Evidence that Cytokine-mediated Immune Interactions Induced by *Schistosoma mansoni* Alter Disease Outcome in Mice Concurrently Infected with *Trichuris muris*

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Summary

In murine models of *Schistosoma mansoni* infection, egg production is associated with a switch from T helper cell (Th)1- to Th2-type responses to both schistosome-specific and unrelated antigens. Polyparasitism is common in human populations within *S. mansoni* endemic areas. We have, therefore, examined whether coinfection with *S. mansoni* could affect the outcome of a second parasitic infection, through Th2 cytokine–dependent modifications to the host immune response. We find that when mice susceptible to infection with the gut nematode *Trichuris muris* are coinfected with *S. mansoni*, they acquire the capacity to resolve *T. muris* infection, thus demonstrating a resistant phenotype. This ability to expel *T. muris* is associated with the production of Th2-associated cytokines, and corresponding antibody isotypes, in response to *S. mansoni* egg antigens. The Th2 response shows that there is no compartmentalization between spleen and mesenteric lymph nodes, and that the expulsion of *T. muris* is not caused by any changes in the host intestine associated with excretion of schistosome eggs. This influence of schistosome infections may be important, not only for the outcome of infections with unrelated pathogens in endemic areas, but also for the efficacy of vaccines in such areas.

During murine infection with *Schistosoma mansoni*, the production of eggs from adult worms stimulates a Th2 type response with corresponding cytokine production, associated antibody isotype profiles, and eosinophilia (1, 2). These Th2 responses can be directed towards both egg-specific and unrelated (nonschistosome) antigens, possibly at the expense of Th1 responses (2–4). This could have significant implications in cases of polyparasitism, in which schistosome infections coincide with parasites such as *Leishmania*, *Toxoplasma*, and *Mycobacteria*, for which production of Th1-associated cytokines may be required for disease resolution. Reciprocal Th1 and Th2 responses have been clearly defined for *Trichuris muris* infection in the mouse (5, 6). Resistance to infection is associated with the expansion of Th2 lymphocyte populations and the in vitro production of IL-4, -5, and -9 (7, 8). In contrast, susceptible strains (e.g., AKR, B10.BR) demonstrate increased levels of IFN-γ production, reduced Th2 responses, and failure to expel the parasite.

We have observed the effects of the dominant Th2 response to *S. mansoni* egg antigens in mice susceptible to *T. muris* and normally unable to produce the Th2-associated cytokines required for protective immunity. The data presented here demonstrate that susceptible (AKR) mice acquire the ability to resolve *T. muris* infection when concurrently infected with *S. mansoni* and that this is associated with the development of a Th2 response, downregulation of Th1-derived cytokines, and subsequent altered *T. muris*–specific antibody isotype profiles.

Materials and Methods

Parasites and Parasite Antigens

Maintenance and production of a Puerto Rican strain of *S. mansoni* have been previously described (9). *S. mansoni* eggs were isolated from outbred TO mice (Tuck & Sons, Battlebridge, United Kingdom) infected with 200 cercariae for 8 wk as previously described (10). For injection, viable eggs were diluted in low endotoxin medium (Sigma Chemical Co., Poole, United Kingdom). Alternatively, eggs were frozen under liquid nitrogen in defined numbers and then thawed and diluted appropriately. Soluble egg antigen (SEA) was derived from viable eggs by homogenization, centrifugation at 110,000 *g* for 60 min, and recovery of the resulting supernatant. The maintenance and method used for *T. muris* infection have also been previously documented (5). *T. muris* worms were isolated from the caeca of infected mice and adult excretory/secreted antigen prepared as described previously (6).

