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NOTES

Mice Lacking the Gamma Interferon Receptor Have an Impaired Granulomatous Reaction to *Schistosoma mansoni* Infection

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The egg-induced granulomatous reaction in *Schistosoma mansoni*-infected individuals develops within the portal system of the liver and is the major pathological finding in schistosomiasis. We have infected mice lacking the gamma interferon (IFN-γ) receptor with *S. mansoni* larvae and studied the development of hepatic granulomas in these mutant mice in comparison to that in control wild-type mice. In the absence of IFN-γ activity, a dramatic reduction in the size and architecture of the granuloma was observed. Granulomas from mutant mice were smaller than those from the control group and showed a significant reduction in the number of infiltrating inflammatory cells. Moreover, they appear to prematurely progress to the chronic phase of the reaction at a time when the control group still has acute inflammation. Our data suggests a pivotal role for IFN-γ in the early events of the granulomatous reaction in vivo.

Schistosomiasis is a chronic disease resulting from infection with schistosome worms. The main pathological alteration observed in paients with schistosomiasis is a granulomatous reaction within the liver, triggered by parasite egg deposition (20). As a consequence of the granuloma formation, an extensive area of the liver is destroyed by the fibrotic reaction during the chronic phase that follows the inflammatory process. The fibrotic reaction also leads to blockage of the mesenteric veins and, consequently, hypertension of the portal system (2, 16). The disease can be accurately reproduced in mice, and egg-induced granulomas in mouse liver and lungs have been used as a model for dissecting the mechanisms regulating the granulomatous reaction (18).

In mice, the formation of the granuloma initiates with egg deposition within the portal vascular system and reaches its inflammatory peak at 8 weeks postinfection. During this phase, the reaction has been characterized as a delayed-type hypersensitivity response, which is dependent on $CD4^+$ T cells (2, 21). After 8 weeks, the granulomatous reaction begins to subside, ending with an intense fibrotic reaction and tissue destruction. $CD8^+$ T cells appear to play a major role in this later phase of the response (6). A significant number of in vivo experiments using the mouse model have been performed, aiming to elucidate the role of Th-1- and Th-2-derived cytokines in the regulation of the granulomatous reaction.

It was demonstrated by Chensue et al. (5) that in mice, interleukin 4 (IL-4) production occurs during days 1 and 2 of granuloma formation, whereas gamma interferon (IFN-γ) is produced mainly on days 4 to 8 postinfection. Moreover, in mice injected with *Schistosoma mansoni* eggs, which leads to granuloma formation within the lungs, administration of anti-IL-4 or anti-IL-2 antibody led to the formation of pulmonary granulomas with a reduced size (5, 12), whereas treatment with anti-IFN-γ antibodies did not alter the size of granulomas but led to a reduction in the number of giant cells (5). In contrast, anti-IL-4 treatment did not alter the size of either hepatic or pulmonary granulomas during natural infection, when eggs are laid by worms (7). In larva-infected mice, administration of anti-IL-2 antibody leads to a reduction in the size of hepatic granulomas and, interestingly, a reduction in the levels of IL-5 (3) followed by a reduced number of eosinophils in peripheral blood and tissues of treated animals. It was suggested that IL-2 might have an important role in the generation of a Th-2-type response rather than in the establishment of a Th-1-associated response (3). Similarly, administration of anti-IFN-γ antibody in *Schistosoma japonicum*-infected mice resulted in granulomas of reduced size, with no effect on the fibrotic reaction that follows the inflammatory response (4).

It was suggested by Pearce et al. (15) that the switch between Th-1- and Th-2-type responses could be a consequence of different antigens presented at different stages of the infection. Those authors proposed that a Th-1-type response would be triggered by antigens from the larval stage of some helminth parasites, whereas egg-derived antigens, appearing later in the infection, would switch the response to a predominately Th-2-type response.

