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Dynamic Analysis of Splenic Th1 and Th2 Lymphocyte Functions in Mice Infected with *Schistosoma japonicum*

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Recent studies indicate that egg granuloma formation in murine *Schistosoma mansoni* infection is associated with Th2-mediated immune responses. The present study was designed to analyze dynamically the Th1 and Th2 responses in *S. japonicum*-infected animals and compare them with the results seen with *S. mansoni*. C3H mice were infected with 10 to 20 cercariae of *S. japonicum* and sacrificed 3 to 22 weeks later. Spleen cells were stimulated with parasite antigens (egg and adult worm) or the mitogen concanavalin A. Interleukin-2 (IL-2), IL-4, IL-5, and gamma interferon (IFN-γ) levels were measured in the culture supernatants by enzyme-linked immunosorbent assay (ELISA) or bioassays. Additionally, cytokine-producing cells were enumerated by ELISPOT. The results show that Th2 cytokine production, characterized by IL-4 and IL-5, represents the major response in the first month after egg laying begins, while the Th1 functions of IFN-γ and IL-2 production are greatly depressed. However, by 22 weeks Th2 responses have diminished and IFN-γ production in response to concanavalin A is apparent. IL-2 responses are minimal at all times. In vitro depletion of T-cell subsets indicates that $CD4^+$ cells are the major subset responsible for production of IL-5 at 7 weeks of infection. These findings suggest that, as in the case of *S. mansoni* infection, *S. japonicum*-induced immunopathology is temporally associated with the host Th2 response, although other experiments indicate that IFN-γ is also involved.

Murine *Schistosoma japonicum* infections are characterized by peripheral eosinophilia, a prominent immunoglobulin E (IgE) antibody response, and eosinophil-rich granulomas in the liver and intestines. Recent investigations suggest that, as in the case of schistosomiasis mansoni, the immune pathogenesis of granulomatous inflammation in schistosomiasis japonica is primarily a cell-mediated immune response (1, 2, 22). Nevertheless, remarkable differences exist between the two infections in the processes of immunopathology and immune modulation. *S. japonicum*-infected mice have marked immediate and delayed-type hypersensitivity to soluble egg antigen (SEA) and require subcutaneous injection of eggs or SEA to obtain sensitization in lung model experiments (15, 33). In contrast, *S. mansoni* SEA mainly elicits delayed footpad swelling in mice, and peritoneal injection of antigen readily sensitizes the animals to induce an accelerated and augmented granulomatous response in the lungs following intravenous injection of eggs (34). Histopathologically, the early tissue reactions to *S. japonicum* eggs are often abscesslike lesions containing predominantly neutrophils and eosinophils. Plasma cells are commonly seen in later stages of the infection (1, 32, 35). In addition, the modulation of egg granulomas around *S. japonicum* eggs develops more rapidly, and the adoptive transfer at 4 to 5 weeks of infection of either serum IgG1 from mice infected for 10 to 30 weeks or splenic T cells from animals infected for 10 weeks leads to a marked reduction of granulomatous inflammation (16, 23, 29), whereas in *S. mansoni* infection, $CD8^+$ cells modulate granuloma size but antibody has no effect (4, 6, 31).

T-cell cloning techniques have resulted in a new subclassification of murine $CD4^+$ T cells into at least two subgroups, i.e., Th1 and Th2 cells, based mainly on their lymphokine synthesis. Th1 cells produce interleukin-2 (IL-2), gamma interferon (IFN-γ) and lymphotoxin, whereas Th2 cells synthesize IL-4, IL-5, IL-6, and IL-10. Although both types of $CD4^+$ cells can help B cells, there are important qualitative differences, such as in the isotypes of Ig induced. Data also suggest that delayed-type hypersensitivity is mediated by Th1 cells (5, 21).

Recent studies on cytokine analysis in a murine model of schistosomiasis mansoni showed that upon mitogenic or antigenic stimulation, the spleen cells, liver slices, or isolated hepatic egg granulomas produced significant amounts of IL-5 and IL-4 but only low levels of IFN-γ (17, 18, 27). Injection of schistosome eggs into mouse footpads initiated similar strong Th2 but minimal Th1 responses (25). In contrast, splenocytes from mice carrying unisexual schistosome infection displayed only marginal Th2 cytokine synthesis but greater Th1 cytokine responses, suggesting that Th2 functions are associated with the immunopathology of the disease and that egg deposition is the major stimulus of the Th2 cytokine response (17). In light of these findings, it was of interest to investigate the differences in immune responses between *S. mansoni* and *S. japonicum* infections by characterization of $CD4^+$ cell functions in infected hosts. The present study was designed to analyze the kinetics of splenic Th1 and Th2 cytokine responses in *S. japonicum*-infected mice and to compare the pattern of the responses with that seen in *S. mansoni* infection in order to gain a better understanding of the roles and regulations of these T-cell subgroups in the immune pathogenesis of schistosome infection.

