acutely infected mice with SC from mice infected for 10 weeks. The cells which suppress appear to be Lyt2$^+$ T cells. The data are consistent with the hypothesis that suppressor T cells inhibit the production of IL-2 and perhaps of other cytokines or lymphokines and that this suppression explains the spontaneous down-regulation of HGI which occurs during schistosomiasis japonica.**

C57BL/6 mice infected with *Schistosoma japonicum* develop hepatic granulomatous inflammation (HGI) (22) and delayed hypersensitivity (DH) (5) 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 weeks 9 and 15 of infection, there is a dramatic, spontaneous regression in hepatic granuloma size (21), portal pressure (21), and DH to SEA (5). At weeks 7 to 8 of infection, several in vitro responses of the hepatic granuloma peak and then decline, including the synthesis and secretion of immunoglobulin M (IgM) and IgG antibody to SEA, production of a lymphokine (eosinophil stimulation promoter), and the incorporation of [^{3}H]thymidine ([^{3}H]TdR) (5). A number of immune responses of spleen cells (SC) of infected animals also show evidence of this spontaneous regression or modulation; in vitro stimulation by SEA of immunoglobulin synthesis and of [^{3}H]TdR incorporation were maximal at 5 weeks of infection and decreased to control values by 12 weeks (5). In vitro and adoptive transfer experiments implicate Lyt2$^+$ splenic T cells in the initial modulation of granulomatous inflammation (22) at 10 weeks and of the SEA-induced SC proliferative response (30). In vivo adoptive transfer experiments implicate serum IgG1 of mice infected for 20 or 30 weeks in the continuing modulation of granulomatous inflammation (22). Thus, there is evidence for early cellular regulation followed by humoral regulation of granulomatous inflammation and other SEA-stimulated cellular immune responses.

In search for a common denominator(s) for these pathogenic and regulatory events, it seemed reasonable to investigate the level of interleukin-2 (IL-2) produced upon addition of SEA to SC of infected mice. It was postulated that the reduced in vitro blastogenic response of SC to SEA (5) and the dramatic and spontaneous down-regulation of HGI (22) and DH to SEA (5) might be due to a deficiency of this hormone, which plays such a central role in the proliferation and differentiation of activated T cells in a variety of cell-mediated immune responses (7). It was also postulated that the inhibition of granulomatous inflammation observed upon adoptive transfer of Lyt2$^+$ splenic T cells from 10-week-infected mice into acutely infected animals (22) or the suppression by these T cells of SEA-evoked proliferation of SC from 5- to 6-week infected mice (29) is indirectly or directly due to inhibition of IL-2 production by suppressor T cells. Further impetus to our study was provided by observations in this (5) and another (23) laboratory of a deficiency of eosinophil stimulation promoter production by hepatic granulomas from mice infected for more than 8 weeks and, especially, by reports of deficiencies in production of IL-2 by the cells of animals and humans infected with a variety of organisms (10, 25, 30).

In our previous study, T cells which suppressed HGI were demonstrated in the spleens of 10-week-infected mice (22). Therefore, it was surmised that these cells homed to the granulomas where they suppressed the cellular immune response which mediates this inflammation. Having postulated that these suppressor cells modulated granulomatous inflammation by suppressing lymphokine, i.e., IL-2, production in the liver, we designed the present study to demon-

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strate the level of IL-2 production by SEA-challenged SC and, especially, how the dynamics of IL-2 production correlated with the dynamics of granulomatous inflammation and of DH to SEA. To establish possible links with our previous findings that splenic suppressor cells inhibited [^{3}H]TdR uptake by SC from acutely infected mice (29), we assayed both SEA-induced IL-2 production and SEA-stimulated [^{3}H]TdR at a number of time points.

The dynamics of the reduction of SEA-induced IL-2 production by SC from infected mice was found to correlate more closely with the dynamics of the drop in granulomatous inflammation and DH induced by SEA, as established in our previous studies (5, 21), than with the decrease in the SEA-induced proliferative response of SC from infected animals. There is some evidence that this reduction in SEA-stimulated IL-2 production may be the result of active suppression by Lyt2$^+$ T cells.

