Parasitological And Immunopathological Responses Balb/C Mice With Concomitant Schistosoma Mansoni And Plasmodium Berghei Infections

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Citation

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Abstract

Schistosomiasis and malaria often occur in the same area. This study investigated the effect in BALB/c mice harbouring a schistosomiasis-malaria co-infection. Mice infected with S. mansoni were divided into three groups, which were super-infected at weeks 4, 5, and 6 post-infection with P. berghei. There were uninfected and single-infected controls. Sampling was at day 4 and 7 post-super-infection. The following assays were carried out: parasitaemia, perfusion for worm recovery, IgG ELISA, gross and histopathology. Co-infected mice had: higher malaria parasitemia, higher IgG responses, lower worm counts, smaller granulomas compared to the single-infected mice. This study showed that co-infections with schistosome and malaria parasites aggravated malaria severity, as shown by increased parasitemia and severe gross pathology of liver and spleen in the co-infected mice. In contrast, malaria conferred protection to S. mansoni infection, as demonstrated by reduced worm counts and granuloma sizes and high schistosme-specific IgG levels in the co-infected mice.

INTRODUCTION

Although polyparasitism is a common phenomenon in human populations living in malaria endemic areas, little is known about how co-infections affect the immune responses against malaria and vice versa. If made available, such knowledge would be important in the design and optimization of vaccines and chemotherapy (Helena et al., 1998).

Some situations are known in which the association of infection by S. mansoni with other pathogens in the same host results in a type of disease, which differs, from the simple summation of the individual effects of each infection (Helena et al., 1998). If the combined responses were determined, this would probably serve as a ground for proper vaccination and treatment.

Concurrent infection with helminth parasites, which are highly prevalent in many areas where malaria is endemic, has been recognized as a possible confounding factor modulating immune responses to other pathogens, including malaria parasites (Nacher, 2001).

Malaria is highly endemic in Sub-Saharan Africa, Southeast Asia, and South America, where there is also a high prevalence of helminth parasite infections. For instance, infections with the major human gastro-intestinal nematodes, including Ascaris lumbricoides, Trichuris trichura, and the hookworm species Ancylostoma duodenale and Necator americanus, are widespread in most of these areas. The combined prevalence of infections with gastro-intestinal nematodes can be as high as 90% in some parts of the African continent (Chan, 1997; Chan et al., 1994; De Silva et al., 2003). Modulation of immune responses to viral, bacterial, and protozoan pathogens by concurrent helminth infection has been observed in many human epidemiological studies and in laboratory animal models. Patients with helminth infections have been observed to have higher loads of Human Immunodeficiency Virus (HIV) in plasma, and reduced delayed-type hypersensitivity responses to Mycobacterium tuberculosis purified protein derivative or house dust mite antigen (Borkow et al., 2001; Van den et al., 2004).

In laboratory animal studies, mice co-infected with S. mansoni and Leishmania major showed impaired ability to resolve L. major infection (LA Flamme et al., 2002; Yole et al., 2007). Similar impairment of protective immunity by concurrent helminth infection has also been observed in

other co-infection models, such as the nematode Nippostrongylus bransiliensis and the bacterium Chlamydophila arbortus (Buendia et al., 2002), the cestode Taenia crassiceps and the protozoan Trypanosoma cruzi (Rodriguez et al., 1999), and S. mansoni and the recombinant Vaccinia virus.

In malaria, it has been reported that mixed Plasmodium falciparum and Plasmodium vivax infections are more frequent in A. lumbricoides-infected patients in Thailand (Nacher, 2002). Epidemiological studies also showed that worm infection in humans alters the development of cerebral malaria (Nacher et al., 2000). Mice co-infected with S. mansoni and Plasmodium chabaudi develop increased malaria parasitaemia (Helena et al., 1998).

This study was performed to examine the parasitological, pathological, and immune responses that occur in mice, during a schistosomiasis-malaria co-infection.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The study was carried out in BALB/c mice, a model for both malaria and schistosomiasis. The study entailed an infection and analysis experiments on the immune, parasitological and pathological responses.

At the beginning of the study, mice were infected with 150 S. mansoni cercariae at day 0 (zero). Randomly selected mice infected with S. mansoni, served as S. mansoni single-infection control (SmC). Another group of mice with S. mansoni infection were picked at random and super-infected with P. berghei at week 4 post-S. mansoni infection (Experiment 1). Each mouse was injected with 5.5 l of blood containing 2.5 x 10^6 P. berghei parasitized red blood cells. At the same time, some naive mice were infected with a similar number of P. berghei parasitized red blood cells, from the same pool as for the co-infection. These served as the P. berghei single-infection control (PbC4). This procedure was repeated for super infecting other mice at weeks 5 and 6 post-S. mansoni infection (Experiments 2 & 3), and also for malaria controls (PbC5 and PbC6).

Sampling for each of the three experiments, was done at Day 4 and Day 7 post-P. berghei infection. At Day 4, sampling entailed preparation of blood smears, heart puncture, gross pathology, and histopathology. At Day 7, perfusion was carried out in addition to all the procedures performed on Day 4. Five mice from each experimental group were sacrificed at each sampling point.

