Lipopolysaccharide Hyperreactivity of Animals Infected with 
Trypanosoma lewisi or Trypanosoma musculi

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Rats and mice infected with Trypanosoma lewisi and Trypanosoma musculi, respectively, showed hyperreactivity to lipopolysaccharide (LPS) from gram-negative bacteria. Fatal shock could be precipitated with a dose of LPS 100 to 1,000 times less in infected compared with noninfected animals. In trypanosome-infected rats and mice, extensive liver damage was evident after LPS challenge. These animals showed a pronounced hypoglycemia, marked elevation of blood aspartate transaminase level, and diffuse severe degeneration and total depletion of glycogen in hepatocytes. Only minor changes were observed in noninfected animals given the same dose of LPS. No mononuclear phagocytic cell infiltration was observed in the liver of infected animals. The most striking change was the great increase in size and the probable increase in hyperactivity and number of sinusoidal Kupffer cells. We suggest that elevated Kupffer cell activity in trypanosome-infected animals may play a role in LPS-induced hepatotoxicity.

Several agents such as Mycobacterium bovis (25), mouse hepatitis virus (13), Corynebacterium parvum (16), Zymosan (3), and Plasmodium species (4) have been shown to render mice hyperreactive to lipopolysaccharide (LPS) from gram-negative bacteria. The effects of LPS on these experimental animals include severe liver parenchymal cell lesions accompanied by hypoglycemia and elevated aspartate transaminase levels in the blood (7, 8, 23). These findings have suggested the possibility that everyday exposure to LPS of gut origin may contribute significantly to the illness accompanying a variety of diseases. Among the properties of agents rendering experimental animals highly sensitive to LPS is the capability to stimulate the mononuclear phagocytic system, and it has been proposed that LPS, by causing the release of a range of factors from activated macrophages, precipitates the toxic effects (1, 7, 8, 12). An increased activity of the mononuclear phagocytic system has been demonstrated during the course of trypanosome infections (11, 15, 20). In rats infected with Trypanosoma lewisi this was shown to be due to both an increase in activity of phagocytic cells and an increase in their numbers in livers and spleens (11). Accordingly, we examined whether trypanosome-infected animals become hyperreactive to LPS.

MATERIALS AND METHODS

Experimental animals. Female Porton strain rats and female C3H/He mice were used in these experiments.

Maintenance and collection of trypanosomes. The T. lewisi isolate was that used in previous studies and was maintained and collected from infected rat blood as described previously (10). Trypanosomes were harvested during the inhibitory (nondividing adult forms) phase of the infection cycle.

Trypanosoma musculi was a cloned isolate, reference no. LUMP 1189, kindly provided by G. A. T. Targett, Department of Medical Protozoology, London School of Hygiene and Tropical Medicine, London, England. The parasites were maintained in C3H/He mice and collected from infected blood as described for T. lewisi.

Parasitism. Since no significant reduction in erythrocyte numbers occurred during the course of a T. lewisi infection in Porton rats (A. Ferrante, unpublished data), the parasitemia was measured from thin blood smears made from blood samples taken from the rat tail vein at various time intervals. The smears were fixed in methanol and stained with May-Grünwald/Giemsa. The number of parasites per ca. 500 erythrocytes (500 to 520) was counted by using a microscope and an eyepiece grid. The values were then expressed as the number of trypanosomes per 10⁶ erythrocytes.

Since T. musculi is known to cause anemia in C3H/He mice (18), parasitemia was estimated by placing 25 µl of blood or blood dilution on a microscope slide, adding a cover slip, and counting trypanosomes in a high-power field (×400).

LPS. Escherichia coli serotype O55:B5 LPS W was obtained from Difco Laboratories, Detroit, Mich. (extracted by the phenol-water method). A stock solution of LPS was made in saline, and samples were stored at −70°C.

Biochemical determinations. Blood was collected into containers in 20 IU of heparin per ml. Blood cells and parasites were sedimented by centrifugation, and plasma was collected and filtered through 0.25-μm filters (Millipore Corp.) to ensure the complete removal of trypanosomes. Plasma glucose levels were measured with a glucose analyzer (YSI model 23 AM; Yellow Springs Instrument Co., Yellow Springs, Ohio). Aspartate transaminase levels in plasma were determined with GOT-S.V.R. reagents (Calbiochem-Behring, La Jolla, Calif.).