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 Phillipine strain of *S. japonicum* (24) (supply contract AI 052590; National Institute of Allergy and Infectious Diseases). These mice consistently develop an infection in which DH to SEA (5) and HGI (21) reach a peak at 9 weeks and then spontaneously diminish to basal levels by 15 weeks.

SC proliferative response. Single-cell suspensions of SC were prepared, washed twice, and suspended to a concentration of 10^6 cells per ml of RPMI 1640 medium (GIBCO, Grand Island, N.Y.) supplemented with 10% fetal calf serum (Hyclone Laboratories, Logan, Utah), glutamine, sodium pyruvate, arginine, folic acid, 5×10^{-5} M 2-mercaptoethanol, penicillin, and streptomycin. SC (0.2 ml) were then added to each well of a 96-well (flat-bottom) microtiter plate (Costar, Cambridge, Mass.) with 2.5, 5, or 10 μg of SEA prepared as described previously (5), and the plates were then cultured at 37°C for 72 h. [^{3}H]TdR (1 μCi per well; 5 Ci/mmol; New England Nuclear Research Products, Boston, Mass.) was 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. The assays were done in duplicate.

Induction of IL-2 production. SC (5×10^6/ml of supplemented RPMI medium) were cultured 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 peak of IL-2 production when SC from acutely infected mice were challenged with antigen.

Production of rat or mouse factor. SC were prepared from 250-g Sprague-Dawley rats of C57BL/6 mice. A crude IL-2 preparation was made from concanavalin A (ConA)-stimulated rat SC (7) and 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 source of mouse IL-2.

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 cells (6). CTLL-20 is an IL-2-dependent cloned murine cytotoxic T-cell line (kindly provided by James Finke, Cleveland Clinic Research Foundation, Cleveland, Ohio). This line was propagated in Dulbecco modified Eagle medium (GIBCO Laboratories) fortified with 2% fetal calf serum, 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 log 2 serial dilutions of the samples in flat-bottomed wells for 18 h and then pulsed for 6 h with 0.5 μCi of [^{3}H]TdR per well. 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 Facility, Frederick, Md., was assayed at levels of 1, 5, 10, and 50 U/ml to permit probit analysis (12) of the data and the determination of the number of units of IL-2 generated. Each assay was done in triplicate.

Other reagents. Ultrapure IL-1 (human) and ultrapure IL-2 (human) were obtained from Genzyme Corp. (Boston, Mass.). Sodium indomethacin trihydrate was a gift from Laurence B. Peterson (Merck Institute for Therapeutic Research, Rahway, N.J.).

Preparation of T-cell subsets. Lyt1.2$^+$ T cells were lysed with 1/4,000 monoclonal antibody (NEI-017; New England Nuclear Corp., Boston, Mass.) and Cedarlane Low-Tox rabbit complement incubated for 60 min at 37°C. The Lyt2.2$^+$ 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. (8). In some experiments, the desired populations were prepared by panning with monoclonal rat IgG2a antibodies to Lyt1$^+$ and Lyt2$^+$ cells produced by hybridomas obtained from the American Type Culture Collection (TIB 104 and 105, respectively) as previously described (29). These antibodies were affinity purified and screened for cytotoxicity before use. These hybridomas were originally produced by Ledbetter and Herzenberg (17). Briefly, the plates were coated with affinity-purified rabbit antibody to rat IgG2a. T cells prepared on nylon columns were suspended in L-15 medium (no fetal calf serum), anti-Lyt1.2 or anti-Lyt2.2 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% Lyt1$^+$ and 96% Lyt2$^+$ cells whether prepared by antibody-complement lysis or by panning.

