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Research Brief

Plasmodium yoelii: Adverse outcome of non-lethal *P. yoelii* malaria during co-infection with *Schistosoma mansoni* in BALB/c mouse model

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ABSTRACT

Plasmodium yoelii and *Schistosoma mansoni* co-infections were studied in female BALB/c mice aged 4–6 weeks to determine the effect of time and stage of concomitant infections on malaria disease outcome. Patent *S. mansoni* infection in BALB/c mice increased malaria peak parasitemia and caused death from an otherwise non-lethal, self-resolving *P. yoelii* malaria infection. Exacerbation of malaria parasitemia occurred during both pre-patent and patent *S. mansoni* infection resulting in a delay of 4–8 days in malaria parasite resolution in co-infected mice. Praziquantel administered to mice with patent schistosome infection protected from fatal outcome during co-infection. However, this treatment did not completely clear the worm infestation, nor did it reduce the peak malaria parasitemia reached, which was nonetheless resolved completely. Hepatosplenomegaly was more marked in schistosome and malaria co-infected mice compared to either infection separately. The results suggest a complex relationship between schistosome co-infection and malaria disease outcome in which the timing of malaria infection in relation to schistosome acquisition is critical to disease outcome and pathology.

1. Introduction

Helminth and malaria co-infections are known to occur commonly and hence polyparasitism is more of "the rule rather than the exception" in human populations living in malaria endemic areas (Buck et al., 1978; Keusch and Migasena, 1982). However, little is known about how these concurrent infections affect disease outcome. Moreover, co-infection with helminths and malaria parasites can also modulate protective immune responses against each other.

The immune response to the intraerythrocytic stages of malaria parasite have been characterized in various rodent models and these studies have provided evidence that cell-mediated immunity and humoral immunity act in concert and sequentially to control and clear a blood stage malaria infection (Langhorne et al., 1989; Stevenson and Tam, 1993; Taylor-Robinson et al., 1993). On the other hand, experimental *Schistosoma mansoni* infections are known to induce a strong Th2 type response, as shown by occurrence of high IgE and eosinophil levels. At the time of patency or egg production, approximately 5 weeks post-infection, a Th2 response is thought to down-modulate the inflammatory response associated with granuloma formation and severe tissue damage induced by egg deposition, mainly in the liver (Brunet et al., 1997; Flores-Villanueva et al., 1996). In the context of Th1–Th2 paradigm, the majority of data from mice and humans have collectively categorized schistosomiasis as a predominantly Th2 modulated disease, implicating Th2 responses as the cause of morbidity and being detrimental to the host. However, recent data from rodent models have challenged the view that schistosome pathology can be clearly categorized as Th2 in nature, and more convincingly that evidence from infected humans implicate pro-inflammatory Th1 type responses as the cause of morbidity (Fallon, 2000).

In order to examine the underlying pathological and immunological mechanisms mediating concurrent helminth and malaria infections, rodent models offer many benefits that permit more appropriate experimental design and the ability to utilize an extremely well characterized rodent immune system (Druilhe et al., 2002; Fallon, 2000). Drawbacks to the use of rodent models as opposed to actual clinical observations in humans include: differences between host species and parasite species, field versus laboratory conditions and presence of other confounding multiple infections as well as differences in response to drugs and varying drug pharmacokinetics. According to a review by Druihle et al., the pattern of infection is not a fixed characteristic for a given pathogen but depends – particularly for the parasite – on the host in which it develops: susceptibility, load of infection, time course and resistance, all of which can vary greatly depending on host species or individuals (Druilhe et al., 2002).

This study describes the outcome of infection by non-lethal *Plasmodium yoelii* malaria parasite during pre- and post-patent *S. mansoni* infection in terms of malaria disease severity, parasitemia,

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anemia or percent hematocrit, the extent of hepatosplenomegaly and mortality.

2. Materials and methods

2.1. Animals

Female BALB/c mice (4–6 week-old) were procured from the National Cancer Institute (Bethesda, MD, USA) and maintained in a clean micro-isolation facility. Experimental mice were determined to be free from a variety of common infections through testing of sentinel mice exposed to cage bedding of the experimental mice as well as occasional direct testing of selected experimental mice for murine hepatitis virus. Mice were supplied with food and water *ad libitum*.

2.2. S. mansoni infections

Schistosoma mansoni cercariae were maintained in *Biomphalaria glabrata* snails fed on a diet of lettuce. To obtain cercariae of both sexes, four infected snails were placed in 50 ml of spring water and exposed to bright light for 30 min to induce cercariae shedding. The snails were removed and the water stirred to mix well before a single drop was transferred to a glass slide, stained with Lugol's iodine, and the number of cercariae counted (three replicates). Suspensions were adjusted to obtain an average of five cercariae per drop. The required number of cercariae (~75) was added into a beaker and each mouse infected individually by dipping the tail for 30 min under anesthesia. After infection, a drop of Lugol's iodine was added into the beaker to stain cercariae in order to estimate the number of penetrating cercariae. Mice were found to be infected with *S. mansoni* cercariae at a level of ~70 cercariae per mouse. Fifty mice were randomly assigned into four experimental groups (A–D) of 10 mice each (Fig. 1), and a fifth control group (E). For each group 5 of the 10 mice were infected with *S. mansoni* and the other five were mock-infected.

