

Schistosome-induced Pathology Is Exacerbated and Th2 Polarization Is Enhanced During Pregnancy

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Abstract. *The aim of this study was to investigate the immunopathological impact of pregnancy on an ongoing experimental schistosomiasis infection. Materials and Methods: Female BALB/c mice were randomly divided into three groups (A, B and C) of 15 animals each. The mice in Groups A and B were infected with 40 *S. mansoni* cercariae, percutaneously. Six weeks post-infection, the mice in Groups B and C (schistosome-naïve controls) were mated. Schistosome-induced morbidity and cytokine recall responses were subsequently evaluated at weeks 7 and 8 post-infection. Results: Hepatic and pulmonary lesions resulting from trapped schistosome eggs were more frequent and more severe in Group B mice than in Group A mice. Group C mice had suppressed mitogen-stimulated interleukin 4 (IL-4) but maintained high interferon gamma (IFN- γ) responses. In contrast, Group A mice had elevated mitogen- and parasite-specific IL-4 but muted IFN- γ responses. Group B mice had an early (week 7) high IL-4 response, even higher than in group A mice. Conclusion: Taken together the data suggest that pregnancy exacerbates schistosome-induced morbidity, probably through up-regulation of parasite-specific IL-4.*

Parasitic infections and pregnancy are two biological events that have a marked impact on the immune system. The primary cause of morbidity in schistosomiasis, a chronic debilitating parasitic disease, is the schistosome egg granuloma, and a CD4+ T-cell-mediated delayed type hypersensitivity (1). The balance between T helper 1 (Th1) and Th2 cytokine expression correlates with the extent of

granulomatous inflammation (2) and hence the severity of the disease. Large exuberant granulomas and severe morbidity are associated with a Th2-dominated cytokine response and minimal lesions are found associated with a Th1-dominant response (3).

Pregnancy is a physiological process that may influence the outcome of a schistosome infection in several ways. First, like schistosomiasis, a successful pregnancy is dependent upon a tight regulation of the Th1/Th2 balance (4). Secondly, steroid hormones of the hypothalamic-pituitary-adrenal (HPA) axis have a regulatory role in the establishment, maturation and oviposition of schistosomes and the progression of schistosomiasis morbidity. Morales-Montor and co-workers (5) have demonstrated that severe schistosomiasis morbidity in the baboon is associated with low levels of these hormones in the circulation. Our own preliminary studies have shown that schistosomiasis has a negative impact on the estrous cycle in baboons (6). Thirdly, pregnancy entails cessation of the cyclical pattern of hormone secretion of the normal estrous cycle. Instead, there is a sustained elevation of progesterone secretion throughout pregnancy. Progesterone and other placental products, such as prostaglandin E₂, suppress Th1 responses (7).

Finally, a unique feature of pregnancy is the enhanced blood circulation to the lower abdomen. There is also increased intra-abdominal pressure with advancing pregnancy and progressive enlargement of the uterus, often resulting in dilatation of blood vessels and possibly formation of collateral circulation. The effects of these circulatory changes on schistosomes and their microenvironment in the mesenteric blood vessels and the distribution of schistosome eggs have not been investigated. The present study investigated the overall immunopathological consequences of pregnancy on schistosomiasis in infected BALB/c mice.

Materials and Methods

Animals. Female nulliparous inbred BALB/c mice, six weeks of age (at the commencement of the infection) and bred at the International Laboratory Research Institute (Nairobi, Kenya) were used in the study. The animals were acclimatized for 2 weeks prior to

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Key Words: Cytokines, morbidity, pregnancy, schistosomiasis, Th1/Th2 balance.

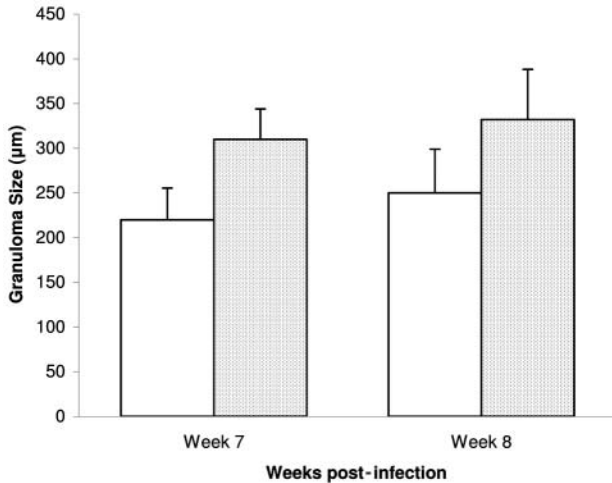


Figure 1. The mean hepatic granuloma diameter for non-pregnant infected mice (Group A, open bars) and pregnant infected mice (Group B, hatched bars) at weeks 7 and 8 post-infection. Group A had significantly smaller granulomas than Group B at both time-points ($p < 0.05$).

infection. The animals were housed in groups of five per cage and maintained on commercial pellets and water *ad libitum*. Animals were under a natural dark/light cycle of ≈ 12 h/12 h, ambient temperatures of $20^\circ\text{C} \pm 1^\circ\text{C}$ and relative humidity of 50%-60%.

