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Chronic Intestinal Nematode Infection Exacerbates Experimental *Schistosoma mansoni* Infection▽

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Mixed-parasite infections are common in many parts of the world, but little is known of the effects of concomitant parasite infections on the immune response or on disease progression. We have investigated the in vivo effects of a chronic gastrointestinal nematode infection on the infectivity and development of the immune response against the common trematode helminth *Schistosoma mansoni*. The data show that mice carrying an established chronic *Trichuris muris* infection and coinfected with *S. mansoni*, had significantly higher *S. mansoni* worm burdens than mice without coinfection. The increase in *S. mansoni* worm burden was accompanied by a higher egg burden in the liver. Kinetic analysis of *S. mansoni* establishment indicate reduced trapping of *S. mansoni* larvae during skin-to-lung migration, with *T. muris*-induced alterations in lung cytokine expression and inflammatory foci surrounding lung-stage schistosomula, suggesting that the immunomodulatory effects of chronic *T. muris* infection elicited at the gut mucosal surface extend to other organs and perhaps specifically to other mucosal surfaces. The data show that a preexisting chronic gastrointestinal nematode infection facilitates the survival and migration of *S. mansoni* schistosomula to the portal system, and as a result, increases the egg burden and associated pathology of *S. mansoni* infection.

Helminth infections are among the most common infections of humans (2, 7, 26). Helminth infections are characteristically chronic in nature, and in order to achieve such long-lasting infections, these parasites have developed sophisticated survival strategies such as secretion of immunomodulatory substances and/or the induction of regulatory immune responses (35). These immunomodulatory activities may alter immune responsiveness to third party antigens such as vaccines (9, 10, 18, 42) and concurrent infections (reviewed in references 4 and 27). Intriguingly, recent studies have also demonstrated that the immunomodulatory effects of helminth infections may have beneficial effects, such as controlling allergy and inflammatory diseases (19, 53).

Individuals living in areas where helminths are endemic often carry more than one species of worm infection; egg output for an individual helminth species is often higher in individuals carrying mixed infections than in individuals carrying single-species infections (3, 5, 20, 38, 45), which may reflect higher intensities of infection and so a higher risk of morbidity. Geographical and environmental factors are known to be of significance in facilitating this type of polyparasitism: for example, similarities in the transmission pathways for certain soil-transmitted nematodes are likely to account for some of the observed associations. However, significant associations have also been demonstrated between helminth infections that do not share obvious transmission pathways and some of these associations are not explained solely by household or environmental effects (20). Furthermore, the intensity of helminth infections alters the risk of multiple-species infection (20, 31, 45) indicating that immunological and/or genetic factors may be involved in regulating resistance and susceptibility to helminth-helminth coinfections. In order to investigate how a chronic helminth infection affects the establishment and outcome of a second concurrent helminth infection, we have used the mouse models of the gastrointestinal nematode *Trichuris muris* (the murine equivalent of human *Trichuris trichiura* [whipworm] infection) together with the trematode *Schistosoma mansoni* (a common human infection in tropical countries). Mice that fail to develop an early protective Th2 response against *T. muris* go on to develop long-term chronic infections (8). However, as the infection progresses beyond the chronic stage, the initial Th1 response is downregulated and a subsequent increase in Th2 response is seen (21, 22). Moreover, production of the regulatory cytokine interleukin-10 (IL-10) has been shown to be critical for host survival (43). Similarly, mice infected with *S. mansoni* respond with a Th1 response in the early phase of infection, switching to a Th2-dominated response once the adult worms commence egg production (41). Some of the eggs produced by the female *S. mansoni* worms are trapped in the microvasculature of the liver, where they induce a strong granulomatous response. Most of the pathology associated with *S. mansoni* infection in both humans and mice is caused by these granulomas.

The data presented here demonstrate that mice with an established chronic *T. muris* infection and challenged with *S. mansoni* developed significantly higher *S. mansoni* worm burdens than mice without a concurrent *T. muris* infection. The higher *S. mansoni* burden resulted in significantly higher egg

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and granuloma burden in the liver. This is the first experimental demonstration that a chronic intestinal nematode infection can exacerbate *S. mansoni* infection.

MATERIALS AND METHODS

Animals and infections. Six- to eight-week-old AKR mice were obtained from Harlan Olac, Ltd. (Bicester, United Kingdom). All experiments were performed under the regulations of the Home Office Scientific Procedures Act (1986). All experiments were performed at least three times. Experimental animals (5 to 10 per group) were infected with 150 embryonated *T. muris* eggs on day 0 by oral gavage and 40 days later infected with 50 or 200 *S. mansoni* cercariae percutaneously. Maintenance, infection, and recovery of the parasites were performed as described previously (17, 28). Briefly, *S. mansoni* worms were recovered by portal perfusion with perfusion buffer (phosphate-buffered saline with 0.02 U/ml heparin). The worms were washed free of erythrocytes and counted using a dissecting microscope. For estimation of the liver egg burden, livers were removed and weighed. Eggs were recovered by incubation of the tissue in 5% KOH overnight at 37°C, and the numbers of eggs in 50-μl aliquots were counted in triplicates. *T. muris* worms in ceca were counted under a dissection microscope.

