

## Effect of Splenectomy on the Size of Amoebic Liver Abscesses and Metastatic Foci in Hamsters

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The role of the spleen in hepatic amoebiasis in hamsters was studied. In hamsters receiving an intrahepatic inoculation of  $10^5$  trophozoites of axenic *Entamoeba histolytica* at 7 or 14 days postsplenectomy, the mean weight of metastatic foci increased significantly when compared with sham-splenectomized or intact controls. In contrast, when both splenectomy and intrahepatic inoculation with amoebae were carried out at the same time, there was not only a significant increase in the mean weight of metastatic foci but also in the liver abscess. It is suggested that the spleen is important for host defense against *E. histolytica* infection, especially in the reduction in the degree of metastatic spread from the primary site.

The role of the spleen in protozoan infections has been studied by a number of workers. Taliaferro (13) in a comprehensive review on the function of the spleen in relation to blood protozoan infections indicated that this organ is essential in their control. The function of the spleen is generally ascribed to the phagocytic activity of spleen cells and to the development of humoral (13) or cellular immunity (10, 12). Haleem and Minton (7) reported that parasitemia due to *Trypanosoma lewisi* increased significantly in splenectomized rats as compared with the control groups (14), and Hof et al. (8) demonstrated that splenectomy significantly affected the course of *Plasmodium berghei* and *Toxoplasma gondii* infections in rats and mice, respectively, and produced very high mortality. Hussein (9) reported that splenectomy increased parasitemia and mortality in mice infected with *Babesia microti* and *B. hylomsci*. Wyler et al. (15) indicated that the spleen exerted a protective role during the acute stage and a suppressive function in the chronic phase of quartan malaria due to *Plasmodium inui*.

Although it has been shown that splenomegaly results from hepatic amoebiasis in hamsters (6a), the effect of splenectomy on the course of experimental amoebiasis has not been investigated.

The present study examines the effect of splenectomy on the establishment of amoebic liver abscesses and on their metastatic dissemination to other organs in hamsters.

### MATERIALS AND METHODS

The IP-106-L2 substrain of *Entamoeba histolytica* used in these experiments was obtained by passaging

the IP-106 strain (5) twice through hamster liver, followed by reisolation in axenic culture in the TPS-1 medium (1).

Six-week-old female inbred Syrian hamsters, strain LHC/LAK, weighing 60 to 65 g, were used in this study.

Hamsters were anesthetized by intraperitoneal injection of Nembutal (Abbott Laboratories). An incision was made below the last rib in the flank. The spleen was exposed on a gauze pad, its major blood vessels were tied with 5-0 surgical silk (Ethicon Sutures Ltd.), and the spleen was removed. The skin was closed with stainless steel autoclips (Clay Adams). The operated animals remained under observation in the operating room for 1 h before they were transferred to individual plastic cages and given mouse chow and water ad libitum. In sham splenectomy, the spleen was exposed and then replaced to the peritoneal cavity. The skin was closed as already described. Amoebae, in a dose of  $10^5$  axenic trophozoites in 0.1 ml of medium, were injected directly into the edge of the liver with a tuberculin syringe and a gauge 26, 3/8-in (ca. 9.45-mm) needle.

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 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 of the surrounding healthy tissue and weighed on a Mettler PL1200 read-out top-loading balance. The weights were used to quantify the degree of the infection.

The differences in the mean weights of liver abscesses and metastatic foci in the experimental and control groups were analyzed by Student's *t* test and were considered significant for probability (*P*) values of  $<0.05$ .

## RESULTS

In a preliminary experiment, three groups of eight hamsters each were used. Animals in group 1 were splenectomized, those in group 2 were sham splenectomized, and those in group 3 were intact controls. All of the animals were inoculated intrahepatically with amoebae and killed 10 days later. The data shown in Table 1 demonstrate that amoebic liver abscesses and metastases in hamsters of group 1 (inoculated with amoebae immediately after splenectomy) were significantly larger than those in the two control groups. Sham operation did not have any effect on the size of the abscesses or metastases as compared with intact controls.

To confirm these preliminary results and to determine whether the length of the time interval between splenectomy and inoculation with amoebae had any effect on the severity of the infection, the following three experiments, each using 24 hamsters, were performed. In each experiment there were three groups of eight hamsters each. Group 1 in each case consisted of splenectomized animals, group 2 consisted of sham-splenectomized animals, and group 3 consisted of intact controls. In experiment 1, splenectomy and sham splenectomy were performed 14 days before amoebic inoculation; in experiment 2, the surgery was performed 7 days before amoebic inoculation; and in experiment 3, the surgery and inoculation were done at the same time.

Table 2 shows the results of experiment 2. Splenectomy performed 14 days before inoculation caused only slightly bigger liver abscesses than those in the two control groups, but significantly larger metastatic foci. The results of experiments 3 (Table 3) shows that splenectomy performed 7 days before amoebic inoculation had an effect similar to that of splenectomy performed 14 days before inoculation, whereas splenectomy performed on the same day as inoculation (experiment 4, Table 4) resulted in significantly bigger liver abscesses and metastatic foci, thus confirming the results of the preliminary experiment.

