Downregulation of Th1 Cytokine Production Accompanies Induction of Th2 Responses by a Parasitic Helminth, *Schistosoma mansoni*

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Summary

In the mouse, infection with *Schistosoma mansoni* results in an egg-producing infection and associated disease, whereas vaccination with attenuated larval stages produces a substantial and specific immunity in the absence of egg-induced pathology. Preliminary data showing enhanced interleukin-5 (IL-5) production by T cells from infected mice and interferon γ (IFN-γ) synthesis by cells from vaccinated animals (7), suggested differential $CD4^+$ subset stimulation by the different parasite stimuli. To confirm this hypothesis, lymphocytes from vaccinated or infected animals were compared for their ability to produce IFN-γ and IL-2 (secreted by Th1 cells) as compared with IL-4 and IL-5 (characteristic Th2 cytokines). After stimulation with specific antigen or mitogen, T cells from vaccinated mice or prepatently infected animals responded primarily with Th1 lymphokines, whereas lymphocytes from patently infected mice instead produced Th2 cytokines. The Th2 response in infected animals was shown to be induced by schistosome eggs and directed largely against egg antigens, whereas the Th1 reactivity in vaccinated mice was triggered primarily by larval antigens. Interestingly, Th1 responses in mice carrying egg-producing infections were found to be profoundly downregulated. Moreover, the injection of eggs into vaccinated mice resulted in a reduction of antigen and mitogen-stimulated Th1 function accompanied by a coincident expression of Th2 responses. Together, the data suggest that coincident with the induction of Th2 responses, murine schistosome infection results in an inhibition of potentially protective Th1 function. This previously unrecognized downregulation of Th1 cytokine production may be an important immunological consequence of helminth infection related to host adaptation.

Eosinophila and elevated serum IgE are immunological hallmarks of infection with parasitic helminths (1). Recently, experimental studies in mice have revealed that both of these responses, along with other manifestations of immediate hypersensitivity, are under the control of cytokines secreted by the Th2, but not Th1, subset of $CD4^+$ T lymphocytes (2–6). This has been clearly illustrated in mice infected with the important human pathogen *Schistosoma mansoni*, where IgE and eosinophil levels are reduced to background by the administration of neutralizing mAbs to IL-4 and IL-5, respectively (7, 8). Although they represent dominant responses in schistosome infected mice, there is no definitive evidence that either IgE or eosinophils are involved in the acquired resistance to superinfection (concomitant immunity) demonstrated by these animals. Indeed, resistance in this system is largely nonspecific in nature (9) and due in part to pathological changes in the hepatic vasculature that result from the T cell–dependent granulomatous inflammation characteristic of schistosomiasis (10, 11). Granulomas develop in response to schistosome eggs, up to 600 of which are produced per day by each male/female worm pair and many of which become trapped in the liver. Some eggs are excreted and initiate the extramammalian phase of the life cycle that culminates in the production of cercariae which, by direct skin penetration, establish infection in a new host. The larval parasites (schistosomula) subsequently require ~6 wk to mature into adult worms living in the hepatic-portal vasculature.

The immunologic sequelae of percutaneous infection with cercariae attenuated by irradiation are quite different from those seen after exposure to normal schistosomes. Irradiated parasites, which die before maturing and never oviposit, induce a strong $CD4^+$ T cell–dependent protective immunity (12–14) in the absence of eosinophilia or elevated serum IgE (7). This resistance to challenge infection is deficient in inbred mice carrying genetic defects in cell-mediated immunity (15) and in animals depleted of the Th1 cell product IFN-γ,

but not in those depleted of IL-4 of IL-5 (16). In addition, preliminary data from this laboratory have indicated that T cells from vaccinated mice predominantly synthesize IFN-γ, in contrast to those from infected animals that produce IL-5 (7). Thus, taken together, these previous studies suggest that infection stimulates a nonprotective Th2-type response while vaccination induces protective Th1 cells.

In the present study, we have systematically assayed T lymphocyte IFN-γ and IL-2 (to detect Th1 function), as well as IL-4 and IL-5 (measures of Th2 activation) responses to formally establish that infection with normal or irradiated cercariae results in the stimulation of different Th subsets. Our results indicate that before the onset of egg laying (the prepatent period), T cells from mice infected with normal parasites secrete a similar pattern of Th1 type cytokines to those seen in vaccinated animals. Only after oviposition (when the infection is considered patent) does a strong TH2 response, which appears to be largely directed towards eggs, become evident. An unexpected finding is that a marked decrease in IFN-γ and IL-2 secretion coincides with elevated IL-4 and IL-5, suggesting a link between Th2 responses and the downregulation of Th1 function. This observation is of fundamental importance from the point of view of Th subset regulation and the immunological control of helminth infections.