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Experimental Design

AKR mice (Harlan-Olac Ltd., Bicester, United Kingdom) were used at 6–8-wk old. For the first experiment (A), mice were infected with either (*a*) 25 *S. mansoni* cercariae percutaneously, (*b*) 400 *T. muris* eggs orally, or (*c*) *S. mansoni* plus *T. muris* (see legend to Fig. 1). In further experiments (B), designed to eliminate gut mucosal damage associated with the passage of *S. mansoni* eggs into the gut lumen, mice were either (*a*) infected with 400 *T. muris* eggs orally and then injected intraperitoneally with two doses of 10,000 viable *S. mansoni* eggs, or (*b*) infected with *T. muris* as before and then injected with two doses of viable *S. mansoni* eggs plus two doses of 10,000 frozen eggs (see legend to Fig. 3). Controls included groups of mice with *T. muris* infection alone, *S. mansoni* infection alone, or no infection. Infection with *S. mansoni* was monitored by fecal egg count analysis. Mice were killed at various time points to evaluate *T. muris* infections through worm burden determination as previously described (11).

Immunological Assays

Production of Cell Culture Supernatants for In Vitro Detection of Cytokine Responses. Murine mesenteric lymph nodes (MLN) and spleens were aseptically removed, pooled within groups, homogenized, resuspended (5×10^6/ml) in RPMI 1640 supplemented with 5% FCS, 100 U/ml penicillin/streptomycin, 2 mM glutamine, 30 mM Hepes and 5×10^5 M 2-ME, and incubated at 37°C in 5% CO_2 in air. A nonmitogenic *Trichuris* antigen suitable for cell stimulation assays is currently unavailable. Cells were, therefore, stimulated with either Con A (as previously described for both *Trichuris*, *Nippostrongylus*, and the maintenance of T cell clones [12, 13]) or SEA, at appropriate dilutions. Dilutions of the SEA and Con A used to stimulate cytokine production are outlined (see legend to Fig. 2). Supernatants for cytokine analysis were harvested after 24, 48, or 72 h and immediately frozen at −20°C until assayed. Results are shown for the 48 h supernatants.

Cytokine Analysis. The presence of cytokines in cell culture supernatants was determined by ELISA for all cytokines investigated, as previously described (12). The following antibody combinations were used: (*a*) IFN-γ, R46A2 and biotinylated XMG1.2 (14); (*b*) IL-4, 11B11 (PharMingen/AMS Biotechnologies, Whitney, United Kingdom), and biotinylated 249.2 (PharMingen); (*c*) IL-5 TRFK-5, and biotinylated TRFK-4 (*d*); (*e*) IL-10, JESS 2A5 (PharMingen), and biotinylated SXC-1 (PharMingen). Binding of biotinylated antibodies was detected using streptavidin peroxidase conjugate (Radiochemical Centre, Amersham, United Kingdom) and ABTS substrate solution (Sigma) in citrate buffer (1 mg/ml). Cell culture supernatants were assayed in duplicate undiluted and at dilutions of 1:2, 1:4, and 1:8. Values greater than the mean of 16 control wells plus 3×SD were considered positive. Results were standardized to appropriate recombinant cytokine standard curves and expressed as units per milliliter.

Detection of Parasite-specific IgG1 and IgG2a by ELISA. *S. mansoni* SEA and *T. muris* E/S antigen were coated onto plates (in carbonate buffer pH 9.6) at 0.5 μg/ml. Test sera were diluted from 1/20 to 1/2,560. Antibody binding was detected using biotinylated anti–murine IgG1 (Serotec Ltd., Oxford, United Kingdom) and biotinylated anti–murine IgG2a (PharMingen). Results were developed using streptavidin peroxidase (Serotec) and ABTS substrate in citrate buffer (1 mg/ml).

Total IgE Detection by ELISA. Rat monoclonal anti–murine IgE (Serotec) was coated on ELISA plates at 1/1,000 in carbonate buffer pH 9.6 as a capture antibody. Serum was again diluted from 1/10 to 1/280. Binding of serum IgE was detected using peroxidase-labeled goat anti–murine IgE (Nordic Immunological Laboratories Ltd., Maidenhead, United Kingdom) at 1/5,000 with an IgE monoclonal to DNP used as a reference standard.