Control groups receiving single infections were infected in parallel for each experiment.

Cell culture and cytokine analysis. Mesenteric lymph nodes (MLN) and spleens were removed from uninfected and infected animals, and single-cell preparations were resuspended in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.05 mM β-mercaptoethanol (all from Invitrogen, Paisley, United Kingdom). Cells were cultured at 37°C and 5% CO_2 in flat-bottom 96-well plates (Nunc, Roskilde, Denmark) at a final concentration of 5×10^6/ml in a final volume of 0.2 ml/well. Cells were stimulated with *T. muris* ES antigen (25 μg/ml), *S. mansoni* egg antigen (SEA) (25 μg/ml), *S. mansoni* worm antigen (25 μg/ml), or plate-bound anti-CD3 antibody (mAb145-2C11, 10 μg/ml [ATCC]). Cell-free supernatants were harvested after 48 h and stored at −80°C.

Cytokine ELISA. Cytokine analyses were carried out using commercially available sandwich enzyme-linked immunosorbent assays (ELISAs) for IL-4, gamma interferon (IFN-γ) (Mabtech AB, Nacka, Sweden), and IL-13 and IL-10 (R&D Systems, Abingdon, United Kingdom).

Histopathological analyses. Tissues were fixed in neutral buffered formalin and histologically processed by standard methods, and sections were stained with hematoxylin and eosin. Liver granulomas surrounding eggs containing visible miracidia were assessed for size, and inflammatory foci in the lungs were assessed for size and cellular composition. The transverse and longitudinal diameters of granulomas and foci were measured using an ocular micrometer, and the mean diameter was calculated. At least 15 granulomas and 20 foci were measured per tissue per mouse, and the mean values were calculated. Individual animal means were then used to derive the group mean values. The cellular composition of lung foci was determined using an eyepiece graticule by counting all of the cells in a rectangular grid spanning the whole diameter of the foci. The percentages of the predominant cell types (eosinophils, fibroblasts, and macrophages) were determined from at least 20 foci per mouse and used to determine the group mean values.

Real-time PCR. Tissues were harvested and stored in RNAlater (Qiagen, Crawley, United Kingdom) at −80C until processing. RNA was purified using an RNeasy minikit from Qiagen according to the manufacturer's instructions, with an additional DNase treatment step (Qiagen). Reverse transcription was performed using the Omniscript reverse transcription kit (Qiagen). Real-time PCR was performed in an ABI 7000 sequence detection system (Applied Biosystems, Warrington, United Kingdom) using Sybr green PCR Master Mix (Qiagen). Primers for hypoxanthine phosphoribosyltransferase (5′-GTTGGATACAGGC CAGACTTTGTTG and 3′-GATTCAACCTTGCGCTCATCTTAGGC), IL-4 (5′-CCTCACAGCAACGAAGAACA and 3′-TGGACTCATTCATGGTGC AG), IL-10 (5′-AGGGTTACTTGGGTTGCCAA and 3′-CACAGGGGAGAA ATCGATGA), and IL-13, IL-5, inducible nitric oxide synthase (iNOS), IFN-γ, and tumor necrosis factor (TNF) (40) were obtained from Invitrogen. Results were normalized to the housekeeping gene coding for hypoxanthine phosphoribosyltransferase and expressed as increase (fold) compared to tissue from naïve, uninfected controls (given an arbitrary value of 1).

Statistical analyses. Significant differences ($P < 0.05$) between experimental groups were determined using the Mann-Whitney U test for worm counts and Students *t* test for all other analyses.

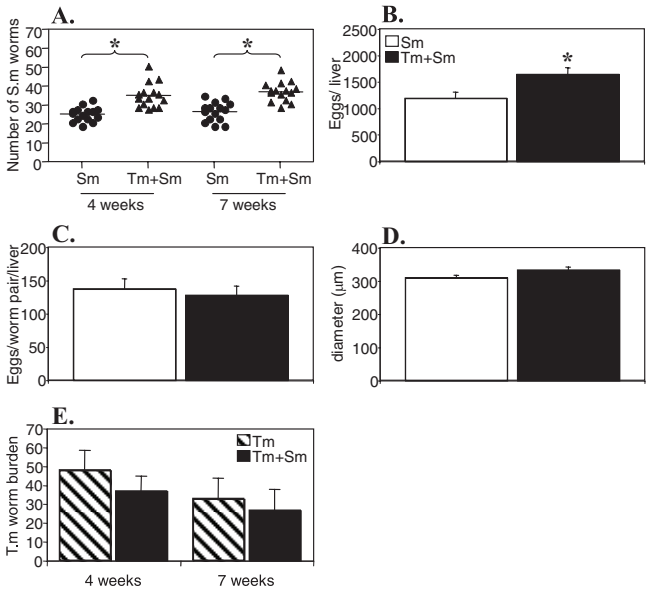

FIG. 1. *Schistosoma mansoni* (Sm) worm burden (A), total egg burden in the liver (B), *S. mansoni* female fecundity (C), mean liver granuloma diameter (D), and *T. muris* (Tm) worm burden during *T. muris*-*S. mansoni* coinfection. AKR mice were infected orally with 150 *T. muris* eggs, percutaneously with 50 *S. mansoni* cercariae, or both. *S. mansoni* infection was started 40 days after *T. muris* infection. Worm burdens were analyzed 4 and 7 weeks post-*S. mansoni* infection, and liver pathology was analyzed 7 weeks post-*S. mansoni* infection. Means and standard errors are shown. Data were pooled from three separate experiments. *, statistically significant difference between groups of mice ($P < 0.05$).

