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Effect of Silica on Resistance of Mice to *Entamoeba histolytica* Infection

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The role of macrophages in intestinal amoebiasis in mice has been investigated. The effect of injuring host macrophages on the course of infection was examined by using strains selected as being either genetically susceptible (C3H/HeJ, C57BL/6) or genetically resistant (A/J) to amoebiasis. Mice were treated with an intravenous injection of silica particles 1 day before infection with 2.5×10^5 or 5×10^5 polyxenic trophozoites of *Entamoeba histolytica*. The animals were killed at various times after infection, and the parasite burden in the cecum was measured. Silica treatment dramatically increased the growth of parasites in the susceptible mice. The same trend was evident, although less marked, in the resistant mice. The effect of silica treatment in experimental amoebiasis was much more pronounced in animals inoculated with 5×10^5 amoebae than in those with 2.5×10^5 amoebae. These data suggest that macrophages play an important role in host defense against amoebiasis in mice.

A murine model of infection with the human parasite *Entamoeba histolytica* has recently been established in this laboratory (E. Ghadirian and P. A. L. Kongshavn, Parasite Immunol. [Oxford], in press). The mechanisms which play a significant role in the defense of the host against this parasite are not well understood. However, it is evident that such mechanisms can be influenced by the genetic background of the host. When surveyed for susceptibility to intestinal amoebic infection, inbred mouse strains fell into two distinct groups: mice classed as susceptible had intestinal parasite burdens 5 days postinfection which were 1,000 times higher than those of mice from strains classed as relatively resistant. These findings imply that the host engenders resistance to the amoebic infection in some fashion as yet unidentified. It is an early response and, therefore, most likely can be ascribed to natural resistance rather than to an acquired, immunologically specific response. In a number of other infections, genetically controlled natural resistance mechanisms have been clearly linked to the activity of macrophages (4, 6, 8, 16). Furthermore, our previous observations have demonstrated the participation of these cells in controlling amoebic liver abscesses in hamsters (7), suggesting that macrophages can provide protection against *E. histolytica*. The administration of silica particles to experimental animals is known to selectively impair the mononuclear phagocyte while leaving the function of other cells intact (2). This treatment has, moreover, been shown to decrease resistance to a variety of infections in which the macrophage is a key cell responsible for host defense (12, 13). In the present study, the role of the macrophage in providing resistance to murine intestinal amoebiasis has been examined by employing silica treatment as a probe for this investigation.

(A preliminary report of part of this work has already been given [E. Ghadirian and P. A. L. Kongshavn, Abstr. Can. Fed. Biol. Soc. 1983, 26, p. 196]).

MATERIALS AND METHODS

Mice. C3H/HeJ, C57BL/6, and A/J mouse strains were purchased from Jackson Laboratory, Bar Harbor, Maine, and were used at 6 weeks of age. All mice used in this study were male.

Parasites. The IP:1182:2 strain of *E. histolytica* used in this study was isolated originally by E. Meerovitch, Institute of Parasitology, McGill University, Quebec, Canada, from the sigmoidoscopic material (blood and mucus) of a patient.

Cultivation and preparation of amoebae for in vivo study. The amoebae were grown at 37°C in Robinson medium (14) in 10-ml screw-capped glass bottles. The technique of amoebic culture was the same as that described by Robinson, except the sheep serum was replaced by heat-inactivated calf serum. The cultures were polyxenic, containing bacterial flora derived from the sigmoidoscopic material obtained from the patient. Two-day-old amoebic cultures were pooled and centrifuged for 10 min at $600 \times g$. The sedimented amoebae were collected, counted with the aid of a hemacytometer, and suspended in Robinson medium to obtain the desired concentration of trophozoites.

Intracecal inoculation of amoebae. In the absence of a suitable experimental procedure in which an amoebic infection can be produced by oral administration of infective cysts, the best available technique to produce intestinal or extraintestinal amoebiasis in experimental animals is by using *E. histolytica* trophozoites. Before inoculation of *E. histolytica* trophozoites, repeated stool examinations were performed to ensure that the mice were free of *Entamoeba muris*. After laparotomy, each cecum was exteriorized and placed on a sterile gauze pad. Amoebic inocula in 0.3 ml of medium were injected into the ceca through a 26-gauge needle, giving 2.5×10^5 trophozoites per mouse unless stated otherwise. The small size of the needle and of the inoculum prevented significant tissue damage and hemorrhage. The incision was closed only when we were certain that no leakage of amoebae had taken place.