TABLE 1. Preliminary data on the effect of splenectomy on liver abscess weight and metastasis of *E. histolytica* in hamsters inoculated on day of surgery

Group <sup>a</sup>	Wt of liver abscess (g) <sup>b</sup>	Wt of metastatic foci (g) <sup>b</sup>
Splenectomized	5.12 ± 0.22	1.92 ± 0.21
Sham operated	2.22 ± 0.23	0.62 ± 0.08
Control	1.97 ± 0.25	0.51 ± 0.11

<sup>a</sup> Eight hamsters per group.

<sup>b</sup> Mean ± standard deviation; *P* < 0.001.

TABLE 2. Effect of splenectomy on liver abscess weight and metastasis of *E. histolytica* in hamsters inoculated 14 days after surgery

Group <sup>a</sup>	Wt of liver abscess (g) <sup>b</sup>	Wt of metastatic foci (g) <sup>c</sup>
Splenectomized	2.8 ± 0.35	1.27 ± 0.15
Sham operated	2.4 ± 0.19	0.52 ± 0.1
Control	2.27 ± 0.17	0.4 ± 0.07

<sup>a</sup> Eight hamsters per group.

<sup>b</sup> Mean ± standard deviation; not significant.

<sup>c</sup> Mean ± standard deviation; *P* < 0.01.

TABLE 3. Effect of splenectomy on liver abscess weight and metastasis of *E. histolytica* in hamsters inoculated 7 days after surgery

Group <sup>a</sup>	Wt of liver abscess (g) <sup>b</sup>	Wt of metastatic foci (g) <sup>c</sup>
Splenectomized	2.4 ± 0.23	1.9 ± 0.21
Sham operated	2.2 ± 0.19	0.62 ± 0.08
Control	1.97 ± 0.21	0.5 ± 0.1

<sup>a</sup> Eight hamsters per group.

<sup>b</sup> Mean ± standard deviation; not significant.

<sup>c</sup> Mean ± standard deviation; *P* < 0.001.

TABLE 4. Effect of splenectomy on liver abscess weight and metastasis of *E. histolytica* in hamsters inoculated on day of surgery

Group <sup>a</sup>	Wt of liver abscess (g) <sup>b</sup>	Wt of metastatic foci (g) <sup>b</sup>
Splenectomized	4.8 ± 0.3	1.6 ± 0.2
Sham operated	1.95 ± 0.23	0.47 ± 0.07
Control	1.68 ± 0.2	0.36 ± 0.07

<sup>a</sup> Eight hamsters per group.

<sup>b</sup> Mean ± standard deviation; *P* < 0.001.

## DISCUSSION

It is generally known that the spleen plays an important role in elaborating cellular or humoral immunity against various infections. Pitney (11), in a review of the literature on the role of the spleen in malaria, suggested that its removal may interfere with both the cellular and humoral defense mechanisms which the host requires for protection against this infection.

The results of the present experiments (Tables 2 and 3) indicate that in hamsters inoculated with amoebae 14 or 7 days after splenectomy, there was a significant increase in the size of metastatic foci as compared with control animals, but not in the size of the liver abscesses. In contrast, when splenectomy and intrahepatic inoculation with amoebae were done at the same time, not only was there a significant increase in the dissemination of amoebae from the initial site of injection, but also a great increase in the size of the liver abscess (Tables 1 and 4). Although no specific explanation can be offered for

the enhancement of liver abscesses in these groups, it is evident that splenectomy and inoculation of amoebae at the same time made the animals much more susceptible to the infection. If this interpretation is correct, it is likely that the splenectomized animals of the other groups required 7 to 14 days after the operation to become immunologically compensated for the loss of spleen.

In previous reports (5, 6) it was shown that intrahepatic inoculation of hamsters with amoebae not only produced abscesses but also metastatic foci in other sites, such as the spleen, kidneys, diaphragm, lungs, and mesentery. It appears that this parasite, under certain conditions, may undergo tumor cell-like metastatic dissemination. The results of the present experiments indicate that in hepatic amoebiasis the spleen may specifically control metastatic dissemination of the amoebae from the primary focus to other sites. Faraci et al. (2), in their study on the effect of splenectomy on metastatic spread of murine reticulum cell sarcoma, have shown that splenectomized animals had the highest number of visceral metastases. Gershon and Carter (3) and Gershon and Kondo (4) demonstrated that splenectomy induced decreased immunity to transplantable lymphoma and increased the number of metastatic deposits in hamsters.

The present results show that splenectomy increases both the size of amoebic abscesses in the liver and dissemination of amoebae to other sites. Thus, the intact spleen appears to be important in the control of the *E. histolytica* infection, but the exact nature of the mechanism and of the effector cells involved is not clear at present.

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