Protective Effect of Glucan Against Visceral Leishmaniasis in Hamsters

JAMES A. COOK,[1]* THOMAS W. HOLBROOK,[2] AND WILLIAM J. DOUGHERTY[3]

Departments of Physiology,[1] Laboratory Medicine,[2] and Anatomy,[3] Medical University of South Carolina, Charleston, South Carolina 29425

The effect of pre- or posttreatment with glucan, a reticuloendothelial stimulant, on the course of Leishmania donovani infection was assessed in highly susceptible hamsters. Intravenous administration of glucan before or after L. donovani infection significantly suppressed proliferation of amastigote-stage parasites in liver and spleen. Glucan-activated peritoneal macrophages in vitro also significantly reduced multiplication of the intracellular parasite. Ultrastructural studies revealed a well-defined hepatic granulomatous response to glucan, with hypertrophic Kupffer cells and reduced numbers of intracellular parasites compared to the control group. In additional studies, groups of hamsters were immunized by intravenous injections of glucan with Formalin-killed promastigote-stage L. donovani cells and challenged 60 days after the last immunizing injection. This treatment regimen significantly prolonged the mean survival time of those hamsters which died after infection, relative to untreated control groups. Hamsters stimulated with the glucan-killed promastigote preparation also exhibited significant reductions in splenic amastigotes on days 10 and 21 postinfection compared with all other control groups, but on day 35, splenic amastigotes did not differ significantly from those of control animals. Our composite observations provide evidence for glucan-enhanced nonspecific resistance of hamsters to visceral leishmaniasis.

Species of the genus Leishmania represent a diverse group of kinetoplastid protozoan parasites transmitted by phlebotamine sandflies (19, 31). Clinical manifestation of the disease in humans may be seen as a spectrum of disorders ranging from localized cutaneous lesions to fatal disseminated infection of the reticuloendothelial system (19, 31). Leishmania tropica and L. mexicana (and subspecies thereof) usually cause localized cutaneous lesions which spontaneously resolve and elicit sustained resistance to reinfection with the same species (19, 31). Cosmetic vaccination against L. tropica is practiced in some geographic areas by inoculation with virulent organisms (9). Such an approach, however, is not feasible with species which may disseminate and produce more severe disease. The most important species of the latter group, against which immunoprophylaxis is not presently possible, are L. braziliensis and L. donovani, the causative agents of mucocutaneous and visceral leishmaniasis (kala-azar), respectively (31).

Recent studies in our laboratories demonstrated that glucan, a β(1→3) glucopyranose derivative from the cell wall of yeast, has significant value as an immunopotentiator in enhancing both nonspecific and specific resistance of mice

to infection with L. donovani (3, 13). Glucan administered alone, either pre- or posttreatment, protected mice against infection with the protozoan, as measured by significantly reduced parasite burdens in organs (3). Importantly, glucan also had an adjuvant effect when inoculated simultaneously with Formalin-killed promastigote-stage parasites (13). Mice immunized with glucan and dead parasites exhibited significant resistance to a challenge infection delivered up to 80 days after stimulation. Injections of glucan alone provided a lesser degree of protection, but mice receiving dead parasites alone were not protected (13).

Mice, however, respond spontaneously against L. donovani infection, and immunoprophylaxis could, therefore, be easier to achieve in the murine host than in more susceptible animal models. Hamsters are relatively unique in that they exhibit a poor immunological response to L. donovani and usually succumb to the infection even after inoculation with low numbers of parasites (26). The hamster infection also resembles human kala-azar in that both may terminate fatally without chemotherapeutic intervention (19, 25, 31). In the present study we evaluated the effect of pre- or posttreatment with glucan on

the course of *L. donovani* infection in highly susceptible hamsters. Specifically, organ parasite counts (i.e., liver and spleen) were monitored during the course of the infection. The characteristics of the hepatic infection in glucan-treated and control hamsters were further assessed by light and ultrastructural examination. In parallel studies, the ability of glucan-activated peritoneal macrophages from hamsters to suppress intracellular proliferation of the parasite was evaluated. Additionally, in a separate study, groups of hamsters were immunized with glucan and Formalin-killed promastigote-stage *L. donovani* cells. After challenge with viable parasites, changes in organ parasite counts and mortality were assessed.