Experimental design. Maintenance of the parasite life cycle has been described before (8). The female mice were randomly divided into three groups of 15 animals each. Groups A and B animals were infected with 40 *S. mansoni* cercariae each, while group C animals remained unexposed and served as controls.

All infections were performed percutaneously by immersing the mouse tail in a suspension of the appropriate number of cercariae in water for 30 min. Seven weeks post infection, mice in Groups A and C were mated by placing each female mouse with a non-infected male mouse.

Necropsy and tissue sampling. The mice were killed in groups of five at week 7 and 8 post infection, by exsanguination, to obtain the spleens for isolation and culture of splenocytes and liver tissue for quantification of granuloma sizes. The vertical and horizontal diameter of granulomas with a visible centrally placed schistosome egg were measured using an ocular micrometer. The average of the horizontal and vertical diameter was taken to be the diameter of the granuloma. A total of 10 granulomata were measured for each animal.

Immunological assays. Preparation of soluble egg antigen (SEA) and soluble worm antigen preparation (SWAP) has been described in detail previously (8). After isolation and quantification, the splenocytes were cultured for cytokine production at 3×10^6 /ml in 48 well tissue culture plates (Falcon, Becton Dickinson Co., Franklin Lakes, NJ, USA) in the presence of 20 $\mu\text{g/ml}$ SWAP, 5 $\mu\text{g/ml}$ SEA, 10 $\mu\text{g/ml}$ concanavalin (Con) A (Sigma Co., St. Louis, MO, USA) or complete RPMI medium.

The cultures were incubated at 37°C in a humidified atmosphere with 5% carbon dioxide. Supernatants were harvested after 96 h for

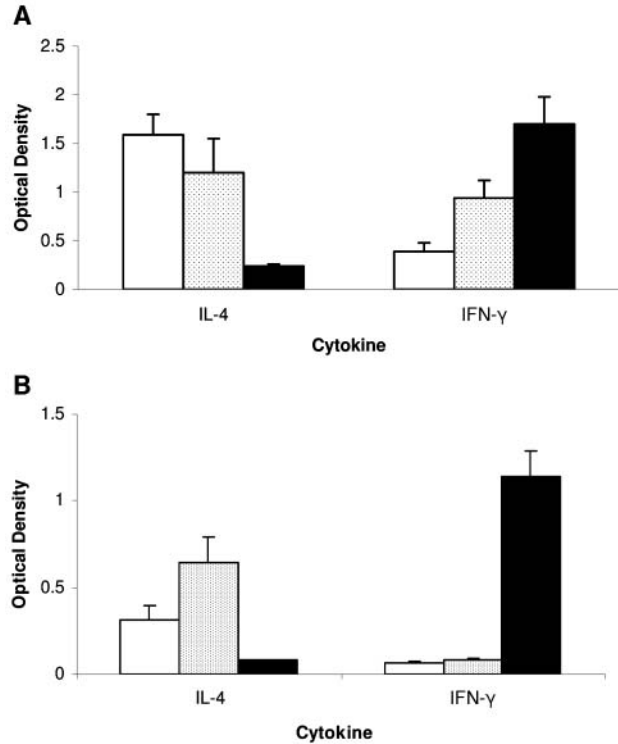


Figure 2. Optical density values at A: 7 weeks and B: 8 weeks after infection for concanavalin (con) A-induced interferon gamma and interleukin 4 production from supernatants of splenocyte cultures harvested after 96 h, in non-pregnant infected (Group A, open bars), pregnant infected (Group B, hatched bars) and pregnant non-infected mice (Group C, dark bars). Group C animals had abundant IFN- γ response in contrast to Groups A and B.

the quantification of the levels of cytokines. IFN- γ and IL-4 were quantified using a capture enzyme-linked immunosorbent assay and the absorbance at 450 nm was measured (8).