Results

Concurrent Infection Alters Parasite-specific Antibody Responses. The effect of Th2 expansion in response to *S. mansoni* egg deposition on antibody responses was evaluated. Susceptible AKR mice were percutaneously infected with *S. mansoni* cercariae followed by 400 *T. muris* eggs 57 d later. Mice were then killed at either 21 or 35 d later and isotype-

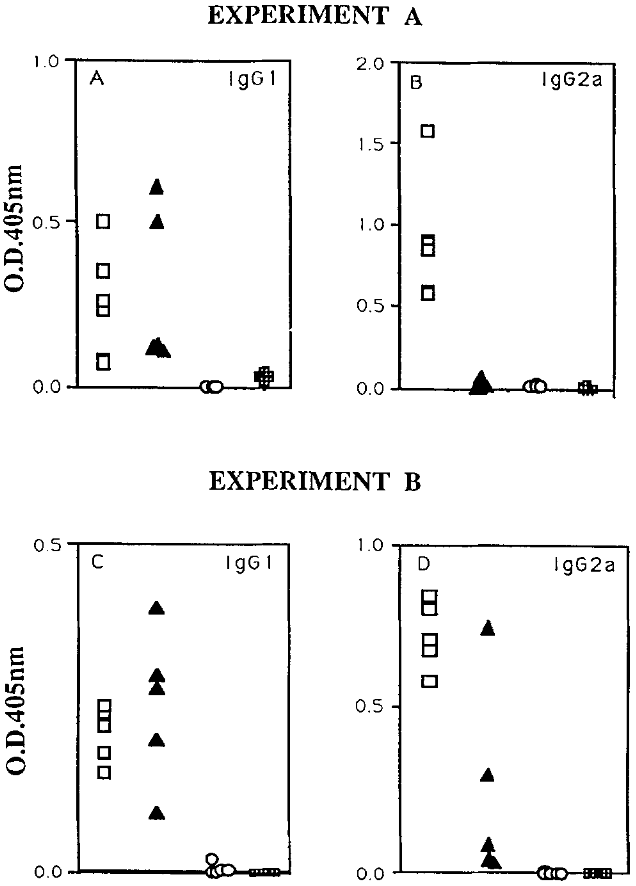

Figure 1. Production of *T. muris*–specific IgG2a and IgG1 antibodies (*Experiment A*) AKR mice were infected with 25 *S. mansoni* cercaria percutaneously on day 0 followed by 400 *T. muris* eggs orally on day 56 (▲). Mice were killed 21 d after challenge with *T. muris*. The presence of *T. muris*–specific IgG1 (*A*) and IgG2 (*B*) was determined by ELISA and are represented as absorbance at 405 nm. Results shown were obtained from a 1/200 serum dilution and compared to levels seen in mice infected with either *T. muris* alone (□) *S. mansoni* alone (O), or uninfected controls (⊞). Results are shown for each of six individual mice in each group. In a second experiment (*Experiment B*), AKR mice were infected with 400 *T. muris* eggs orally and then injected intraperitoneally with 10,000 *S. mansoni* eggs 3 and 7 d later, followed by a further intraperitoneal injection of 10,000 frozen *S. mansoni* eggs on days 27 and 31 (▲). Mice were killed at day 35 and the levels of *T. muris*–specific IgG1 (*C*) and IgG2a (*D*) determined. Relative levels were again compared to those from mice infected with either *T. muris* alone (□), *S. mansoni* alone (O), or uninfected controls (⊞). Results are shown for each of five individual mice in each group.

specific antibody responses determined. The levels of *T. muris*–specific IgG1 were comparable in the *T. muris* alone and concurrently infected groups (Fig. 1 *A*). High levels of IgG2a were found in mice infected with *T. muris* alone (Fig. 1 *B*), reflecting the Th1 response in vivo. These levels were completely abrogated when animals were coinfected with *S. mansoni*. *S. mansoni*–specific IgG1 and IgG2a levels were unaltered by concurrent infection (data not shown). No cross-reactivity was observed between *T. muris*– and *S. mansoni*–specific isotypes. Analysis of total IgE at day 21 revealed negligible values (mean value of 0.3 μg/ml) from the *T. muris* alone group but substantial levels (mean value of 40.4 μg/ml) from *S. mansoni*–infected animals. Similarly, high levels of IgE antibody were observed when mice were concurrently infected (mean value of 21.3 μg/ml).

