

Role of Macrophages in Host Defense Against Hepatic Amoebiasis in Hamsters

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The role of macrophages in hepatic amoebiasis in hamsters has been investigated by means of antimacrophage serum prepared in rabbits. Animals treated with normal rabbit serum or antimacrophage serum, as well as untreated controls, were inoculated intrahepatically with 10^5 axenic trophozoites of *Entamoeba histolytica*. In hamsters treated with antimacrophage serum before intrahepatic inoculation of amoebae, the mean weight of the metastatic foci was significantly greater than in normal rabbit serum-treated or untreated controls. Treatment of hamsters with antimacrophage serum both before and after administration of amoebae not only increased significantly the size of the abscess in the liver but also allowed dissemination of metastatic foci to other organs.

Although monocytes/macrophages have been shown to play an important role in host resistance to various protozoan infections (4, 5, 12), their involvement in defense against *Entamoeba histolytica* infection is not well known. Stemberger (14) and Gold et al. (11) reported that cellular immunity plays a role in human and experimental amoebic infections, but the effector cells involved in this reaction have not been studied.

Preliminary experiments in our laboratory have indicated that macrophages play a role in host resistance against amoebic infections (10). Therefore, the following experiments were performed to confirm and extend our preliminary observations on the effect of macrophage depletion on the host defense against hepatic amoebiasis in hamsters.

MATERIALS AND METHODS

Parasites. The IP-106-L2 substrain of *E. histolytica* used in these experiments was obtained by passaging the IP-106 strain (8) twice through hamster livers followed by reisolation in axenic culture in TPS-1 medium (7).

Animals. Inbred female Syrian golden hamsters, strain LHC/LAK, weighing 65 to 70 grams, were used in all experiments.

Preparation of antimacrophage serum (AMS). Antiserum to hamster macrophages was prepared by the method of Stinnett et al. (15), with some modifications. Peritoneal exudate cells were obtained from five hamsters 7 days after intraperitoneal injection with glycogen. The cells were washed three times with cold Hanks balanced salt solution at $1,500 \times g$ for 10 min and then suspended in medium 199 without serum. The cells were counted in a hemacytometer and diluted in medium 199 to a concentration of 35×10^7 cells

per 15 ml. This suspension was distributed into five plastic petri dishes (60 by 15 mm) and incubated at 37°C under 5% CO_2 for 1 h. The dishes were washed with medium 199 to remove nonadherent cells. Adherent cells were removed with the aid of a rubber policeman and washed three times with the medium. The concentration of these cells was adjusted to 2×10^7 cells per ml. A rabbit was injected intravenously with 2 ml of the cell suspension. Two weeks later the rabbit was injected in a similar manner with the same number of cells. The rabbit was exsanguinated by cardiac puncture 10 days after the second injection. AMS and normal rabbit serum (NRS) were heat inactivated at 56°C for 30 min. The antiserum was absorbed three times with thymocytes by incubating 2×10^7 cells per ml of serum for 30 min per absorption. Sera were sterilized by filtration through 0.22- μm Millipore membranes and stored at -20°C until used.

Cytotoxicity. The specificity of the rabbit AMS and control NRS was examined by means of complement-dependent cytotoxicity on peritoneal exudate cells in a trypan blue exclusion test. After 1 h of incubation of AMS with peritoneal exudate cells, 65% of the cells were killed. It was found that the viable cells remaining after AMS treatment resembled lymphoid cells. Therefore, it was concluded that this antiserum was specifically cytotoxic to macrophage cells. NRS had no specific effect on macrophages.

Intrahepatic inoculation of amoebae. The hamsters were anaesthetized with Nembutal and laparotomies were done. A dose of 10^5 *E. histolytica* trophozoites was injected directly into the edge of the liver with a tuberculin syringe and a 26-gauge, 0.95-cm needle. After inoculation, the peritoneum was closed with surgical sutures and the skin was closed with stainless-steel surgical clips. The operation took about 5 min per hamster.

Evaluation of gross pathology. All of the hamsters were killed 10 days after amoebic inoculation. A macroscopic examination was made of the liver and

other organs which could have been affected by the amoebae. Direct smears were made from the infected tissues to demonstrate microscopically the presence of amoebae. The presence of amoebic abscesses in the liver and of metastatic foci in other sites was noted. The organs affected were the diaphragm, peritoneum, kidneys, and spleen.

The abscesses in the primary and secondary sites were carefully dissected out from the surrounding healthy tissue and weighed on a Mettler PL1200 balance. The weights were used to quantify the degree of the infection.

Statistical analysis. The significance of differences in the mean weights of amoebic liver abscesses and metastases in animals receiving different treatments was determined by Student's *t* test, and the level of significance was selected to $P < 0.05$.