From these observations, it becomes clear that the egg-induced granuloma reaction in *Schistosoma*-infected mice is regulated by a complex balance of cytokines and should not be classified as a classical Th-1-based delayed-type hypersensitivity response. Th-2-type cells clearly play a critical role in the development of this inflammatory process.

In order to better understand granuloma formation and the role of Th-1-derived cytokines in regulating the granulomatous reaction in vivo, we infected mice with a homologous disruption of the IFN-γ receptor with *S. mansoni* larvae and investigated the development of hepatic granulomas.

Infection of mice. Mice with a targeted deletion within the gene coding for the α-chain of the IFN-γ receptor ($IFN\text{-}\gamma R^{0/0}$) were generated by Huang et al. (11). Control and mutant mice

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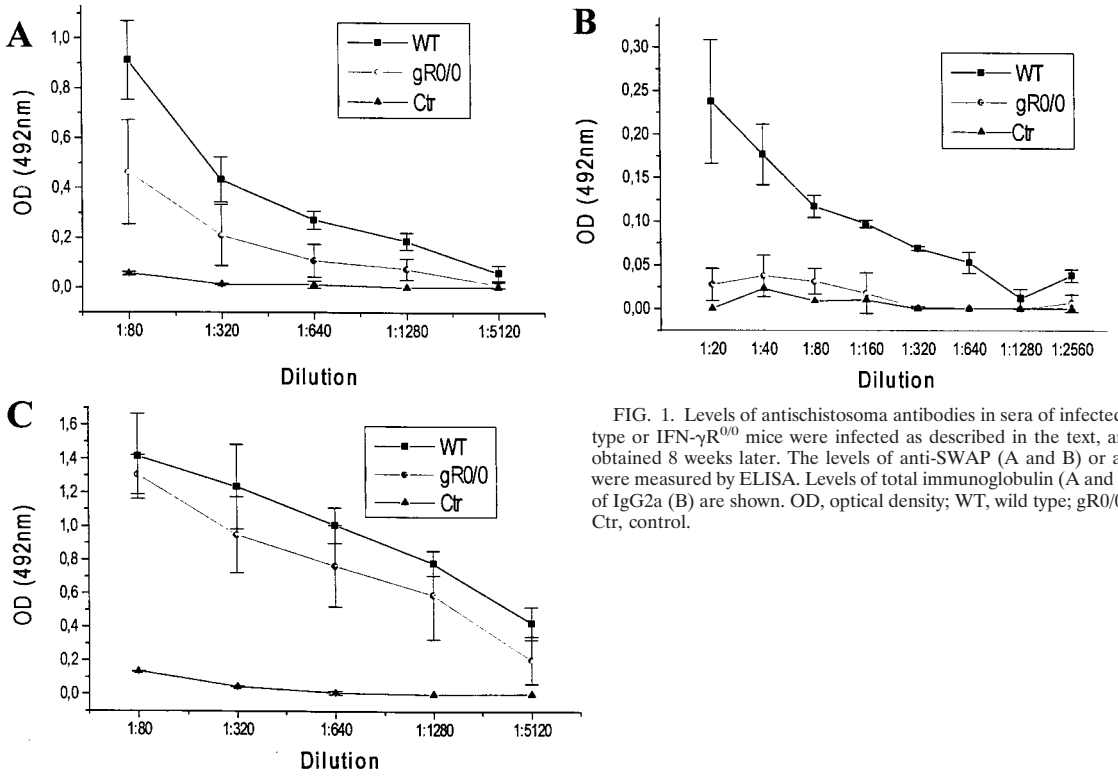

FIG. 1. Levels of antischistosoma antibodies in sera of infected mice. Wild-type or IFN-$\gamma R^{0/0}$ mice were infected as described in the text, and sera were obtained 8 weeks later. The levels of anti-SWAP (A and B) or anti-SEA (C) were measured by ELISA. Levels of total immunoglobulin (A and C) and levels of IgG2a (B) are shown. OD, optical density; WT, wild type; gR0/0, IFN-$\gamma R^{0/0}$; Ctr, control.