HOSTS AND PARASITES

Six-week-old BALB/c mice bred at the Institute of Primate Research (Nairobi, Kenya), were caged in groups of 10 and fed on commercial pellets and water provided ad libitum. They were kept under a natural light-dark cycle of 12/12 hours, at an ambient temperature of 25°C and relative humidity 50-60%.

A Kenyan isolate of S. mansoni derived from human patients and maintained using Biomphalaria pfeifferi and baboons (Papio anubis) was used to infect mice. Mice were anaesthetized intra-peritoneally with a 1:3 mixture of Xylaxine (Rompun® 2%) and Ketamine (100 mg/ml; Rotex Medica GMBH Tritau-Germany) at 0.02 ml of anaesthesia per 30 g body weight. Each mouse was infected on the abdominal skin with 150 cercariae of S. mansoni (Smithers and Terry, 1965)

P. berghei-parasitized blood suspension (from IPR parasite bank) was used to infect a BALB/c mouse intraperitoneally. After 6 days, the parasitemia of the mouse blood was established to be 15%. Three mice were infected via the tail vein each with a volume of blood containing 2.5 x 10^6 parasitized red blood cells. These were used as a source of parasites for the experiments.

PREPARATION OF GIEMSA STAINED BLOOD FILMS FROM TAIL BLOOD

Two separate thin blood films from tail blood were made on standard microscope slides, and air-dried before fixing the films in methanol for 5 seconds. They were stained with fresh Giemsa solution (10% v/v in distilled water). The stained blood films were observed under a standard light microscope using the x100 objective lens with immersion oil. Infected and uninfected erythrocytes in different fields of view were identified and counted. A total of at least 2000 erythrocytes were counted per slide. The percentage parasitemia was then determined.

SERUM PREPARATION

Blood was collected from anaesthetized mice using the heart puncture technique. At each specified sampling point, blood from a particular group was pooled. Serum was prepared from the pooled blood and stored at –20°C for analysis.

ANTIGEN PREPARATION

Soluble worm antigen (SWAP) was prepared from S.

mansoni worms obtained from infected mice perfused at week five post-infection. The protein estimation was based on the Bio-Rad method of Bradford 1976. The protein was sterilized by exposure to UV light (10 minutes, 5 cm from a 30 watt ultra violet OSRAM bulb) before use in in vitro assays. The aliquots were stored at -20°C.

0-3 hr antigen was prepared from S. mansoni cercaria following artificial transformation of cercariae (Ramalho-Pinto et al., 1974). Heads and tails were separated on a discontinuous Percoll gradient (Lazdin et al., 1982). The schistosomula were cultured at 37°C, 5% CO_2, in RPMI 10 (RPMI 1640 in 10% foetal serum, 0.1% gentamycin and 5x 10^{-5} β-mecarptoethanol). The protein was sterilized and stored as for SWAP.

Soluble P. berghei antigen was prepared from blood obtained by heart puncture from P. berghei infected mice with a parasitemia of 4%. Red blood cells were obtained by washing at 450g, in complete culture medium (RPMI 1640, pH 7.3, containing 25% FCS v/v). The cells were re-suspended in 40 ml complete culture medium and the flasks were gassed for 2 minutes using 5% CO_2, 5% O_2, and 90% N_2 and incubated for 18 hrs at 37°C. The culture was centrifuged at 1000g for 10 minutes and the supernatant containing soluble P. berghei antigen harvested. The supernatant was aliquoted and stored at -20°C until use in assays.

CRUDE ANTIGEN

Crude P. berghei antigen was prepared from infected blood at 50% parasitemia. The blood was centrifuged at 1000g for 10 minutes and the supernatant discarded. The pellet was mixed with 0.15 % saponin in RPMI (w/v) in the ratio 1:4 and mixed thoroughly. This was incubated in ice (4°C) for 10 minutes shaking every two minutes to enhance lyses of the red blood cell. The suspension was washed three times at 1000g, 10 min. The pellet was re-suspended in 4.5 ml of RPMI, aliquoted and stored at -20°C awaiting use.

GROSS PATHOLOGY AND PERFUSION

Perfusion was done using a modified method of Yole et al 1996. Each mouse was anaesthetized with 0.02 ml per 30 g body weight Ketamine/Rompun® (2:1). The abdominal wall was opened up without cutting the viscera. Gross pathology of the liver and spleen was observed. Observations included: liver enlargement, adhesions and presence of granulomas. Inflammation of the spleen was also considered. The gross pathology of the liver was then subjectively categorized as none (no granulomas), few (1-3 granulomas per lobe) moderate (4-10), and severe (>10).

After the observation of the gross pathology, the thoracic cavity was opened and a perfusion needle containing perfusion fluid (0.85% Sodium chloride and 1.5% Sodium Citrate) was inserted in the left ventricle of the heart and perfusion carried out until the liver and the mesenteries were clear. Worms were recovered from the perfusate using the method of Yole et al (1996).

The worms were counted and the mean and S.E.M for each group calculated. The livers were collected and fixed in 10% buffered formalin awaiting histological examination.