Characterization of *E. histolytica* infection. The cecal contents were flushed into a tube containing 5 ml of normal saline and enumerated microscopically with a hemacytometer. This technique resulted in significantly higher recovery of amoebae than that reported previously (Ghadirian and Kongshavn, in press). It was confirmed in two ways that the amoebae were *E. histolytica* and not *E. muris*. First, cecal

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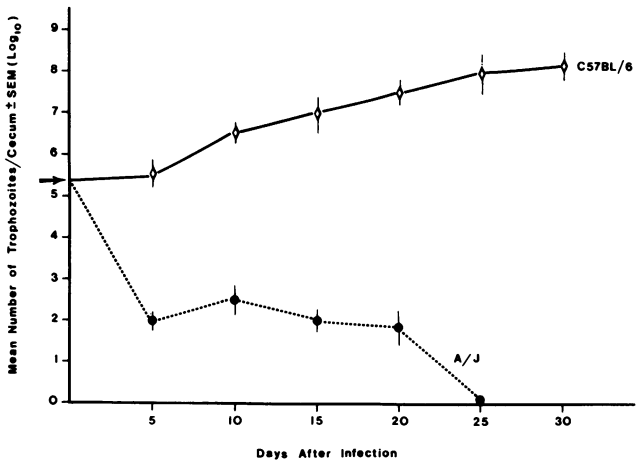

FIG. 1. Course of infection in C57BL/6 and A/J mice. Experiments were performed with a mean of 4 to 5 mice per group ± standard error of the mean.

contents of infected mice were cultured successfully in Robinson medium, which is recognized as being specific for *E. histolytica* (14, 15). Second, fixed stain smears were prepared to differentiate *E. histolytica* from *E. muris* (15).

Treatment of mice with silica. Silica (no. 216 Min-U-Sil), purchased from Whittaker, Clark and Daniels, Inc., Plainfield, N.Y., was prepared for inoculation by the method of Allison et al. (3). It was suspended in saline and sonicated for 4 min immediately before administration. Each mouse received an intravenous injection of 3 mg of silica particles of < 5 μm, 1 day before infection with amoebae. Control mice received saline.

Test for efficacy of silica treatment. To test the efficacy of the silica treatment in impairing macrophage function, silica-treated and control groups of mice were tested for their resistance to the bacterium *Listeria monocytogenes*. The macrophage is the effector cell which protects the host against infection with listeria (11). Therefore, lowered resistance to this infection would provide experimental evidence that the silica treatment is, in fact, effective against the macrophage (13). Accordingly, two groups of 5 to 6 A/J strain mice were treated with either silica or saline and, 24 h later, were inoculated intravenously with 10^4 CFU of *L. monocytogenes*. The bacterial burden was measured in the liver 3 days later by the method described previously (5). Briefly, 10-fold dilutions of liver homogenates in saline were plated onto tryptose agar, and the colony counts were performed 18 h later. Student's *t* test was used to analyze the data. A probability level of $P < 0.05$ was considered significant.

RESULTS

(i) Test for efficacy of silica treatment. Bacterial colony counts of *L. monocytogenes* in the liver of A/J mice 3 days postinfection indicated that the bacteria reached a level 2 to 3 logs higher in the silica-treated mice ($\log_{10}$ CFU per liver, 9.2 ± 0.6 standard error of the mean) than in the control mice ($\log_{10}$ CFU per liver, 6.7 ± 0.4 standard error of the mean), showing that this treatment greatly impairs macrophage function.

(ii) Course of *E. histolytica* infection in C57BL/6 and A/J mice. In the first set of experiments, the course of infection was followed in two different mouse strains, the genetically susceptible C57BL/6 strain and the genetically resistant A/J strain. Accordingly, 35 C57BL/6 and 30 A/J mice were inoculated intracecally with *E. histolytica*, and the parasite burden in the cecum was measured at various times thereafter with groups of four to five mice per time point. The results show that the number of amoebae in the ceca of C57BL/6 mice increased with time and persisted at a high level until the experiment was terminated at day 30 (Fig. 1). In the A/J mice, the parasite burden was 1,000-fold lower, and over 50% of the mice were cleared of *E. histolytica* 25 days after inoculation. Diarrhea was often observed but only in mice from the susceptible strain. No amoebic cysts were found in any of the animals infected with *E. histolytica* trophozoites. Eight control mice of each strain, inoculated with a suspension of bacterial flora obtained from the amoebic culture, showed no signs of sickness or pathological damage in the cecum.

(iii) Effect of silica treatment on intestinal amoebiasis in mice. C57BL/6 and A/J mice (8 to 10 mice per group) treated with silica or saline were inoculated with *E. histolytica* and the parasite burden in the cecum was measured 5 days later. Figure 2 shows that silica treatment allowed a significant increase in the growth of parasites in the ceca of susceptible C57BL/6 mice when compared with that in the saline-treated control group ($P < 0.001$). The same trend was evident, although less marked, in the resistant A/J mice ($P < 0.05$). In silica-treated C57BL/6 mice, ulcers were scattered over the mucosal surface of the ceca. The lesions were shallow, irregular in shape, and packed with yellowish fluid and pus. The materials obtained from the cecal contents contained very active *E. histolytica* trophozoites. A control group of 5 silica-treated animals were given a suspension of bacterial flora obtained from the amoebic culture; they showed no signs of sickness and had normal intestinal pathology upon gross examination 5 days after treatment.

