Immunological Determinants of the Granulomatous Response in *Schistosoma Mansoni*-Infected Baboons

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ABSTRACT

The present study highlights the roles of exposure, intensity and treatment on the outcome of schistosomiasis in baboon with particular reference to the immune and granomalosous responses. The study demonstrates that baboons, despite their high cost of maintaince, are suitable models for the study of s. masoni host-parasite interactions: they are susceptible to natural and experimental infections, high cross-reactivity between human and baboon immune response markers (antibodies, cytokines etc.) permit human reagents to be used for dissecting baboon responses. The use of this model may advance the development of a vaccine against human schistosomiasis mansoni infections.

Key words. Baboon, cytokines, granuloma, Schistosoma mansoni

INTRODUCTION

Schistosomiasis is a widespread chronic helminth infection that contributes to the death of over 500,000 people annually (1). The major form of disease results from the chronic granulomatous response to parasite ova trapped in host tissues. One notable characteristic of *schistosome* infections is that most infected individuals tolerate chronic infection without debilitating illness. This has been postulated to be due to mechanisms that lead to down-modulation of host granulomatous response (1). In about 10% of infected persons, failure to modulate can ultimately lead to hepatic periportal fibrosis, portal hypertension and death. The mechanisms associated with modulation of the granulomotous inflammation in liver and other tissues are not well understood. The precise role of antibodies and cytokines remain to be worked out. Studies in the murine model show that granuloma formation correlates with increased production of *schistosome* egg antigen (Ag)-specific interleukin 4 (IL-4), IL-5 and IL-13 (2-6) and that its downmodulation is partially mediated by IL-10 and parasite antigen-mediated antibodies (7,8). It is not clear whether the same mechanisms that regulate granulomatous responses in murine model, also operate in humans. Human studies are limited because of the difficulty in obtaining tissue samples in the acute phase of the disease. Despite this, some observations of the immune response in chronically infected humans have been made (9-13). The mechanisms involve poor IFN-γ production in asymptomaytic *S. mansoni*-infected patients, when compared to acutely infected patients, subjects undergoing chemotherapy or patients with clinically apparent disease. There seems to be an important role for IL-10 in the active suppression of IFN-γ production (14,15), while TNF-α has been associated with the development of clinically overt hepatosplenic disease. The evidence is less clear for Ag-specific IL-4 and IL-5 whose role appear variable (16-18).

We don't know why the immune responses to schitosomiasis are highly variable and why only some infected people progress to clinically overt disease. It seems plausible that factors such as exposure, intensity and duration of infection as well as treatment may contribute to the heterogeneity of the observed responses. Our lab has embarked on detailed studies of exposure, treatment, and re-infection for the immune and granulomatous response using the baboon model of *S. mansoni* infection and we will highlight some of these studies here.

Baboon as a model of Schistosomiasis

It is uncertain to what degree the mechanisms of granuloma formation and modulation observed in mice are relevant to human schistosomiasis. Humans are repeatedly infected for years with unknown numbers of parasites, and most parasite eggs are deposited in the submucosa and mucosa of the large intestine and few in the liver (19), unlike in the mouse where most eggs are deposited in the liver. Less than 10% of chronically infected humans develop fibrosis of the liver while infection of mice with *S. mansoni* is invariably associated with liver fibrosis (20-22). Lastly, even a single worm pair in a mouse is equivalent to a large parasite burden in humans relative to the body size (22).