HISTOPATHOLOGICAL EXAMINATION OF TISSUES

The fixed (in 10% buffered formalin) livers were dehydrated using ethyl alcohol, and embedded in paraffin wax. Sections, 7μm thick, were cut using a rotary microtome. Tissue sections were placed on slides and stained with haematoxylin and counter-stained with eosin. Slides were observed under X25 and X40 objective lenses. The number of granulomas was noted and the sizes established by measuring the length and width of each granuloma with a centrally placed schistosome egg. The average of the length and width was taken as the granuloma size (Farah et al., 2000). Ten granulomas were measured for each of the two schistosome-infected groups: the co-infection and S. mansoni control.

IGG ELISA

Specified wells of Nunc-Immuno™ plates (MaxiSorp ™ Surface) ELISA plates were coated overnight with 50 μl of SWAP, 0-3 hr antigen (10 μg/ml), soluble P. berghei antigen (4 % parasitemia), or crude P. berghei antigen (50% parasitemia) and incubated overnight at 4°C. Non-specific binding sites were blocked by incubation with 100 μl 3% BSA. Diluted (1:200) serum samples (50 μl) were dispensed into specified wells in duplicates and incubated for 1 h at 37°C. IgG binding was detected using 50 μl of 1:2000 Goat anti-mouse IgG conjugated to hoarse radish peroxidase (Jackson's Immunoresearchs laboratories INC). After each incubation, the plate was washed 6 times with washing buffer (0.05 % Tween 20 in PBS). The substrate was 50 μl orthophenyldiamine (0.4 μg/ml) in citrate buffer. The plates were incubated for 30 min at 37°C in the dark and colour development read at 630 nm in an ELISA microplate reader.

STATISTICAL ANALYSIS

Experimental and control values were analyzed for significant differences by Student's t-test. A probability of less than 0.05 was considered significant.

RESULTS

0-3 HR ANTIGEN- SPECIFIC IGG RESPONSES

Experiment 1: Mice in this experiment were super-infected with P. berghei at 4 weeks post-infection with S. mansoni. The results (Fig.1) indicate that IgG antibody response to 0-3 Hr antigen was low in the malaria-only-infected group at both sampling points. It was however higher at day 4 than at day 7. IgG level in both the S. mansoni-only and co-infected groups was higher at day 4 than at day 7, day 4 having similar levels in both groups, but co-infected group having a lower level at day 7. Malaria-only group had lower responses as compared to the two groups harbouring S. mansoni infection. All the treatments showed a higher response than the negative group except for malaria-only group at day 7.

Experiment 2: Mice in this experiment were super-infected with P. berghei at week 5 post-S. mansoni infection (Data not shown). The malaria-only-infected group showed a low IgG response, with similar responses in both days. The S. mansoni-only group had similar responses on both day 4 and 7, although day 7 was slightly higher. In S. mansoni-only and co-infected groups, IgG response was higher at day 7 than at day 4. The S. mansoni-only group had a slightly higher response on day 4, while the co-infected group had a higher response on day 7.

All the treatments showed a higher IgG response than the negative, and day 7 responses were higher than day 4 in all the groups.

0-3 hr-specific IgG levels were generally lower than in experiment 1 and the malaria-only-infected group still showed a lower IgG level than both groups infected with S. mansoni.

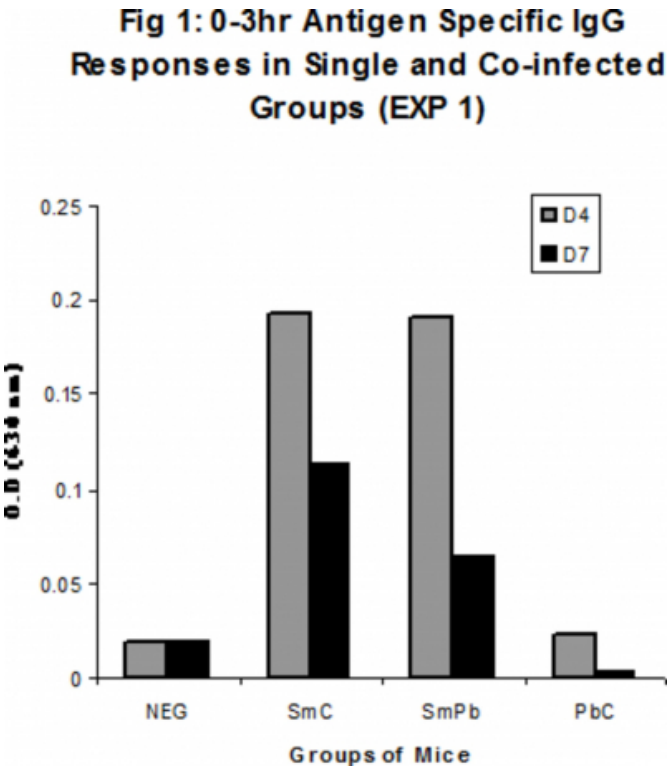
Experiment 3: Mice in this Experiment were super-infected with P. berghei at week 6 of S. mansoni infection (Data not shown). For the malaria-only group, the IgG levels were low and similar for both day 4 & 7. The co-infected group showed the highest IgG level at day 4, and a lower level at day 7. For the S. mansoni-only group, the IgG level was lower at day 4 than at day 7. The co-infected group showed higher IgG level at day 4 than the S. mansoni-only group on the same day. However, on day 7, the S. mansoni-only group showed a higher IgG level than the co-infected group.

