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INTERFERENCE WITH HOMOLOGOUS IMMUNITY AND FAECAL EGG EXCRETION IN SCHISTOSOMA INFECTIONS IN MICE CONCURRENTLY INFECTED WITH TRYPANOSOMA BRUCEI AND SCHISTOSOMA MANSONI

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Abstract. The effects of the blood protozoan, *Trypanosoma brucei*, was assessed on the homologous immunity to *Schistosoma mansoni* infection and the distribution of *S. mansoni* eggs in the tissues and faeces of dually infected mice. The trypanosome infection reduced the homologous protection induced by a primary *S. mansoni* infection against a challenge infection and the faecal excretion of eggs during the primary schistosome infection. The findings are discussed in relation to the immunosuppressive action of *Trypanosoma* infection.

It is well known that a pre-existing infection with *Schistosoma* species in mice induce acquired resistance to subsequent homologous challenge infections and that this immunity which is predominantly directed against the adult worms of the challenge infection can also be demostrated to a lesser extent during the migratory stages in the skin and lungs (Dean 1983). Furthermore, it has been amply demonstrated that primary infections with unrelated helminths such as other trematodes (Hillyer 1976, Christensen et al. 1981, Siraq et al. 1980) and nematodes (Crandal et al. 1966, Hunter et al. 1967) can induce significant protection in mice against subsequent infection with *Schistosoma* species. However, the effects of protozoan parasites on secondary infections with *Schistosoma* species are equivocal. While *Toxoplasma gondii* infection given at the appropriate time has protected mice against *S. mansoni* infections (Mahmoud et al. 1976), *Plasmodium chabaudi* infection had no effects on worm recovery of secondary *S. mansoni* infections (Long et al. 1980). Mixed results were obtained as regards the effect of *Trypanosoma cruzi* on resistance to *S. mansoni* challenge infections (Kloetzel et al. 1973).

Apart from the effects of heterologous parasites, the immune status of the host has been found to affect the longevity of schistosomes and the pattern of egg excretion in experiments involving *S. mansoni* infection in T-cell deprived mice (Doenhoff et al. 1978, 1979).

In this study, the effect of *Trypanosoma brucei* on the migrating schistosomules in lungs, liver and messenteric veins during secondary *Schistosoma mansoni* infections in mice harbouring existing primary infections of both *S. mansoni* and *T. brucei* was investigated. In addition, the effect of *T. brucei* on *Schistosoma* egg distribution in tissues and excretion in faeces was also studied in primary *S. mansoni* infections in mice with concurrent infections with *T. brucei*.

MATERIALS AND METHODS

Mice and parasite materials: The mice used in these experiments were outbred albino mice and they weighed 25 to 30 grams at the beginning of the experiment. The *S. mansoni* was the Puerto Rican strain and was maintained in the laboratory by transmission between mice and *Biomphalaria glabrata* snails. *Trypanosoma brucei* (Treu 858) was obtained from the Centre for Tropical Veterinary Medicine, University of Edinburgh and maintained in the laboratory by syringe passage in mice.
Parasite infections: Infection of experimental mice with *S. mansoni* was done by the 'ring' method after anaesthesia with pentobarbitone (Smithers and Terry 1965). Blood containing *T. brucei* was obtained from donor mice and the experimental mice were infected with *T. brucei* (10^4 trypanosomes per mouse) intraperitoneally after the donor blood was diluted with phosphate buffered saline (pH 7.2). All the infected mice were invariably parasitaemic as judged by regular examinations of blood by the wet mount method.
The recovery of schistosomules and adult worms: For schistosomule recovery from lungs, mice were killed with an overdose of pentobarbitone containing 10 units of heparin. The lungs were dissected out into a conical flask and macerated with sharp, fine-pointed scissors. After the addition of Hanks Balanced Salt Solution (HBSS) the flask was incubated for 3 hours at 37 °C. The contents of the flask was then filtered through a 150 μm sieve into a 10 ml centrifuge tube. The preparation was washed by centrifuging in heparinised HBSS and the sediment was re-suspended in 1 ml HBSS before being transferred into a Sedgewick Rafter counting cell. The living schistosomules in each sample were counted under a dissecting microscope.