Preparation of tissues for histological studies. Livers removed from rats or mice were cut into slices (thickness, 3 mm) and fixed in 10% (vol/vol) buffered isotonic Formol-saline for 24 h. They were then embedded in paraffin, sectioned at 5-μm, and stained by hematoxylin and eosin and the periodic acid Schiff (PAS) reaction for glycogen.

Experimental design. Sixty-seven rats were each infected...
with 2 x 10^5 adult *T. lewisi* by the intraperitoneal (i.p.) route. From these, five rats were removed and kept as a group for monitoring the parasitemia. At various time intervals after infection, groups of rats were challenged i.p. with a range of LPS doses. In another aspect of this study, rats were divided into four groups of six. Those in groups 1 and 2 were injected with saline i.p., and the remaining rats (groups 3 and 4) were infected with 2 x 10^5 adult trypanosomes i.p. Five days later, rats in groups 2 and 4 were challenged with 500 µg of LPS i.p., whereas the remaining two groups (groups 1 and 3) were given saline. All rats were bled at ca. 3 h after LPS challenge, and plasma was prepared and examined for glucose and aspartate transaminase levels. Also, the livers of two rats taken at random from each group were removed, cut into slices, and fixed for histological studies.

Thirty-two mice were infected with 10^5 adult *T. musculi* i.p. Five mice were retained and used for estimating the parasitemia. From the remaining mice, two to three were removed at random on the days indicated and challenged intravenously with LPS. In a second set of experiments, a group of 10 mice were infected with *T. musculi*, and a second

**FIG. 1.** Effects of LPS on blood aspartate transaminase levels in rats infected with *T. lewisi*. Infected (■) or noninfected (□) animals were injected with either saline or 500 µg of LPS. Each bar represents means ± standard error of the mean of six rats. Significantly different: noninfected animals treated with LPS when compared with noninfected saline injected gave 0.01 < P < 0.02; infected animals injected with saline when compared with noninfected animals injected with saline gave 0.02 < P < 0.05; infected animals treated with LPS when compared with non-infected, saline-treated; infected, saline-treated; or noninfected, LPS-injected animals gave 0.001 < P < 0.01.

**FIG. 2.** Effects of LPS on blood glucose levels in rats infected with *T. lewisi*. Infected (■) or noninfected (□) animals were injected with either saline or 500 µg of LPS. Each bar represents mean ± standard error of the mean of six rats. Statistical difference: infected animals treated with LPS when compared with noninfected saline-injected; infected, saline-injected; or noninfected, LPS-treated animals gave P < 0.001.

**FIG. 3.** Liver from uninfected rat challenged with LPS 3 h before death. Centrilobular region shows normal morphology. Hematoxylin and eosin stain; ×280.
LPS-INDUCED LIVER DAMAGE IN TRYPANOSOMA INFECTIONS

FIG. 4. Liver from the same animal as that shown in Fig. 3. Periportal area shows normal morphology and normal quantities of darkly staining glycogen granules in hepatocytes. PAS reaction: ×224.

FIG. 5. Liver from infected rat not challenged with LPS. Kupffer cells in this field are very prominent and probably increased in number. Hematoxylin and eosin stain; ×280.

FIG. 6. Liver from the same animal as that shown in Fig. 5, showing moderate depletion of glycogen in the periportal area (compare with normal appearance in Fig. 4). PAS reaction: ×224.

FIG. 7. Liver from infected rat challenged with LPS 3 h before death. Periportal area shows swollen liver cells, complete depletion of glycogen (c.f. Fig. 4 and 6), prominent Kupffer cells, and small nonphagocytic mononuclear cells in sinusoids and portal area. PAS reaction: ×280.

A group of 10 animals was injected with saline. On day 7, each of these groups were divided into two subgroups of five animals and injected intravenously with LPS or saline. Mice were bled 3 h after challenge, and the plasma was examined for glucose and aspartate transaminase levels. The livers of two mice from each subgroup were prepared for histological examinations.

In these studies, the doses of LPS shown were based on a number of factors. These included the number of animals available, the expected size of the parasitemia, and the established time course of increases in activity of the reticuloendothelial system (11). In addition, for each challenge day, the injections for each group (and dose) were staggered. The first treated group was carefully scrutinized for the first hour after injection. If animals became severely ill, a further lower dose(s) was usually tried.