Generally, the IgG levels in Experiment 3 were similar to those in Experiment 2, except for the co-infected group where in Experiment 3, day 4 recordings were higher than in Experiment 2. However, the malaria-only group showed higher IgG levels in Experiment 3 than in Experiments 1 and 2 but still showed the lowest recording just like in the other experiments. The S. mansoni-infected groups showed lower recordings than in Experiments 1 and 2.

The IgG response to the other antigens (SWAP, Crude and Soluble P. berghei) was consistent with these results but the data is not shown here.

Figure 1

Figure 1: Schistosome-specific IgG responses



Key: NEG - Negative control; SmC - Schistosoma mansoni only infected group; SmPb - S. mansoni-P. bherghei co-infected group; PbC - P. berghei only infected group.

ANALYSIS OF PARASITEMIA

Experiment 1: The malaria-only group recorded significantly higher parasitemia at day 7 (Fig. 2; 4.82 ± 0.846%) than at day 4 (0.728 ± 0.184%, $p<0.05$). The co-infected mice also developed a significantly higher malaria parasitemia, at day 7 (17.12 ± 0.871%) than at day 4 (5.38 ± 0.626%, $p<0.01$).

The co-infected mice developed a significantly higher malaria parasitemia than mice infected with P. berghei alone at both sampling points ($p<0.01$).

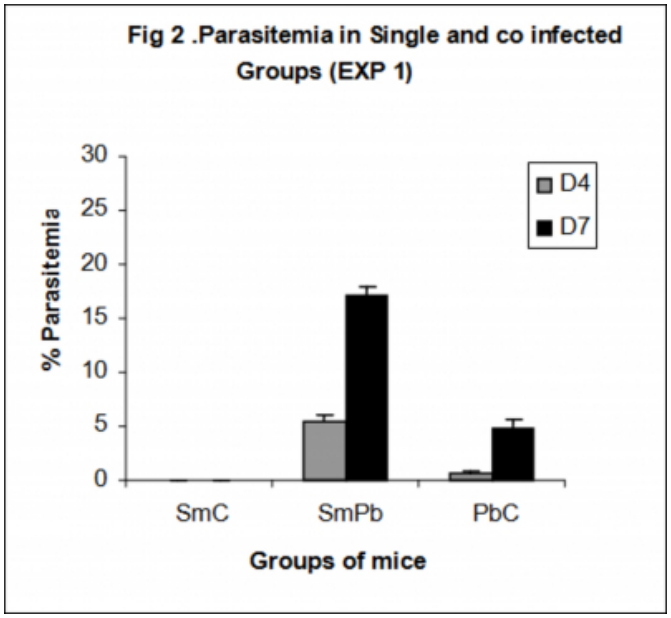
Experiment 2: In the malaria-only group, parasitemia at day 7 (Fig.3; 19.66 ± 1.465%) was significantly higher than at day 4 (0.535 ± 0.056%, $p<0.01$). In the co-infected group, three out of 6 mice had died before day 7, so the data given is only for 3 co-infected mice. The parasitemia in the co-infected group was also higher at day 7 (6.03 ± 3.086%) as compared to day 4 (1.395 ± 0.395%). However, the difference was not significant ($p>0.05$). A comparison between the two groups showed that the P. berghei-only mice developed significantly higher parasitemia than the co-infected group at day 7 ($p<0.05$). At day 4, although the co-infected group showed higher parasitemia than the malaria-only group, the difference was not significant ($p>0.05$).

Experiment 3: The parasitemia in the co-infected group (Fig 4), was significantly higher at day 7 (23.94 ± 3.575%), as compared to day 4 (1.308 ± 0.193%, $p<0.01$). In the single infected group, parasitemia at day 7 (19.018 ± 2.887%) was significantly higher than at day 4 (4.68 ± 0.741%, $p<0.05$).

The parasitemia in the co-infected group was higher at day 7 than in the malaria-only group although the difference was not significant ($p>0.05$). At day 4, the malaria-only group exhibited a significantly higher parasitemia than the co-infected group ($p<0.05$).