Materials and Methods

Parasites and Experimental Infections. *S. mansoni* (NMRI strain) parasites were obtained from the Biomedical Research Institute (Rockville, MD). 6–8-wk-old female C57BL/6 mice from the National Cancer Institute (Frederick, MD) were percutaneously infected via the tail with ~40 or 400 normal or radiation attenuated (50 krad, ^{60}Co) cercariae. Adult schistosomes were perfused from the hepatic vasculature of mice infected 6 wk previously with normal cercariae (17). Schistosomula were prepared from cercariae by mechanical transformation as described previously (18). Both of these life cycle stages were used for preparing antigen (see below). Eggs were recovered from the livers of 8-wk infected mice as described (19, 20). After washing in PBS containing penicillin (100 U/ml) and streptomycin (100 μg/ml), some of the eggs were frozen for preparing soluble antigen (see below) while others were counted and injected into vaccinated mice at doses of 5×10^3 intravenously (via the tail vein) plus 3.5×10^4 intraperitoneally. This dose represents a conservative estimate of the number of eggs produced by eight worm pairs over the course of 2 wk and thus mimics the egg antigen stimulus to which mice infected with 40 normal cercariae are exposed between weeks 6 and 8.

Antigens. Soluble extracts of schistosomula, adult worms and eggs, in PBS, were prepared as described in detail previously (19–21), sterile filtered through 0.45-μm membranes (Millipore, New Haven, CT), assayed for protein content using the Biorad method (Biorad Laboratories, Richmond, CA) and stored at −40°C until use.

Footpad Inoculations. Mice were injected in the rear footpads with either 50 μg of soluble egg antigen, 5×10^3 living or frozen and thawed (dead) eggs or 5×10^3 living or frozen and thawed schistosomula, suspended in 50 μl of PBS. Popliteal lymph nodes draining the footpads were collected 7–10 d later.

T Cell Cytokine Response Assays. Mice were killed by cervical dislocation and their spleens or lymph nodes were removed under aseptic conditions. Single cell suspensions were prepared by forcing lymphoid tissues through fine wire mesh, lysing splenic erythrocytes with ACK lysing buffer (NIH media kitchen), and washing extensively with DME. Viable cells (those that excluded trypan blue) were suspended in tissue culture medium (TCM; DME, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 30 mM Hepes, 5×10^{-5} 2-ME and 10% FCS) at 10^7/ml and incubated alone or with antigen at 5 or 50 μg/ml, or Con A (Pharmacia Fine Chemicals, Piscataway, NJ) at 5 μg/ml (all final concentrations) in 24-well plates (Costar, Cambridge, MA) at 37°C in an atmosphere containing 5% CO_2. In some experiments, purified recombinant human IL-2 (the generous gift of Cetus Corp., Emeryville, CA) was added to the cultures. After 24 or 72 h, supernatants were removed and either assayed for LK immediately or frozen at −40°C until needed. IL-5 and IFN-γ in 72-h culture supernatants were assayed by highly specific two-site ELISAs (22–24) with reference to standard curves constructed using known amounts of recombinant lymphokines.

IL-2 and IL-4 in 24-h culture supernatants were measured using the HT2 cytotoxic T lymphocyte line that proliferates in response to either of these cytokines (24, 25). Mabs S4B6 (anti-IL-2, ref. 25) and 11B11 (anti-IL-4, ref. 27), at neutralizing concentrations, were used to establish monospecificity. Proliferation was assayed by measuring the incorporation of [^{3}H]TdR (New England Nuclear, Boston, MA) and compared with the proliferation induced by known amounts of recombinant IL-2 or IL-4.

T cells secreting IL-5 or IFN-γ were enumerated using the ELISPOT assay (26). Cells used in the LK secretion cultures described above were removed from the 24-well plates at 72 h, counted, and resuspended in TCM at 10^6 viable cells per milliliter plus 50 U/ml recombinant human IL-2. 100-μl or 10-μl aliquots of this suspension were then pipetted into wells of cellulose acetate–bottomed filter plates (Millipore) precoated with mAb HB170 anti–IFN-γ (22) or TRFK5 anti-IL-5 (23). After 20 h of culture, the plates were washed and processed as for the ELISAs, except that for the IFN-γ assay binding of rabbit anti-IFN-γ was detected by the addition of biotinylated goat anti–rabbit antibody (Vector Laboratories, Burlingame, CA) followed by streptavidin-peroxidase. IFN-γ and IL-5 ELISPOTS were developed with 4-chloro-1-naphthol and spots of deposited substrate, each of which represent an LK-secreting cell, counted under a dissecting microscope.