Statistical analysis. Differences between groups were analyzed with the two-tailed t test.

RESULTS

As early as 4 to 5 days after infection with *T. lewisi*, rats displayed increased sensitivity to LPS (Table 1). Although death was scored 8 h after challenge, some animals died as early as 2 h, and occasional one died after 8 h. At the beginning of the infection (day 0), rats had to be given ca. 5 mg of LPS to cause death. However, concomitant with a rise in parasitemia was an increased sensitivity of animals to
LPS. On day 6, as little as 50 μg of LPS produced shock and death in this group of animals. Rats demonstrated seizures, and diarrhea was found to accompany LPS-induced shock in these animals. By day 12, infected rats demonstrated reduced sensitivity to LPS, although they still harbored high numbers of parasites in their blood.

A small, but significant, elevation of aspartate transaminase was observed in the blood of trypanosome-infected rats (Fig. 1), but the blood glucose level remained relatively unchanged (Fig. 2). However, LPS injected into infected rats resulted in a greatly elevated level of aspartate transaminase (Fig. 1) and a marked reduction of glucose in the blood (Fig. 2). When the same dose of LPS was injected into noninfected rats, only a slight effect on the aspartate transaminase levels was observed (Fig. 1).

Similar marked differences were found by histological examination of the livers of the animals. In uninfected animals challenged with LPS, the livers were morphologically normal and contained abundant amounts of glycogen (Fig. 3 and 4). In the infected animals not challenged with LPS, focal hyperplasia of phagocytic (Kupffer cells) and occasional individual necrotic hepatocytes were seen (Fig. 5), and there was a slight depletion of glycogen in the outer two-thirds of the lobules (Fig. 6). In the infected animals challenged with LPS, all the hepatocytes were swollen and glassy (hydropic degeneration) and completely depleted of glycogen (Fig. 7), and occasional ones were frankly necrotic. Kupffer cells were markedly hyperplastic, many being seen

![FIG. 8. Liver from the same animal as that shown in Fig. 7, showing a Kupffer cell in mitosis near the center of the field. A piece of phagocytosed debris is present in its cytoplasm. PAS reaction after diastase digestion; ×560.](http://iai.asm.org/)

![FIG. 10. Liver from the same animal as that shown in Fig. 7 to 9. Phagocytic Kupffer cells, small nonphagocytic mononuclear cells, and a plasma cell are present within sinusoids. PAS reaction after diastase digestion; ×560.](http://iai.asm.org/)

![FIG. 9. Liver from the same animal as that shown in Fig. 7 and 8. Enlarged Kupffer cells containing phagocytosed debris are present, and their cytoplasm stains are diffusely positive with PAS after diastase digestion. PAS reaction after diastase digestion; ×560.](http://iai.asm.org/)
in mitosis (Fig. 7 and 8), and contained much phagocytosed cellular debris as well as much amorphous material which stained positively with the PAS technique after diastase digestion (Fig. 8 and 9) (a small amount of similar material also was present in the infected unchallenged animals). In addition to the Kupffer cells, sinusoids contained small numbers of small nonphagocytic mononuclear cells and plasma cells, and similar cells were present in small numbers in portal areas (Fig. 7 and 10).

Analogous to rats infected with *T. lewisi*, mice infected with *T. musculi* died after challenge with relatively low concentrations of LPS (Table 2). Mice subjected to *T. musculi* infections did not demonstrate significantly depressed glucose or increased aspartate transaminase levels (Fig. 11 and 12). Although noninfected mice challenged with LPS showed decreased glucose levels and increased aspartate transaminase levels (ca. 2-fold), similarly treated infected animals showed a 30-fold decrease in blood glucose levels and a ca. 7-fold increase in blood aspartate transaminase levels. Histological studies confirmed the marked liver damage as a result of LPS challenge after a *T. musculi* infection. Details are not presented as these were similar to those made with rats infected with *T. lewisi*.

**DISCUSSION**

Rats and mice infected with *T. lewisi* and *T. musculi*, respectively, became highly sensitive to LPS. Death occurred with much lower doses (1,000 times less) of LPS in infected compared with noninfected animals. In infected LPS-challenged animals, extensive liver damage was evident from the pronounced hypoglycemia and elevated blood aspartate transaminase levels. Furthermore, histological studies demonstrated diffuse severe degeneration and total depletion of glycogen in the hepatocytes of these animals.