Altered Antibody Response Correlates with Induction of a Th2 Response. Production of IgG2a and IgG1 has been used as markers of Th1 or Th2 responses in vivo (7, 16–19). The production of Th1 and Th2 related cytokines in vitro was, therefore, measured and compared to the antibody isotypes described above. To avoid bias of results due to differential compartmentalization of the immune response in the different infection models, both the MLN cell (MLNC) and splenic lymphocyte populations were isolated and restimulated with parasite antigens and mitogen in vitro. Supernatants were removed and the relative levels of IL-4, -5, -10, and IFN-γ determined by ELISA. Similar cytokine profiles were found between splenic and MLNC populations, indicative of the disseminated effect of the *S. mansoni* response distal from the primary sites of egg deposition. Representative results of MLNC populations are shown in Fig. 2. High levels of production of the Th2-associated cytokines IL-4, -5, and -10 in response to SEA were found in animals infected with *S. mansoni*, either as a single or concurrent infection. Such responses to SEA were not observed in animals infected with *T. muris* alone, indicating a lack of cross-reactivity. The production of IFN-γ, in response to Con A, was low in mice infected with *S. mansoni* alone. These levels were further decreased when mice were concurrently infected. No IFN-γ was produced in response to *S. mansoni* worm or egg antigens. In contrast, in vitro stimulation of MLNC and splenic populations from mice infected with *T. muris* alone resulted in significant levels of IFN-γ in response to mitogen, but not *S. mansoni* antigens. These results strongly suggest that the altered antibody response to *Trichuris* antigens in concurrently infected animals is attributable to an increased capacity to produce Th2-associated cytokines after exposure to *S. mansoni* egg antigens.

T. muris Worm Burdens Are Reduced in Susceptible Mice When Concurrently Infected with S. mansoni. The results outlined in Table 1 (experiment *A*) demonstrate that altered cytokine and antibody responses to *S. mansoni* egg antigens in mice concurrently infected with *T. muris* is associated with the ability of these mice to expel *T. muris* with an immune response comparable to that observed in resistant strains. This can be contrasted to mice infected with *T. muris* alone which still harbored adult worms at day 21.

Worm Expulsion Is Not Attributable to S. mansoni Egg-associated Intestinal Changes. Infection with *S. mansoni* and passage of parasite eggs into the lumen of the intestine are associated with inflammation and damage to mucosal surfaces. Given that *T. muris* is a gut-dwelling nematode it was possible that the reduction in worm burdens observed above was attributable to *S. mansoni*–induced pathological changes. AKR mice were,