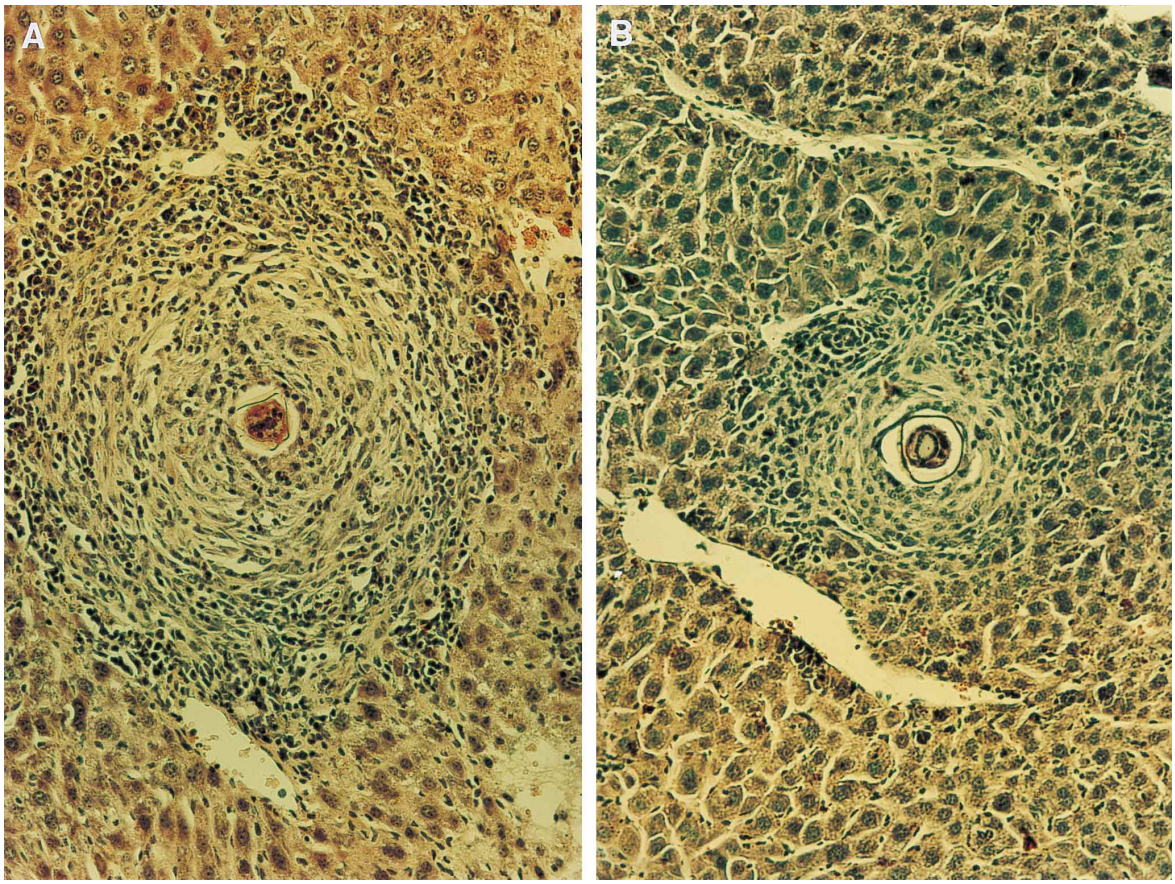

FIG. 2. *S. mansoni* egg-induced granulomas in the livers from wild-type or IFN-$\gamma R^{0/0}$ mice. Mice were infected with *S. mansoni* larvae and sacrificed 8 weeks after infection. Liver sections were prepared and stained with hematoxylin-eosin as described in the text. (A) Section from wild-type animal. (B) Section from IFN-$\gamma R^{0/0}$ animal. Magnification = ×170.