Removal of macrophages by adherence. Macrophages were removed from SC 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 macrophages 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, we utilized the following reagents (obtained from Becton Dickinson and Co., Mountainview, Calif.): phycoerythrin-labeled rat IgG2b antibody to L3T4 (GK 1.5 clone) and fluorescein-labeled rat IgG2b antibody to Thy1.2 (30-H12 clone). The percentages of Lyt1$^+$ and Lyt2$^+$ cells were determined by treatment of the cells with monoclonal antibodies (TIB 104 and 105, respectively) followed by fluorescein-conjugated antibody to rat IgG (heavy and light chains; Caltag Laboratories, South San Francisco, Calif.). For analysis of B cells, a fluorescein-conjugated affinity-purified goat antibody to mouse IgG and IgM (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) was used. Erythrocytes were lysed with NH_4Cl-Tris

before analysis. Measurements of fluorescence were made 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 the mice were originally infected with cercariae, an occasional mouse was found not to be infected; neither adult worms nor granulomas could be found. Therefore, to distinguish infected from noninfected mice during the early weeks before granulomas, adult worms, or antibodies to SEA were detectable, we took advantage of our previous observation of the early development of immediate hypersensitivity and DH responses to SEA (5). SEA (50 μg) in 0.05 ml of phosphate-buffered saline was injected into the left hind footpad and 50 μg of bovine serum albumin was injected into the right hind footpad of infected mice. Immediate hypersensitivity and DH were measured 1 and 24 h later, respectively, by measuring the thickness of each hind footpad with vernier calipers. 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 and DH were observed as early as 3 weeks after infection.

RESULTS

Background IL-2 activity. Before determining the dynamics of the SEA-induced IL-2 activity in SC cultures from infected mice, we performed a large number of IL-2 assays on supernatants of cultures of SC from uninfected mice as well as from mice infected for 4 to 20 weeks. In all instances, these supernatants contained less than 1 U of IL-2.

Dynamics of induction of IL-2 activity and of proliferative responses upon addition of SEA to SC from mice infected for different lengths of time. In preliminary experiments, assayable IL-2 activity was first found upon addition of SEA to SC from mice infected for 3 to 4 weeks, and low or undetectable levels of IL-2 activity were generated when SEA was added to SC from mice infected for 15 to 22 weeks. Inasmuch as both the proliferative response of SC and granulomatous inflammation induced by SEA were at very low levels by 15 weeks of infection (5, 22), the remaining experiments employed SC from mice infected for 3 to 15 weeks. To determine whether there was any correlation between the dynamics of SEA-induced [^{3}H]TdR uptake and the dynamics of SEA-stimulated IL-2 response, in a number of experiments at 3, 4, 7, 12, and 15 weeks of infection the extent of both of these SEA-induced immune responses was determined. In other experiments at 6, 8, 9, and 10 weeks, only one set of determinations was done.

Figure 1 presents the results of these experiments. The level of IL-2 activity was high when SC from mice infected for 7 to 8 weeks were challenged with SEA. The activity began to drop significantly ($P < 0.05$ by Student's t test) when SC from animals infected for 10 weeks or longer were challenged with antigen and remained low for the remainder of the time frame which was examined. In confirmation of our previous results (5), the SEA-stimulated uptake of [^{3}H]TdR was first detected when SC from 3-week-infected mice were stimulated with antigen, rose to a peak with cells from 5-week-infected mice, and declined thereafter. Thus, the SEA-stimulated proliferative response dropped before the IL-2 response, and therefore the deficiency of IL-2 by itself cannot account for the drop in proliferation.