MATERIALS AND METHODS

Experimental animals and infection. Female C3H/HeN mice aged 6 to 10 weeks were purchased from the Division of Cancer Treatment, National Cancer Institute, Frederick, Md. *Oncomelania hupensis quadrasi* snails exposed to a Philippine strain of *S. japonicum* were obtained from Lowell

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University, Lowell, Mass. The snails were crushed to release cercariae. The mice were exposed to between 10 and 15 cercariae by subcutaneous injection. Uninfected mice of the same age were used as controls.

Antigens. SEA prepared from *S. japonicum* eggs was generously provided by Thomas Kresina and G. Richard Olds, Brown University, Providence, R.I. Soluble worm antigen preparation (SWAP) was prepared by homogenization of adult worms followed by centrifugation at 10,000 × *g* for 2 h to remove insoluble components. The protein concentration of the antigens was determined by using a Micro BCA kit (Pierce, Rockford, Ill.).

Proliferation assay. At 3 to 22 weeks after infection, noninfected and infected mice were killed at intervals by peritoneal injection of 10 mg of sodium pentobarbital with 50 U of heparin and the spleens were aseptically removed. Single-cell suspensions were prepared by forcing the splenic tissue through a fine wire mesh. The dispersed cells were washed twice with RPMI 1640 supplemented with penicillin and streptomycin and finally resuspended at 10^6 cells per ml with complete RPMI 1640, i.e., RPMI 1640 containing 10% heat-inactivated fetal calf serum, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 2 mM glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml (all from Biofluids, Inc., Rockville, Md.), and 5×10^{-5} M 2-mercaptoethanol (J. T. Baker Inc., Phillipsburg, N.J.). Cells were added to 96-well flat-bottomed tissue culture plates (Costar, Cambridge, Mass.) in triplicate along with various dilutions of concanavalin A (ConA; Chemical Dynamics Corp., South Plainfield, N.J.) (from 5 μg/ml), SEA (from 20 μg/ml), and SWAP (from 40 μg/ml). The plates were cultured at 37°C for 72 h in a 5% humidified CO_2–air atmosphere. [^{3}H]thymidine (1 μCi per well; 61 Ci/mmol; American Radiolabeled Chemicals Inc., St. Louis, Mo.) was added to each well for the final 6 to 16 h of culture. The cells were then harvested with an automatic cell harvester (Skatron, Sterling, Va.) onto glass filter paper (Printed FiltermatA; Pharmacia, Turku, Finland), dried, and added to an aqueous counting solution (Beta plate scint; LKB) for scintillation counting in an LKB Wallac 1205 beta counter.

Cytokine assays. Samples (2 ml) of dispersed spleen cells from each animal were cultured at 5×10^6 cells per ml in 5-ml polystyrene tissue culture tubes (Falcon 2063; Becton Dickinson, Lincoln Park, N.J.) and stimulated with ConA (5 μg/ml), SEA (20 μg/ml), or SWAP (40 μg/ml) at 37°C. Supernatants were collected after 24 h (for IL-2 and IL-4) and 72 h (for IFN-γ and IL-5), and cytokines were measured as described below.

The production of IFN-γ and IL-5 by the cultured spleen cells was measured by a two-site sandwich enzyme-linked immunosorbent assay (ELISA) as described by Curry et al. (7) and Schumacher et al. (26), respectively. The assay for IFN-γ involved a rat anti-murine IFN-γ (anti-MuIFN-γ) monoclonal antibody (MAb) (HB170) and a polyclonal monospecific rabbit anti-MuIFN-γ antibody as the two sandwich sites and a final reaction with peroxidase-conjugated donkey anti-rabbit IgG (Jackson Immune Research Laboratories, Inc., West Grove, Pa.). The assay for IL-5 involved coating the microtiter plate wells with the rat anti-murine IL-5 (anti-MuIL-5) MAb TRFK-5 and then biotinylated TRFK-4 as the secondary reaction site. Peroxidase-conjugated streptavidin (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) was used as the secondary layer, and the reactions were visualized by incubating the plates with substrate solution ABTS (Kirkegaard & Perry). The plates were then read on an ELISA plate reader (Titertek Multiskan MC) by measuring the optical density at 415 nm. The concentration of cytokines was calculated by reference to standard curves, using known concentrations of recombinant IFN-γ or IL-5 (Genzyme Corp., Boston, Mass.).