(pure 129/Sv/Ev background) were used at the age of 6 to 10 weeks. Cercariae of the LE strain (Belo Horizonte, Brazil) of *S. mansoni* were obtained from infected *Biomphalaria glabrata* snails by use of artificial light (17). Anesthetized mice were infected subcutaneously by exposure to 25 cercariae with three animals in each group, in a total of three independent experiments. Infected animals were sacrificed 8 weeks postinfection.

Antibodies against parasite antigen. The levels of anti-soluble egg antigen (anti-SEA) and anti-soluble worm antigen preparation (anti-SWAP) antibodies in sera of infected animals at 8 weeks postinfection were measured by enzyme-linked immunosorbent assay (ELISA) as described elsewhere (9). Sera of six animals from each group were tested individually, and the results are presented as the averages for three animals from each group. For isotype determination, sera from three animals from each group were analyzed by ELISA using subtype-specific antibodies. The results are presented as the average optical density for each group.

Analysis of the granuloma. Livers from 8-week-infected control or mutant animals were collected and fixed with 4% paraformaldehyde in phosphate buffer. Histologic sections were prepared and stained with hematoxylin-eosin or with Heidenhein's azan as described by Hirata et al. (10). The area of the granulomas surrounding single, mature eggs were measured along the longer and the shorter axes with the aid of an ocular micrometer. A total of 270 granulomas from nine animals of each genotype were measured in three independent experiments (three animals per group in each experiment). Results of a representative experiment are shown below.

The numbers of adult worms recovered from control and mutant mice were similar (data not shown), suggesting that IFN-γ is dispensable for the immune response against invasive forms of *S. mansoni*. A pronounced reduction in the levels of anti-SWAP immunoglobulin G2a (IgG2a) antibody was observed in mutant mice compared to that of the control group (Fig. 1). No significant reduction in the levels of IgG1, IgG3, or IgM was observed (data not shown). This result confirms the earlier observation by Akhiani et al. (1), using the same IFN-$\gamma R^{0/0}$ mice as in this study, of a significant reduction in the number of IgG2a-producing cells in mice infected with *S. mansoni* and suggests that the decrease in IgG2a-producing cells is biologically relevant.

It was recently reported by Wilson et al. (22) that mice vaccinated with irradiated cercariae can be protected against challenge with normal parasites but that, under these conditions, IFN-$\gamma R^{0/0}$ mice appear to be less sensitive to the protective effects of vaccination. This suggests that IFN-γ may play an important role in the protective immune response elicited by vaccination. However, in agreement with previous studies (19), Wilson et al. found that vaccine-induced protection is a cell-mediated rather than an antibody-mediated immune response. It remains to be determined whether the reduced level of anti-SWAP IgG2a production leads to an altered immune response during secondary infection in mutant mice.

To investigate the number, structure, and architecture of the granulomas, liver samples were obtained from mice of each genotype and sections were prepared. We investigated the number of granulomas present within the livers of each individual animal and found no significant difference between control or mutant mice, suggesting that IFN-γ does not influence egg deposition by adult female worms (data not shown).

However, a dramatic difference in both the size and the architecture of the granulomas from the two groups was observed. Figure 2 shows the hematoxylin-eosin staining of a typical granuloma from a wild-type and a mutant animal. Granulomas from IFN-$\gamma R^{0/0}$ mice were significantly smaller