Figure 2

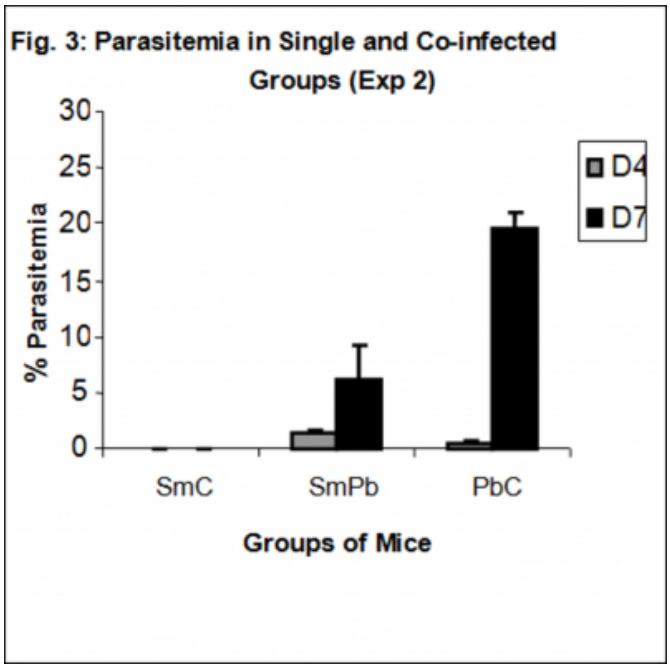
Figure 2: Experiment 1: Parasitemia



Key: NEG - Negative control; SmC - Schistosoma mansoni only infected group; SmPb - S. mansoni-P. bherghei co-infected ; PbC - P. berghei only infected group.

Figure 3

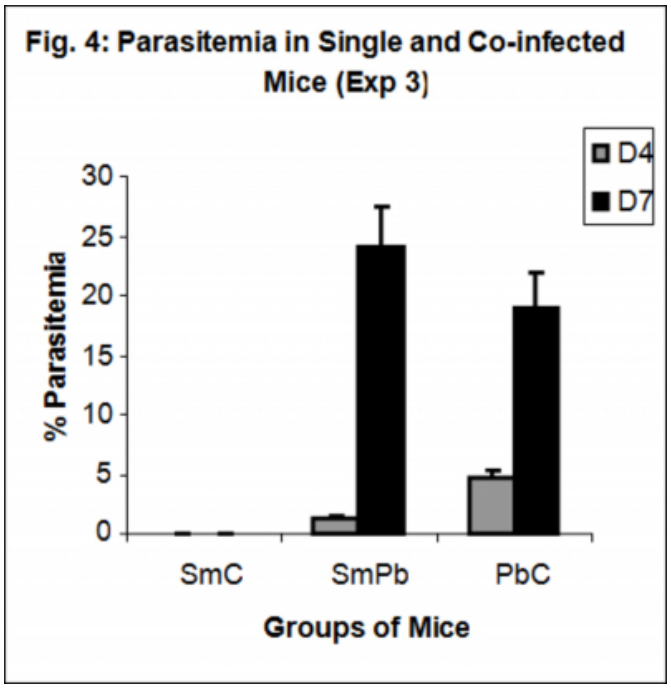
Figure 3: Experiment 2: Parasitemia



Key: NEG - Negative control; SmC - Schistosoma mansoni only infected group; SmPb - S. mansoni-P. bherghei co-infected ; PbC - P. berghei only infected group.

Figure 4

Figure 4: Experiment 3: Parasitemia



Key: NEG - Negative control; SmC - Schistosoma mansoni only infected group; SmPb - S. mansoni-P. bherghei co-infected group; PbC - P. berghei only infected group.

ANALYSIS OF WORM LOAD

The number of worms present in the S. mansoni only and co-infected groups was monitored through perfusion and counting carried out 7 days post-P. berghei infection, beginning at the fourth week of S. mansoni infection. Mean and standard error were worked out (Fig.5).

Experiment 1: In the mice super-infected with malaria at week 4 of S. mansoni infection, and their controls, the S. mansoni-only group showed a slightly higher mean worm load of 13.6± 2.223, as compared to 13 ± 2.041 in the co-infected group. The difference, however, was not significant ($p>0.05$).

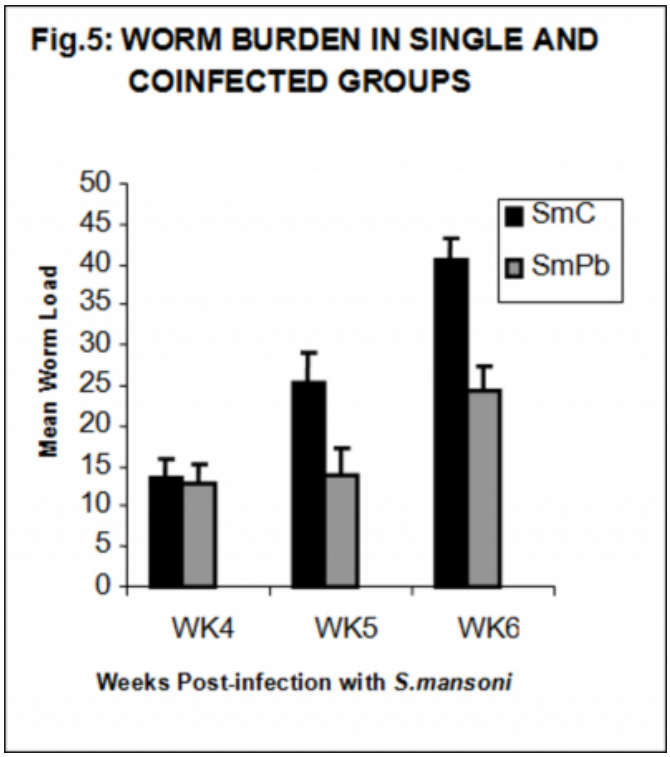
Experiment 2: The mice super-infected with malaria at week 5 of S. mansoni infection, had a significantly higher the mean worm load in the S. mansoni-only group (25.2 ± 3.815), than in the co-infected group (14 ± 3.206, $p<0.05$).