To deplete T cells, splenocytes were incubated with rabbit anti-Thy-1 (Cedarlane Laboratories, Westbury, NY) diluted 1/1,000 in TCM for 1 h at 4°C, washed three times in TCM, and then incubated with fresh guinea pig serum (1/12 in TCM) for 1 h at 37°C. CD4$^+$ splenic T lymphocytes were quantitated by immunofluorescent staining with mAb anti-L3T4 (Becton Dickinson & Co., Sunnyville, CA) and flow cytometry using EPICS IV (Coulter Electronics, Hialeah, FL).

Results

Lymphocytes from Vaccinated and Prepatently Infected Mice Secrete a Different Pattern of Cytokines to those Produced by Cells from Patently Infected Animals. The Th1 and Th2 cytokine responses of vaccinated and infected mice were initially compared by assaying IFN-γ and IL-5. Infections with 400 normal or irradiated parasites, the dose of irradiated cercariae normally used to induce protective immunity in the mouse, were utilized. However, since mice exposed to 400 normal cercariae were killed by the infection at 6–7 wk, all 8-wk analyses of infected mice used animals exposed to only 40 normal parasites. Some experiments were performed at 3 wk on mice vaccinated or infected with 40 cercariae.

Schistosomulum antigen–stimulated cells from mice vaccinated or infected with 400 cercariae 3 wk previously produced 3.2 ng/ml and 1.6 ng/ml of IFN-γ, respectively (Fig. 1, *A* and *B*). By comparison, very little IL-5 was made by these same cell populations (Fig. 1, *A* and *B*). Surprisingly, similar results were obtained with spleen cells from mice that received 40 cercariae inocula (Fig. 1, *E* and *F*), except that 40 attenuated cercariae failed to induce a detectable IL-5 response.

In both vaccinated and infected mice there was a hierarchy in the ability of different antigen preparations to stimulate IFN-γ production, in the order som>adult>egg. 8 wk after vaccination with 400 attenuated parasites, the amounts of IFN-γ and IL-5 produced in response to all three antigen preparations were quite similar to those published in preliminary form previously (7) and those seen at 3 wk (Fig. 1 *C*). However, this was not the case for mice harboring 8-wk infections. In agreement with preliminary data (7), antigen-stimulated splenocytes from these animals demonstrated a significantly heightened production of IL-5, particularly in response to egg antigens (3.8 ng/ml) (Fig. 1 *D*). In marked contrast, after antigen stimulation, these cells produced levels of IFN-γ that were barely detectable by the ELISA used (Fig. 1 *D*). While the amounts of cytokines produced by mitogen-stimulated cells were greater than those seen after antigen stimulation, the overall patterns obtained were comparable (Fig. 2). Thus, the amounts of IFN-γ and IL-5 produced by splenocytes from vaccinated mice remain similar over the period of 3–8 wk after immunization, whereas in infected mice the level of IFN-γ produced after stimulation with Con A drops significantly between weeks 3 and 8, while mitogen-induced IL-5 production increases substantially over the same period. Treatment of splenic cells from vaccinated or infected mice with anti-Thy-1 plus complement before antigen or mitogen stimulation resulted in undetectable IFN-γ or IL-5 in culture supernatants, confirming that T lymphocytes were responsible for the production of these lymphokines in our experiments (data not shown).

Flow cytometric analysis revealed that only 13.5% of splenic

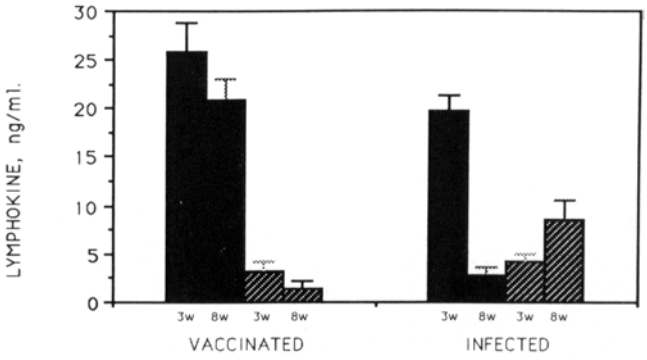

Figure 2. Con A–stimulated IFN-γ and IL-5 production by spleen cells from mice vaccinated or infected 3 or 8 wk previously. Bars represent mean values (± SE) of data from the animals used to obtain the results shown in Fig. 1. (■) IFN; (▨) IL-5.