The olive baboon (*Papio cynocephalus anubis*) is an excellent host for *S.mansoni* parasites and in many respects represent a better model to study mechanisms of immunity and pathology to schistosomiasis (summarized in Fig 1). First, our own work and that of others indicate that baboons represent a model of disease and acquired immunity with more close parallels to humans than mice (reviewed (23) and have other advantages in monitoring infection and disease. They better resemble humans anatomically and phylogenetically. Baboons, for example, are natural hosts for *S. mansoni* in East Africa (24,25), and are highly susceptible to experimental infection (26). Maturation of infecting larvae often exceeds 90% (27,28). This contrasts to 30-40% maturation rate observed in mice. Baboon can be natural host for *S. mansoni* and they maintain infection in communities removed from human contact (24). They can also act as reservoirs for human transmission to humans (24,29). Due to their greater body mass, an experimental infection of sufficient size to induce granuloma development does not represent such a massive antigen exposure (as would even a single worm pair in a mouse), and allows chronic infection to develop. It is also possible to monitor disease in multiple organs throughout the course of infection by surgical manipulation (27,30). Many of the necessary reagents to evaluate the fine specificity of immune responses to experimental infections are now available for baboons and a few published studies have demonstrated that immunization of baboons with recombinant schistosome antigens produced significant levels of protective immunity (31,32)

Granuloma size determinations in singly and multiply-infected animals

We have examined the contribution of cytokines to the granulomatous response in baboons infected with *S. mansoni* in a number of independent experiments (30,33,34). In these experiments olive baboons were either repeatedly infected (multiple infections, MI) or received a single exposure (SI) to a comparable number of cercariae and were allowed to develop a chronic infection (> 19 weeks) before treatment with

Parasite Biology

- High maturation of larvae into adults [27] [28]
- Larvae migrate rapidly from skin to lung two to five days after infection [26]
- 80 – 90 % of ova in a chronic infection recovered in guts [27]

Pathology

- Dysentry and fevers with acute infection [27]
- Hepatic and intestinal granuloma down modulate in size with chronic infection [27] [30] [33]
- Periportal fibrosis develops with repeated exposures and/or chronic infections [34] [25]
- Significant intestinal pathology with chronic infection [27] [30]
- Porto-systemic shunting <u>not</u> observed

Immune Response

- IgE and eosinophilia are consistently observed [39]
- PBMC, splenocytes aand mesenteric lymph nodes make IL-2, IL-4, IL-5 IL-10 and IFN-g with acute infection (6-9 weeks post-infection) that all decrease with chronic infection (>16 weeks post-infection) [30] [33] [39]
- Persistently elevated TGF-B correlates with down-modulation of granuloma formation and increased risk of fibrosis [30] [34].

Acquired Immunity

- 50-90%protection to RA cercariae vaccine correlates with serum anti-parasite IgG levels [28].
- 60-80% protection with repeated natural infection and subsequent PZQ cure [39].
- 37-39% protection with ova plus IL-12 immunization [39].
- Protection with repeated natural infection and PZQ uniquely correlates with serum levels of parasite-specific IgE [39].

Numbers in superscript correspond to references in text.

Fig. 1: Summary of key features of *S. mansoni* infection in baboons

Praziquantel (PZQ). After treatment animals were re-infected in similar fashion with *S. mansoni* cercariae. Animals underwent serial liver biopsies to evaluate the granulomatous response during the acute and chronic phases of infection, using procedures previously described (27). Hepatic granuloma sise was serially examined at 6, 9, and 16 weeks post-infection in the same animals corresponding to acute (6 and 9 weeks) and chronic phases (16 weeks) of infection following PZQ treatment. Prior to treatment peak granuloma size occurred at 6 weeks post-infection, and granulomas diminished in size as the infection became chronic in animals exposed to a single infection. In multiply infected animals, peak granuloma size occurred at 9 weeks after primary infection (Fig.1). However no granulomas that warranted measurement were observed at 16 weeks post-infection. We have observed this pattern of granuloma development and modulation in all three independent experiments following either a single or multiple infection. In one experiment (30), the mean worm burden at perfusion were 901+/-89 in the singly infected control group, while it was lower for the multiply-infected group (767+/-65 $P<0.05$). Infection following treatment resulted in a two fold reduction in acute granuloma size in both groups ($P<0.01$) and few hepatic granuloams were found in most chronically infected animals both after the primary infection and with re-infection after treatment. There was no significant difference in granuloma size among chronically infected animals. Infection following treatment resulted in significantly fewer adult worms in the previously multiply exposed baboons (161+/-89) than in baboons in singly infected group (466+/-182; $P<0.05$)