MATERIALS AND METHODS

Parasite and experimental host. *L. donovani* 2S (27) was kindly provided by J. P. Farrell of the University of Pennsylvania, Philadelphia. The parasite was maintained axenically as the promastigote stage in medium 199 supplemented to 10% with a filtered lysate of sterile defibrinated rabbit blood and 14.6 mg of L-glutamine/100 ml. Cultures were initiated from infected hamster spleen cells and passaged at approximately 5 to 7-day intervals.

Male outbred golden hamsters, LVG:LAK (Syr), were obtained commercially (Charles River Breeding Laboratories) and kept in air-conditioned quarters with food and water provided ad libitum. At the start of the experiment the animals (7 to 8 weeks old) weighed 80 to 100 g.

Treatment protocol. Glucan was prepared by a modification of the method of Hassid et al. (12), described in detail by Di Luzio et al. (7). In studies assessing nonspecific protection, hamsters were injected intravenously via the dorsal vein of the penis with glucan (3.0 mg) on days 4, 7, 10, and 13 before inoculation with viable parasites. An additional group received glucan as a posttreatment modality on days 2, 6, and 10 after challenge. Control groups received isovolumetric dextrose or were untreated. Promastigote-stage cells were harvested from axenic cultures during log-phase growth by centrifugation (900 × g for 30 min) and suspended in Earle balanced salt solution (EBSS). Hamsters were challenged by intravenous injection of 2.5 × 10⁷ promastigotes. At designated intervals after injection, at least five hamsters in each treatment group were killed with chloroform vapor. Body, spleen, and liver weights were recorded, and impression slides were made from liver and spleen tissue. Slides were fixed with methanol, stained with Giemsa, and the parasite burdens in organs were estimated by the method of Stauber (25).

For immunization, log-phase promastigotes were washed three times in EBSS and killed by suspension in 0.1% Formalin for 30 min at room temperature; they were kept overnight at 4°C. Parasites were then washed three times, counted in a hemacytometer, and suspended to the desired concentration in EBSS. A sample of each promastigote preparation was inoculated into culture medium, held at 25°C, and examined for at least 21 days to ensure that the treatment

effectively killed all parasites. For intravenous immunization, 2.5 × 10⁷ Formalin-killed promastigotes were suspended in 0.2 ml of EBSS and combined with 3 mg of glucan/0.2 ml. Animals injected with glucan or dead parasites alone received the same isovolumetric concentration.

Hamsters were immunized with 2.5 × 10⁷ Formalin-killed parasites, either with or without intravenous glucan (3.0 mg) for three injections at 4-day intervals. On day 60 after the last injection, hamsters were challenged intravenously with 0.2-ml suspensions of 6.4 × 10⁶ amastigote-stage cells obtained from infected hamster spleen. Subsequent changes in organ parasite counts and mortality were monitored.

Ultrastructural studies. Liver tissue for electron microscopic study was prepared by perfusion of 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) via the portal vein of ether-anesthetized animals. After 5 min of perfusion, small samples of liver tissue were taken and immersed in fixative for an additional 2 to 3 h at room temperature. Tissue samples were then rinsed in buffer, treated with 1% OsO₄ in 0.1 M cacodylate buffer for 1.5 h, rinsed in buffer, dehydrated in an acetone series, and infiltrated and embedded in low-viscosity epoxy resin (24). Ultrathin sections were stained with uranyl acetate and lead citrate and examined with Hitachi HU-11E2 and HS-9 electron microscopes.

In vitro macrophage-parasite interaction. Interaction of peritoneal macrophages from glucan-treated and dextrose-treated hamsters infected with *L. donovani* was evaluated in vitro. Animals received a single intraperitoneal injection of glucan (3.0 mg) or dextrose 4 days before harvesting of peritoneal exudate cells. Cells were collected by washing the peritoneal cavity with 5.0 ml of RPMI 1640-heparin (10 U/ml) supplemented with penicillin (50 U/ml) and streptomycin (50 μg/ml). The cells were centrifuged and suspended in RPMI 1640–10% heat inactivated fetal calf serum without antibiotics. Cultures were initiated in 35 mm² plastic petri dishes with 1.4 × 10⁶ cells and incubated in 5% CO₂–95% air at 37°C. Cultures were washed 1 day later and inoculated with fresh medium containing 4 promastigotes per macrophage. At 4 hr, 1 day, and 3 days postinoculation, three cultures in each group were air dried, fixed with methanol, and stained with Giemsa. The percentage of cells infected and the mean number of parasites per infected cell were used as comparative parameters.