The assays for IL-2 and IL-4 in the culture supernatant involved the IL-2- and IL-4-dependent cell line CTLL-2. The concentration of IL-2 in the supernatants was measured by its ability to maintain the cell growth after addition of MAb against IL-4 (11B11), whereas IL-4 was tested in the presence of S4B6, a rat MAb against murine IL-2. The growth of the cells was measured by a modified MTT method (30), and the cytokines were quantitated by reference to standard curves derived from known concentrations of recombinant IL-2 and IL-4 (Genzyme Corp.).

Enumeration of cytokine-producing spleen cells by ELISPOT assay. To evaluate the dynamics of populations of cytokine producing cells, we performed an enzyme-linked immunospot (ELISPOT) assay to enumerate IFN-γ- and IL-5-producing cells in the spleens. The procedure was similar to that described by Czerkinsky et al. (8) with slight modifications. In brief, individual wells of sterile nitrocellulose-bottomed 96-well Millipore HA plates (Millipore, Bedford, Mass.) were coated with either 50 μl of rat anti-MuIFN-γ (HB170) or rat anti-MuIL-5 (TRFK-5) at a concentration of 10 μg/ml at 4°C overnight. The spleen cells, stimulated with mitogen and antigens for 72 h, were washed twice with warm medium and resuspended in complete medium at 2×10^6 to 5×10^6/ml. Cell suspension (100 μl) was added to each well of the plate, and the mixtures were incubated at 37°C for another 20 h. The plates were rinsed twice with phosphate-buffered saline (PBS), and specific primary antibodies (rabbit anti-IFN-γ or biotinylated TRFK-4) were added. The secondary layer of reagents used were either biotinylated goat anti-rabbit IgG (for IFN-γ) or peroxidase-conjugated streptavidin (for IL-5). The spots representing single IFN-γ- or IL-5-producing cells were visualized by incubating the plates with 3-amino-9-ethylcarbazole in 0.05 M citrate buffer (pH 5.0). The spots were counted by using a dissecting microscope.

In vitro depletion of T-cell subsets. To correlate the phenotypes of T-cell subsets with cytokine production and to determine the subset responsible for the production of the Th2 type of cytokine, i.e., IL-5, we performed in vitro depletions of total T cells, $CD4^+$ cells, and $CD8^+$ cells as previously described (3). In brief, spleen cells were prepared as above and erythrocytes were lysed with ACK lysing buffer (B & B Scott Laboratories, Inc., Fiskeville, R.I.). The cells were resuspended at a concentration of 10^7 cells per ml in complete RPMI 1640 containing 10% rabbit complement (Low-Tox-M Rabbit Complement; Cederlane Laboratories, Ltd., Hornby, Canada) and antibodies against T-subset surface antigens (mouse polyclonal antiserum against mouse Thy1.2 [Cederlane Lab.] for Thy1.2; RL1.72, a rat MAb provided by R. Gazzinelli, for $CD4^+$ cells; and 3-155, a mouse MAb provided by Fred Ramsdell, for $CD8^+$ cells). The suspension was incubated at 37°C for 30 min and washed twice with medium. A second incubation with antibodies was done under the same conditions but with a higher cell concentration. The effectiveness of depletion was checked by immunofluorescence staining of antibody-treated samples followed by flow-cytometric analysis with an Epics C flow cytometer (Coulter Electronics). After being washed twice with medium, the cells were counted and diluted either to the original volume or to the predepletion cell number. In the latter case, the values of cytokine concentration measured