This study was repeated in the genetically susceptible C3H/HeJ mouse strain. The results confirm the previous observation on the effect of silica treatment on the susceptibility of these hosts 5 days after amoebic infection (Fig. 3). (Comparison of the saline-treated versus silica-treated groups: $P < 0.01$ for C3H/HeJ and $P < 0.05$ for A/J.)

(iv) Effect of increasing dose of *E. histolytica* trophozoites on

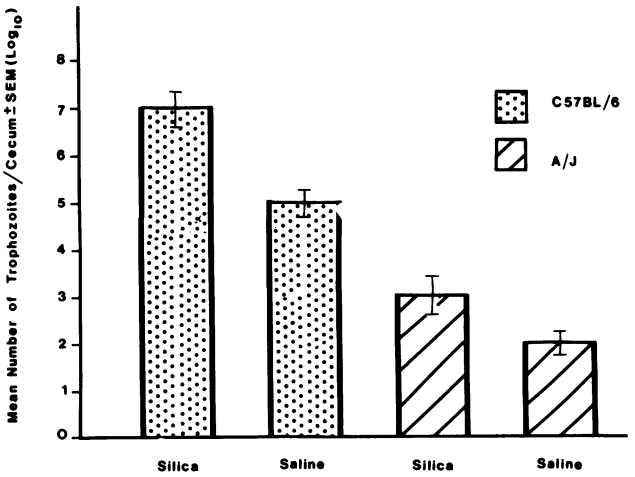

FIG. 2. Effect of silica treatment in A/J and C57BL/6 mice. Experiments were performed with a mean of 8 to 10 mice per group ± standard error of the mean (bars).

efficacy of silica treatment. Further studies were performed, in which the efficacy of silica treatment was examined in A/J and C3H/HeJ mice inoculated with double the number of parasites used previously. The silica-treated C3H/HeJ mice showed severe illness about 3 days after infection, and all were dead within 6 days (Table 1). Three of the eight animals treated with silica developed hepatic amoebiasis, thus showing metastatic dissemination of amoebae from the primary site of injection to the liver. On the other hand, no deaths occurred in the silica-treated A/J mice for at least 10 days, at which time all of the animals were sacrificed for autopsy. Although silica treatment in A/J mice failed to cause hepatic amoebiasis or death in any of the animals, this treatment enhanced the growth of the parasites in the ceca of these hosts as measured by the cecal parasite burden (data not shown).

This observation indicated that the effect of silica treatment in experimental amoebiasis was much more pronounced in animals inoculated with 5×10^5 amoebae than in those given 2.5×10^5 amoebae.

(v) Effect of silica treatment on long-term course of infection in A/J and C3H/HeJ mice. The following experiment was performed to examine the effect of silica treatment on the long-term course of infection. Accordingly, the numbers of amoebae in the ceca of silica-treated and control A/J and C3H/HeJ mice were determined 5, 10, 15, and 20 days after infection. The results demonstrate that the mean number of trophozoites in the ceca of silica-treated animals remained dramatically higher than the number in the ceca of control mice for the duration of the experiment (Fig. 4).

DISCUSSION

The model of murine intestinal amoebiasis developed in our laboratory has been investigated in relation to the role of macrophages in providing resistance to the infection. Silica has been employed as a tool for the study. This is an agent which is selectively toxic to macrophages but not to lymphocytes or polymorphonuclear cells (9). The direct toxic effect of silica on macrophages has been shown both in vitro (1, 12) and in vivo (10). The evidence suggests that this substance acts by inducing macrophage organelle membrane instability and reticuloendothelial system dysfunction (9, 10). Mice treated with a single dose of silica particles one day before

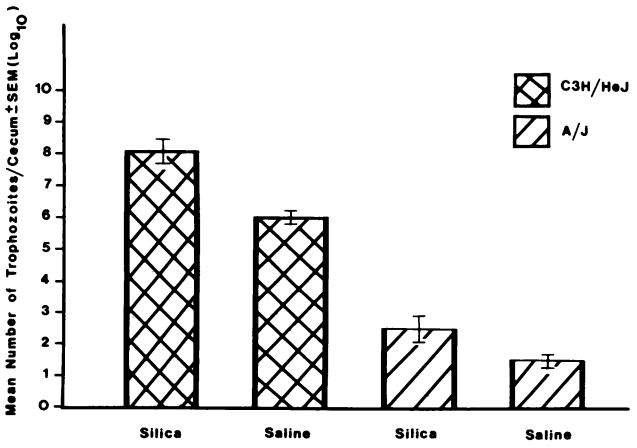

FIG. 3. Effect of silica treatment in A/J and C3H/HeJ mice. Experiments were performed with a mean of 4 to 5 mice per group ± standard error of the mean.