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

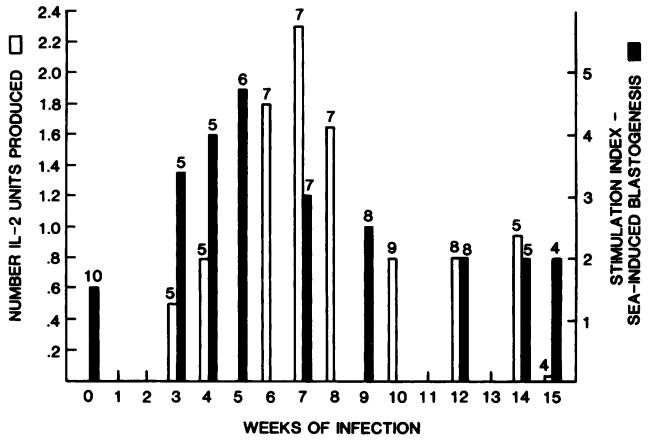

FIG. 1. Dynamics of induction of IL-2 activity and proliferative responses upon addition of SEA to SC from mice infected for different periods of time. IL-2 activity was induced by the addition of 1 or 5 μg of SEA per ml to 5×10^6 SC from uninfected mice or mice infected for 3 to 15 weeks. Blastogenesis was induced by the addition of 2.5, 5, or 10 μg of SEA per ml to 2×10^5 SC in 0.2 ml of medium for 72 h, at which time [^{3}H]TdR was added. The IL-2 assays were performed in triplicate, and the values shown are the means of the highest values at each time point. The proliferative results are represented as a stimulation index which is the ratio of counts per minute in the presence of SEA/counts per minute in the absence of SEA. The numbers at the top of each column indicate the number of experiments done at each time point. Standard error of the mean (SEM) bars 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 was a significant increase in the proliferative response between 3 and 5 weeks of infection and a significant drop in this response between 5 and 7 weeks of infection ($P < 0.05$). There was a significant increase in the production of IL-2 between 3 and 6 or 7 weeks of infection and a significant decrease in this production between 7 and 9 or 10 weeks of infection ($P < 0.05$).

to altered antigen concentration requirements for maximal induction or to both. However, increasing the SEA concentration to 25 μg and lengthening the time of incubation of SC with antigen to 48 and 72 h did not increase the IL-2 activity generated from SC from chronically infected mice (data not shown).

It was also possible that the supernatants generated by the culture of SC from mice infected for 10 weeks and 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 SC from 10-week-infected mice to the assay cells in the presence and absence of 1, 10, and 50 U of IL-2 (Jurkat). The assays results were identical in the presence and absence of these supernatants (data not shown).

Effect of exogenous IL-2 on SEA-stimulated proliferation of SC from infected mice. The results shown in Fig. 1 indicated that it was not necessary to have an IL-2 deficiency for the SEA-induced proliferation of SC from infected mice to be depressed. Nevertheless, we did examine whether there was any effect of the addition to SC from chronically infected mice of IL-2 in the form of the purified molecules or as one of a mixture of molecules induced by the addition of ConA to SC from uninfected rats or mice. Table 1 presents the typical data showing that the addition of 10 U of human Jurkat IL-2 or 10 U of IL-2 mouse or rat SC ConA supernatants did not reconstitute the depressed SEA-induced proliferation of SC from mice infected for 9 to 15 weeks. In other experiments,

TABLE 1. Effects of exogenous IL-2 on the SEA-induced proliferation of SC from chronically infected mice[a]

Expt	SEA (μg)	IL-2 species added[b] (U)	[^{3}H]TdR cpm (10^3) incorporated by 2×10^5 SC[c]					
			10-wk SC		14-wk SC		9-wk SC	
			−IL-2	+IL-2	−IL-2	+IL-2	−IL-2	+IL-2
9-9	0	Rat (10)	14[d]	13	19[d]	20[d]		
	2.5		26[d]	14	39[d]	38[d]		
	5.0		37	16	37	29		
12-12	0	Mouse (10)					17[d]	17[d]
	2.5						38[d]	32
	5.0						45[d]	23
12-12	0	Human (10)					17	20
	2.5						38	30
	5.0						45	27

[a] At each time interval, SC from individual mice were used as a source of cells.
[b] Rat IL-2 is the ConA-induced supernatant from culture of rat SC; mouse IL-2 is the ConA-induced supernatant from culture of mouse SC; human IL-2 is Jurkat preparation.
[c] SC were cultured in microtiter plates with flat-bottomed wells with indicated amounts of SEA for 72 h. [^{3}H]TdR was added for the final 18 h of culture.
[d] Value significantly different ($P < 0.05$) from culture without SEA. No significant increase noted upon addition of IL-2. SEM less than 10% of indicated value for all determinations.

1, 2, and 5 U of Jurkat IL-2 and mouse and rat ConA supernatants containing these concentrations of IL-2 also failed to reconstitute the reduced SEA-evoked proliferative response of SC from mice infected for 9 to 15 weeks.