Ethical review. The study was approved by the Institutional Ethical and Scientific Review Committee at the Institute of Primate Research, Karen, Nairobi, Kenya.

Statistical analysis. All p -values < 0.05 as determined with Student's t -test were considered significant.

Results

Morbidity. Schistosome-induced hepatic lesions included hepatomegaly, increased friability and discoloration, roughened surface and presence of whitish focal to multifocal nodules protruding over the liver capsule. A subjective assessment of the different groups revealed that the infected pregnant mice (Group B) developed more severe hepatic lesions than did the infected non-pregnant mice (Group A) at both weeks 7 and 8 post-infection. This was further confirmed when a quantitative assessment of the granuloma diameter was carried out (Figure 1). The mean

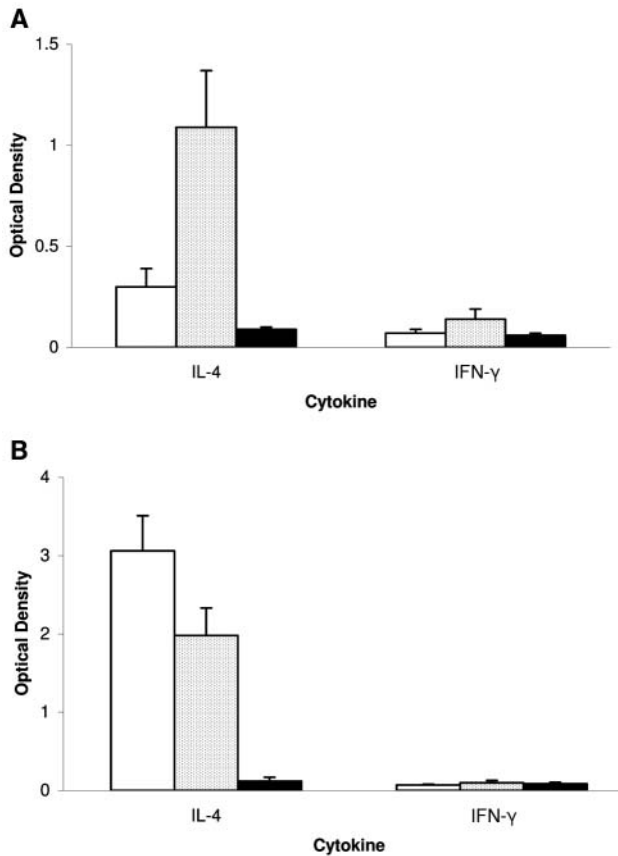


Figure 3. Optical density values at A: 7 weeks and B: 8 weeks after infection for soluble adult worm antigen preparation (SWAP)-induced interferon gamma and interleukin 4 production from supernatants of splenocyte cultures harvested after 96 h, in non-pregnant infected (Group A, open bars), pregnant infected (Group B, hatched bars) and pregnant non-infected mice (Group C, dark bars). Group B animals had an early SWAP-induced IL-4 response.

granuloma diameter was significantly larger for Group B than Group A at both weeks 7 and 8 ($p < 0.05$).

Pulmonary lesions comprised either mononuclear cellular infiltration, inter-alveolar thickening, expanded bronchial associated tissue and on rare occasions granulomatous inflammation. These lesions were more frequently observed in the Group B animals than in Group A animals. At week 7, none of the Group A mice had pulmonary lesions while 4/5 of Group B had lesions. By week 8, 2/5 of Group A and all the animals in Group B had these lesions.

In vitro cytokine secretion. ConA-stimulated lymphocytes from the animals in the three groups yielded different amounts of IL-4 and IFN- γ . In the non-infected pregnant mice (Group C), ConA-stimulated-IL-4 production was significantly suppressed while IFN- γ production was not significantly affected at week 7 (Figure 2A) or 8 (Figure 2B).