Recovery of early adult worms was by the method of portal perfusion described by Smithers and Terry (1965) but since the worms were still small in size, counting was done under a dissecting microscope after the perfuseates were transferred into Sedgewick Rafter chambers.
Faecal egg counts: Mice were placed individually in shoe-box cages on metal grids which allowed faeces to pass through to the floor of the cages. Faecal collection was for 18 hours after which all the faeces from each mouse were airdried and weighed separately. The schistosome eggs were extracted from the faeces by filtration and stained with ninhydrin solution as described by Bell (1963). The faecal egg count was expressed as the number of eggs per adult worms pair recovered during portal perfusion.
Tissue egg counts: After the perfusion of infected mice, the small intestine and liver were removed separately into 100 ml conical flasks and macerated with scissors. The tissues were then digested in 50 ml of 3 % potassium hydroxide solution at 37 °C for 16 hours (Cheaver 1968) before the contents of the flask were diluted by adding 50 ml water. 1 ml of this mixture was pipetted into a petri dish and the number of eggs contained in this aliquot was counted under a dissecting microscope. The number of eggs counted was multiplied by 50 and expressed as the number of eggs per worm pair recovered by perfusion.
Experimental plan: The first experiment was set up to investigate the effect of *T. brucei* on the distribution of *S. mansoni* eggs in tissues and their excretion in faeces in mice harbouring primary *S. mansoni* and *T. brucei* infections. Two groups of mice were infected with *S. mansoni* initially. After two weeks of the *S. mansoni* infection, *T. brucei* was superimposed in one group while the second group served as the *S. mansoni* mono-infected control. All the *T. brucei* — infected mice was parasitaemic throughout the experiment as judged by regular examination of blood by the wet mount method. Tissue and faecal egg evaluations were done on days 38, 42, 46, 50, 54 and 58 post-infection with *S. mansoni*. At least three mice were used from each group during each day of evaluation. After the collection of faecal samples, the mice were immediately killed and perfused for adult worm recovery and their tissues were stored at —20 °C for tissue egg counts at the end of the experiment. The number of mice in each group was 24 and each mouse received 120 cercariae of *S. mansoni*.

A second experiment was designed to study the effect of *T. brucei* on the attrition of the worms of a challenge *S. mansoni* infection in mice harbouring a primary schistosome infection. Two groups of mice were each infected with 120 *S. mansoni* cercariae per mouse. Two weeks after this schistosome infection one of these groups was infected with *T. brucei*. Six weeks after the primary *S. mansoni* infection, these two groups of mice and a third group which was previously parasite-free were infected with *S. mansoni* (150 cercariae per mouse). From day 4 to day 10 of the secondary *S. mansoni* infection, at least 3 mice were killed daily from each of the three groups and the number of schistosomulae recovered from the lungs were determined. The number of young adult worms of the challenge *S. mansoni* infection was determined by portal perfusion three weeks after the challenge infection.

RESULTS

The effects of *T. brucei* infection on the distribution of *S. mansoni* eggs in tissues and in faeces are shown in Fig. 1. *S. mansoni* eggs were present in liver and small intestine as from day 38 after infection. The distribution of eggs in tissues was comparable for both the *S. mansoni* — infected mice and the *S. mansoni* plus *T. brucei* infected mice throughout the experimental period (Fig. 1A, B). Fig. 1C shows that *S. mansoni* eggs were detectable in faeces from about day 42 onwards and reached a peak on day 50. However, the number of faecal eggs per worm pair was consistently and significantly lower ($P < 0.05$) in the *S. mansoni* plus *T. brucei* infected mice than in the *S. mansoni* mono-infected mice. The wet and dry weights of faecal material per mouse in 18 hours were not significantly different between the two groups of mice throughout the experimental period.

In Fig. 2, the peak recovery of challenge schistosomules from mice harbouring primary *S. mansoni* infection was delayed relative to naive mice indicating a delay of schisto-