Uptake of IL-2 by SC from uninfected mice and mice infected for different lengths of time. Although the results in Fig. 1 and Table 1 clearly dissociated the IL-2 deficiency from the depressed SC proliferation, we were interested in determining the mechanism(s) of the IL-2 deficiency. Broadly considered, the mechanisms might include hyperutilization of IL-2, including the simple possibility of greater uptake of IL-2 (which was produced) by the cells of mice infected for 10 weeks or longer, or underproduction of IL-2, or both. Failure to enhance the proliferative response with many more units of IL-2 than were produced at the height of the response strongly suggested that excessive binding of IL-2 did not account for the deficiency. Nevertheless, the uptake of IL-2 by the SC or T cells from mice infected for 4 to 15 weeks was examined under conditions which included those under which IL-2 production was measured. Thus, various numbers of SC or T cells were incubated with 5 to 25 U of murine, rat, or human (Jurkat) IL-2 for 24 h, and residual IL-2 activity then measured. Usually, there were no significant differences in IL-2 uptake between the SC and T cells from mice infected for different lengths of time (Table 2). Occasionally, (Table 2, experiments 1-30 and 4-18), cells from acutely infected mice bound more IL-2 than those from chronically infected animals.

Flow cytometric analysis of cell populations and enumeration of phagocytic cells in spleens of uninfected and infected mice. We next considered the possible mechanisms of underproduction of IL-2 by the SC of infected mice. A possibility was a numerical deficiency of one or more of the cell populations required for this response. The deficiency might be due to actual destruction of cells by autoantibodies (13) and/or to the emigration of cells from the spleen to other sites, especially to the hepatic granulomas which contain SEA (28). The cell populations required for the induction of cellular immune responses in general and granulomatous inflammation in particular include antigen-presenting cells (APC) such as macrophages and B cells expressing major histocompatibility complex surface class II molecules and L3T4$^+$ T cells which actually produce the IL-2 and are required for the expression of granulomatous inflammation (18). The number of macrophages was determined by phagocytosis of fluorescein-labeled microspheres. The numbers of the other cell populations were determined by flow cytometry. Table 3 summarizes the data obtained upon analysis of these cell populations in the spleens of uninfected mice and mice infected for different lengths of time. No numerical deficiencies were observed in the cell populations which were analyzed. Indeed, there was a rather substantial in-

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

Expt	No. of cells	IL-2 species added[b] (U)	Incubation (temp, h)	Amt of IL-2 (U) in supernatant after incubation with:				
				SC[c]			T cells[c,d]	
				6	10	14	7	14
9-17	1×10^7	Mouse (5)	37°C, 24	4.5[e]	4.5			
1-30	2.4×10^6	Rat (20)	4°C, 1	15	18			
1-23	2×10^7	Rat (20)	4°C, 2				18	18
5-23	1×10^7	Rat (20)	4°C, 1	17	17	17		
4-18	1×10^7	Human (25)	4°C, 2		10	22[f]		

[a] In each experiment, SC from only one mouse were used.
[b] Mouse and rat IL-2, ConA-induced supernatant from SC of these species; human, Jurkat IL-2.
[c] Numbers refer to number of weeks mice were infected.
[d] T cells from spleen.
[e] SEM less than 10% of indicated value for all determinations.
[f] Value significantly greater ($P < 0.05$) than 10-week value.

TABLE 3. Cell populations in the spleens of uninfected C57BL/6 mice and mice of this strain infected for different lengths of time with *S. japonicum*

Wk of infection	% of each cell population[a]			
	Thy1.2$^+$	L3T4$^+$	Immunoglobulin$^+$	Phagocytic
0	45 ± 4 (5)	22 ± 1 (2)	36 ± 2 (3)	6 ± 2.6 (5)
5	47 ± 9 (3)	23 ± 3 (2)		23 ± 4.7 (3)[b]
6	49 ± 10 (3)		30 ± 3 (2)	25 ± 4 (2)[b]
7	44 ± 2 (2)		46 ± 1 (2)	
8		22 ± 2 (3)		26 ± 5 (2)[b]
10	46 ± 11 (4)		24 ± 2 (3)	28 ± 4 (2)[b]
11	44 ± 6 (2)	24 ± 3 (3)	25 ± 2 (3)	
14		20 ± 2 (3)	54 ± 6 (3)	15 ± 5 (3)[b]

[a] Thy1.2$^+$, L3T4$^+$, and immunoglobulin$^+$ cells were determined by flow cytometry; phagocytic cells were assayed by the phagocytosis of fluorescein-labeled microspheres. Means ± SEM are shown. The number in parentheses is the number of mouse spleens assayed at each time point.
[b] Significant increase ($P < 0.05$) over control (0 week) value.

crease in the number of phagocytic cells, predominantly macrophages, in the spleens of infected compared with uninfected mice.