TABLE 1. Effect of silica treatment in C3H/HeJ and A/J mice after administration of 5×10^5 trophozoites of *E. histolytica*

Mouse strain	Pre-treatment	Observation record (days post-inoculation)	No. dead/ no. inoculated	Mortality (%)	No. positive for trophozoites/no. inoculated
C3H/HeJ	Silica	6	8/8[a]	100	8/8
C3H/HeJ	Saline	10	2/8	22	8/8
A/J	Silica	10	0/8		8/8
A/J	Saline	10	0/8		6/8

[a] Including three mice with liver abscesses.

inoculation of *E. histolytica* trophozoites had a marked increase in the cecal parasite burden during the ensuing infection, indicating that silica-induced macrophage toxicity allowed increased multiplication of the amoebae. This increased susceptibility of the silica-treated mice was seen early in the course of infection, by day 5, and persisted throughout the whole period of observation (20 days postinfection) (Fig. 4). These findings provide good evidence that macrophages are important in controlling the multiplication of the trophozoites and, therefore, that these cells participate in host defense against murine amoebiasis. Further support for this notion comes from our previous observations that macrophages play a similar role in host defense against hepatic amoebiasis in hamsters (7).

Since the detrimental effect of silica treatment is evident in the early stages of the infection, a reasonable assumption would be that this treatment interferes with natural resistance, which normally provides a certain degree of protection to the host before specific immunity develops. If so, it can be inferred that the macrophage is important in providing natural resistance to amoebiasis. Amoebiasis resembles a number of other infections in which the macrophage plays a key role in natural resistance (4, 6).

The host defense mechanisms controlling intestinal infection with *E. histolytica* in mice are under genetic regulation (Ghadirian and Kongshavn, in press). For this reason, the effect of silica treatment on the course of infection has been explored in both genetically resistant and genetically susceptible strains of mice to determine whether the effect might be different in each case. However, it was found that genetically susceptible C57BL/6 and C3H/HeJ mice became more susceptible after silica treatment as, also, did genetically resistant A/J mice (Fig. 2 through 4).

The cumulative effects of genetic susceptibility and silica treatment resulted in the development of very severe infection. Thus, in the C3H/HeJ mice followed for 20 days after infection, the parasite burden became extremely high, and the cecal lesions were observed to increase in severity from superficial necrosis to abscess formation as the disease progressed. Additionally, when the dose of trophozoites was increased, silica treatment proved fatal to these mice (Table 1), some of which were found to have developed liver abscesses. These data suggest that there is a natural barrier in mice which normally prevents dissemination of amoebae from the primary site into extraintestinal foci, such as the liver. In the present study, this barrier was broken down by administration of the macrophage toxin silica.

In the present study, bacteria-associated amoebae were used rather than the axenically cultivated trophozoites administered in our earlier work, the reason being that much smaller numbers of amoebae are required to produce infection in mice when amoebae are bacteria associated. Since inoculation of bacterial flora alone failed to produce any

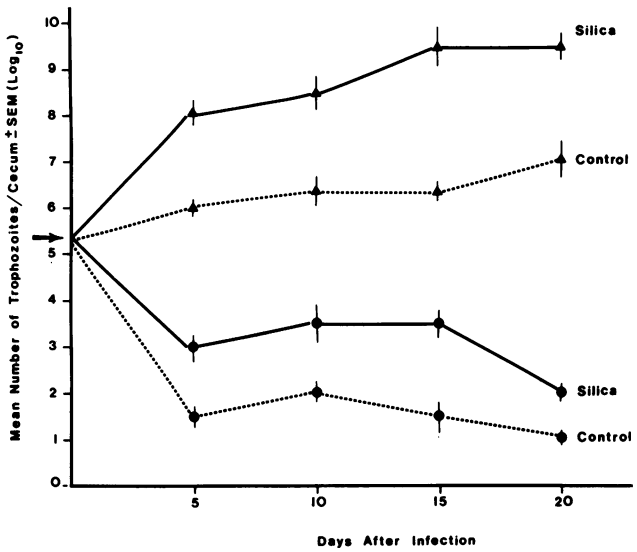

FIG. 4. Effect of silica treatment on long-term course of infection in A/J and C3H/HeJ mice. Experiments were performed with a mean of 5 mice per group ± standard error of the mean. Symbols: ▲, C3H/HeJ mice; ●, A/J mice.

signs of illness in mice, the use of bacterized amoebae was considered acceptable.

To summarize, we conclude that the cell population which apparently provides natural resistance to amoebiasis and is depleted by silica treatment is the macrophage. Histological studies to confirm this hypothesis are currently under way and will form the subject of a further communication.

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