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Schistosoma co-infection protects against brain pathology but does not prevent severe disease and death in a murine model of cerebral malaria

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ABSTRACT

Co-infections of helminths and malaria parasites are common in human populations in most endemic areas. It has been suggested that concomitant helminth infections inhibit the control of malaria parasitemia but down-modulate severe malarial disease. We tested this hypothesis using a murine co-infection model of schistosomiasis and cerebral malaria. C57BL/6 mice were infected with *Schistosoma mansoni* and 8–9 weeks later, when *Schistosoma* infection was patent, mice were co-infected with *Plasmodium berghei* ANKA strain. We found that a concomitant *Schistosoma* infection increased parasitemia at the beginning of the *P. berghei* infection. It did not protect against *P. berghei*-induced weight loss and hypothermia, and *P. berghei*-mono-infected as well as *S. mansoni*–*P. berghei*-co-infected animals showed a high case fatality between days 6 and 8 of malarial infection. However, co-infection significantly reduced *P. berghei*-induced brain pathology. Over 40% of the *S. mansoni*–*P. berghei*-co-infected animals that died during this period were completely protected against haemorrhaging, plugging of blood vessels and infiltration, indicating that mortality in these animals was not related to cerebral disease. *Schistosoma mansoni*–*P. berghei*-co-infected mice had elevated plasma concentrations of IL-5 and IL-13 and on day 6 lower levels of IFN-γ, IL-10, monocyte chemoattractant protein-1 (MCP-1) and monokine induced by IFN-γ (MIG) than *P. berghei*-mono-infected mice. We conclude that in *P. berghei* infections, disease and early death are caused by distinct pathogenic mechanisms, which develop in parallel and are differentially influenced by the immune response to *S. mansoni*. This might explain why, in co-infected mice, death could be induced in the absence of brain pathology.

1. Introduction

Polyparasitic infections in humans are common in many tropical regions of the world (Raso et al., 2004; Hamm et al., 2009). As helminths and malaria parasites are often co-endemic, helminth-malaria co-infections are frequently observed. Several studies have demonstrated that helminth infections can modulate the immune response to malaria parasites. Nevertheless, the outcomes of these studies have been contradictory, with some suggesting that co-infections can ameliorate (Briand et al., 2005; Lyke et al., 2005) and others showing that co-infections can exacerbate (Nacher et al., 2002; Le Hesran et al., 2004) disease severity in the host. Co-infection studies using well-defined animal models indicate that these contradictory results might be explained by variables such as the species and developmental stage of the parasites as well as the genetic background of the host (Lwin et al., 1982; Graham et al., 2005; Fernandez et al., 2009).

In malaria, a balance between pro- and anti-inflammatory cytokines plays a central role in the regulation of the immune response and pathogenesis. During early stage malaria, a strong Th1-like immune response to this intracellular parasite can prevent its uncontrolled multiplication, but an over-production of pro-inflammatory cytokines can lead to severe immunopathology, such as cerebral malaria (CM) (Hartgers et al., 2008). In humans, CM is a form of life-threatening malaria with CNS involvement. It is a major cause of death in *Plasmodium falciparum* infections with a high case fatality rate (Snow et al., 2001). Prominent histopathological features of CM are microhaemorrhages and oedema in the brain, and the cytoadhesion of host cells, such as parasitised red blood cells

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(pRBCs), leucocytes and platelets, to the brain microvasculature (Hunt and Grau, 2003).

The most important helminths in humans are trematodes, cestodes and nematodes. Although they live in extracellular body compartments and are exposed to the immune system, they are able to avoid immune destruction and can persist in their host for decades (Chabasse et al., 1985). Their long-term survival is facilitated by immuno-regulatory mechanisms specific for the different parasitic life cycle stages. For example, schistosomal parasites in the egg-laying phase (patent infection) modulate dendritic cells and macrophages and induce regulatory T cells (reviewed in Dunne and Cooke (2005)). This generates Th2 and anti-inflammatory cytokine responses. Thus, a regulatory network evolves which protects the parasites from elimination, but also prevents severe inflammation and resulting immunopathology.

Immune responses in chronic helminth infections can dampen the immune response to third party antigens (Maizels and Yazdanbakhsh, 2003). It has been proposed that anti-inflammatory and Th2 responses to patent schistosomiasis might inhibit parasite control of early stage malaria, but down-modulate the malaria-induced pro-inflammatory response and limit severe pathology at later stages of malaria (Hartgers and Yazdanbakhsh, 2006). The present study investigates this hypothesis using a co-infection model of schistosomiasis (*Schistosoma mansoni*) and CM (*Plasmodium berghei* ANKA strain) in C57BL/6 mice. These mice were selected as they develop cerebral malaria at a high rate, probably due to their pro-inflammatory/Th1 predisposition (Lou et al., 2001). To investigate the impact of patent schistosomiasis on *P. berghei*-induced malaria, the course co-infection was followed longitudinally and quantified in terms of parasitemia, changes in body mass, body temperature, plasma lactate levels, brain histopathology and survival. To analyse the influence of co-infection on the systemic cytokine response, plasma concentrations of IFN-γ, TNF-α, IL-5, IL-10, IL-13, IFN-inducible protein (IP-10), monocyte chemoattractant protein-1 (MCP-1), monokine induced by IFN-γ (MIG) and macrophage inflammatory protein-1 (MIP-1α) were determined at different time points before and after infection with *P. berghei*.