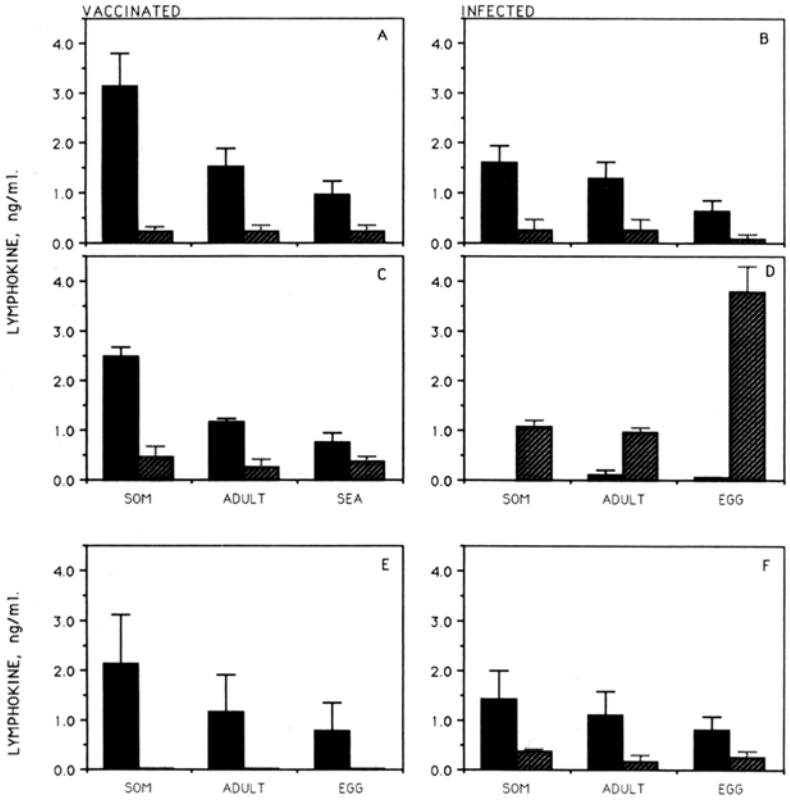

Figure 1. The influence of time post infection on LK production by vaccinated versus infected mice. Schistosomula (SOM), adult schistosome (ADULT), and egg antigen (EGG)–stimulated IFN-γ and IL-5 secretion by spleen cells from mice 3 (*A*) or 8 wk (*C*) after vaccination with 400 irradiated cercariae; 3 wk after infection with 400 normal cercariae (*B*); 8 wk after infection with 40 parasites (*D*), or 3 wk after vaccination (*E*) or infection (*F*) with 40 cercariae. The bars in *A*, *B*, *E*, and *F* represent the mean values (± SE) of data pooled from a total of seven individual mice per group from two separate experiments. In *C* and *D*, bars represent mean values (± SE) of data from four separate experiments in each of which spleens from groups of three mice were pooled to obtain results. SOM, soluble extract of schistosomula. (■) IFN, (▨) IL-5.

cells from patently infected mice were $CD4^+$, compared with 21% before oviposition. However, this change is not responsible for the reduced IFN-γ levels produced at week 8 (Fig. 1), since when spleen cells from patently infected mice were adjusted to include the same number of $CD4^+$ lymphocytes per milliliter as for cells from 3-wk infected mice, levels of IFN-γ produced remained barely detectable (not shown).

Since the pattern of mitogen-stimulated production of IFN-γ and IL-5 mimicked that seen with antigen, we used Con A–stimulated cells to assay for two additional cytokines that discriminate between Th1 and Th2 cells, namely IL-2 and IL-4. At 3 wk, splenocytes from both vaccinated and infected animals produced comparable levels of IL-2 and IL-4 (Fig. 3). However, whereas production of these cytokines was similar at 3 and 8 wk in vaccinated mice, cells from 8-wk infected mice produced significantly more IL-4 and less IL-2 than cells from 3-wk infected or vaccinated animals (Fig. 3). In general, IL-2 production in response to antigen stimulation was low compared to that seen with mitogen, with the only detected responses being to schistosomulum antigens by cells from mice infected for 3 wk (3.3 ± 0.4 U/ml, $n = 2$), or cells from mice vaccinated 3 or 8 wk previously (5.4 ± 0.3 U/ml, $n = 2$, and 6.3 ± 1.6 U/ml, $n = 4$, respectively). Antigen-driven IL-4 responses were measurable only in mice with 8-wk-old infections, where egg and schistosomulum antigens stimulated production of 129 ± 25 U/ml ($n = 4$) and 33.5 ± 6.8 U/ml ($n = 4$) of IL-4, respectively.