Patency of *S. mansoni* infection was confirmed by the miracidial hatching technique at 6 and 7 weeks post-schistosome infection. Briefly, fresh fecal pellets were collected in a small volume of saline solution in a 15 ml Pyrex tube. The tube was exposed to a strong beam of light and spring water added to cause the *S. mansoni* eggs to hatch by osmosis, releasing the phototactic miracidia, which then swam towards the light. The miracidia could be easily seen due to their swimming movement by use of a hand held magnifying lens within 30 min of initiation of hatching.

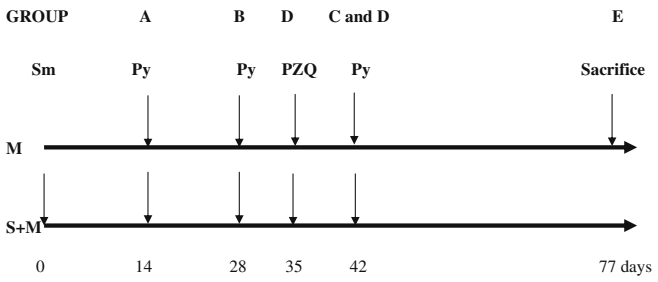

Fig. 1. Schematic of the study design. Mice were infected with *S. mansoni* (Sm) on day 0. *P. yoelii* (Py) infection was initiated on day 14 (Group A), day 28 (Group B), day 42 (Groups C and D). Mice in Group D were treated with praziquantel (PZQ) on day 35. All the surviving mice were sacrificed on day 77. Each group had schistosome and malaria (S + M) co-infected and malaria only (M) infected sub-groups of mice represented by the two horizontal arrows. The control group (E) had schistosome only infected (S) and uninfected (N) sub-groups of mice. (All groups were subjected to same treatment by mock-infection.)

2.3. P. yoelii infections

At 14 days post-schistosome infection (DPSI) (group A), 28 DPSI (group B) and 42 DPSI (groups C and D) mice were infected with 10^5 *P. yoelii* (Strain 17XNL – a non-lethal self-resolving malaria parasite) infected red blood cells (iRBCs) (Fig. 1). Each of groups A, B, C and D of mice was then made up of two sub-groups, i.e., schistosome and malaria co-infected (S + M) and malaria only infected (M). The control mice (group E) consisted of five mice infected with schistosomes only (S) and five uninfected (mock-infected) mice (N). In order to ensure uniformity of iRBC, parasite stabilates frozen from a single batch of *P. yoelii* were used for the infection of various groups. At each time of infection, frozen parasites were thawed and injected intraperitoneally into a single donor BALB/c mouse and parasitemia allowed to develop to 5–10% before infected blood from donor was taken and diluted to give a dose of 10^5 iRBCs per mouse. Malaria-free control groups received the same volume (200 μl) of 1× PBS instead of iRBCs. Malaria infections were monitored by Giemsa-stained thin blood smears prepared from tail bleeds starting on the 3rd day post-malaria infection and subsequently on every other day thereafter. Percent parasitemia was determined by examining at least 1000 RBCs on the slide and averaged for each group of mice.

2.4. Antihelminthic treatment

Mice in group D were treated perorally with praziquantel (PZQ) (Sigma, St. Louis, Missouri) suspended in 2% cremophor EL (Sigma) at a dose of 500 mg/kg of body weight at 35 DPSI, when *S. mansoni* infection is considered to be patent (Fig. 1). Fresh fecal pellets were tested by the miracidial hatching technique to check for worm clearance.

2.5. Other measurements

Total body mass was measured for each individual mouse on a weekly basis, whereas hematocrit (Hct) was measured after *P. yoelii* infection in individual mice at 1 and 2 weeks following malaria infection in all groups. Approximately 50 μl of blood were drawn via tail bleeds into heparinized microcapillary tubes (Fisher) and then centrifuged at 1200 rpm for 15 min. After 77 DPSI, all surviving mice were sacrificed, liver, spleen masses determined and compared among groups.

2.6. Data analysis

Mean parasitemia of schistosome and malaria co-infected (S + M) and malaria only infected (M) groups of mice were compared using a non-parametric test, the Mann–Whitney test, whereas percent hematocrit, liver and spleen masses were compared based on standard errors of mean (SEM) differences. Differences in parasitemia between groups were considered significant when p value was less than 0.05.

3. Results

3.1. Increased susceptibility of pre-patently ***S. mansoni***-infected mice to malaria

To determine if pre-patent schistosome infection altered mouse susceptibility to malaria, mice in groups A (14 DPSI) and B (28 DPSI) and their age-matched worm-free counterparts, respectively, were injected intraperitoneally with the same dose of 10^5 iRBCs of non-lethal *P. yoelii* strain (17XNL). Overall, pre-patently co-infected (S + M) mice developed higher group mean malaria parasitemia