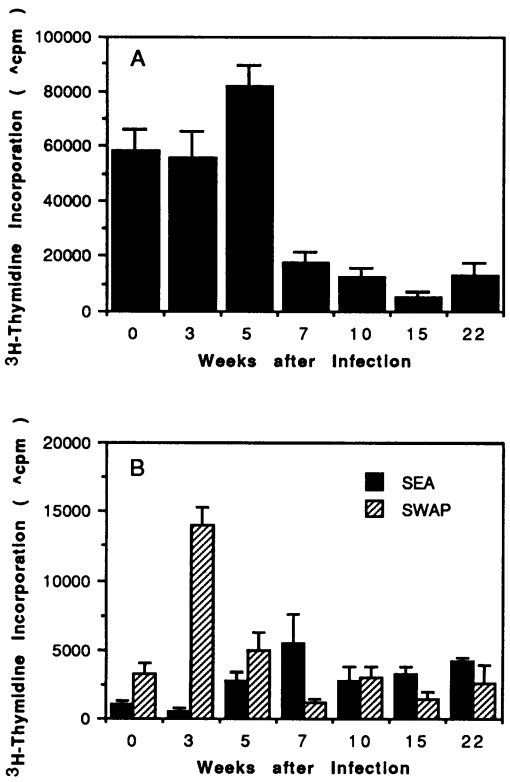

FIG. 1. Proliferative response of spleen cells from infected mice to ConA (A) or to SEA and SWAP (B). Background values were subtracted before plotting. In Fig. 1 to 5, time zero is the mean value averaged from age-matched uninfected controls and vertical bars represent 1 standard error.

were corrected to represent the same number of cells of undepleted subsets as were contained in the original sample.

Data analysis. The experiment was repeated twice. At each time interval, data from eight animals (five infected animals and three age-matched controls) were collected. Values at each time point in graphs of ELISA and the ELISPOT assay for IFN-γ and IL-5 were averaged from two sets of experiments (10 animals). Data from IL-2 and IL-4 assays and in vitro depletion assays were from one experiment (five animals). Student's two-tailed *t* test was used to compare values at two different time points. $P < 0.05$ was considered significant.

RESULTS

Proliferative responses and Th1 and Th2 cytokine profiles of spleen cells after stimulation with mitogen or schistosome antigens. The proliferative responses of spleen cells to ConA and schistosome antigens are summarized in Fig. 1. The maximal proliferative response of spleen cells to ConA was observed in mice infected for 5 weeks, and the response then declined abruptly ($P < 0.01$) and remained low. SEA first stimulated spleen cells to proliferate significantly 7 weeks after infection ($P < 0.05$), at which time the response was at its peak. SWAP stimulated a relatively strong response at 3 weeks and dropped to almost background level after 5 weeks ($P < 0.01$ compared with 3 weeks). As expected, the responses to SEA and SWAP were lower than those to ConA (Fig. 1).

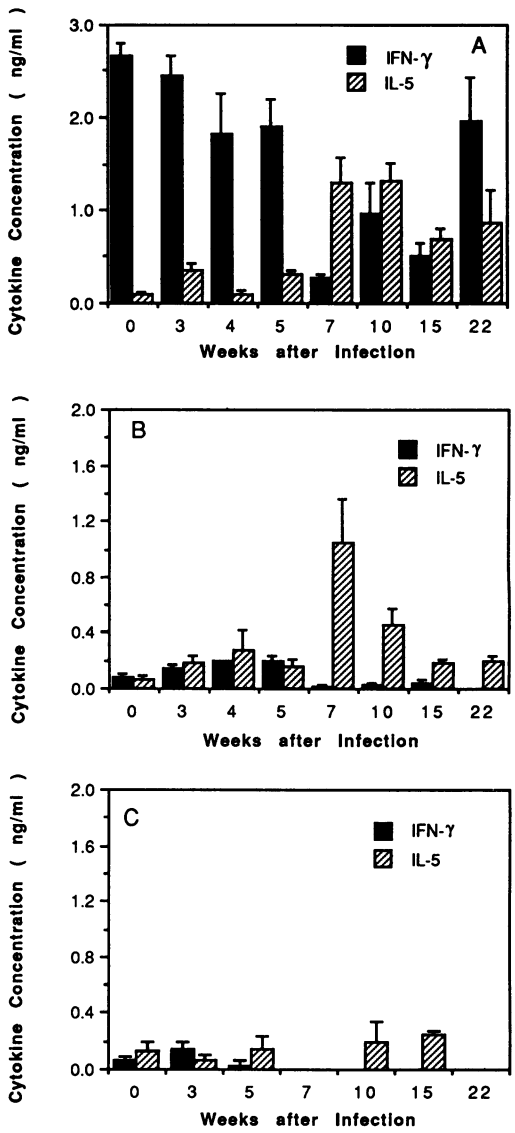

FIG. 2. IFN-γ and IL-5 production by spleen cells from mice infected from 3 to 22 weeks when stimulated by ConA (A), SEA (B), or SWAP (C).