RESULTS

A preexisting chronic *T. muris* infection result in increased *S. mansoni* worm burden. To investigate if a chronic intestinal nematode infection can alter the outcome of *S. mansoni* infection in vivo, we infected female AKR mice (10 to 20 per group) with 150 embryonated *T. muris* eggs by oral gavage. Forty days later, the mice were infected with 50 *S. mansoni* cercariae percutaneously. Age-matched control animals receiving single infections were infected in parallel for each experiment. Four and 7 weeks post-*S. mansoni* infection, the animals were sacrificed and the livers were perfused to obtain *S. mansoni* worm counts. The results in Fig. 1A show that mice with a preexisting *T. muris* infection had significantly higher *S. mansoni* worm burdens than mice infected with only *S. mansoni* at both 4 and 7 weeks post-*S. mansoni* infection ($P < 0.05$ at both time points). The *T. muris* worm burdens were not affected by *S. mansoni* infection (Fig. 1E).

Increased liver egg burden in coinfected mice. Analysis of the *S. mansoni* liver egg burdens in singly infected and coinfected mice 7 weeks post-*S. mansoni* infection revealed that the higher number of adult schistosomes found in *T. muris*-coinfected animals was reflected in a significant increase in the total number of *S. mansoni* eggs in the liver (Fig. 1B). When the egg output per female worm was calculated, the data show that the fecundities of female schistosomes were comparable between the two groups, confirming that the egg output is directly correlated to the number of worms (Fig. 1C). Further-

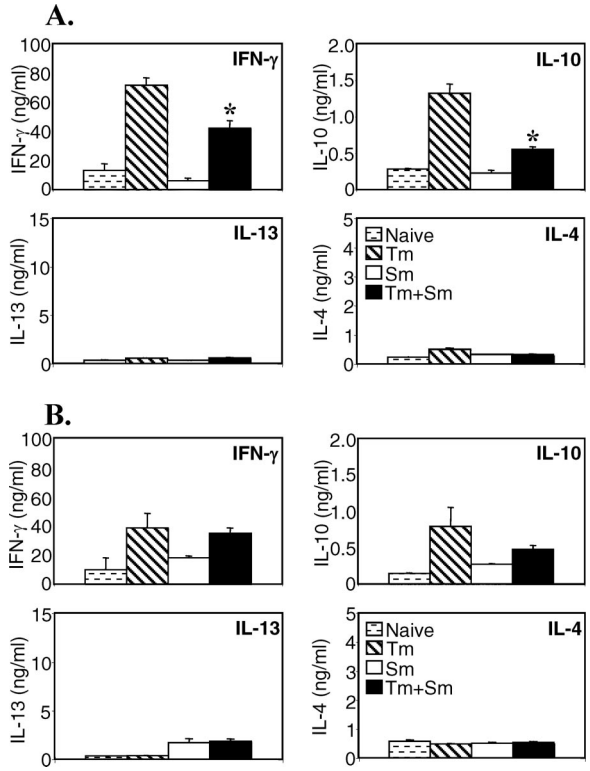

FIG. 2. Concurrent *S. mansoni* (Sm) infection modulates the cytokine response to *T. muris* (Tm) in the spleen. AKR mice were infected orally with 150 *T. muris* eggs, percutaneously with 50 *S. mansoni* cercariae, or both. *S. mansoni* infection was started 40 days after *T. muris* infection. Mice were sacrificed 7 weeks after *S. mansoni* infection. Spleen (A) and MLN (B) cells from uninfected (stippled bars), *T. muris*-infected (hatched bars), *S. mansoni*-infected (white bars), or *S. mansoni*- and *T. muris*-coinfected (black bars) mice were removed and stimulated in vitro with *T. muris* antigen. Supernatants were analyzed by sandwich ELISA for the presence of IFN-γ, IL-10, IL-13, and IL-4. Means of five mice per group and standard errors of the mean are shown. Data from one representative experiment out of three are shown. *, statistically significant difference between *T. muris* singly infected and coinfected groups ($P < 0.05$).