The increased liver damage induced by LPS in animals subjected to a variety of agents could possibly be attributed to mononuclear phagocytes which infiltrate the liver (6-9). Some evidence supporting such a hypothesis is shown by the amelioration of liver damage by anti-inflammatory drugs (9). However, in *T. lewisi*-infected rats, a mononuclear phago-

![Figure 12](http://iai.asm.org/)  
**FIG. 12.** Effect of LPS on blood aspartate transaminase levels in mice infected with *T. musculi*. Infected (□) or noninfected (□) animals were injected with either saline or 10 μg of LPS. Each bar represents mean ± standard error of the mean of five mice. Significant difference: infected animals treated with LPS compared with noninfected, saline-injected; infected, saline-injected; or noninfected LPS-injected animals gave *P < 0.001*.

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![Figure 11](http://iai.asm.org/)  
**FIG. 11.** Effect of LPS on blood glucose levels in mice infected with *T. musculi*. Infected (□) or noninfected (□) animals were injected with either saline or 10 μg of LPS. Each bar represents mean ± standard error of the mean of five mice. Significantly different: noninfected animals treated with LPS when compared with noninfected, saline-injected animals gave *P < 0.001*; infected animals treated with LPS when compared with noninfected, saline-injected; infected, saline-injected; or noninfected LPS-injected animals gave *P < 0.001*.

cyte liver infiltration was not observed although mononuclear cells (nonphagocytic) were found lining liver sinusoids (11). In the present study, this observation has been confirmed and extended to mice infected with *T. musculi*. The nonphagocytic mononuclear cells (lymphocytes and plasma cells) were quite small, and the most striking change was the great increase in size of sinusoidal Kupffer cells. These cells also appeared to be increased in numbers, and several were in mitosis, but this was not quantitated. Thus it is most likely that in the LPS-induced liver damage of trypanosome-infected rats and mice, the Kupffer cells, which show elevated activity during infection (11), are responsible for the hepatocyte damage. LPS may mediate the damage by causing the release from macrophages (Kupffer cells) of a range of mediators-enzymes which may directly or indirectly cause hepatocyte damage. Natural killer cells, which may comprise a portion of the nonphagocytic mononuclear cell infiltrate, could contribute to the liver damage. The cytotoxicity of these cells could be effectively increased by levels of interferons normally arising during the course of trypanosome infections (2) or by elevated levels of interferons possibly associated with an increased release from macrophages in infected animals treated with small doses of LPS (5, 20, 21). LPS has also been shown to induce high levels of circulating gamma interferon in *Propionibacterium acnes*-infected mice, possibly by inducing the release of mediators from macrophages which provide help in the response of gamma interferon-producing cells to inducers other than LPS (19).

Death in trypanosome-infected animals challenged with low concentrations of LPS may be mainly a result of the marked hypoglycemia and accompanying depletion of glycogen stores, but a generalized endotoxin shock syndrome may contribute. Factors involved could be prostaglandins producing vasodilation, increased vascular permeability induced by mediators such as histamine and bradykinin, and
thromboplastin release by activated macrophages which could trigger intravascular coagulation.

Our preliminary studies have shown that infection with other trypanosome species also renders animals sensitive to relatively low doses of LPS; mice infected with *Trypanosoma congolense* (J. Ferluga, A. Ferrante, and A. C. Allison, unpublished data) or *Trypanosoma brucei brucei* (I. Ljungström and A. Ferrante, unpublished data) became hyperreactive to LPS. Thus, daily exposure to LPS of gut origin or exposure to higher levels as a result of superinfection with a gram-negative bacterium may in part be responsible for the illness observed during trypanosome infections. Furthermore, trypanosomes may contain LPS or LPS-like material, or both, which may play a role in the illness. LPS has been shown to be present in *Trypanosoma cruzi* (14). Glucocorticosteroids with their action as membrane stabilizers can limit the exudation or leakage of deleterious lysosomal substances (17), and hence it may be advantageous to combine glucocorticosteroids with treatment for trypanosomiasis, especially considering that trypanosomal drugs such as suramin and melarsoprol B have been shown to inhibit hepatic mixed-function oxidase activity (24). However, the use of glucocorticosteroids should be tempered with caution (22).

**LITERATURE CITED**