Figures 2 and 3 present results of ELISA analysis of Th1 and Th2 cytokines synthesized by cultured spleen cells after stimulation with ConA or parasitic antigens. After ConA stimulation, spleen cells from mice infected for 3 to 5 weeks produced levels of IFN-γ comparable to those from normal spleen cells. The production of this cytokine, however, decreased significantly to almost background concentration at 7 weeks ($P < 0.001$ compared with 5 weeks) and remained at low levels until 22 weeks after infection, when, upon ConA stimulation, the cells synthesized a significantly higher concentration of IFN-γ than at 15 weeks ($P < 0.005$), a level comparable to that produced by cells from 3- to 5-week-infected mice. Neither SEA nor SWAP stimulated a significant IFN-γ response throughout that period. The production of IL-2 after ConA stimulation was reduced from the first interval significantly when compared with normal controls ($P < 0.05$) and remained low at all times tested. SEA and

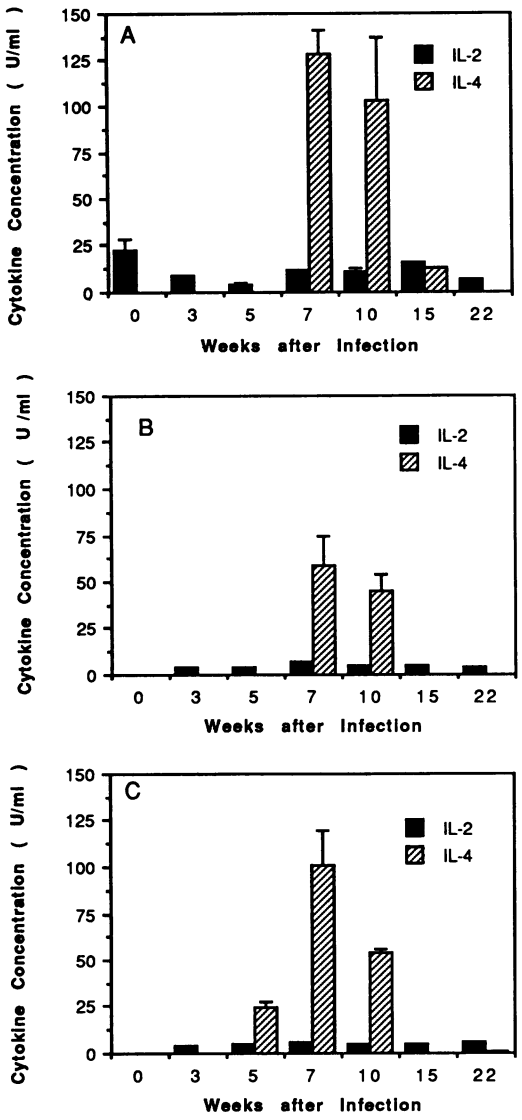

FIG. 3. IL-2 and IL-4 production by spleen cells from mice infected from 3 to 22 weeks when stimulated by ConA (A), SEA (B), or SWAP (C).

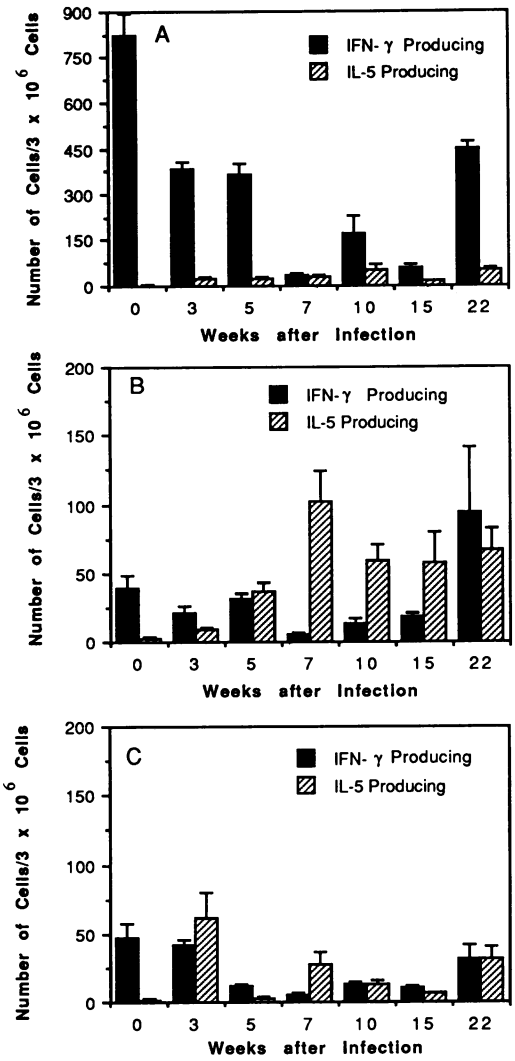

FIG. 4. Kinetics of IFN-γ- and IL-5-producing cells in spleens from infected mice after stimulation by ConA (A), SEA (B), or SWAP (C). Data are expressed as the number of spots per 3×10^6 cells. The numbers of IFN-γ- and IL-5-producing cells without stimulation were 16 and 5 per 3×10^6 cells, respectively, and did not vary significantly at different time points.