ELISPOT analysis of antigen-stimulated cells from mice vaccinated or infected 8 wk previously concurred with the results obtained from supernatant measurements, indicating significantly more cells secreting IFN-γ in the spleens of vaccinated versus infected mice, while the reverse is true for IL-5–secreting cells (Fig. 4).

IL-2 Does not Augment IFN-γ Secretion by Antigen-stimulated Cells from 8-wk Infected Mice. Since T cells from infected mice make little IL-2, it was possible that the observed reduced secretion of IFN-γ by these cells in vitro was due indirectly

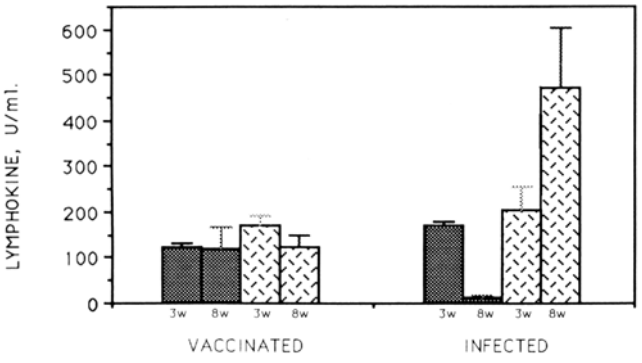

Figure 3. Con A–stimulated IL-2 and IL-4 production by spleen cells from mice vaccinated or infected 3 or 8 wk previously. Values (± SE) shown are pooled from two separate experiments consisting of three to four animals per group per experiment.

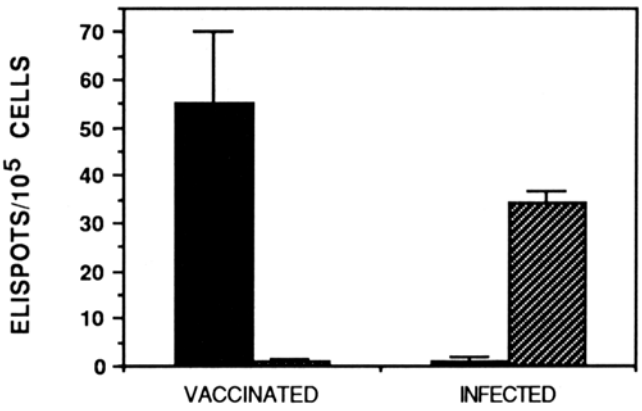

Figure 4. ELISPOT analyses of IFN-γ and IL-5 producing cells from antigen stimulated splenocyte populations of mice vaccinated with 400 irradiated cercariae or 40 normal parasites 8 weeks previously. Data are pooled from two separate experiments consisting of 3 mice per group each and are shown as means ± SE. (■) IFN, (▩) IL-5.

to this deficit, rather than a direct effect on IFN-γ production. Nevertheless, addition of exogenous IL-2 to cells from infected mice had no overall effect on the amount of IFN-γ secreted in response to antigen, but did increase IFN-γ secretion from mitogen-stimulated cells (Table 1). In contrast, IL-2 caused an increase in IFN-γ secretion by egg and to a lesser extent adult and larval antigen and mitogen driven splenocytes from vaccinated mice (Table 1).

Injected Eggs Induce Th2 Response and Suppress IFN-γ Secretion by T Cells from Vaccinated Mice. A major difference between vaccinated or 3-wk infected mice and 8-wk infected

Table 1. *Effect of IL-2 on IFN-γ Secretion by Splenic T Cells from 8-wk Vaccinated versus 8-wk Infected Mice*

		IFN-γ with the antigens:			
	IL-2 added	Som	Adult	Egg	Con A
	U/ml	*ng/ml*			
Vaccinated	0	2.20	1.00	0.75	16.00
	35	2.54	1.35	1.54*	22.00
	350	3.25*	1.50	2.70*	23.50
8-Wk infected	0	0.05	0	0	0.54
	35	0	0	0	1.00
	350	0.25	0	0	5.40*

Spleen cells from vaccinated or 8-wk infected mice were incubated with antigens or mitogen for 72 h in the presence or absence of exogenous IL-2 in the amounts indicated, following which IFN-γ levels in culture supernatants were determined. Values represent means of quadruplicate assays.