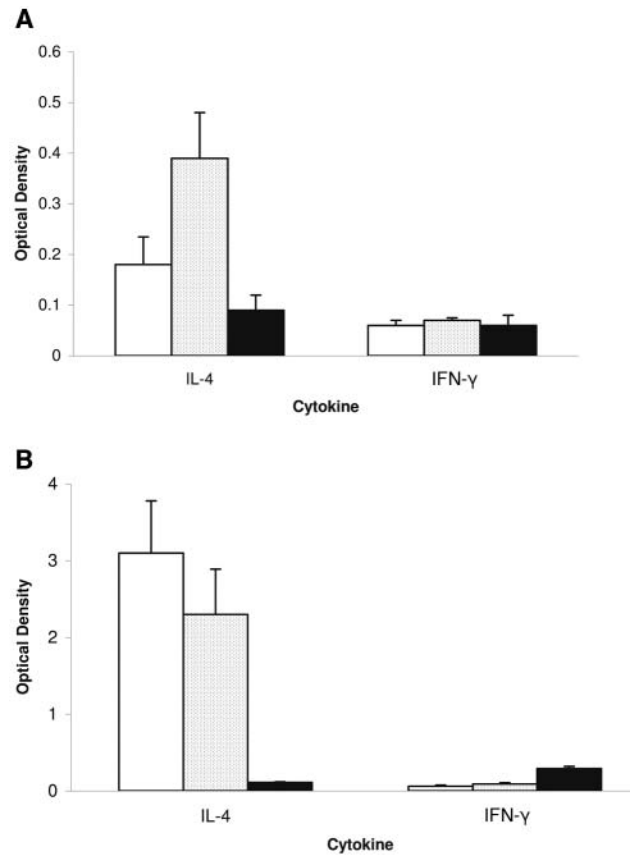


Figure 4. Optical density values at A: 7 weeks and B: 8 weeks after infection for schistosome egg antigen (SEA)-induced interferon gamma and interleukin 4 production from supernatants of splenocyte cultures harvested after 96 h, in non-pregnant infected (Group A, open bars), pregnant infected (Group B, hatched bars) and pregnant non-infected mice (group C, dark bars). Group A animals had the highest SEA-induced IL-4 response.

Schistosomiasis infection had the reverse effect, suppressing ConA-stimulated IFN- γ production and upregulating IL-4 production. Despite the pregnancy, Group B animals had significant ConA-stimulated IL-4 production and by week 8, IFN- γ production was significantly suppressed.

Group B animals had an early (week 7: Figure 3A) SWAP specific IL-4 production that persisted to week 8 (Figure 3B). By contrast, Group A animals had low SWAP-specific IL-4 production at week 7, but by week 8 they exhibited significantly elevated IL-4 levels. SWAP-specific IFN- γ responses were muted in the animals of both infected groups. As expected no SWAP-specific IL-4 or IFN- γ was detected in the non-infected group.

Similar to SWAP responses, an early (week 7: Figure 4A) response to SEA specific IL-4 was detected. This persisted to week 8 (Figure 4B) in the infected pregnant mice (Group B).

Discussion

Pregnancy is associated with some degree of maternal immunosuppression (4, 9), hence it is considered an immunologically vulnerable period when susceptibility to parasitic infections is tremendously increased (10). In this study, we have demonstrated that pregnant mice suffer a more severe acute phase schistosomiasis as compared to non-pregnant infected control mice, as evidenced by the severe hepatic pathology and the large hepatic granulomata in the pregnant animals.

When a specific immune response is elicited during pregnancy, the response is biased towards the less damaging antibody-mediated Th2 immune response (11). This is evidenced by the preponderance of Th2-associated cytokines at the maternal-fetal interface (12). Furthermore, a direct role for natural killer cells, macrophages, inducible nitric synthase (iNOS) and tumor necrosis factor α in early embryo loss and fetal resorption has been demonstrated in the murine model (13). Similarly, in this study the severe schistosome morbidity was correlated to an early parasite specific IL-4 response, a cytokine pattern that has been associated with exacerbated lesions (3).

Interestingly, the non-infected pregnant mice had an elevated mitogen-stimulated IFN- γ , a Th1 status, both at weeks 7 and 8 post-infection. Accumulating data suggest that Th1 cytokines, although harmful during mid-gestation, are necessary during the early stages of pregnancy and during parturition (14). Thus, kinetics experiments in mice have shown peaks of IFN- γ and TNF- α production on days 7, 9 and 12 of gestation in maternal serum followed by a decline thereafter (15).

Pulmonary lesions including schistosome egg granulomas were more frequent in the pregnant infected mice than in the non-pregnant infected controls, and by week 8 all the mice in this group had pulmonary lesions. This was attributed to the development of collateral circulation from the mesenteric blood vessels that by-passes the portal circulation into the pulmonary circulation, hence eggs embolize into the lungs rather than the liver. This confirms previously findings in mice (16) including those undergoing repeated schistosome infection and chemotherapy (8).

In schistosomiasis endemic areas women typically become infected during early childhood and usually maintain infection into middle-age, a time-span that includes child-bearing years. In view of the present results, it will be highly relevant to study the overall effect of pregnancy on the severity of the schistosomiasis in individuals suffering from an already established schistosome infection.

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