SWAP did not stimulate significant IL-2 production throughout the experiment (Fig. 3).

When Th2 cytokines were measured, the kinetics were very different from those of Th1 cytokines. Spleen cells from unsensitized mice did not respond to mitogen or antigen to produce IL-4 and IL-5. However, spleen cells from infected mice produced IL-5 from week 3 of infection after ConA stimulation ($P < 0.01$ compared with uninfected mice) and from week 7 after SEA stimulation ($P < 0.001$). IL-4 production began from week 5 to 7 after mitogen and antigen stimulation ($P < 0.001$). IL-4 and IL-5 levels peaked at 7 weeks and then gradually declined. By 15 to 22 weeks the levels of IL-4 and IL-5 became very low or undetectable (Fig. 2 and 3).

Dynamics of Th1 and Th2 cell number in mouse spleens as determined by the ELISPOT assay. The ELISPOT assay was used to enumerate the cells producing IFN-γ and IL-5. In unsensitized normal spleen a small number of IFN-γ- or IL-5-producing cells was detected. SEA and SWAP did not stimulate an increase in this background level.

Spleen cells from mice infected for 3 to 5 weeks contained significantly fewer IFN-γ-producing cells than did normal spleens after ConA stimulation (Fig. 4A; $P < 0.001$). The number of IFN-γ-producing cells became very small at 7 weeks ($P < 0.001$) and stayed small until 22 weeks, when there was again an increase compared with the number at 15 weeks ($P < 0.001$). SEA did not stimulate a significant increase of IFN-γ-producing cells until 22 weeks after infection, at which point SEA stimulated a slight but significant increase of IFN-γ-producing cells (Fig. 4B; $P < 0.05$), but stimulation of IFN-γ-producing cells by SWAP never exceeded levels produced from normal spleen cells (Fig. 4C).

ConA stimulated a slight increase of IL-5-producing cells from week 3 of infection ($P < 0.01$). The number of IL-5-producing cells peaked at 7 to 10 weeks, fell slightly by 15 weeks, and remained significantly larger than that for normal

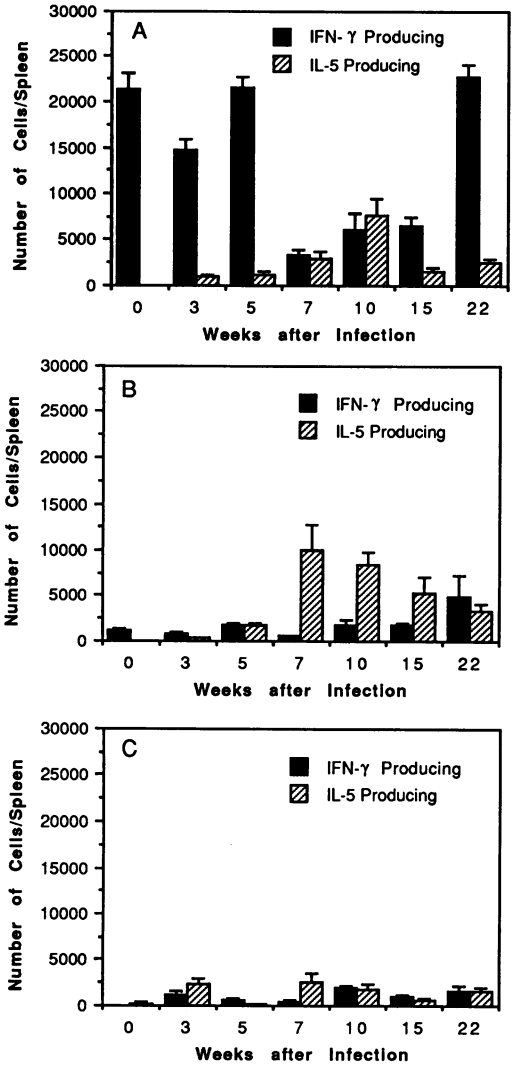

FIG. 5. Kinetics of IFN-γ- and IL-5-producing cells in spleens from infected mice after stimulation by ConA (A), SEA (B), or SWAP (C). Data are expressed as the number of spots per spleen. The numbers of IFN-γ- and IL-5-producing cells without stimulation were 1,537 and 318 per spleen, respectively.

controls at 22 weeks (Fig. 4A; $P < 0.001$). SEA stimulated a significant increase in IL-5-producing cells from week 5 ($P < 0.001$ compared with spleen cells from uninfected mice), and the number peaked at week 7 and did not drop significantly thereafter (Fig. 4B). SWAP stimulated minor but significant increases in IL-5-producing cells at 3, 7, and 22 weeks of infection (Fig. 4C; $P < 0.005$, $P < 0.01$, and $P < 0.001$, respectively).