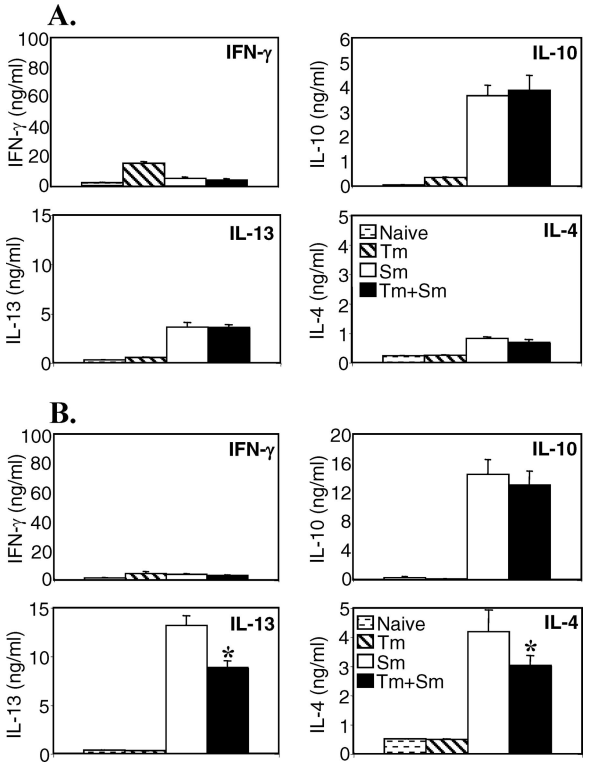

FIG. 3. Concurrent *T. muris* (Tm) infection does not modulate the cytokine response to SEA in the spleen. AKR mice were infected orally with 150 *T. muris* eggs, percutaneously with 50 *S. mansoni* (Sm) cercariae, or both. *S. mansoni* infection was started 40 days after *T. muris* infection. Mice were sacrificed 7 weeks after *S. mansoni* infection. Spleen (A) and MLN (B) cells from uninfected (stippled bars), *T. muris*-infected (hatched bars), *S. mansoni*-infected (white bars), or *S. mansoni*- and *T. muris*-coinfected (black bars) mice were removed and stimulated in vitro with SEA. Supernatants were analyzed by sandwich ELISA for the presence of IFN-γ, IL-10, IL-13, and IL-4. Means of five mice per group and standard errors of the mean are shown. Data from one representative experiment out of three are shown. *, statistically significant difference between *S. mansoni* singly infected and coinfected groups ($P < 0.05$).

more, the mean diameters of liver granulomas were similar between the two groups (Fig. 1D).

Concurrent *S. mansoni* infection modulates the cytokine response to *T. muris* antigen in the spleen but not in local lymph nodes. Previous studies have demonstrated that an established *S. mansoni* infection can markedly skew the cytokine response to unrelated antigens toward a Th2 profile. In order to investigate if this ability to skew cytokine responses could also alter an established *T. muris* response, we analyzed *T. muris*-specific cytokine production in single and coinfected animals. In vitro restimulations with *T. muris* antigen were performed on spleen and MLN cells, and cytokine secretion was analyzed by ELISA. The data in Fig. 2 show that spleen and MLN cells from mice singly infected with *T. muris* secreted high levels of IFN-γ and IL-10 and low levels of IL-4 and IL-13, in agreement with their susceptible phenotype (8). Spleen cells from mice that were coinfected with *T. muris* and *S. mansoni*, however, secreted significantly lower levels of *T. muris*-specific IFN-γ and IL-10 (Fig. 2A). Interestingly, the reduction in IFN-γ and IL-10 secretion was not associated with an increase in IL-4 and IL-13 secretion, indicating that the *S. mansoni* infection had not shifted the *T. muris* response toward Th2, but caused a general inhibition of the *T. muris* response. No significant difference in the cytokine response in the MLN could be detected in coinfected mice, showing that the *T. muris*-specific cytokine response nearest to the site of the nematode infection was intact.

Chronic *T. muris* infection alters the response to SEA in the lymph nodes but not in the spleen. When we analyzed the cytokine secretion profile in response to SEA in vitro, we found that cells from mice singly infected with *S. mansoni* secreted high levels of IL-4, IL-13, and IL-10 and low levels of IFN-γ, as expected (Fig. 3). There were no differences in the levels of spleen cell cytokine secretion between mice with only *S. mansoni* infection and those that were coinfected with *T. muris* (Fig. 3A). In the MLN, however, coinfection resulted in a significant reduction in SEA-specific IL-13 and IL-4 secretion ($P < 0.05$), while the SEA-specific IL-10 and IFN-γ responses remained unaffected (Fig. 3B). Thus, a chronic *T. muris* infec-

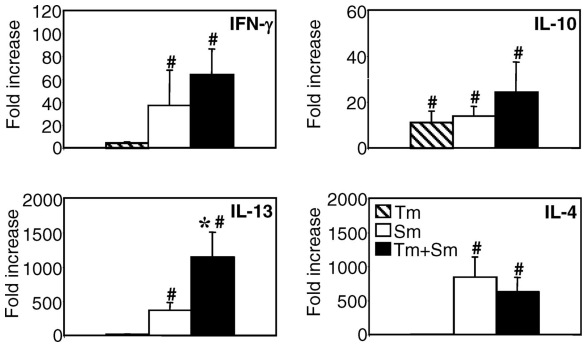

FIG. 4. Liver cytokine mRNA expression during *T. muris-S. mansoni* coinfection (Tm + Sm). AKR mice were infected as described above. RNA was prepared from freshly isolated liver tissue 7 weeks after *S. mansoni* infection and reverse transcribed, and cytokine mRNA expression was analyzed by real-time PCR. Means of four to six mice per group and standard errors of the mean are shown. Data from one representative experiment out of three are shown. *, statistically significant difference between *S. mansoni* singly infected and coinfected groups ($P < 0.05$); #, statistically significant difference between infected and uninfected groups ($P < 0.05$).

tion is able to inhibit the SEA-specific Th2 response in the local lymph node but not in the spleen.