These studies demonstrated that a challenge infection with *S. mansoni* following treatment resulted in granulomas that were significantly smaller than those produced during a primary infection.(30). This proves that

there is persistence of partially modulated granulomas and implies that treatment followed by re-infection in areas endemic for schistosomiasis is unlikely to enhance morbidity. This finding is supported by studies of the granulomatous response in both mice (35,36) and humans' (37)

Cytokines associated with a primary *S. mansoni* infection

To examine the immunological mechanisms responsible for the initiation and modulation of the granulomatous response before and after infection, peripheral venous blood was collected approx. every 3 weeks throughout the course of the experiments to examine parasite Ag and mitogen-induced cytokine production by PBMC. The mean net *schistosome* egg antigen (SEA)-specific cytokine responses for 7 animals corresponding to an acute and chronic phase of a primary infection are shown in Table 1(reprinted with permission from ref.(30)). The sampling points were grouped into acute (6-9 weeks) and chronic (>13 weeks post-infection). IFN-γ peaked during the acute phase and then became undetectable during the chronic phase of a primary infection in representative animals. Similarly, egg antigen-driven IL-2, IL-4, IL-5, TGF-β and IL-10 release peaked during the acute phase and declined, as the infection became chronic. We have compared the relative contribution of both PBMC and splenocytes to the immune response in baboon schistosomiasis; PBMC is the tissue available in human studies while spleen is the most frequently used tissue. Both splenocyte proliferative and cytokine responses to parasite antigens and mitogens mirrored those of PBMC (33). We also know that antigen-specific cytokine responses in PBMCs accurately reflect cytokine responses in granulomas, since we have shown by PCR analysis that the cytokine pattern in both tissues is similar (Kariuki et al manuscript in prep)

Table1: Geometric mean SEA-specific cytokine production from baboons (n = 7) during the acute and chronic phases of the primary infection and re-infection (***Reprinted with permission from (30)***)

Cytokine	Infection[a]	Geometric mean production (pg/ml) ± SEM in[b]			
		Acute phase		Chronic phase	
		Primary	Reinfection	Primary	Reinfection
IFN-γ	SI	297 ± 32	203 ± 72	28 ± 8	120 ± 42
	MI	291 ± 59	270 ± 61	31 ± 17	190 ± 93
IL-2	SI	85 ± 14	784 ± 173**	119 ± 42	704 ± 316**
	MI	163 ± 51	1,483 ± 186**	242 ± 83	727 ± 124**
IL-4	SI	137 ± 28	251 ± 113*	21 ± 14	173 ± 63*
	MI	62 ± 25	639 ± 217**	20 ± 8	68 ± 31
IL-5	SI	285 ± 52	129 ± 64	17 ± 4	117 ± 46
	MI	31 ± 14	616 ± 76*	219 ± 58	160 ± 57
IL-10	SI	224 ± 38	496 ± 81**	126 ± 61	138 ± 21
	MI	71 ± 41	348 ± 136*	130 ± 55	28 ± 3
TGF-β	SI	1,141 ± 217	1,850 ± 261**	767 ± 164	1,712 ± 312*
	MI	751 ± 192	2,852 ± 310***	862 ± 250	1,785 ± 351*

[a] SI, single primary infection of 1,000 cercariae; MI, multiple primary infection of 100 cercariae per week for 10 weeks. All animals were reinfected singly with 1,000 cercariae after treatment.