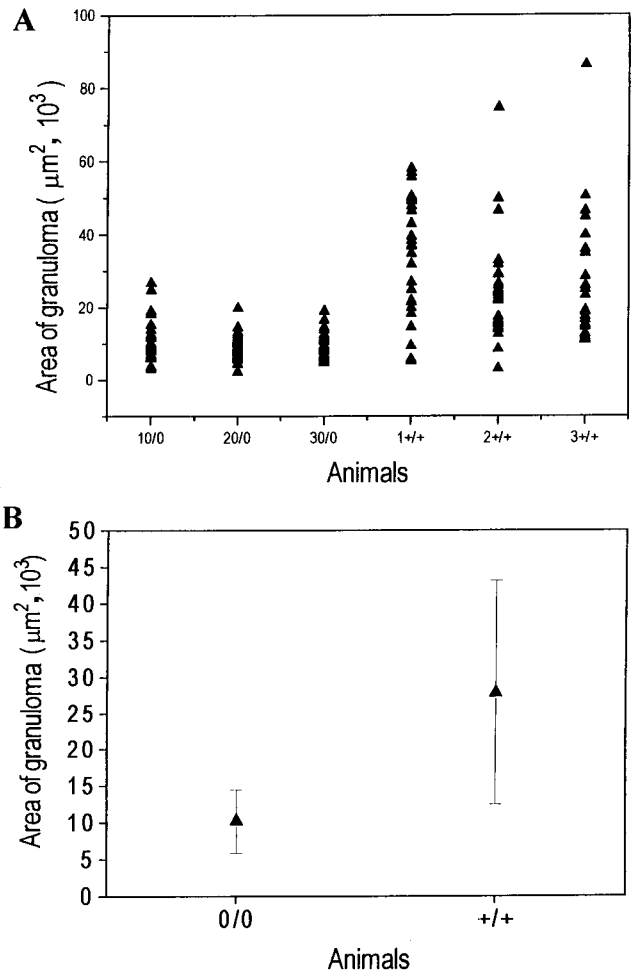

FIG. 3. Size distribution of granulomas in the livers of *S. mansoni*-infected control or mutant mice. Animals were infected as described in the text, and histological analyses of stained sections were performed. The size of each granuloma surrounding a mature egg was measured with the aid of an ocular micrometer. (A) Size distribution of granulomas from three mutant ($1^{0/0}$, $2^{0/0}$, and $3^{0/0}$) or control ($1^{+/+}$, $2^{+/+}$, and $3^{+/+}$) animals. (B) Averages of 30 granulomas from three animals from each group ($n = 90$ per group). 0/0, mutant; +/+, wild type.

than those from the control group. We have analyzed a total of 270 individual granulomas from three experiments, with three animals in each of the two groups (for a total of nine animals in each group). The sizes of 30 granulomas from three individual IFN-$\gamma R^{0/0}$ and wild-type animals are shown in Fig. 3A as a representative example of one such experiment. Since the deposition of eggs in the liver is not synchronized, a rather large variation in the sizes of the granulomas is evident. The contrast in the size distribution between granulomas from mutant or control animals (90 granulomas of each genotype) is most apparent when the sizes are averaged across genotypes as depicted in Fig. 3B.

The hematoxylin-eosin staining also revealed another striking difference between the granulomas of mutant and control mice. There is an obvious reduction in the number of inflammatory cells in the IFN-$\gamma R^{0/0}$ mice compared to that in the granulomas from wild-type mice. The reduction of inflammatory cells is especially apparent in the outer layer of the granulomas. In addition, a reduced number of epithelioid cells at the center of the granuloma can be observed in sections of granulomas from IFN-$\gamma R^{0/0}$ mice (Fig. 2B), suggesting that