* Significant difference ($p < 0.05$) from value obtained without exogenous IL-2, as determined using Student's t test.

animals, is the exposure of the latter to schistosome eggs. To test whether these eggs are responsible for the high Th2/low Th1 responses observed in patently infected mice, we artificially introduced an egg antigenic stimulus into animals vaccinated 1 wk previously by injecting them with living eggs purified from the tissues of 8-wk infected mice. When assayed 2 wk later, spleen cells from vaccinated recipients of eggs secreted significantly less IFN-γ than noninjected vaccinated controls and showed fewer cells secreting IFN-γ (Fig. 5; Table 2). In addition, these cell made significantly more IL-5 than the controls, particularly in response to egg antigens (Table 2). Since the percentages of $CD4^+$ cells in spleens from recipients of eggs and vaccinated controls were indistinguishable (Table 2), these differences in cytokine production were not due to gross alterations in spleen cell populations. Eggs killed by two cycles of freezing and thawing, but not soluble egg antigens, were similar to live eggs, inducing Th2-type responses and suppressing IFN-γ production (not shown). Further, injection of live eggs into mice with prepatent normal infections resulted in a similar inhibition of IFN-γ secretion and stimulation of IL-5 production to that seen in similarly treated vaccinated mice (not shown).

Requirement for Whole Eggs in the Preferential Induction of Th2 Responses. Since the egg stimulus had a profoundly negative effect on Th1 responses while simultaneously stimulating strong Th2 responses, we asked whether, in comparison with other schistosome antigens, eggs were unusual in the cytokine responses they induced. For this, an experimental model was used, in which mice were injected in the footpad with antigen and 7–10 d later the popliteal lymph node cells were assayed for their ability to produce IFN-γ and IL-5 in response to antigenic restimulation in vitro. Cells from mice inoculated with live or dead eggs produced little IFN-γ in response to antigen, but did produce high levels of IL-5, particularly when stimulated with egg antigens (Table 3). In contrast,

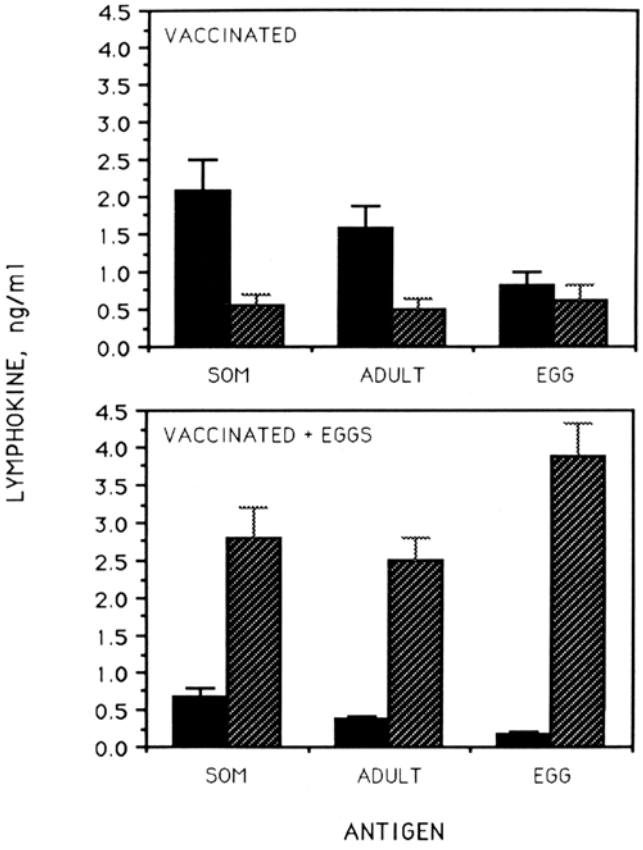

Figure 5. The effect of eggs on vaccine-induced LK responses. Antigen-stimulated IFN-γ and IL-5 secretion in vaccinated mice and vaccinated mice injected with 4×10^4 living eggs. Bars represent mean values (± SE) of data from four separate experiments consisting of three mice per group per experiment. (■) IFN, (▨) IL-5.