Experiment 3: In the mice super-infected with malaria at week 6 of S. mansoni infection, the trend in Experiment 2 was seen, with the single-infected group having a significantly higher worm load (40.4 ± 2.948) than the co-infected ones (24.2 ± 3.234, $p<0.05$).

A comparison between the weeks showed a significant difference for the S. mansoni-only group between week 4 and 5 (<0.05), and between week 4 and 6 ($p<0.01$), However, the difference was no significant between week 5 and 6 ($p>0.05$). For the co-infected group, the difference between the weeks was not significant. In both groups, there was an increase in number of worms recovered, as the number of weeks before super infection was increased. At all time points S. mansoni-only groups had higher numbers of worms recovered when compared with the co-infected groups.

Figure 5

Figure 5: Worm Burdens in Single ad Co-infected Groups



Key: NEG - Negative control; SmC- Schistosoma mansoni only infected group; SmPb: S. mansoni-P. bherghei co-infected group.

GROSS PATHOLOGY

Gross pathology examination was focused on the general and overt appearance of the liver and spleen. The observations considered in the liver were presence or absence of granulomas, presence or absence of adhesions, and whether the liver was inflamed or not. The spleens were checked for inflammation or lack of it. Granulomas appeared as whitish spots on the surface of the liver lobes. The presence of granulomas affected the colour and texture of

the liver. The usually smooth surface of the liver was replaced with a rugged surface and the red-pink hue of normal livers became pale.

Experiment 1: In the mice super-infected with malaria at week 4 of S. mansoni infection, gross pathology of the livers from both the single infected and co-infected groups showed no presence of granulomas though they all showed slight inflammation. The spleens from all the groups also showed some enlargement.

Experiment 2: In the mice super-infected with malaria at week 5 of S. mansoni infection, the spleens from the co-infected group were grossly enlarged, while those with single infections of S. mansoni and P. berghei showed slight inflammation. The livers of the co-infected mice showed presence of few granulomas while the single infected ones had no visible granulomas.

Experiment 3: In the mice super-infected with malaria at week 6 of S. mansoni infection, spleens from the co-infected group were severely inflamed while the single-infected ones showed only slight inflammation. The livers from the co-infected group had moderate (4-10 granulomas per lobe) to severe (>10) granulomas. The livers were enlarged and oedematous with adhesions connecting the different lobes. The mice in the S. mansoni-only group had few (1-2), to moderate (3-10) granulomas per lobe, and the livers were inflamed and had adhesions. The malaria-only group had slightly inflamed livers.

HISTOPATHOLOGY

Granulomas appeared as schistosome eggs surrounded by immunological cells and fibrotic changes. Apart from the granulomas, the histopathological sections also showed the presence of portal triads with the accumulation of immune cells around them.

Granuloma sizes were determined by measuring the length and width of a granuloma with a centrally placed egg. The average of the length and the width was taken as the granuloma size. Sizes of 10 granulomas were taken and their averages worked out.

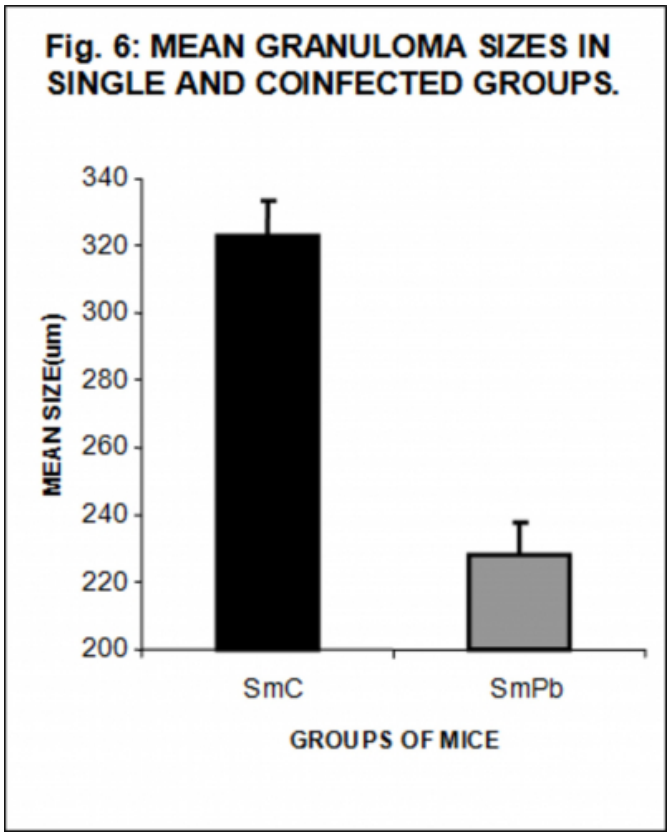
Experiment 1: In the mice super-infected with malaria at week 4 of S. mansoni infection, only two out of five co-infected mice, and one out of five single-infected mice showed small granulomas (125 µm). The rest did not show any granulomas.