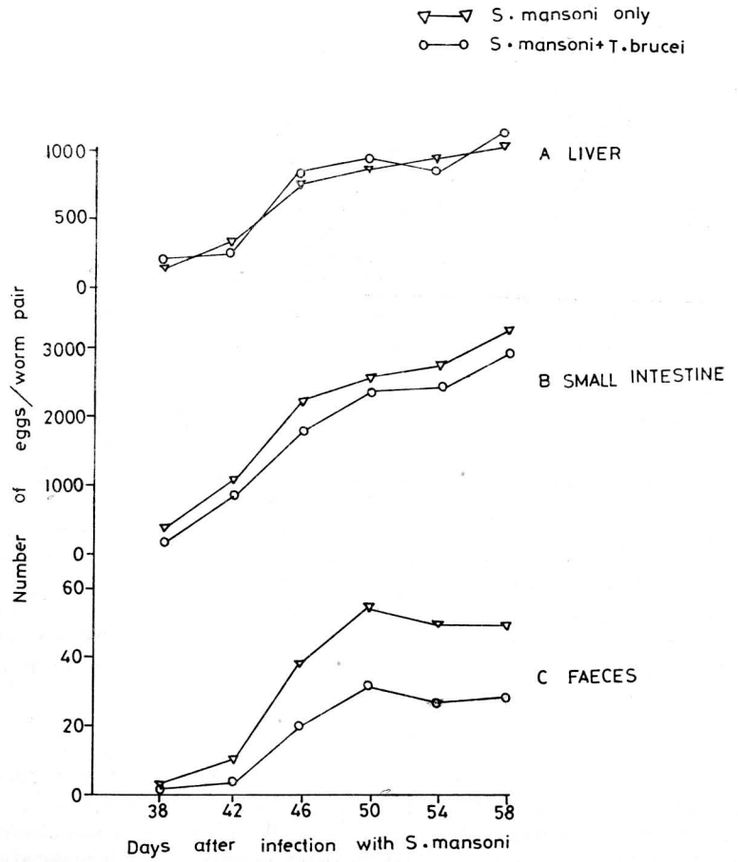

Fig. 1. *S. mansoni* egg counts in the liver, small intestine and faeces of mice harbouring concurrent infections of *S. mansoni* and *T. brucei* and in mice infected with *S. mansoni* only.

moular migration from the skin to the lungs of the immune mice. Furthermore, it seems that the superimposition of *T. brucei* infection on the primary *S. mansoni* infection annulled the delay of the migration of the challenge schistosomules in the *S. mansoni* plus *T. brucei* infected mice so that the schistosomular migration through the lungs of these mice follows a similar pattern to the situation in the naive mice. Besides, it seems that although the peak schistosomulae recovery is delayed and lower in the *T. brucei*-free mice harbouring primary *S. mansoni* infection, the migration spread over a longer period,

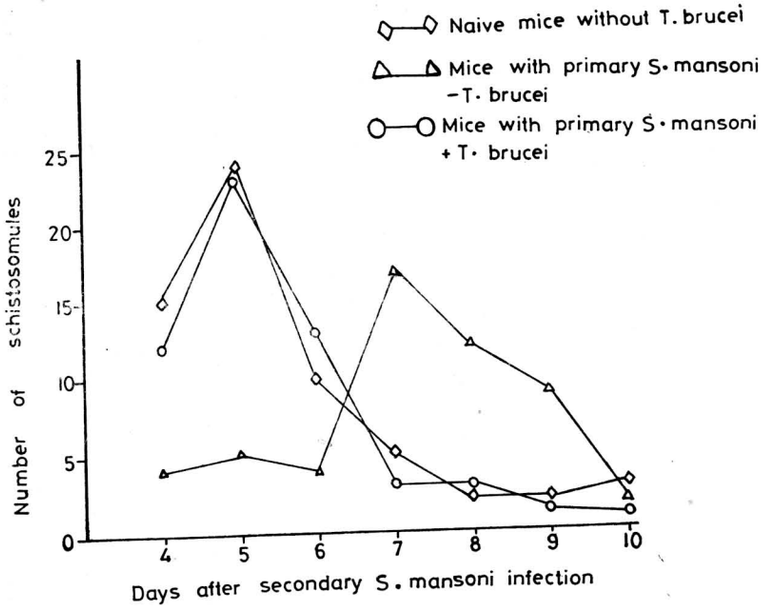

Fig. 2. The effect of *T. brucei* infection on schistosomule recovery from the lungs of mice harbouring patent primary *S. mansoni* infection.

so that the effect of immunity at this stage seems to be manifested only as a delay in, schistosomular migration. The total number of schistosomules recovered from naive *T. brucei* — free mice; *S. mansoni* plus *T. brucei* infected mice; and the mice with primary *S. mansoni* infection were 59 ± 4.0, 56 ± 2.9 and 51 ± 4.7, respectively. The number of young adults recovered from the same groups by portal perfusion were 31 ± 6.8, 25 ± 4.2 and 18 ± 3.7, respectively, indicating a 41 % reduction in worm recovery in the *T. brucei*-free mice harbouring *S. mansoni* infection and a reduction of 28 % in the *T. brucei* — infected mice harbouring primary *S. mansoni* infection relative to naive mice.