Role of macrophages or macrophage factors in deficiency of SEA-induced IL-2. The flow cytometric studies indicated that infected mice did not have reduced numbers of B lymphocytes or of L3T4$^+$ T cells. However, there was an increased number of macrophages. In view of reports that macrophages or macrophage products such as suppression factor (4) and prostaglandin E_2 (PGE_2) (31) could suppress mitogen- or antigen-induced cell proliferation of IL-2 production, this possibility was studied with respect to schistosomiasis japonica.

Table 4 presents the typical results of one of three experiments in which the reduction in the number of macrophages by 20 to 70% in the cultures failed to enhance IL-2 production by SEA-stimulated SC from infected mice. Indeed, IL-2 production was almost always lowered, presumably owing to the lack of enough macrophages to present antigen to T cells for IL-2 production or to a lack of IL-1 (15), or both. The possible role of PGE_2 in the IL-2 deficiency was investigated by the addition to the cultures of indomethacin, an inhibitor of PGE_2 production. As shown in a typical experiment in Table 5, the addition of this drug consistently failed to enhance IL-2 production; indeed, IL-2 production was usually reduced.

Suppression of SEA-induced IL-2 production by SC from chronically infected mice. In the absence of evidence for reduction of SEA-stimulated IL-2 production by macrophages, macrophage products such as PGE_2, or deficiency of APC or L3T4$^+$ T cells, attention was next directed toward the possibility of suppression of IL-2 production by T cells from chronically infected mice. This possibility was suggested by our previous demonstrations that Lyt2$^+$ T cells from the spleens of mice infected for 10 weeks inhibited SEA-induced proliferations of SC from acutely infected mice (30) and that these T cells inhibited granulomatous inflammation upon adoptive transfer into acutely infected animals (22). Therefore, different numbers of SC or T cells from mice infected for 9 to 15 weeks were mixed with different numbers of SC from acutely infected mice, and the resulting IL-2 activity was assayed. As controls, SC or T cells from uninfected mice were mixed with SC from acutely infected mice because in our previous study some suppression of SEA-induced SC proliferation resulted from coculture of normal SC with SC from acutely infected mice (29).

The conditions which were used successfully in our previous study (29) to demonstrate suppression, i.e., coculture of SEA plus 2×10^5 SC from 5- to 6-week-infected mice plus 2×10^4 SC from chronically infected animals, could not be used to show suppression of IL-2 production because little or no IL-2 was generated by 2×10^5 SEA-challenged SC. However, suppression of IL-2 production consistently occurred in four experiments when equal numbers (2.5×10^6) of SC from acutely infected mice and from 10-week-infected animals were cocultured in the presence of SEA (Table 6). When 2.5×10^6 normal SC were substituted for the SC from the 10-week-infected animals, suppression was not observed. Nor was suppression observed with SC from mice infected for 15 or 20 weeks (data not shown). However,

TABLE 4. Effect of removal of macrophages on the SEA-induced production of IL-2 by SC from chronically infected mice

SEA (μg)	IL-2 (U) produced upon incubation of SEA with[a]:	
	14-wk SC[b]	14-wk SC, macrophage depleted[c]
0	0.8	0.6
1	0.8	0.7
5	0.75	0.6

[a] SEM less than 10% of indicated value for all determinations.
[b] 5×10^6 SC plus indicated amount of SEA.
[c] 5×10^6 SC after depletion of 70% of macrophages as judged by phagocytosis of fluorescein-conjugated microspheres. Values are not significantly ($P > 0.1$) different from values for 14-week SC.