Table 2. *Effect of Injected Eggs on Cytokine Production by T Cells from Mice Vaccinated with Irradiated Cercariae*

	Cytokine with the antigens:						IFN-γ ELISPOT with the antigens:			
	Som		Egg		Con A					
	IFN-γ	IL-5	IFN-γ	IL-5	IFN-γ	IL-5	Som	Egg	Con A	$CD4^+$
	ng/ml						*No. cells*			%
Vac	2.98	0.61	1.35	0.83	21.98	5.10	67.0	72.8	111.0	21.6
	±0.61	±0.06	±0.69	±0.29	±5.04	±1.10	±12.0	±9.9	±18.7	±2.1
Vac + eggs	0.83	2.32	0.17	3.75	13.63	7.95	11.3	29.8	40.8	20.2
	±0.19	±0.70	±0.27	±0.31	±1.79	±2.94	±5.9	±2.9	±8.7	±2.9
p Value	0.001	0.001	0.01	0.001	0.01	NS	0.001	0.01	0.001	NS

Cells from vaccinated mice (vac) and vaccinated recipients of eggs (vac + eggs) were stimulated with schistosomulum (som) or egg antigens, or with mitogen, and cytokine levels in culture supernatants were measured. Means (± SD) of duplicate analyses on supernatants for three individual mice are presented. Statistical significance of differences between vac and vac + egg values were determined using Student's *t* test ($n = 6$). NS; $p > 0.05$. ELISPOTS are per 10^5 cells removed from culture at 72 h.

Table 3. *The Differential Induction of Th1- and/or Th2-type Responses by Various Schistosome Antigen Preparations*

		Cytokine with the antigens:							
		Som		Adult		Egg		Con A	
Inoculum	Exp.	IFN-γ	IL-5	IFN-γ	IL-5	IFN-γ	IL-5	IFN-γ	IL-5
					ng/ml				
Live eggs	1	0.56	3.20	0	1.60	0.30	4.96	4.00	10.4
	2	0	2.00	0	3.40	0	6.08	0	10.4
F/T eggs	1	0.25	4.16	0.25	5.20	0	6.10	0.50	6.40
Soluble egg	1	2.00	3.00	2.50	4.00	3.25	7.00	8.50	8.00
extract	2	0.88	1.56	ND	ND	1.30	3.10	12.00	4.00
F/T somula	1	2.00	2.84	1.90	2.48	1.80	2.70	5.00	1.20
	2	1.70	5.20	1.90	2.68	2.00	1.24	10.00	1.20

Popliteal lymph node cells draining footpads inoculated with 500 living or frozen and thawed (F/T) eggs or schistosomula (somula), or 50 μg of egg extract 7–10 days previously were stimulated in vitro with soluble schistosomulum (SOM), adult or egg antigens or mitogen, and cytokines in 72 h culture supernatants measured. Values are means of duplicate analyses on supernatants harvested from cells pooled from 3–4 mice. ND; not done.

cells from mice injected with soluble egg antigens produced reproducibly higher levels of IFN-γ than those inoculated with whole eggs, but similar levels of IL-5 (Table 3). Antigen-stimulated cells from mice inoculated with dead schistosomula produced both IFN-γ and IL-5, but the latter in lower amounts than after egg or soluble egg antigen injection (Table 3).

Discussion

In this paper we describe a previously undocumented immunological feature of helminth infection: the downregulation of Th1 cytokine responses. Specifically, our studies indicate that in *S. mansoni*–infected mice parasite eggs have a profound negative effect on schistosome antigen or mitogen-driven IFN-γ and IL-2 production. Before oviposition, T cells from infected animals produce quantities of IFN-γ and IL-2 similar to those made by lymphocytes from vaccinated mice. However, by 2 wk after the initiation of egg laying, the capacity of T cells from these same animals to secrete IFN-γ in response to antigenic stimulation is reduced to barely detectable levels. Even mitogen stimulation resulted in levels of IFN-γ and IL-2 production 10–100 times lower (respectively) than those observed with similarly treated spleen cells from vaccinated mice or animals carrying prepatent infections. Together these results suggest a marked general downregulation of Th1 responses in patently infected mice. Our hypothesis that this suppression is due directly to the egg stimulus is supported by the observation that the injection of physiologically appropriate numbers of eggs into vaccinated or prepatently infected mice, to simulate the onset of egg laying, severely effects the subsequent ability of T cells from these animals to secrete IFN-γ. As the capacity of T lymphocytes from infected mice to produce IFN-γ and IL-2 decreases, there is a coincident 10-fold increase in antigen-stimulated IL-5 secretion. Since mitogen- and antigen-driven IL-4 responses by cells from 8-wk infected mice are also greater than those from mice carrying prepatent or attenuated infections (where antigen-driven IL-4 production was not detectable), it is clear that the overwhelming T cell response, once egg production has begun, is within the Th2 subset. In vitro, egg antigens stimulate a stronger IL-4 and IL-5 response than either schistosomula or adult worm antigens, suggesting that the increase in Th2 function is largely induced by eggs and directed specifically towards egg antigens. Since many proteins are common to different schistosome stages, it is possible that the antigens in schistosomula and adults that stimulate IL-4 and IL-5 secretion are shared with the egg, but are more highly expressed in the latter. That certain parasite antigens preferentially stimulate different Th subsets is suggested by previous studies in the *L. major*/mouse model (28).