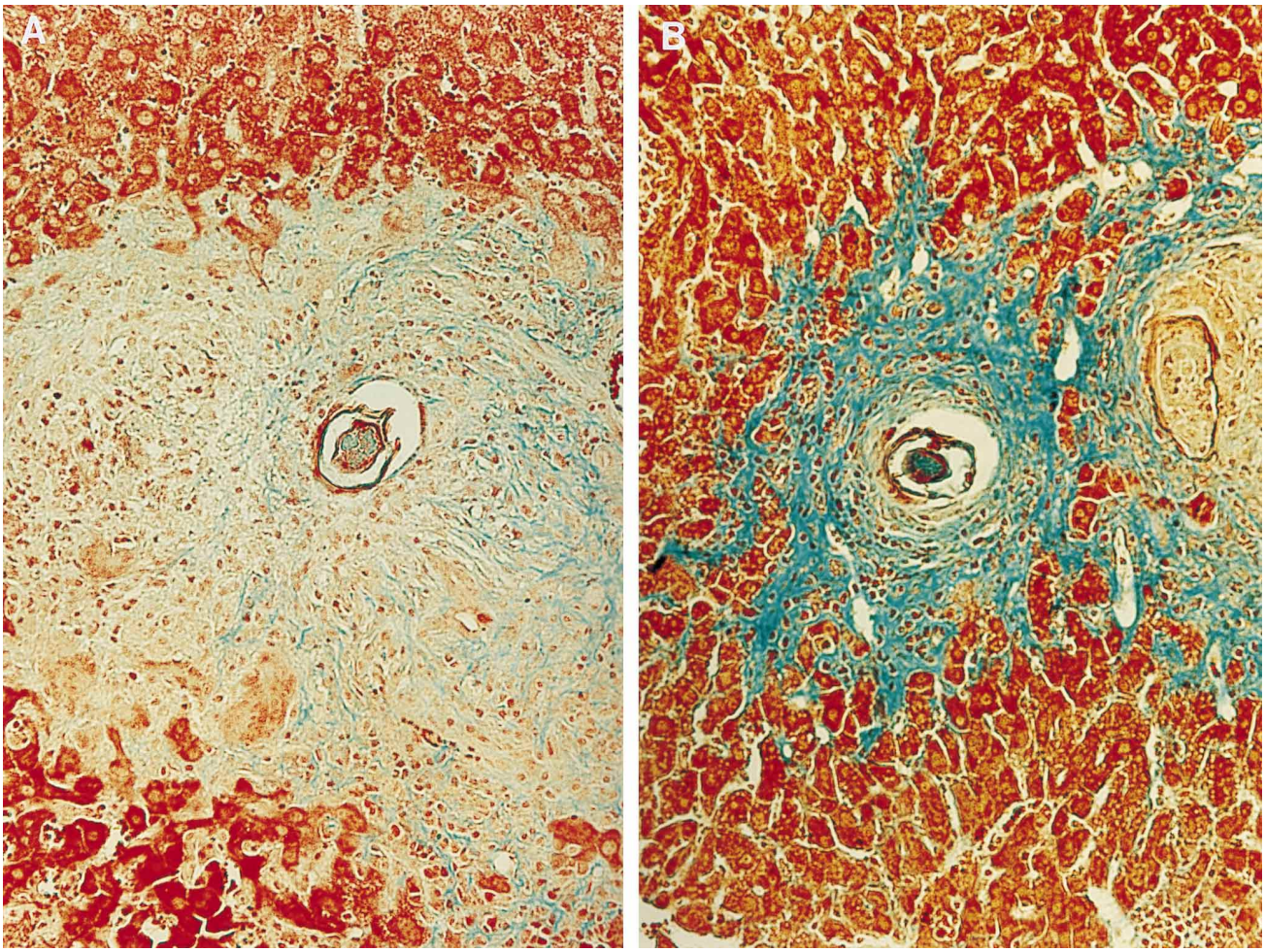

FIG. 4. *S. mansoni* egg-induced granulomas in the livers of wild-type or IFN-γR$^{0/0}$ mice. Mice were infected with *S. mansoni* larvae and sacrificed 8 weeks after infection, and liver sections were prepared and stained as described in the text. Sections were stained by Heidenhein's azan procedure (14). (A) Section from wild-type animal. (B) Section from IFN-γR$^{0/0}$ animal. Magnification = ×170.

IFN-γ not only modulates the size of the granuloma, but also influences its composition.