Altered cytokine mRNA expression in the livers of coinfected animals. Cytokine mRNA expression in liver tissue was analyzed 7 weeks post-*S. mansoni* infection, by quantitative real-time RT-PCR. Mice with only *S. mansoni* infection had high expression of IFN-γ, IL-4, IL-10, and IL-13 in liver tissue compared to uninfected controls (Fig. 4). Mice with only *T. muris* infection had significantly increased expression of IL-10 compared to naïve tissue, but no increase in IFN-γ, IL-4, or IL-13. The levels of IL-10 mRNA in livers from *T. muris*-infected mice were comparable to that detected in livers from *S. mansoni*-infected mice. Mice coinfected with *T. muris* and *S. mansoni* had significantly higher expression of IL-13 compared to the group infected with *S. mansoni* only, while the levels of IFN-γ, IL-10, and IL-4 were comparable between the two groups. This piece of data shows that the coinfection resulted in increased liver mRNA levels of the profibrotic cytokine IL-13, correlating with the increase in liver egg burden. However, the increase in IL-13 was not accompanied by an increase in the levels of the antifibrotic cytokine IFN-γ, suggesting a possibility of more severe disease progression during coinfection.

Chronic *T. muris* infection increases the success rate of *S. mansoni* larval migration. Since the coinfected mice had significantly higher *S. mansoni* worm burdens at 4 and 7 weeks post-*S. mansoni* infection, we investigated if this was due to a higher survival rate of schistosomula during the migration phase. The *S. mansoni* larvae migrate from the skin, through the lungs, to the hepatic portal system during the first few weeks of infection, and it was possible that the higher worm burden observed in the coinfected group resulted from increased survival during the skin-to-lung migration or by increased survival of adult worms once established in the portal system. We therefore superinfected mice harboring a chronic *T. muris* infection with 200 *S. mansoni* cercariae percutaneously and perfused them for *S. mansoni* worm counts at 2, 4,

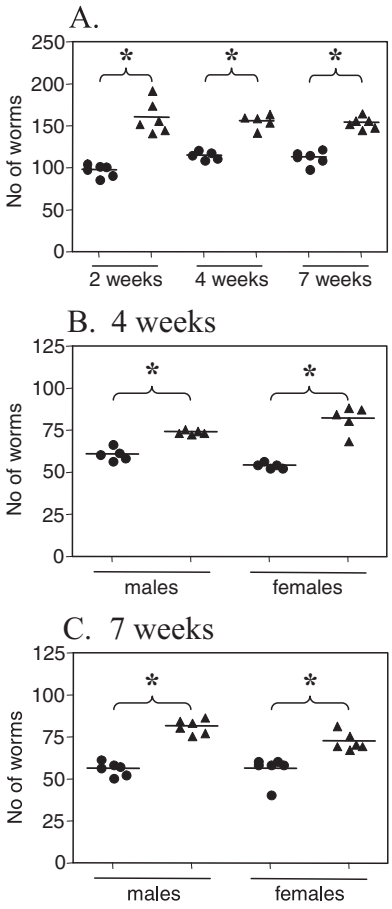

FIG. 5. Kinetics of *Schistosoma mansoni* total worm burden (A) and sex-differentiated *Schistosoma mansoni* worm burden at 4 (B) and 7 (C) weeks post-*S. mansoni* infection in mice with only *S. mansoni* infection (circles) or *T. muris-S. mansoni* coinfection (triangles). AKR mice were infected orally with 150 *T. muris* eggs, percutaneously with 200 *S. mansoni* cercariae, or both. *S. mansoni* infection was started 40 days after *T. muris* infection. Worm burdens were analyzed 2, 4, and 7 weeks post-*S. mansoni* infection. Means of five or six mice per group and standard errors of the mean are shown. Data from one representative experiment out of three are shown. *, statistically significant difference between groups of mice ($P < 0.05$).

and 7 weeks postinfection. Age-matched littermates were infected with *S. mansoni* alone in parallel. The data in Fig. 5 show that a significantly higher *S. mansoni* worm burden was already present in the hepatic portal system of coinfected mice at 2 weeks postinfection, demonstrating that the higher worm burden during coinfection was due to enhanced survival of schistosomula during the larval migration phase. The data also show that the increase in worm burden applied to both male and female worms at both 4 and 7 weeks postinfection (Fig. 5).