The role of eggs in the induction of Th2 type responses was further illustrated by the observations that: (*a*) after antigen stimulation in vitro, T cells from vaccinated recipients of eggs produce more IL-5 than vaccinated controls; and (*b*) popliteal lymph node cells from mice injected with eggs in the footpad respond to antigen or mitogen stimulation by secreting predominantly IL-5 and little IFN-γ. These properties are shared by both living and dead eggs but not by soluble egg antigens, suggesting that both the induction of Th2 responsiveness and the concomitant suppression of cytokine production by Th1 cells are due to components absent in the soluble preparation and/or to the nature of the egg itself (a tanned protein shell that releases antigen slowly; 29). How such physical properties might influence T cell re-

sponse is unclear, but could involve the preferential use of different accessory cells for antigen presentation.

One interpretation of the observed coincident increase in Th2 and reduction in Th1 effector functions is that Th2 cells are inhibiting secretion of lymphokines by Th1 cells. Studies with T cell clones have shown that, upon antigen or mitogen stimulation, Th2 (but not Th1) cells synthesize a protein, designated cytokine synthesis inhibitory factor (CSIF), or IL-10, which inhibits the production of IFN-γ, IL-2, IL-3, lymphotoxin/TNF and granulocyte/macrophage CSF by Th1 cells (30). Recently, IL-10 has been shown to be secreted by $CD4^+$ T cells from patently infected mice in response to egg antigen or mitogen stimulation (Sher, A., P. Caspar, D. F. Fiorentino, and T. R. Mosmann, manuscript in preparation), suggesting that this cytokine may be responsible for the suppression of Th1-type responses reported here. The failure of exogenous IL-2 to promote IFN-γ production in antigen-stimulated spleen cell cultures from 8-wk infected mice is also consistent with a role for IL-10, since this cytokine exerts its suppressive effect in the presence or absence of IL-2 (30). An additional possibility, which we are currently investigating, is a role for transforming growth factor β (TGF-β), which has been reported to inhibit IFN-γ production by Th1 cells in an IL-10–independent manner (30, 31) and is markedly elevated in the diseased livers of schistosome infected mice (32). A final possibility, not formally excluded, is that eggs somehow suppress Th1 function directly. Soluble egg antigens do stimulate cultured human endothelial cells to proliferate (33) and thus can interact with mammalian cells in a manner exclusive of antigen binding to antigen receptor.

The production of IFN-γ in an antigen-specific fashion by T cells from vaccinated mice, is consistent with previous reports that immunity in these mice is mediated in part by the action of this cytokine on macrophages (15, 34), whereas the Th2 cytokines IL-4 and IL-5 appear to play no role in vaccine-induced resistance (16). The som>adult>egg hierarchy in the ability of schistosome antigens to restimulate Th1 responses probably reflects initial priming of T lymphocytes by schistosomula, the earliest stage of the parasite to which the host is exposed. However, it also suggests that the antigens that stimulate Th1 cells are more highly represented in schistosomula than in either adults or eggs, possibly making this the most likely stage to yield a defined Th1-inducing protective immunogen.

While activation of macrophages to kill schistosomula is clearly not the only mechanism capable of conferring protection against schistosome infection (35), the comparative absence of an IFN-γ response in patently infected mice may help to explain why specific immunity is difficult to demonstrate in these animals. Although it is unknown whether the Th1-like responses seen in pre-patently infected mice would be sufficient to mediate protection if the egg stimulus never appeared, it has been reported that single sex infections, which result in ongoing infections without oviposition, can confer immunity (36). This is particularly interesting in light of recent studies from our laboratory showing that single sex infections fail to induce the strong Th2 responses seen in normally infected mice (Grzych, J.-M., A. Cheever, E. J. Pearce, Z. Caulada, P. Caspar, S. A. Hieny, F. Lewis, and A. Sher, manuscript submitted for publication). Further, that drug-abbreviated infections also induce $CD4^+$ T cell–dependent immunity (13, 37) also argues that the responses induced by prepatent infections are protective in nature.

Taken together, the observations indicate that egg-stimulated Th2 cells downregulate potentially protective immune responses induced by schistosomula in *S. mansoni* infection. In addition, the recent observation (38) that Th1 cytokine production in mice infected with the parasitic nematode *Nippostrongylus braziliensis* is reduced below normal levels suggests that the suppression of Th1 function may be a general manifestation of the immune response to helminths.

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