Since the number of spleen cells varied greatly during the infection, e.g., at week 10, the spleen cells from infected mice reached a peak of about five times the number of normal controls, ELISPOT results were also expressed as cytokine-producing cells per spleen. Thus, after ConA stimulation, the number of IFN-γ-producing cells per spleen at week 5 was almost comparable to that of normal controls. The number then dropped to the lowest point 7 weeks after infection ($P < 0.001$ compared with 5 weeks) and again increased at 22 weeks when compared with 15 weeks (Fig. 5A; $P < 0.001$). SEA stimulation induced only a slight increase in the number of IFN-γ-producing cells at 22 weeks (Fig. 5B; $P < 0.05$). The peaks for IL-5-producing cells after ConA stimulation and after SEA stimulation were at 10 and 7 weeks, respectively (Fig. 5A and B).

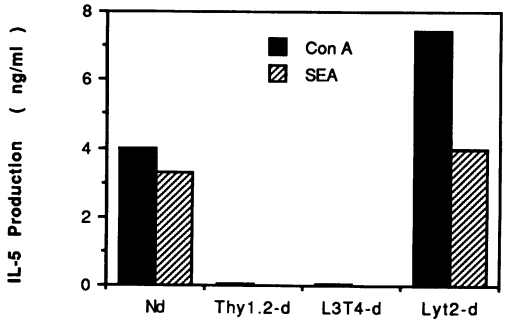

FIG. 6. IL-5 production by spleen cells from 7 weeks of infection before depletion (Nd) and after Thy1.2$^+$ depletion (Thy1.2-d), CD4$^+$ depletion (L3T4-d), or CD8$^+$ depletion (Lyt2-d).

Effect of T-lymphocyte subset depletion on cytokine secretion. Before depletion, the spleen cells from mice infected for 7 weeks produced high concentrations of IL-5 after either ConA or SEA stimulation. Depletions of Thy1.2$^+$ or CD4$^+$ cells completely abrogated this response (Fig. 6). The removal of CD8$^+$ cells, however, seemed to augment the production of this cytokine.

DISCUSSION

In this communication we present data contributing to delineation of the kinetics of Th functions in the course of experimental infection with *S. japonicum*. Thus, when spleen cells taken from acutely infected animals (7 to 10 weeks) were stimulated in vitro with mitogen or antigens, they produced high concentrations of Th2 cytokines (IL-4 and IL-5), but low levels of Th1 cytokines (IL-2 and IFN-γ). These Th2 responses reached their peak at 7 weeks and subsequently declined. Th1 responses to antigen were low throughout, although ConA stimulated moderate IFN-γ production prior to oviposition and at 22 weeks of infection. The kinetics of Th2 cytokine responses correlate well with the egg-induced granulomatous response in the hosts. However, as indicated below, the relation to granuloma regulation appears to be complex.

The kinetics of Th1 and Th2 responses in *S. japonicum* infection are similar to those in *S. mansoni*-infected mice (17), although certain differences were noted. In *S. mansoni* infection, SEA stimulated high levels of IL-2 production before egg deposition and SWAP stimulated a strong IL-5 response from weeks 7 to 15 of infection. In our experiments, however, no significant IL-2 production to mitogenic or antigenic stimulation was evident in infected mice. *S. japonicum* adult worm antigen (SWAP) stimulated strong IL-4 production but not IL-5 production. It is not clear whether these differences represent real distinctions in the mechanisms of cell-mediated responses between the two infections. However, these dissociations of Th1- and Th2-type cytokine expression in the course of infection suggest that polyclonal T-cell activation in vivo involves complex regulatory mechanisms which might be different from in vitro systems.