Lung histology during *S. mansoni* pulmonary migration. Inflammatory foci develop as the schistosomula migrate through the lungs, possibly as a result of tissue damage caused by the migrating larvae (13). We evaluated the size and cellular composition of the inflammatory reactions in singly infected and coinfected mice on days 10 and 17 post-*S. mansoni* infection. The data in Fig. 6 show that the mean diameters of the pulmonary reactions containing schistosomula were significantly

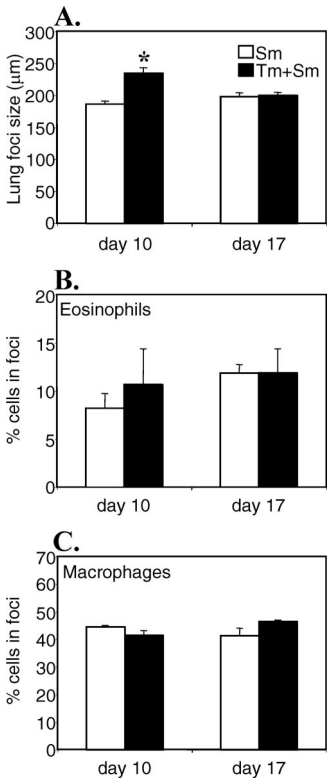

FIG. 6. Size and composition of inflammatory foci in the lungs during the migration phase of *S. mansoni* (Sm). AKR mice were infected orally with 150 *T. muris* (Tm) eggs, percutaneously with 200 *S. mansoni* cercariae, or both. *S. mansoni* infection was started 40 days after *T. muris* infection. At days 10 and 17 post-*S. mansoni* infection, lungs were fixed and embedded and sections were cut and stained with hematoxylin and eosin according to routine methods. The mean diameters of inflammatory foci (A) containing schistosomula and the percentage of eosinophils (B) and macrophages (C) in inflammatory foci were determined for 20 to 40 foci per mouse. Means of four to six mice per group and standard errors of the mean are shown. *, statistically significant difference between groups of mice ($P < 0.05$).

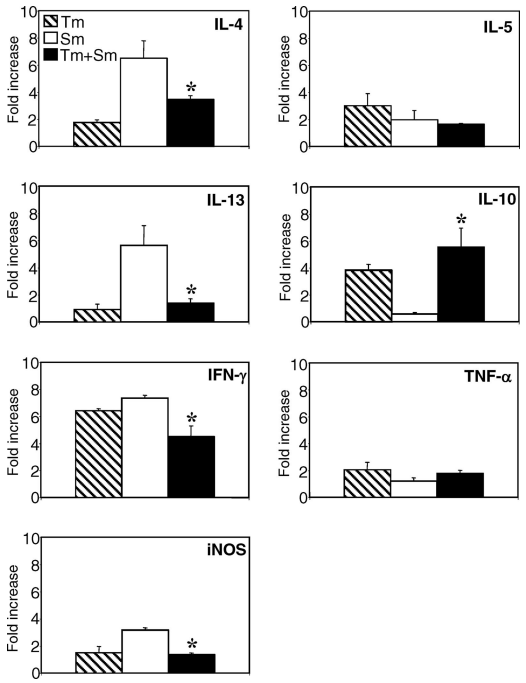

FIG. 7. Lung cytokine mRNA expression during the migration phase of *S. mansoni* (Sm). AKR mice were infected orally with 150 *T. muris* (Tm) eggs, percutaneously with 200 *S. mansoni* cercariae, or both. *S. mansoni* infection was started 40 days after *T. muris* infection. RNA was prepared from freshly isolated lung tissue 7 days post-*S. mansoni* infection and reverse transcribed, and cytokine mRNA expression was analyzed by real-time PCR. Means of four to six mice per group and standard errors are shown. *, statistically significant difference between *S. mansoni* singly infected and coinfected groups ($P < 0.05$).

larger in coinfected than in singly infected mice on day 10 post-*S. mansoni* infection (216.83 ± 13.07 μm versus 173.35 ± 2.64 μm, $P < 0.05$). On day 17 post-*S. mansoni* infection, there was a trend toward the opposite, with the inflammatory foci in mice with only *S. mansoni* infection becoming larger, while the foci from coinfected mice were shrinking. However, the size difference on day 17 was not statistically significant. There was no difference in the cellular compositions of the inflammatory foci, with similar percentages of infiltrating eosinophils and macrophages in the two groups (Fig. 6B and C). No inflammatory responses were observed in the lungs of mice with only *T. muris* infection (data not shown).

Altered cytokine responses in the lungs of coinfected animals. Percutaneous infection with *S. mansoni* in normal mice typically results in the failure of approximately 50% of the worms to reach the portal system. Most of this natural elimination occurs during the lung phase (15, 36). Studies using the irradiated *S. mansoni* vaccine model have demonstrated that attrition of schistosomula in the lungs of vaccinated animals is dependent on IFN-γ-producing $CD4^+$ T cells (44, 48) and correlates with macrophage activation (33) and nitric oxide production (32, 52). In order to investigate the influence of *T. muris* infection on the cytokine environment in the lungs during early *S. mansoni* larval migration, we analyzed cytokine mRNA expression in lungs on day 7 post-*S. mansoni* infection by real-time PCR. This time point was chosen since it represents the peak for larval passage through the lungs (16, 50). The data in Fig. 7 show that the migration of *S. mansoni* larvae is associated with an upregulation of IFN-γ, iNOS, IL-13, and IL-4 mRNAs in the lungs of singly infected mice. Interestingly, mice infected with only chronic *T. muris* infection had increased expression of IFN-γ and IL-10 in lung tissue compared to uninfected mice, demonstrating that although this parasite is living only in the intestinal mucosa, increased cytokine responses can be detected at other mucosal surfaces such as the lungs. Coinfected mice, however, expressed significantly lower levels of IFN-γ, iNOS, IL-13, and IL-4 and significantly higher levels of IL-10, compared to mice with only *S. mansoni* infection. The high level of IL-10 in the coinfected group was comparable to that seen in the *T. muris*-infected group. There was little or no increase in IL-5 or TNF-α expression in any of the groups. Thus, these data show that a chronic *T. muris* infection alters the cytokine environment in the lungs, resulting in an IL-10-dominated response which appears to inhibit innate antilarval responses during *S. mansoni* lung-stage migration.

