PATHOGENIC ASPECTS OF PYOGENIC LIVER ABSCESS ASSOCIATED WITH EXPERIMENTAL SCHISTOSOMIASIS

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Abstract. Schistosomiasis mansoni infection that occurs concurrently with Staphylococcus aureus bacteremia favors the formation of pyogenic liver abscess. The present experimental study in mice evaluated the following aspects of the relationship between infection with Schistosoma mansoni and liver abscess caused by S. aureus: a) the role of the eggs of S. mansoni in the genesis of the abscesses; b) the influence of different phases of schistosomiasis in the development of liver abscesses; and c) the effect of the treatment of schistosomiasis on the development of the abscesses. Macroscopic and histopathological study showed multiple liver abscesses around granulomas of S. mansoni in the acute and chronic phases of schistosomiasis. Treatment of acute schistosomiasis before experimentally-induced bacteremia did not prevent the formation of liver abscess. The study findings indicate that granulomas around S. mansoni eggs and worms lodged in the liver provide a focus and substrate for pyogenic abscesses caused by S. aureus.

INTRODUCTION

Pyogenic liver abscesses are relatively uncommon, despite the frequency of cholecystitis, appendicitis, and diverticulitis, which are the main sources of bacterial infection to the liver. Liver abscesses have also been described as an infectious complication of chronic granulomatous disease and liver transplantation. When present in children, they occur up to 5 years of age and are associated with thrombophlebitis of the umbilical vein, abdominal trauma or immunosuppressive disease.

In adults, pyogenic liver abscesses are frequently polymicrobial, usually due to enteric Gram-negative bacilli (e.g., Escherichia coli) and anaerobic bacteria. In general, Staphylococcus aureus liver abscesses are associated with infection in other organs or related to generalized hematogenous dissemination in children with impaired host defenses.

In South America, parasitic diseases have been described as a predisposing factor for pyogenic liver abscess. Amebic liver abscesses may be secondarily infected with bacteria, usually of enteric origin. Hepatic hydatid cysts (Echinococcus granulosus) also become secondarily infected. In addition, migrating Ascaris lumbricoides and, more recently, acute schistosomiasis have been implicated as predisposing factors in the genesis of pyogenic liver abscess.

We previously reported a study of the association between schistosomiasis and pyogenic liver abscess. The clinical and experimental study demonstrated that acute schistosomiasis concurrent with S. aureus bacteremia favors the colonization of the liver by bacteria causing pyogenic liver abscesses.

The following aspects of the pathogenesis of the pyogenic liver abscess in mice infected with Schistosoma mansoni are evaluated herein: a) the role of the eggs of S. mansoni in the genesis of the abscesses; b) the influence of the acute and chronic phases of schistosomiasis in the development of the abscesses; and c) the effect of schistosomiasis treatment on the development of the abscesses.

MATERIALS AND METHODS

Schistosomiasis animal model. Five-week-old male albino outbred mice (Mus musculus) were infected with 40 S. mansoni cercariae of the LE strain. The LE strain has been maintained for more than 30 years at the Schistosomiasis Research Unit (Laboratory of Dr. José Pellegrino, Federal University of Minas Gerais, Brazil) by serial passage through Biomphalaria glabrata and hamsters (Mesocricetus auratus). Another group of mice were infected with 250 LE cercariae irradiated with 3 Kr of gamma irradiation of Cobalt-60, to sterilize the female worms and to prevent the deposition of eggs in the liver parenchyma.

Treatment of schistosomiasis. After 60 days infection with non-irradiated cercariae, one group of 12 mice was treated with 400 mg/kg of oxamniquine, by mouth. This dose is sufficient to eliminate all the adult worms of S. mansoni.

Bacterial infection. Mice were injected intravenously with Staphylococcus aureus strains isolated from staphylococcal pyoderma of patients. The strains were cultivated for 24 hours at 37°C on nutrient agar. After incubation, the bacteria were re-suspended in 0.85% saline. Forty thousand colony forming units (CFU), as determined by nephelometer, were injected into the tail vein, according to the method previously described. The bacterial infection was done after 60 days (acute phase) and 120 days (chronic phase) of being infected with cercariae, and 30 days after the treatment of schistosomiasis.

General proceedings. All animals received care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals,” by the United States National Institutes of Health.