2. Materials and methods

2.1. Mice, parasites and infections

Female 3-week-old C57BL/6 mice were obtained from Harlan-Winkelmann (Borchen, Germany). They were kept in the animal facility of Institute of Tropical Medicine, University of Tübingen, Germany. Mice were maintained in individually ventilated cages and supplied with heat-sterilized food and autoclaved water ad libitum. Mice were 4 weeks of age at the time of infection with *S. mansoni*.

The Puerto Rico PR-1 strain of *S. mansoni* is routinely bred at the Institute of Tropical Medicine, University of Tübingen. It is maintained by cyclical passage between pulmonate fresh water snails (*S. mansoni*-susceptible M-line of *Biomphalaria glabrata*) and BALB/c mice. Experimental mice were infected with *S. mansoni* by exposure to 50 cercariae percutaneously (Löhning et al., 1999).

The rodent malaria parasite, *P. berghei* ANKA strain, was originally obtained from D. Walliker, University of Edinburgh, United Kingdom. Parasites were stored as frozen stabilates at −80 °C. To obtain experimental inocula of *P. berghei* ANKA, pRBCs were sequentially passaged through three homologous donor mice. Experimental mice were inoculated i.p. with 10^6 pRBCs. Control mice were injected with blood from an uninfected mouse. All animal experiments were conducted in accordance with German laws after approval by the local authorities.

2.2. Recovery of adult worms and verification of liver granulomas

Establishment of helminth infection was confirmed by the presence of worms and liver granulomas. Each mouse that had been exposed to cercariae underwent necropsy. Whenever possible, worms were obtained via portal perfusion (Smithers and Terry, 1965), and livers were examined for the presence of granulomas under a stereo microscope.

2.3. Determination of reticulocyte counts and erythrocyte counts

Reticulocytes were counted microscopically using brilliant cresyl blue-stained (Merck, Darmstadt, Germany) thin-blood smears of tail blood. To determine erythrocyte numbers, tail blood was diluted 1:200 in Hayem's solution (Merck, Darmstadt, Germany) and erythrocytes were counted in a Neubauer chamber.

2.4. Malaria parasitemia and disease severity

Parasitemia was quantified as the percentage of pRBCs by microscopic examination of Giemsa-stained (Merck, Darmstadt, Germany) thin-blood smears of tail blood. Slides were coded and pRBCs were counted microscopically in at least five microscopic fields, each approximately 300 cells.

Disease severity after infection with *P. berghei* ANKA was measured in terms of change in body mass, body temperature and in terms of behavioural and functional alterations in the animals (Lackner et al., 2006). Body mass was measured using a top-pan electronic balance. Rectal body temperature was measured with a digital thermometer (GTH 1170, Greisinger electronic GmbH, Regenstauf, Germany). Additionally, the colour of ears and feet of the animals were examined daily. White ears and pale colour of plantar surface and digits of forelimbs served as signs for severe anaemia and were recorded accordingly. To evaluate clinical experimental CM and terminal illness, mice were monitored for the following signs: hypothermia below 31 °C, abnormal respiration, abnormal body position (hunching, laying prone, laying on side), impaired motility (limited movement, incapacity of movement), paralysis, convulsions and coma. Alternative behaviour listed after body position and motility indicates increasing disease severity (Lackner et al., 2006) and was assessed with increasing score values ranging from 1 to 3 and from 1 to 2, respectively. Animals with cumulative scores of $\geqslant 4$ were sacrified. For survival analysis, the day of death was recorded as the day when the animal was killed.

2.5. Histology

Immediately after death, the brains of the mice were removed and fixed in 4% buffered formalin for at least 48 h. After that, brain tissue was dehydrated by a series of graded alcohols and xylene, and embedded in paraffin. Then, 7-µm-thick coronal sections from two different brain regions with the stereotaxic co-ordinates bregma 0 and bregma −6 were cut on a microtome (Jung RM 2045, Leica, Bensheim, Germany). The serial sections were placed onto SuperFrost/Plus slides (Microm International, Walldorf, Germany), deparaffinized and hydrated, and routinely stained with haemalaun and eosin (both products Merck, Darmstadt, Germany). Slides were blinded and eight randomly selected sections per brain region were subjected to histological examination. Analysis was done on a Nikon Eclipse E800 research microscope (Nikon, Melville, NY, USA). At least 100 visual fields per section were analysed at 400-fold magnification. They were examined for haemorrhage, sequestration of blood cells to brain microvasculature and infiltration of immune cells into the perivascular space in a semi-quantitative manner. Cerebral score values (CSV) ranging from 0 to 2