[b] Acute-phase results are peak cytokine responses at either 6 or 9 weeks in each animal after primary or secondary infection. Chronic-phase results are peak cytokine responses at 19 weeks in the primary infection and at either 13 or 16 weeks after reinfection. Asterisks indicate a significant difference compared to primary infection (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Cytokines associated with a secondary *S. mansoni* infection, post-chemotherapy

Following re-infection of baboons with either a single or multiple infection with *S. mansoni*, levels of cytokines were again examined. Like for a primary infection, re-infection produced a similar rise in IFN-γ release during the acute infection while the levels tended to drop with chronic infection. Geometric mean SEA-driven IFN-γ production levels for all animals had similar profiles; they were elevated during the acute phase and declined during the chronic phase of reinfection, same as for a primary infection (Table 1and REF 30). Mitogen driven IFN-γ was generally higher with re-infection than with primary infection. Re-infection resulted in 2-8 fold more IL-2 production in both the SI and MI groups than the primary phase. Mitogen driven IL-2 was higher overall with re-infection than at primary infection, particularly in multiply infected animals. SEA-driven IL-4 production showed a pattern similar to that observed for IL-2. It declined dramatically with chronic infection. Production of IL-5 was more variable than for the other cytokines. While it peaked at 6 weeks after the primary infection, the peak of production after reinfection was 9 weeks. Egg Ag-driven IL-10 production increased two to five-fold with reinfection after treatment. It was notable that egg-Ag-induced IL-10 remained elevated during chronic infection after the primary infection but not after reinfection. Egg Ag-driven TGF-β production was also higher with re-infection after treatment than it was during the primary infection. Unlike the other cytokines SEA-driven TGF-β remained elevated during the chronic phase of infection both before and after treatment, indicating a role for this cytokine in downmodulation of the granulomatous response (30,34)

We have consistently observed that the pattern of Ag specific cytokine production in baboons infected with *S. mansoni* do not show a clear separation of Th1 and Th2 type immune polarization, which has also been observed for human schistosomiasis (38); nor do they show consistent evidence of cytokine cross-regulation, for example a reciprocal decrease in IFN-γ with a rise in IL-4/IL-5.

Effect of exposure patterns on cytokine production

The pattern of exposure, either SI or MI did not alter the pattern of SEA-induced IFN-γ production. Similarly, mitogen driven IFN-γ was equivalent between the MI and SI groups. For IL-2 and IL-4 cytokines, mitogen-driven production was higher after re-infection in multiply infected animals. In multiply infected animals peak SEA-driven IL-5 production occurred during the chronic phase of the first infection and 9 weeks after re-infection. This pattern was observed in all animals. SEA-driven IL-5 was significantly higher in the MI than in the SI group of animals during the acute phase but not in the chronic phase after treatment. SEA-driven IL-10 levels were equivalent for MI and SI groups throughout the course of the experiments. We have reached the conclusion, made from these observations, that the frequency of exposure to S. mansoni parasites affects the hosts immune responses and that repeated natural infection of baboons with S. mansoni generates higher levels of immunity than does a single exposure (30,39)

Role of serum antigen-specific antibodies

We have examined in these independent experiments (30,31,33) the humoral correlates of granuloma modulation by determining the levels of Ag-specific IgG, IgM and IgE throughout the course of S. mansoni infection in baboons. Egg antigen specific IgM levels increased rapidly with the initial infection and remained at roughly the same levels throughout the course of experiment. In contrast egg antigen-specific IgE and IgG antibodies increased significantly with re-infection after treatment and continued to rise with ongoing infection. Chronically infected animals that were repeatedly infected with S. mansoni had significantly higher levels of SEA-specific IgG and IgE in serum after treatment than singly infected animals ($p<0.05$). Thus it seems that re-infection after treatment significantly boosts parasite-specific antibody responses, demonstrating the persistence of parasite specific B-cell memory after praziquantel treatment. Antibodies have also been shown to be important in regulating the granulomatous response in mice (40,41,54) and they may do so by the formation of immune complexes or anti-idiotypic antibody (8). The relevance of these antibodies to protection has also been demonstrated in our Laboratory, where we examined immune correlates of protection in S. mansoni infected baboons, and showed that in baboons and unlike mice, adult worm specific IgE is uniquely associated with acquired immunity to S. mansoni infection (39)

Immunological basis for *S. mansoni*-induced fibrosis

Severe peri-portal fibrosis develops in <10% of chronically infected individuals (42) but the reasons for this are not well understood. Studies in mice infected with schistosomiasis demonstrate that the development of fibrosis requires the production of the profibrotic cytokines IL-2, and IL-4 (43-45), correlates with TGF-β1 synthesis (46,47) and is suppressed by IL-12 and IFN-γ (48,49). Whether these cytokines participate in development of *schistosome* induced hepatic fibrosis in humans or non-human primates have never been

directecly tested.