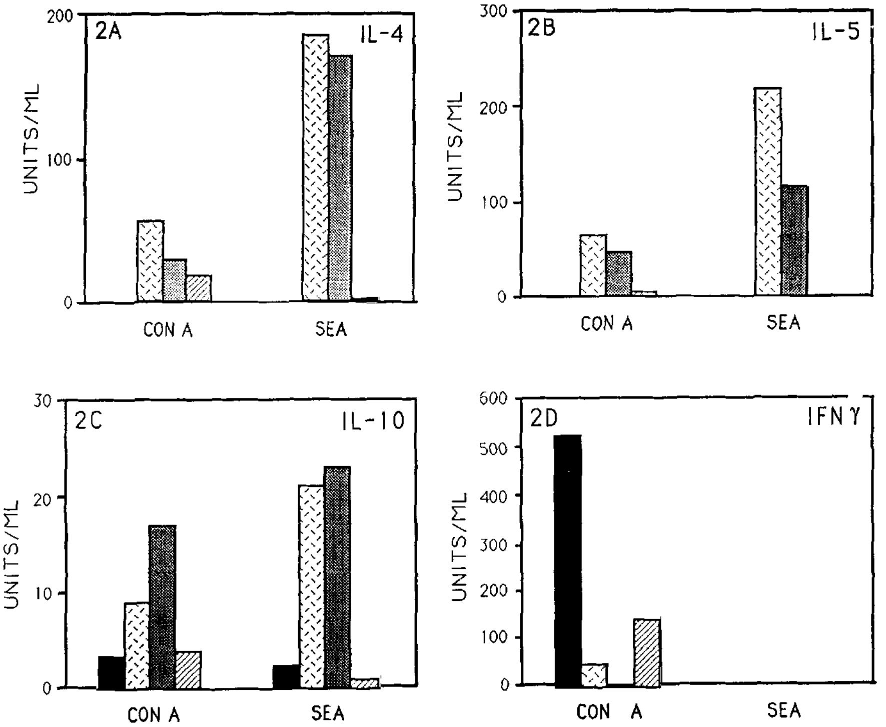

Figure 2. Cytokine production from mesenteric lymph node populations after in vitro stimulation with either Con A or soluble egg antigen. AKR mice were infected as follows: (*a*) 25 *S. mansoni* cercariae percutaneously on day 0 (▧), (*b*) 400 *T. muris* eggs on day 56 (■), (*c*) 25 *S. mansoni* cercariae on day 0 followed by 400 *T. muris* eggs on day 56 (▩), (*d*) uninfected controls (▨). Mice were killed 21 d after infection with *T. muris*. Mesenteric lymph node populations were stimulated in vitro with either Con A (5 μg/ml), or SEA (25 μg/ml). Supernatants were removed after 48 h and assayed for the presence of IL-4 (*A*), IL-5 (*B*), IL-10 (*C*), and IFN-γ (*D*). Cytokine levels observed were standardized by reference to appropriate standards and are expressed as units per milliliter. Results represent the mean obtained from pooled lymph node populations of the six mice in each experimental group.

Table 1. *T. muris Worm Burdens in Mice Infected with Either T. muris Alone or with a Concurrent Infection of T. muris plus S. mansoni*

Experiment	A		B			
Challenge	*T. muris*	*T. muris* + *S. mansoni*	*T. muris*	*T. muris* + *S. mansoni*	*T. muris*	*T. muris* + *S. mansoni*
Day	21	21	21	21	35	35
Worm burden	129	0	108	103	111	0
	260	0	111	128	158	0
	155	0	134	58	122	0
	219	0	80	87	110	0
	186	0	179	12	81	0
	96	0				

(Experiment *A*) AKR mice were infected orally with either (*a*) 400 *T. muris* eggs, or (*b*) 25 *S. mansoni* cercariae percutaneously, followed by 400 *T. muris* eggs after 56 d. Mice were killed 21 d after infection with *T. muris*, and the number of adult *T. muris* worms present in the six mice of each experimental group determined. (Experiment *B*) AKR mice were infected with *T. muris*, as above, and then injected with either (*a*) 10,000 live *S. mansoni* eggs (intraperitoneally) on days 3 and 5 (with mice killed 21 d after the infection with *T. muris*), or (*b*) 10,000 live *S. mansoni* eggs, as above, followed by a further two intraperitoneal injections of frozen eggs on days 27 and 31 with mice killed on day 35 after infection with *T. muris*. Five mice were present in each experiment group.

therefore, infected with *T. muris* and then injected with both viable and freeze/thawed eggs as outlined in the legend to Fig. 3. The ability of egg injection to elicit a Th2 response was confirmed by the cytokine responses to antigen and mitogen in vitro. Cytokine profiles were comparable between MLNC and splenic populations and those of MLNC are shown in Fig. 3 (day 35). Control animals produced IL-4, IL-10, and IFN-γ in response to Con A. High levels of IL-5 and IL-10, but not IFN-γ, were seen in response to both *S. mansoni* egg antigens and Con A from mice infected with *S. mansoni* alone. Infection with *T. muris* alone, however, stimulated a predominant Th1 type response (i.e., IFN-γ production, but little IL-4, IL-5, or IL-10). Injection of *S. mansoni* eggs into *T. muris*–infected animals also resulted in high levels