TABLE 5. Effect of addition of indomethacin on the SEA-induced production of IL-2 by SC from chronically infected mice[a]

SEA (μg)	IL-2 (U) produced upon incubation of SEA with[b]:	
	9-wk SC	9-wk SC + 2 ng of indomethacin
0	1.0	0.5[b]
1	0.8	0.6
5	0.8	0.8

[a] The experiment utilized cells from the spleen of a single mouse. SEM less than 10% of indicated value for all determinations.
[b] Significantly lower ($P < 0.05$) than value for 9-wk SC alone.

TABLE 6. Suppression of SEA-induced IL-2 production upon addition of SC from 9-week-infected mice to SC from 6-week-infected animals[a]

SEA (μg)	IL-2 (U) produced upon culture of the following SC for 24 h with SEA[b]:				
	NSC	6-wk SC	9-wk SC	6-wk SC + NSC	6-wk + 9-wk SC
0	0	0	0	0	0
1	0	1.8	1.0[c]	1.6[d]	0.7[e]
5	0	2.0	1.2[c]	1.8[d]	0.8[e]

[a] At each time interval, SC from a single mouse were cultured.
[b] NSC, SC from a normal mouse; 6-wk SC, SC from a 6-week-infected mouse; 9-wk SC, SC from a 9-week-infected mouse. For each cell type, 2.5×10^6 cells were used.
[c] Significantly different ($P < 0.05$) from 6-week-infected mice.
[d] Not significantly different ($P > 0.1$) from 6-week-infected mice.
[e] Significantly different ($P < 0.05$) from 6-week-infected mice.

TABLE 7. Suppression of SEA-induced IL-2 production by Lyt2$^+$ splenic T cells from mice infected for 10 weeks with *S. japonicum*[a]

SEA (μg)	IL-2 (U) produced upon culture of the following cells for 24 h with SEA[b]:					
	5-wk SC	10-wk SC	5-wk + 10-wk SC	5-wk SC + 10-wk T	5-wk SC + 10-wk Lyt1$^+$ T	5-wk SC + 10-wk Lyt2$^+$ T
0	0	0	0	0	0	0
1	1.2	0.6[c]	0.5[c]	1.0[d]	1.4[d]	0.5[c]

[a] Two spleens from 5- and 10-week-infected mice were used in this experiment.
[b] The Lyt1$^+$ and Lyt2$^+$ T cells were prepared by panning of splenic T cells (See Materials and Methods). For each cell type, 2.5×10^6 cells were used.
[c] Significantly different ($P < 0.05$) from 5-week SC.
[d] Not significantly different ($P > 0.1$) from 5-week SC.

T-cell preparations from the spleens of 9- to 14-week-infected mice suppressed IL-2 production in only 5 of 10 experiments. Further analysis of these populations revealed that they were mixtures of Lyt1$^+$ and Lyt2$^+$ cells. The numbers of Lyt1$^+$ cells were consistently around 30% of the population, but the numbers of Lyt2$^+$ cells varied from 10 to 20% in the different preparations and did not show any consistent relationship to the duration of infection. However, in four experiments in which Lyt2$^+$ cells were removed either by panning or by lysis with antibody and complement, suppression of SEA-induced IL-2 production was abolished (Table 7). Thus, it appears that Lyt2$^+$ splenic T cells from chronically infected mice can suppress SEA-evoked IL-2 production in vitro.

DISCUSSION

The deficiency of IL-2 in SEA-challenged cultures of SC from infected mice clearly does not by itself account for the reduced antigen-induced proliferation of SC from these mice because the reduced blastogenic response is observed at least 2 weeks before the diminished IL-2 production. Although attempts to reconstitute the diminished blastogenesis with exogenous IL-2 were unsuccessful, this type of reconstitution experiment is open to the criticisms that exogenous IL-2 itself may regulate proliferation through its effects on IL-2 receptors (2) and that the proliferation of T lymphocytes in response to IL-2 depends on their state of activation (1). The dichotomy between lymphokine production and cell proliferation suggested by our results is in keeping with the recent demonstration that distinct signals are required for proliferation and lymphokine gene expression in T cells (9).