The reduced size of the granulomas in the mutant mice could be a consequence of delayed kinetics in the initiation or progression of the inflammatory phase of the granulomatous reaction. Alternatively, the reduced size could result from the premature termination of the inflammatory phase. To determine which of these two mechanisms is responsible for the reduction in the size of granulomas from mutant mice, slices from the livers of animals from both groups were stained with Heidenhein's azan as described by Hirata et al. (10), which reveals the presence of collagen. Collagen deposition is a hallmark of the terminal phase of the inflammatory phase of the granulomatous reaction. Granulomas from mutant mice developed an intense fibrotic reaction 8 weeks after infection (Fig. 4B), whereas the granulomas from control mice continued the inflammatory process, with minimal and discrete collagen deposition (Fig. 4A). These results suggest that in the absence of IFN-γ actions, the inflammatory phase of the granuloma in mutant mice ends prematurely. This observation does not support the idea that granulomas from mutant mice are smaller due to delayed kinetics of inflammation.

Our observation that granulomas from cercaria-infected IFN-γR$^{0/0}$ mice are smaller than those from wild-type mice contrasts with results reported by Akhiani et al. (1), who failed to detect any significant change in the size of granulomas even though they were using the same mutant mice. One major disadvantage of the in vivo model of hepatic granuloma triggered by *S. mansoni* is that eggs are continuously released and their deposition in the liver is unsynchronized. As a consequence, not all granulomas within the liver of infected mice are of the same age, which results in a large size variation (Fig. 2B). Since the report by Akhiani et al. (1) does not describe the number of granulomas measured in each group, we cannot directly compare our findings. As demonstrated by the data presented in Fig. 2, there are some granulomas of comparable sizes in the two groups, which most likely are granulomas surrounding recently deposited eggs. In addition, some granulomas from mutant mice have little collagen deposition, comparable to that of the small, immature granulomas from wild-type mice (data not shown). Nevertheless, the analysis of the average size of 90 individual granulomas from mutant mice compared to that of the control group showed a clear and distinct size difference.

Our results corroborate the data reported by Cheever et al. (4) for *S. japonicum*-infected mice. Those investigators demonstrated a reduction in the sizes of granulomas when mice were treated with anti-IFN-γ antibody. More recently, Flores-

Villanueva et al. (8) showed that in mice treated with IL-10, there was a significant inhibition of hepatic granuloma formation. Lymph nodes from IL-10-treated mice have reduced levels of both IL-2 and IFN-γ expression. The authors hypothesized that the reduced granuloma formation resulted primarily from the specific down-regulation of the Th-1-type response.

Granulomas from mutant mice also showed a reduced number of infiltrating inflammatory cells, as well as a reduced number of epithelioid cells at the center of the granuloma. This result confirms the earlier in vitro observation by Möst et al. (14) that IFN-γ and LFA-1 expression is required for proper generation of giant cells. We are performing experiments using labeled antibodies and in situ hybridization in order to identify specific cell types within the individual granulomas and their cytokine production profiles.

Our results suggest that IFN-γ plays a critical role in controlling the granulomatous reaction in response to *S. mansoni* infection in vivo. It was suggested by Pearce et al. (15) that the immune response elicited by schistosomula in *S. mansoni*-infected mice is preferentially of the Th-1 type, whereas the response triggered by the *S. mansoni* egg is of the Th-2 type. Accordingly, in natural infection, mice would initially have a Th-1-type response up until the stage when eggs are released by the adult females in the liver. Deposition of eggs in the liver would then switch the profile of secreted cytokines to a Th-2-type response. We propose that the secretion of IFN-γ triggered by larvae and adult worms during the initial phase of the immune response is essential for the proper initiation of the inflammatory reaction that leads to granuloma formation in the liver. Recently, Metwali et al. (13) reported that IL-4-deficient mice infected with *S. mansoni* have hepatic granulomas of reduced size and also a reduced number of infiltrating eosinophils and mast cells compared to those of wild-type controls. These results support the idea that both Th-1- and Th-2-dominated phases are necessary for proper granuloma formation.

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