DISCUSSION

Helminth infections are the most common parasitic infections in the world, and epidemiological studies show that individuals infected with multiple species of helminths often have higher-intensity infections than individuals with single-species infections (3, 5, 45). The associations observed between soil-transmitted nematode species may largely be due to the similarity in transmission of these nematodes, which is closely related to poor hygiene and the lack of adequate sanitation (20). Thus, it is difficult to evaluate the relative contributions of environmental hygiene, socioeconomic, genetic, and pathogen-driven factors in determining rates and prevalence of polyparasitism in humans and it is likely that some or all of these are contributing to polyparasitism to some degree. However, associations between helminths transmitted by different routes (soil versus nonsoil) are more difficult to explain purely based on environmental, geographical, or socioeconomic factors. Nonetheless, increased intensity of infections has been reported in *S. mansoni* and hookworm coinfection (6, 23, 34) as well as in *Schistosoma japonicum*- and *T. trichiuria*-coinfected individuals (20). Interestingly, lower infection intensities have also been reported in *S. mansoni* and *Ascaris lumbricoides* coinfections (23). Such lower infection intensities may be explained by reciprocally enhanced Th2-mediated resistance mechanisms, as lower *Ascaris* worm burdens correlate with increased Th2 responses in humans (46). This is supported by experimental evidence, as mice harboring an established *S. mansoni* infection are more resistant to concurrent *Strongyloides venezuelensis* (54) and *T. muris* (14) infections.

Using the mouse models of *T. muris* and *S. mansoni*, we have investigated the impact an established chronic *T. muris* infection has on resistance to *S. mansoni* infection. We observed significant increases in schistosome worm burden in mice previously infected with *T. muris* (ranging between 64 and 81% of the cercariae applied). Following primary exposure of normal mice to *S. mansoni* infection, typically only 30 to 50% of the cercariae survive to adulthood. The migration from the skin to the portal system involves a complex series of events in which the parasites spend an average of 2 to 5 days penetrating the skin before entering the blood vessels, where they are transported through the lungs before arriving in the hepatoportal system. Approximately 50 to 70% of cercariae never reach the hepatoportal system and are cleared in the lungs (15, 16, 49). While in the lung, the parasite is of such a size that it may completely occlude the vessels and will have to squeeze its way through the capillaries, distending them, and in some cases causing endothelial damage (13), and it is likely that the elasticity of the capillaries may be of importance in permitting the migration of schistosomula through the lungs. Studies using the irradiated cercarial vaccine have shown that following challenge infection in vaccinated animals, focal aggregates of inflammatory cells quickly develop in the lungs and are believed to be a crucial feature of the resistance mechanism (12). Such inflammatory foci also develop in normal unvaccinated mice after infection, although they are less pronounced and slower to develop. We found that the foci were significantly larger in the lungs of coinfected animals on day 10 post-*S. mansoni* infection, but there was no difference in the cellular composition of foci between the groups. It is possible that the larger and more diffuse foci observed in the lungs of the coinfected mice may be less efficient in trapping the parasites, and although the significance of this finding is yet to be established, it bears some resemblance to previous studies in anti-IFN-γ-treated vaccinated animals. These animals have reduced protection against *S. mansoni* (i.e., higher worm recoveries than vaccinated controls) but larger and more diffuse pulmonary foci (44), suggesting an important role for IFN-γ in effector focus formation. In agreement with this, we found that coinfected mice expressed significantly lower IFN-γ and iNOS mRNA levels in the lungs than mice with only *S. mansoni* infection. The reduction in IFN-γ corresponded to an increase in IL-10 expression, which was also evident in mice infected with only *T. muris*. Thus, it is clear that although the *T. muris* worms are entirely restricted to the intestine, their effects on immunoreactivity also extend to other mucosal sites. Lung tissue from animals with *S. mansoni* infection alone expressed increased levels of IL-4, IL-13, iNOS, and IFN-γ, in agreement with previous studies (51, 52).