There have been considerable differences in the literature in the measurement of IL-2 in the immune responses during

schistosome infection. Our findings conflict somewhat with results reported by Stavitsky and Harold, who found that an *S. japonicum* SEA-induced IL-2 production by spleen cells peaked at 7 weeks and then modulated (28). Our results, however, are consistent with the observations by Yamashita et al. that IL-2 production in *S. japonicum*-infected mice was significantly inhibited even at week 2 of infection (36). Measurements of IL-2 levels in *S. mansoni*-infected animals have also varied; low levels have been reported from this laboratory (17, 25), but Mathew et al. (20) reported that spleen cells at 6 to 8 weeks produced high levels of IL-2 after ConA and SEA stimulation. Administration of recombinant IL-2 to acutely infected, anti-L3T4 MAb-treated, or chronically infected mice reversed the diminished or modulated granulomatous response (20). Our present results are therefore comparable to those seen in *S. mansoni* infection in mice in this laboratory (17, 25). Possible explanations for these discrepancies may be the difference in assay conditions or even the mouse strains used. Recent in vitro analysis of cytokine mRNA in *S. mansoni* infection demonstrated that IL-4 mRNA was abundant in the granulomatous livers, mesenteric lymph nodes, and spleens of infected mice but that IL-2 mRNA was not detected (17a). Clearly, the relative significance of different cytokines in the immune responses in schistosome infection must be further clarified.

Our study also provides evidence confirming that egg deposition is a major factor responsible for the increased Th2 response and depressed Th1 response in schistosome infection, since Th2 responses were switched on and Th1 switched off at the time when the worms began to deposit eggs in the host tissues. Recent studies showed that the regulation of Th subsets involves more complex mechanisms. It has been shown that Th2 cells may secrete factors that inhibit cytokine production by Th1 cells (11). One of these factors, IL-10, is produced in large quantities in *S. mansoni* infection (27a). Other studies suggest that the pattern of antigen stimulation or the lymphoid tissue microenvironment exerts regulatory influences over helper T-cell function (9, 30). In addition, macrophages, major components of granuloma formation, might participate in the regulation of Th functions through either antigen presentation or IL-1 production. The latter cytokine has been shown to be important in regulating the pattern of cytokines produced by T-cell subsets during activation (12), to be able to cause a reduction in IL-2 or IFN-γ production by activated T cells although increasing IL-4 production (9). Macrophages have been shown to be responsible for the depression of proliferative response of spleen cells to mitogens in *S. mansoni*-infected mice at 7 to 8 weeks of infection (10); this is also the time when Th2 responses switch on and Th1 responses switch off.

As the infection progressed to immune-modulated status, we observed that the production of IL-4 and IL-5 became low or undetectable by 15 to 22 weeks after infection, indicating that Th2 functions are downregulated by some factors in immune-modulated hosts. It has been well documented that although the immune modulation in mice infected with *S. mansoni* is mediated by a suppressor cell cascade involving several well-defined T-cell subpopulations, modulation in *S. japonicum* infection is mediated partly by suppressive serum components. The active serum components for both in vivo and in vitro suppression were found to be of the IgG1 isotype (15, 19), whose production is preferentially enhanced by IL-4. In vitro analysis showed that the production of antibodies to *S. japonicum* SEA by spleen cells after SEA stimulation was first detected at 4 weeks, remained elevated between 6 and 12 weeks, and decreased only moderately by 22 weeks (15). The overlapping of IL-4 production and antibody responses during the infection may suggest a possible link between the two phenomena.

It is of interest that at the time of Th2 function depression, there is a recovered production of IFN-γ by mitogen-stimulated spleen cells. At the same time, SEA and SWAP also stimulated a minor increase in the number of IFN-γ-producing cells. Our findings indicate that the total number of $CD8^+$ cells reached its maximum at this stage (data not shown), and other reports show that $CD8^+$ cells might also be responsible for the downregulation of IL-4 and IL-5 production by strongly influencing the Th1/Th2 ratio and secreting IFN-γ (13, 14). It is reasonable to postulate a role for IFN-γ in the regulation of Th2 functions in immune modulation. We are currently investigating the possible interrelations between Th2 cytokine downregulation and the recovered IFN-γ response in this process.

The relation of Th1 and Th2 cytokine responses to the induction and regulation of granulomatous reactions is more complex than is suggested by the augmentation of Th2 cytokines during granuloma formation and their diminution during granuloma modulation. Owhashi et al. have reported a decrease in granuloma size in IgE-deficient *S. japonicum*-infected mice (24), thus implicating an IL-4-dependent response in the reaction to eggs. However, we have found that depletion of Th2-related IL-5 or Th1-related IFN-γ decreases the size of granulomas around *S. japonicum* eggs (3a).

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