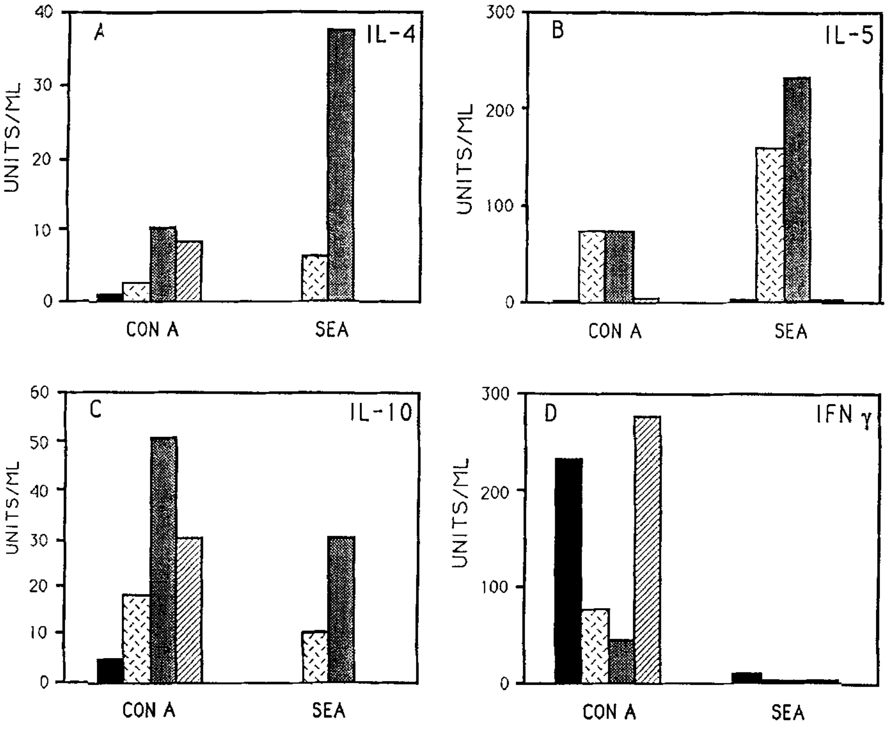

Figure 3. Cytokine production from mesenteric lymph node populations after in vitro stimulation with either Con A or soluble egg antigen. AKR mice were infected as follows: (*a*) 400 *T. muris* eggs on day 0 (■); (*b*) 10,000 live *S. mansoni* eggs injected intraperitoneally on days 3 and 10, followed by 10,000 dead (frozen) *S. mansoni* eggs injected intraperitoneally on days 27 and 31 (░), (*c*) 400 *T. muris* eggs on day 0, followed by 10,000 live *S. mansoni* eggs on days 3 and 10, plus 10,000 dead eggs on days 27 and 31 (▓), (*d*) uninfected controls (▨). Mice were killed 35 d after infection with *T. muris*. Mesenteric lymph node populations were stimulated in vitro with either Con A (5 μg/ml), or SEA (25 μg/ml). Supernatants were removed after 48 h and assayed for the presence of IL-4 (*A*), IL-5 (*B*), IL-10 (*C*), and IFN-γ (*D*). Cytokine levels observed were standardized by reference to appropriate standards and are expressed as units per milliliter. Results represent the mean obtained from pooled lymph node populations of the six mice in each experimental group.

of IL-4, IL-5, IL-10, and reduced IFN-γ production in vitro indicative of a Th2 response. These cytokine profiles correlate with the *T. muris*–specific antibody responses observed, which are shown in Fig. 1, *C* and *D*. Significant reduction in IgG2a (but not IgG1) was observed in mice infected with *T. muris* and injected with *S. mansoni* eggs compared with mice infected with *T. muris* alone. *S. mansoni* IgG1 and IgG2a responses were unaltered by concurrent infection (data not shown). In one mouse, the switch from Th1-Th2 was incomplete, as represented by both worm burden analysis (Table 1, experiment *B*) and corresponding antibody profiles (Fig. 1 *D*). IgE profiles again correlated with the strong Th2 response observed. The mean IgE levels after infection with *T. muris* alone, *S. mansoni* alone, and *T. muris* plus *S. mansoni* eggs were 0.4, 21.7, and 23.7 μg/ml, respectively. Finally, the ability of *S. mansoni*–injected eggs to induce expulsion of *T. muris* can be seen by the decrease in worm burden, as shown in Table 1 (experiment *B*).