Our data suggest that the preexisting *T. muris* infection is dominating the cytokine response in the lung during coinfection, and it is tempting to speculate that the high levels of *T. muris*-induced IL-10 are responsible for the suppression of IL-4, IL-13, iNOS, and IFN-γ which is normally observed in the lungs during *S. mansoni* migration. Attempts to block IL-10 in coinfected mice in order to confirm the function of IL-10 during the lung-stage migration of *S. mansoni* were unsuccessful, however. Blockade of IL-10 during the chronic stage of *T. muris* infection rapidly leads to severe intestinal pathology and mortality, preventing us from testing this hypothesis experimentally (data not shown). These findings confirm the requirement for host-protective IL-10 during *T. muris* infection (43). As such, it is currently not possible to test this hypothesis experimentally without affecting the disease progression dramatically in singly infected animals. It is worth noticing, however, that studies using the irradiated schistosome vaccine have shown that IL-10-deficient mice are more resistant and have increased in vitro schistosomulicidal capacity (30), indicating that high levels of IL-10 are unfavorable in the resistance against migrating lung-stage schistosomula.

With respect to protective antischistosomula mechanisms, in vitro studies have shown that IFN-γ-activated macrophages or endothelial cells can kill larval schistosomes through an arginine-dependent mechanism involving production of reactive nitrogen oxide (NO) (32, 39). Furthermore, it is possible that occlusion of pulmonary vessels by the activation of endothelial cells might impede the migration of larvae in the lungs or force them into long-term contact with activated endothelial cells producing toxic mediators such as NO. Studies using the irradiated schistosome vaccine have shown that peak IFN-γ and iNOS responses in the lungs occur at the time when challenge parasites are believed to be eliminated, and iNOS can be identified in the pulmonary inflammatory foci around the migrating larvae (52). Interestingly, previous studies have shown that *S. mansoni* infection in nonvaccinated mice given hemiguanidine sulfate to block production of NO (52) or infection of iNOS-deficient mice (11) results in significantly increased *S. mansoni* worm burdens compared to those in normal mice. This may be due to cytotoxic effects of NO, or it could simply reflect the function of NO as a vascular relaxing factor (37), affecting

smooth muscle tone and vessel diameter and thus affecting the parasites' migration through the capillaries. Significantly, IL-10 is a major negative regulator of NO production (25). Thus, our findings of increased IL-10 and reduced IFN-γ and iNOS levels in the lungs of *T. muris*-coinfected mice may, at least in part, provide an explanation for the enhanced survival rate of *S. mansoni* observed in *T. muris*-coinfected animals. In this context, it is also worth emphasizing that both Th1 and Th2 cytokines are known to play different roles during different stages of schistosome infection and that an excessive IFN-γ-dominated response during the egg-laying stage leads to severe host pathology (29). Thus, different cytokines play different roles during different stages of the infection. Other factors than IFN-γ and iNOS may also be involved in regulating the migration success for *S. mansoni* larvae. Studies in rats have demonstrated that sublethal irradiation of normal rats before infection resulted in higher worm burdens (24). This was also the case for complement (47) and mast cell depletion (24), indicating that a number of factors may be contributing to innate resistance. Of relevance is also the fact that immunodeficient rats or mice do not develop higher worm burdens, showing that the mechanisms regulating resistance to pulmonary migration are indeed part of the innate system (1, 24).

Analysis of the cytokine response to SEA in the spleen 7 weeks post-*S. mansoni* infection revealed no differences between singly infected and coinfected animals. In the MLN, however, SEA-specific levels of IL-4 and IL-13 production were significantly reduced, although this was not accompanied by a shift toward a Th1 response. Conversely, the *T. muris*-specific cytokine response in the MLN was intact in coinfected animals, while the antigen-specific IFN-γ and IL-10 responses were significantly reduced in the spleen. Again, there was no shift in T helper cytokine profile. Thus, it appears that the two infections are dominating the immune response in separate anatomical and lymphoid compartments and suppressing antigen-specific responses without skewing the cytokine profiles. Interestingly, we also found that the *T. muris* worm burdens were unaffected by the *S. mansoni* infection. This is in contrast to results from a previous study where *S. mansoni* infection preceded *T. muris* infection (14). As such, it is clear that the timing and sequence of infections are of vital importance for the outcome of coinfections.

The most striking finding from our present study is the demonstration that a preestablished chronic *T. muris* infection resulted in significantly increased *S. mansoni* worm burden. Crucially, this increase in adult worm burden also resulted in significantly increased schistosome egg burden in the liver as well as increased levels of the profibrotic cytokine IL-13. Since there was no corresponding increase in the antifibrotic cytokine IFN-γ (29), this raises the possibility that *Trichuris* coinfection may increase the risk or level of liver fibrosis during chronic schistosomiasis. Although several human studies have reported increased intensity of infection in schistosome-infected individuals during intestinal nematode coinfection (6, 20, 23, 34), we have not been able to find any studies reporting on parameters of clinical morbidity, such as periportal fibrosis. Further studies are needed to confirm whether such an increase in schistosome infection intensity is of clinical importance in human schistosome-associated morbidity.

In conclusion, we have provided the first demonstration that a preexisting infection with a gastrointestinal nematode can promote the infectivity and establishment of the trematode helminth *S. mansoni*, resulting in higher worm burdens and increased egg-induced pathology. The underlying immunological mechanisms appear to involve nematode-induced alterations in lung cytokine responses and reduction in innate resistance mechanisms. We believe this study may represent the first experimental insight into the aggregation of multiple helminth infections seen in nature, providing a foundation for future investigations into the immunology of helminth-helminth coinfections.

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