DISCUSSION

The results obtained from the present studies show that *T. brucei* infection reduced *S. mansoni* faecal egg excretion during the primary schistosome infection in mice although the pattern of egg distribution in the liver and small intestine were not altered. Furthermore, this trypanosome infection annulled the delay in schistosomular migration through the lungs which occurred in the immune mice and tended to reduce the homologous protection induced by a primary *S. mansoni* infection against a secondary infection.

Adequate *S. mansoni* faecal egg excretion surprisingly depends on immunological competence of the host in mice. Thus Doenhoff et al. (1978), have shown that mice deprived of T. lymphocytes by means of thymectomy and anti-thymocyte serum and subsequently infected with *S. mansoni* had substantially fewer eggs in their faeces than in immunologically intact control mice although the pattern of egg distribution in tissues remained unchanged. Since the effect of *T. brucei* on the schistosome egg excretion in faeces is similar to the effects obtained after experimentally induced immunosuppression, it is possible the effect produced by the trypanosome is a consequence of its immunosuppressive action. Impairment of T. and B. lymphocyte function during trypanosomiasis has been shown by several workers (Eardly and Jayawardena 1977, Moulton and Coleman 1977, Pearson et al. 1978, Mansfield and Bagastra 1978). This suppression of T. and B. cells during trypanosomiasis may also be responsible for the interference with homologous resistance in *S. mansoni* plus *T. brucei*-infected mice as the overall result of such lymphocyte dysfunction is a generalised suppression of cellular and humoral immune responses (Greenwood 1974).

The present findings also shed some light on the point of attrition of the schistosomes of a challenge infection in mice harbouring a primary homologous infection. Although a delay in schistosomular migration through the lungs of mice with a primary *S. mansoni* infection occurred, it seems that the main point of attrition of the worms of the challenge infection in the mice was after they had left the lungs. The investigations by Dean and Mangold (1983) using autoradiographic techniques have proved this view.

It seems obvious from these experiments that a concurrent infection with *T. brucei* and *S. mansoni* in mice could have great consequences for both the host and the schistosome and this provides another example of the interactions that occur between parasites in concurrent infections.

ВЗАИМНОЕ ВЛИЯНИЕ ГОМОЛОГИЧНОГО ИММУНИТЕТА И ЭКСКРЕЦИИ ЯЙЦ ПРИ ШИСТОСОМОЗНОЙ ИНФЕКЦИИ У МЫШЕЙ КОНКУРЕНТНО ИНФИЦИРОВАННЫХ *TRYPANOSOMA BRUCEI* И *SCHISTOSOMA MANSONI*

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Резюме. Изучалось влияние протозойной инфекции *Trypanosoma brucei* на гомологичный иммунитет против *Schistosoma mansoni* и на распределение яиц *Schistosoma mansoni* в тканях и экскрементах у мышей с двойной инфекцией. Инфекция трипаносомами снижает степень гомологичной защиты индуцированной первичной инфекцией *Schistosoma mansoni* против повторного заражения и выделение яиц с экскрементами в ходе первичной инфекции. Результаты исследования дискутируются в отношении с иммуносупрессивным влиянием трипаносомной инфекции.

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This is an outstanding work compiled by a team of about 500 specialists covering all branches of biology including those developing intensively in the last years. It contains nearly 7,600 items the more important of which are accompanied by references to special publications. Included are many black and white drawings depicting various biological objects. The extinct organisms and many plant and animal species are represented on plates 42 of which are in colours. The proper text is followed by a list of scientists, index of Latin names, index of Russian terms and list of synonymes of several Russian names of plants and animals. The dictionary is closed by a list of biological literature divided into 21 sections, one of which deals with parasitology. In each section the most important monographs, journals, encyclopaedias and reference journals are given. It is a very useful work, destined for pedagogues, students and all those interested in biological sciences. It was edited with a great care and the authors are to be warmly congratulated. *Dr V. Černý, C.Sc.*