RESULTS

AMS treatment before administration of amoebae. In this experiment, three groups, each consisting of eight hamsters, were used. All of the animals were treated intraperitoneally with either NRS or AMS. Group 1 was injected with 0.2 ml of AMS on days -3, -2, and -1 before intrahepatic inoculation of amoebae. Animals in group 2 received the same treatment with NRS, and group 3 received no special treatment. All of the animals were killed 10 days after amoebic injection.

The results of this experiment (Table 1) show that AMS treatment affected the susceptibility of hamsters to amoebic infection. In AMS-treated animals, the mean weight of the metastatic foci was significantly greater ($P < 0.01$) than in NRS-treated or untreated controls. However, there was no significant difference between the groups in the size of primary liver abscesses.

AMS treatment before and after administration of amoebae. Since AMS treatment on 3 consecutive days before inoculation of amoebae did not change the development of abscesses in the liver, AMS treatment was given 3 days after administration of amoebae. For this purpose, three groups, each consisting of eight hamsters, were used. Group 1 was treated with 0.2 ml of AMS on days -3, -2, -1, 1, 2, and 3 relative to

TABLE 1. Effect of AMS treatment before *E. histolytica* injection in hamsters

Group ^a	Treatment	Mean abscess wt (g) ± SE	Mean metastatic focus wt (g) ± SE
1	NRS	3.8 ± 0.3	1.1 ± 0.1
2	AMS	4.1 ± 0.4 ^b	2.5 ± 0.4 ^c
3	Untreated	3.6 ± 0.2	0.9 ± 0.12

^a Each group consisted of eight animals. The length of infection was 10 days for all groups.

^b Not significant.

^c $P < 0.01$.

TABLE 2. Effect of AMS treatment before and after *E. histolytica* injection in hamsters

Group ^a	Treatment	Mean abscess wt (g) ± SE	Mean metastatic focus wt (g) ± SE
1	NRS	3.6 ± 0.2	0.8 ± 0.1
2	AMS	5.2 ± 0.4 ^b	2.2 ± 0.3 ^c
3	Untreated	3.9 ± 0.3	1 ± 0.15

^a See Table 1, footnote a.

^b $P < 0.02$.

^c $P < 0.001$.

the inoculation of amoebae on day 0. Animals in group 2 received NRS on the same treatment schedule, and those in group 3 served as controls. The animals in all groups were injected intrahepatically with amoebae and killed 10 days later.

Table 2 shows the results of the experiment. AMS-treatment before and after administration of amoebae not only enhanced ($P < 0.001$) the mean weight of metastatic foci but also induced a significant increase in the mean weight of primary amoebic liver abscesses ($P < 0.02$) when compared with NRS-treated or normal hamsters.

DISCUSSION

The experiments described in this study were done to investigate the role of macrophages in hepatic amoebiasis, by depletion of these cells from the host. The data presented in this study show that macrophages play a role in host defense against *E. histolytica* infection (Tables 1 and 2). The enhancement of the size of abscesses in the liver and of metastasis in the animals treated with AMS can tentatively be linked to depression of macrophage activity since in vitro evidence indicated that the AMS used was specifically cytotoxic for these cells. Although AMS treatment for 3 days before amoebic injection failed to enhance the growth of abscesses in the liver, there was a significant difference between AMS-treated and control groups in the size of metastatic foci in other sites (Table 1). On the other hand, treatment of hamsters with AMS shortly before and after administration of amoebae induced a significant increase in both the mean weight of abscesses in the liver and also metastatic foci in other organs (Table 2). The results of the first experiment suggested that AMS treatment for a short period before injection of amoebae was not sufficient to be effective, since there was no difference in the size of primary abscesses in the liver unless the AMS treatment was extended for 3 days after administration of amoebae.

In previous experiments (10a) it was clearly

demonstrated that passive peritoneal exudate cell transfer had a significant effect on abscess growth and metastatic spread of amoebae. Furthermore, our previous observation (9) on amoebicidal activity of macrophages from immune animals provides evidence for the role of macrophages in the control of amoebic infections. In another study (10), it was found that silica-treated animals receiving an intrahepatic inoculation of amoebae developed abscesses in the liver and metastatic foci significantly greater in mean weight as compared with control animals. The severity of infection after silica treatment may be ascribed to the depletion of macrophages, since silica particles are known to be cytotoxic to macrophages (1-3, 6, 13). The cytotoxic effect of silica on macrophages in other protozoan infections has been shown, as Kierszenbaum et al. (12) reported that injection of silica into mice decreased their resistance to infection with *Trypanosoma cruzi*.

These results suggest that at least one of the effector cells responsible for the control of hepatic amoebiasis in experimental animals is the macrophage. However, the mechanism of macrophage involvement in this reaction is not fully understood.

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