All mice were sacrificed 28–30 days after bacterial infection. Worms were recovered by perfusion of portal tract, according to the technique previously described. The thoracic and abdominal organs were examined macroscopically and samples of blood were collected for culture. Purulent material from some liver abscesses was also cultured. Livers were fixed in 10% formalin and processed for histopathological study. The slices were stained with hematoxylin-eosin and gomoritrichrome.
(IrSm) of S. mansoni, in the acute phase of schistosomiasis, were infected with bacteria (Sa) after 60 days of cercariae infection. Mice were divided into 6 groups as follows: Group 1 = Sm + Sa (20 mice infected with 40 non-irradiated cercariae and bacteria); Group 2 = IrSm + Sa (20 mice infected with 250 irradiated cercariae and bacteria); Group 3 = Sm (20 mice infected with 40 non-irradiated cercariae and bacteria only); Group 4 = IrSm (20 mice infected with 250 irradiated cercariae and bacteria only); Group 5 = Sm (10 mice infected with bacteria only); and Group 6 = UNF (10 uninfected mice kept as controls of the acute phase. All groups were sacrificed 30 days after inoculation with the bacteria. Five mice of groups 4 and 5 were hemoperfused.

Experiment 2. In the acute (A Sm) and chronic (C Sm) phases of schistosomiasis, mice that had been infected with 40 non-irradiated cercariae were inoculated with S. aureus. Eight groups were formed as follows: Acute phase: Group 1 = A Sm + Sa (20 mice infected with 40 cercariae and bacteria); Group 2 = A Sm (10 mice infected with 40 cercariae only); Group 3 = A Sa (15 mice infected with bacteria only); and Group 4 = A UNF (10 uninfected mice kept as controls). Chronic phase: Group 1 = C Sm + Sa (15 mice infected with 40 cercariae and bacteria); Group 2 = C Sm (10 mice infected with cercariae only); Group 3 = C Sa (14 mice infected with bacteria only); and Group 4 = UNF (10 uninfected mice kept as controls of the chronic phase).

Experiment 3. After 60 days of 40-cercariae infection, one group of 12 mice was treated with oxamniquine (T). As a control, another group of 10 mice infected with 40 cercariae was kept without treatment. After 30 days of the treatment of the first group, both groups were infected with bacteria. Two groups were formed: Group 1 = Sm + T + Sa and Group 2 = Sm + Sa. Both groups were sacrificed 30 days after the bacterial infection.

Statistical analysis. The frequencies among groups were compared using the Fisher’s exact test and $\chi^2$ test. A P value of 0.05 or less was considered statistically significant. The sample sizes were calculated on the basis of an expected frequency of liver abscesses of more than 50% in the group infected with S. mansoni and S. aureus and less than 1% in the control group, as previously demonstrated.18

RESULTS

Experiment 1 (non-irradiated versus irradiated cercariae + S. aureus). Sixteen out of 20 mice of group 1 (Sm + Sa) presented with innumerable granulomas in the liver; 14 (87%) developed liver abscesses measuring five to ten millimeters in diameter. Staphylococcus aureus was recovered from abscesses and blood culture. Granulomas were rarely found on histological examination of the liver of mice in groups 2 (IrSm + Sa) and 4 (IrSm). Two out of twenty mice in group 2 had small liver abscesses (less than 3 millimeters). The livers of mice of group 3 (Sm) presented innumerable granulomas but no abscesses. The histology of the livers of mice in groups 5 (Sa) and 6 (UNF) was completely normal. Statistical analysis showed a significant difference between the formation of abscesses in groups infected with non-irradiated and irradiated cercariae ($P = 0.00001$) (Table 1). Two per cent of irradiated worms and 12 per cent of non-irradiated worms were recovered from the portal tracts (Table 2).

Experiment 2 (acute versus chronic schistosomiasis + S. aureus). Acute phase: Ten (50%) of 20 mice of group 1 (A Sm + Sa) presented with innumerable granulomas and multiple abscesses up to one centimeter in diameter. Histological examination of the hepatic areas with abscesses showed focus of necrosis with predominance of inflammatory infiltrate of polymorphonuclear cells and colonies of S. aureus. Eggs of S. mansoni were observed in the abscesses. No abscesses or granulomas were found in livers from mice of group 3 (A Sa) and 4 (UNF), but granulomas without abscesses were found in livers of group 2 (A Sm). Statistical analysis showed a strong association between staphylococcal liver abscesses and infection with S. mansoni, in the acute phase of schistosomiasis ($P = 0.0032$).