These results confirmed that the acquired ability of these susceptible mice to expel *T. muris* when coinfected with *S. mansoni* was attributable to the induction of a Th2–associated immune response and was not a consequence of local gut inflammation caused by deposition of *S. mansoni* eggs.

Discussion

The results presented here demonstrate that expansion of the Th2 type population in response to *S. mansoni* egg antigens can markedly influence the outcome of a secondary, concurrent parasitic infection. We have shown that when AKR mice, normally susceptible to *T. muris* infection, are coinfected with *S. mansoni*, they acquire the ability completely to expel *T. muris* worms by generating the Th2 type response observed in resistant mouse strains. This response is characterized by (*a*) the production of Th2-associated cytokines such as IL-4, -5, -10, and downmodulation of IFN-γ, and (*b*) elevated levels of total IgE.

With many parasites, disease outcome has been associated with expansion of Th1 or Th2 $CD4^+$ populations and their associated cytokine production (17, 18, 20). Within *S. mansoni* infections, the production of Th2-associated cytokines has been shown in response to parasite-specific and nonrelated antigens (2, 3, 21). This could influence the outcome of a second infection, as previously suggested with clearance of vaccinia virus (22) and altered antibody responses after hepatitis vaccination (23).

Production of IgG2a has been correlated with Th1 expansion (and IFN-γ production), whereas Th2 expansion (and production of IL-4 and IL-5) is associated with IgG1 and IgE production and immunoglobulin class switching (16–18). Herein, cytokine response to *S. mansoni* eggs was reflected by reduced *T. muris*–specific IgG2a levels in concurrently infected mice. Previous studies (13) have shown similar modulation in AKR mice after administration of either IL-4 or anti-IFN-γ. No reduction in *S. mansoni* IgG1 or IgG2a antibody levels was observed, and concurrent infection with *T. muris* failed to reduce levels of IL-4, IL-5, or IL-10 production in vitro. Levels of IFN-γ were, however, completely abrogated. Furthermore, the elevated IgE responses, characteristic of *S. mansoni* infections, were also unaffected by concurrent infection.

Chronic infection with *S. mansoni* is associated with degenerative pathological changes in the intestine, which could influence the establishment of *T. muris* in concurrently infected mice. Intravenous and subcutaneous injections of *S. mansoni* eggs have been used to stimulate Th2 responses in the spleens of vaccinated mice and the draining lymph nodes of naive mice, respectively (2, 3, 18, 21). We adapted these methods, using intraperitoneal injection of both live and dead *S. mansoni* eggs, to exclude the possibility that the migration of *S. mansoni* eggs through the gut damaged the mucosa in such a way as to disrupt *T. muris* infection. The results obtained parallel those of the mice with natural *S. mansoni* infection. High levels of Th2-associated cytokines (IL-4, -5, -10) were found in mice injected with *S. mansoni* eggs alone, or injected with *S. mansoni* eggs and also infected with *T. muris*. This was accompanied by downregulation of IFN-γ and *T. muris*–specific IgG2a responses, and complete worm expulsion by day 35. Worm burdens were significantly reduced at day 21 and fully eliminated by day 35. Parasite-specific antibody and cytokine production were also modulated by day 21, but were significantly more affected at day 35. This delay could reflect differences in the antigenic stimulation between natural infection (with constant egg deposition from adult worms) and the restricted stimulation when large numbers of eggs were injected intraperitoneally on a weekly basis.

We have demonstrated therefore that the cytokine and antibody production accompanying the Th2 type response to *S. mansoni* egg antigens in vivo dramatically affects the outcome of infection with a coinfecting pathogen. The strong Th2 response to *S. mansoni* egg antigens is systemic, modulating both antibody isotypes and cytokine production. This can result in a susceptible host phenotype acquiring the ability to resolve *T. muris* infection. This ability of *S. mansoni* egg antigens to downregulate Th1 cytokine responses could have implications for vaccine efficacy (20, 23) in diseases such as cutaneous leishmaniasis and mycobacterial infections, where an early Th1 response is critical to disease resolution, and in viral infections such as hepatitis B. The prevalence of these three diseases within schistosomiasis endemic areas has promoted current investigations into the impact of *S. mansoni* on such infections.

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