Experiment 2: In the mice super-infected with malaria at week 5 of S. mansoni infection, none of the single-infected mice showed the presence of granulomas. One out of five of the co-infected mice showed a small granuloma (160 µm).

Experiment 3: In the mice super-infected with malaria at week 6 of S. mansoni infection, the granulomas were large and florid with a conspicuous eggs. The single-infected group had significantly larger granulomas (Fig.6; 323 ± 10.21 µm), than the co-infected group (245 ± 9.081 µm, $p<0.01$).

Figure 6

Figure 6: Mean Granuloma Sizes in Single and Co-infection Grops



Key: NEG - Negative control; SmC- Schistosoma mansoni only infected group; SmPb: S. mansoni-P. bherghei co-infected group.

DISCUSSION

The effects of concomitant infections on the development as well as the maintenance of an immune response remain largely unknown (Helena et al., 1998). The murine models of P. berghei and S. mansoni have been well studied and characterized in terms of both cell-mediated immunity and humoral immunity. Thus, BALB/c mouse is a good model

for studying the interaction between the two different parasitic infections with regard to parasite-specific immune responses. Little information regarding concurrent S. mansoni and malaria infection has been published (Helena et al., 1998).

The present study was undertaken to investigate the effect of an S. mansoni infection on P. berghei infection in BALB/c mice, and vice versa.

IGG RESPONSES

In this study, 4-week, 5-week, and 6-week S. mansoni-infected mice were super infected with P. berghei and the IgG response monitored during the development of the malaria infection. Parasite specific antibody analysis was carried out using the antigens: 0-3 hr, SWAP for S. mansoni and soluble P. berghei, and crude P . berghei.

The results revealed that co-infected mice generally exhibited a high IgG response to all antigens as compared to the S. mansoni-only and malaria-only infected groups. The malaria-only-infected groups showed the lowest IgG response to all the antigens. In the responses to the different antigens, there was no clear difference between day 4 and day 7.

These findings indicate that there is no general decrease in B-cell activity in the co-infected mice. This might suggest that malaria infection boosts the IgG response against S. mansoni. It can therefore be deduced that malaria has got a protective effect against schistosomiasis.

The low P. berghei and crude P. berghei antigen-specific IgG levels in the malaria-only-infected mice could be suggestive of a strong immune suppression seen during malaria infection. The decrease in P. berghei, and crude P. berghei antigens-specific IgG, may be due to IFN-□ production, known to influence B-cell differentiation and immunoglobulin production (Snapper & Paul, 1987), or an increased turnover of plasma cells or antibodies.

Mutapi et al., (2000) reported that children with schistosomiasis who were also infected with malaria parasites had higher levels of schistosome-specific IgG3 than did children who were free of malaria. They suggested that a plausible explanation for this would be that malaria infection influences the cytokine environment and generally favours the production of IgG3.

The observation that mice infected with S. mansoni had responses towards malaria parasites indicates cross-shared antigens between schistosome and malaria. This was also evident in P. berghei single infection though at a lower magnitude as compared to S. mansoni. The occurrence of cross-reactive antibodies to P. berghei and S. mansoni most probably has not been reported elsewhere, although Naus et al., (2003) reported cross-reactivity between P. falciparum and S. mansoni.

PARASITEMIA

Co-infected mice super-infected with malaria at week 4- and 6-post S. mansoni infection had higher parasitemia than the single infected ones ($p<0.01$ for week 4). The lower parasitemia in this group at week 5-post S. mansoni infection was probably due lower parasitemia of the three surviving mice sampled; the others had died before sampling. The deaths could have been caused by severe anaemia associated with increased malaria parasitemia. Hence, mice carrying a patent S. mansoni infection and infected with blood-stage P. berghei parasites developed a more rapid and severe course of malaria as demonstrated by increased parasitemia, indicative of a defect in the initial control mechanism. Some co-infected mice died before day 7 post-superinfection with P. berghei. The increased malaria parasitemia in the co-infected mice could be due to a defect in the capacity of the macrophages to produce TNF-□, known to reduce malaria parasitemia in both mice and humans and to enhance survival in mice (Jacobs et al., 1996). Another likely explanation for the increased parasitemia seen in the co-infected mice might be a S. mansoni-induced suppression of macrophage activation, probably through IL-10 and possibly also IL-4 and/or transforming growth factor □ (Oswald et al., 1992). This would lead to an inability of the macrophages to respond to IFN-□ and thus a defect in their capacity to kill parasitized red blood cells at an early stage.

A number of previous studies have also demonstrated higher parasitemia and severe conditions in mice co-infected with various strains of rodent malaria and other parasites than mice infected with Plasmodium alone. A study by Yoshida et al., (2000) demonstrated higher susceptibility to P. chabaudi, increased mortality and elevated P. chabaudi parasitemia in S. mansoni-P. chabaudi co-infected resistant strain mice than mice infected with P. chabaudi alone. Helena et al., (1998) also observed remarkably higher parasitemia in S. mansoni-P. chabaudi co-infected mice, than in mice infected with P. chabaudi alone. Strickland et al. (1972) observed higher mortality and persistent parasitemia

in mice co-infected with Toxoplasma gondii and P. berghei yoelii than in mice infected with P. berghei yoelii alone.