Chronic phase: Seven (47%) of 15 mice of group 1 (C Sm + Sa) had liver abscesses and multiple fibrosed granulomas confirmed by histological study. Mice of group 2 (C Sm) had innumerable liver granulomas already fibrosed, but without abscesses. No granulomas or abscesses were found in the livers from mice of group 3 (C Sa) and 4 (C UNF). There was a strong association between staphylococcal liver abscesses and infection with S. mansoni, in the chronic phase of schistosomiasis ($P = 0.006$). Nonetheless, there were no appreciable differences between acute and chronic schistosomiasis with regard to the development of liver abscesses in mice infected with S. mansoni and S. aureus ($P = 0.90$).
schistosomiasis). Innumerable granulomas and multiple small abscesses were noted in the liver of 6 mice in group 1 (Sm + T + Sa) and 4 mice of group 2 (Sm + Sa). Additionally, colonies of S. aureus were found attached to degenerated worms in the livers of mice previously treated with oxamniquine (Fig 1). Statistical analysis showed that the treatment of acute schistosomiasis carried out up to 30 days before experimentally-induced bacteremia did not prevent the formation of liver abscesses ($P = 0.69$) (Table 3).

**DISCUSSION**

The results of this experimental study suggest a strong association between staphylococcal liver abscesses and acute and chronic schistosomiasis. We demonstrated that granulomas of S. mansoni and dead worms lodged in the liver provide a focus and substrate for the development of liver abscesses caused by S. aureus. Data presented here also suggest that the necrotic-exudative granulomas formed around the eggs in the liver are a probable basis for bacterial colonization.

**Table 3**

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<tr>
<th>Group</th>
<th>Liver abscesses (%)</th>
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<tr>
<td></td>
<td>Yes</td>
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<tr>
<td>1. Infected with <em>Schistosoma mansoni</em> and treated 30 days before the infection with <em>Staphylococcus aureus</em> (n = 12)</td>
<td>6 (50)</td>
</tr>
<tr>
<td>2. Infected with <em>S. mansoni</em>, not treated, and infected with <em>S. aureus</em> (n = 10)</td>
<td>4 (40)</td>
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Fisher exact test $P$ value = 0.691.

There were marked differences in the macroscopic appearance of the liver between mice infected with non-irradiated and those infected with irradiated cercariae in both phases of schistosomiasis. Livers of mice infected with irradiated cercariae were almost normal, whereas the livers of mice infected with non-irradiated cercariae were pale and covered with granulomas and abscesses. The histology confirmed the absence of granulomas and the rarity of even small abscesses in the livers of mice infected with irradiated cercariae. Likewise, the multiple abscesses in the livers of mice infected with non-irradiated cercariae and bacteria suggest that the granulomas formed around the eggs of *S. mansoni*, or associated products, promote bacterial adhesion and the development of liver abscesses. It may be that the staphylococci are translocated to the site of inflammation in phagocytes (e.g., the lodging of bacteria at the site of microfractures in vertebral osteomyelitis in older patients). The small abscesses found in the liver of mice infected with irradiated cercariae were probably formed around the scarce number of eggs laid by a few worms that were resistant to irradiation. In fact, the irradiation is expected to provide around 4% of non-sterile worms, as previously demonstrated.14,16

Although investigating the molecular basis of the adherence of *S. aureus* to compounds of the granulomas of *S. mansoni* and the immune response to schistosomiasis infec-
tion were not the aim of this study, our data provide convincing evidence that the adhesion of *S. aureus* to perivascular material within granulomas is implicated in the pathogenesis of this association since pyogenic abscess is rare in the absence of granulomas.

It has been demonstrated that Gram-positive, but not Gram-negative organisms avidly bind to matrix-protein-coated surfaces, in particular, in the presence of fibronectin, laminin, and type IV collagen. As for collagen-associated proteoglycans, fibronectin is abundant during the more active stages of the granuloma. This suggest that compounds of the extracellular matrix of the granulomas of *S. mansoni*, e.g., collagen, fibronectin and proteoglycans, could be involved in adherence of *S. aureus*. In addition, some compounds of the chronic granulomas formed by the balance between the formation and degradation of extracellular matrix and laminin might also be implicated in the pathogenesis of the abscesses.

Besides a local adhesion mechanism, the immune response in schistosomiasis may play a role in pathogenesis. It is possible that the inhibition of Th1 response noted after the deposition of eggs affect the normal response against bacterial infection. Moreover, the eosinophilia and the elevation in IgE that occur in acute schistosomiasis might be implicated in the pathogenesis of the liver abscesses, as previously demonstrated with other worms (e.g., *Ascaris lumbricoides*). The various mechanisms of the pathogenesis of this association suggested here might explain the variable number and size of the abscesses found in the experiments.

In summary, the findings described here provide further support for the concept that the eggs, granulomas and degenerated worms of *S. mansoni* lodged in the liver parenchyma are the focus for bacterial colonization and development of pyogenic liver abscesses. More intensive clinical investigation in endemic areas of schistosomiasis and studies of the molecular basis of these diseases may lead to further understanding of this association.

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