An increased in vitro uptake of IL-2 by the cells of chronically infected mice compared with those of acutely infected mice or uninfected mice was not demonstrated. This low binding of IL-2 is in accord with our findings of low numbers (5 to 10%) of IL-2-receptor-bearing cells in the spleens and hepatic granulomas of acutely or chronically infected mice (A. B. Stavitsky and W. H. Harold, unpublished observations). Low numbers of IL-2-receptor-bearing cells were also found in mice recently antigen sensitized (2) and in the granulomas of human leprosy (19). Thus, the reduced IL-2 activity in SEA-challenged cultures of SC from mice infected for 10 weeks or longer cannot be attributed to increased uptake of IL-2 but is rather due to underproduction of this lymphokine.

Examination of the percentage of potential APC and of L3T4$^+$ T cells did not reveal a deficiency in the numbers of these cell types; indeed, there were more than twice as many macrophages in the spleens of infected mice as in noninfected mice. However, there are no markers to distinguish the L3T4$^+$ T_H1 T-cell subset which produces lymphokines such as IL-2 and mediates cell-mediated immune responses such as granulomatous inflammation from the L3T4$^+$ T_H2 helper subset which produces IL-4 and enhances IgE and IgG1 antibody production (20). Therefore, it is not known whether there are alterations in the relative or absolute numbers of these two different T-cell subsets in the course of murine schistosomiasis japonica. The fact that both immediate hypersensitivity (5) and IgG1 antibody production (16) occur in this disease suggests that at least the T_H2 subset is functional.

The possibility of functional defects rather than numerical deficiencies in one or another cell population required for IL-2 production remains. The induction of a nonresponsive state in IL-2-producing T-helper-cell subsets as has been found for helper T-lymphocyte clones stimulated with carbodiimide-treated APC is also a possibility (11).

Previously, we found that Lyt2$^+$ splenic T cells inhibited SEA-induced proliferation in vivo (29) and granulomatous inflammation in vivo (22). In the present study, this same subset of T cells inhibited SEA-induced IL-2 production in vitro. There is some precedent for T-cell suppression of IL-2 production in studies of other diseases such as trypanosomiasis (26) and of granuloma-bearing mice (14).

The temporal coincidence of the drop in SEA-induced IL-2 production by the spleen cells and the spontaneous modulation of SEA-induced HGI (22) and DH (5) is striking. A causal relationship between the reduced IL-2 production and reduced HGI is supported by additional evidence. Thus, the dynamics of SEA-induced IL-2 production by isolated hepatic granulomas and by cells isolated enzymatically from these granulomas is quite similar to the dynamics of this response in the spleens of infected mice; by 10 to 12 weeks of infection, there is diminished production of this lymphokine compared with that in granulomas and granuloma cells obtained from more acutely infected mice (A. B. Stavitsky and W. H. Harold, submitted for publication). The finding of IL-2 production by granulomas and granuloma cells is not surprising because APC and L3T4$^+$ cells as well as IL-2-receptor-bearing cells have been found in these preparations (A. B. Stavitsky and W. H. Harold, unpublished observations).

A deficiency in an eosinophile chemotactic lymphokine in the course of schistosomiasis japonica was shown previously in this (5) and another (23) laboratory. Recently, BALB/c mice infected with *S. japonicum* showed deficient ConA-induced IL-2 production by their SC (32). IL-1 production was normal. Neither suppressor macrophages nor PGE_2 caused this deficiency. The response to exogenous IL-2 was markedly suppressed by 4 weeks of infection. We have made similar observations in *S. japonicum*-infected C57BL/6 mice (A. B. Stavitsky and G. R. Olds, submitted for publication). Thus, not only is IL-2 deficient during schistosomiasis, but a number of other cytokines involved in the cascade (3) which

presumably are needed for the induction of granulomatous inflammation are also present in low concentration. The deficiency in IL-2 has also been observed in other infectious and granulomatous diseases (10, 25, 30).

The findings in this and a number of other studies have led to the hypothesis (27) that local production of cytokines and lymphokines by antigen-stimulated T cells is required for the development of granulomatous inflammation and that this inflammation can be down-modulated by local T-cell suppression of the T_H1 subset of $L3T4^+$ T-helper cells which produce these lymphokines (18). Experiments to test this hypothesis are in progress.

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