WORM COUNT

In mice super-infected with P. berghei at week 4, the S. mansoni-only group had a higher worm load than the co-infected group. The difference in worm maturation between the co-infected and single-infected mice was however not significant ($p>0.05$).

The worm count in the mice super-infected with P. berghei at week 5 and 6 revealed that the single-infected mice had significantly higher worm loads ($p<0.05$) than the co-infected ones. There was also a general increase in the number of worms present in both groups over time. The delay between super-infections seemed to contribute to this increase in the number of worms.

These results imply that malaria reduces the number of worms in the host during a co-infection with S. mansoni. The presence of malarial parasites in the co-infected group could have had an effect on the immune response of the host against S. mansoni. The high antigen-specific IgG levels seen in co-infected mice might have led to inhibited maturation, establishment, and/or death of the worms.

Yole et al., 2007 reported reduction in S. mansoni worm counts as a result of S. mansoni and Leishmania major co-infection. Ashford et al. (1992) showed a positive correlation between egg load of Ascaris lumbricoides and Plasmodium density, while Briand et al., (2005) observed no association between intestinal helminth infection (A. lumbricoides, Hymenolepis nana, strongyloides stercoralis, and Trichuris trichura), and P. falciparum density.

GROSS PATHOLOGY

In this study, the general and overt appearance of the liver and spleen was observed in all groups of mice.

In mice super-infected at week 4, the liver and spleen were slightly inflamed but no granulomas were seen. This was just the beginning of egg-laying and therefore only a low grade immune response had been mounted. This explains the absence of granulomas and the presence of slight inflammation in the liver and spleen.

At the week 5 super-infection, the livers and spleens of the co-infected mice were grossly enlarged and a few granulomas were present, while those of the single infected ones showed only slight inflammation and no visible granulomas. This indicates that the combined effect of malaria and schistosomiasis elicited a greater immune response than the separate single infections, thus the gross inflammation and presence of granulomas in the co-infected group.

At week 6, the inflammation of the livers and spleens was severe in the co-infected group, with an equally severe case of granulomas. The S. mansoni-only mice had less inflamed livers and had few to moderate granulomas. The results show that the severity of inflammation and granulomas increased over time. This could again be explained in terms of increased immune responses due to the wide array of antigens exposed to the host by the parasites.

Evidently, malaria is aggravating the gross pathology, but interestingly, reduces the granuloma sizes (see histopathology below). The increase in the humoral immune responses as evidenced by the increased IgG levels and increased inflammation seems to counter the cellular responses against schistosome eggs, thus reduced granuloma sizes.

Booth et al., (2004) observed in children that the degree of splenomegaly was greatest among children living in an area where both S. mansoni egg counts and IgG3 responses to malaria schizont antigen were significantly elevated. The children also had hardened spleens, which were associated with relatively high egg count and a greater degree of splenomegaly. The study suggested that children with relatively high levels of exposure to S. mansoni and malaria were worse affected in terms of spleen enlargement. This observation lends support to the present study.

HISTOPATHOLOGY

Hepatic granulomas sizes were measured in S. mansoni single-infected and co-infected groups.

At week 4 super-infection, which is the beginning of egg deposition, only one single-infected mouse and two co-infected ones were found to have granulomas.

At week 5 super-infection none of the single-infected mice showed presence of granulomas, but one of the co-infected mice did. This suggests that the presence of malaria during an S. mansoni infection leads to an early initiation of granuloma formation. It also suggests that malaria boosts the cellular response against S. mansoni.

At week 6 super-infection, there was an increase in number

of granulomas as well as a significant increase ($P<0.01$) in size of granulomas in the single-infected group as compared to the co-infected ones. Concurrent S. mansoni-P. berghei infection led to reduced granuloma sizes. P. berghei seems to modulate granuloma sizes in co-infection. This agrees with a report that P. yoelii infections reduced granuloma formation in the lungs of mice injected with S. mansoni eggs (Abdel-Wahab et al., 1974). Furze et al., (2005) also noted that co-infection of mice with influenza virus during the early phase of trichinosis resulted in a reduced inflammatory infiltrate in the lungs (pulmonary pathology). They explained that the pulmonary pathology and cellular infiltration observed in their study could be due to a variety of factors, such as immune exhaustion as a result of ongoing intestinal inflammation.

CONCLUSION

The present study shows a co-infection with P. berghei and S. mansoni in BALB/c reduced the severity of S. mansoni infection, by reducing the worm load as well as the granuloma sizes and increasing schistosome-specific IgG levels. Even though inflammation of the liver and spleen seems to be exacerbated by the malaria infection, the end result was reduced severity of S. mansoni infection, based upon reduction of granuloma sizes. Fewer worms in the host means fewer eggs will be laid and conceivably less pathology. Reduced pathology means reduced severity of schistosomiasis!

On the other hand, S. mansoni exacerbates malaria parasitemia. S. mansoni worsened malaria infection as shown by the increased parasitemia as well as severe gross pathology of the liver and spleen in the co-infected mice.

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