In a recent experiment, (Ref. 34), we have examined the relationship of various cytokines to development of fibrosis in the olive baboon. As already pointed out baboons are natural hosts for *S. mansoni* in East Africa (24) and wild caught baboons with *schistosomiasis mansoni* have been reported with periportal fibrosis (25). Experimental infections of baboons with *S. mansoni*, however, have not been previously reported to stimulate development of periportal fibrosis (50,51,27). As outlined above, we previously observed that multiple compared to singly infected animals that are subsequently cured and re-infected produced increased levels of SEA-driven TGF-β, IL-4 and IL-2 production by PBMC. Based on these observations, we hypothesized that repeatedly infected and treated animals are at an increased risk for development of hepatic fibrosis that correlates with increased IL-2, IL-4 and TGF-β production. To test this hypothesis, baboons were repeatedly infected or received an equivalent dose of cercariae and allowed to develop a chronic infection (>19 weeks). Animals were subsequently cured with PZQ and re-infected once or multiple times. Serial liver biopsies were obtained on individual animals following re-infection and examined for the presence and extent of fibrosis using trichrome staining of fixed tissue (34). Egg antigen-induced cytokine production by PBMC was examined at 3week intervals after re-infection and correlated with the risk of developing fibrosis. The presence and amount of fibrosis directly correlated with the frequency of exposure. Fibrosis was not associated with adult worm or tissue egg burden. The amount of fibrosis correlated with increased *schistosome* egg Ag-driven TGF-β at 6, 9, and 16 weeks post-infection (r^2= 0.9, 0.8, and 0.54, respectively, all $p<0.01$) and IL-4 production ($p=0.02$) at 16 weeks post-infection and not IFN-γ, IL-2, IL-5, or IL-10. These data suggest that repeated exposure is a risk factor for periportal fibrosis by a mechanism that primes lymphocytes to produce increased levels of profibrotic molecules that include TGF-β and IL-4 (Ref 34).

CONCLUSION

These studies on the granulomatous responses in S. mansoni-infected baboons have shed light on the association between infection and chemotherapy and the effects of long term chronic infections vis-a-vis the development of the modulated granuloma and fibrosis. They also serve to illustrate why the baboon is the most frequently used non-human primate in schistosomiasis research, emphasizing the multiplicity of qualities that make them more relevant models than rodents; baboons maintain natural infections in the wild, are susceptible to experimental infections, develop hepatic and intestinal lesions, modulate these lesions and acquire protective immunity (summarized in Fig 1). However, there are a number of constraints limiting their use, such as the high cost involved in trapping, breeding and maintaining them in captivity as well as animal welfare consideration (reviewed in ref.52). Despite this the availability of human reagents that work well in baboons has enabled us to dissect more accurately the immune mechanisms associated with the granulomatous response which more closely resemble the situation in humans. This is because the genes encoding the baboon cytokines and the cytokines proteins themselves, show 93 to 99% homology at the nucleic acid and protein levels respectively (53). Future efforts are being directed at a deeper understanding of these phenomena and will open up avenues aimed at developing new strategies for the control of schistosomiasis mansoni especially by the vaccination approach.

ACKNOWLEDGEMENTS

The studies described in this review were made possible by the support of NIH grants AI-35935 and AI-01202. We are indebted to J. Nyaundi, M. Njenga, and M. Suleman for surgical expertise and to Simon Kiarie, Fred Nyundo, and Sammy Kisara for excellent technical assistance.

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