Statistical analysis. Evaluation of results was by comparison of group mean values with Student's *t* test. Mortality data was evaluated with the chi-square test.

RESULTS

Effects of pre- and posttreatment with glucan. Groups injected with promastigotes exhibited no significant difference in hepatic parasite burdens at 1 h after challenge (Table 1). By days 8 and 16, postinfection hepatic parasite counts increased (*P* < 0.01) in hamsters receiving dextrose and in untreated animals. Total amastigote burdens in the liver continued to increase in the dextrose-treated group (*P* < 0.05) on day 30. The glucan pretreatment regimen significantly suppressed

TABLE 1. Effect of glucan pretreatment or posttreatment on hepatic parasite counts in hamsters

Group[a]	Postinfection hepatic parasite count ($\times 10^6$) after[b]:			
	1 h	Day 8	Day 16	Day 30
Glucan pretreatment	9 ± 1.2	36 ± 0.3[c]	53 ± 0.6[c]	31 ± 0.5[c]
Glucan posttreatment			63 ± 11[c]	170 ± 39[c]
5% Dextrose	14 ± 2.1	120 ± 8.0	247 ± 16[d]	814 ± 139[d]
Untreated	11 ± 1.5	91 ± 5.5	480 ± 32	389 ± 53

[a] Hamsters were challenged (day 0) with 25.0×10^6 promastigotes intravenously. In the pretreatment group, glucan (3 mg) was injected on days 4, 7, 10, and 13 before challenge inocula. In the posttreatment group, glucan (3 mg) was injected on days 2, 6, and 10 after challenge inocula. The 5% dextrose group was injected as a pretreatment regimen.

[b] Values are expressed as the mean ± standard error of the mean (N = 5 to 7 per group).

[c] Significant value ($P < 0.05$) versus that for the 5% dextrose or untreated group.

[d] Significant value ($P < 0.05$) versus that for the untreated group.

hepatic parasite counts on day 8 ($P < 0.05$), day 16 ($P < 0.005$), and day 30 ($P < 0.01$) relative to both dextrose-treated control animals and untreated control animals. Similarly, the glucan postinfection treatment reduced hepatic parasite burdens on day 16 ($P < 0.005$) and day 30 ($P < 0.01$).

Although parasites in spleen tissue were too few to count at 1 h, proliferation of parasites was also noted in the spleen of untreated and dextrose-treated control groups on days 8, 16, and 30 postinfection (Table 2). These counts were compared with lesser splenic parasite counts ($P < 0.05$) in the glucan pretreatment group on days 16 and 30. The posttreatment glucan regimen, however, did not significantly reduce splenic parasite counts during the same time intervals.

At the initiation of challenge with promastigotes (day 4 after the last glucan injection) there was no significant difference in spleen weight, liver weight, or body weight ratios relative to control groups. Liver/body weight ratios remained unchanged throughout the course of the study. A significant parasite-induced splenomegaly, however, was evident in all groups as

TABLE 2. Effect of glucan pretreatment and posttreatment on the course of splenic parasite counts in hamsters

Group[a]	Postinfection splenic parasite count ($\times 10^5$) on day[b]:		
	8	16	30
Glucan pretreatment	3.1 ± 0.5	31 ± 4.2[c]	182 ± 19[c]
Glucan posttreatment		75 ± 11	452 ± 59
5% Dextrose	2.3 ± 0.1	95 ± 13	337 ± 45
Untreated	6.3 ± 1.1	51 ± 3.9	389 ± 42

[a] Hamsters were challenged (day 0) with 25.0×10^6 promastigotes intravenously. Glucan treatment regimens are described in Table 1, footnote a.

[b] Values are expressed as the mean ± standard error of the mean (N = 5 to 7 per group).

[c] A significant value ($P < 0.05$) versus that for the 5% dextrose group or the untreated group.

the infection progressed. At day 30 postinfection, spleen/body weight ratios (mg/gram) increased in the glucan-treated group from 1.2 ± 0.1 to 4.5 ± 0.2 ($P < 0.01$), the dextrose-treated control group increased from 1.0 ± 0.1 to 4.1 ± 5 ($P < 0.025$), and untreated hamsters increased from $0.89 \pm .05$ to 3.9 ± 0.6 ($P < 0.01$). The degree of splenomegaly, however, did not vary among treated and untreated groups.

Ultrastructural studies. Each photomicrograph (Fig. 1 and 2) was from a representative sample of tissue from hamsters in the dextrose-treated control group or the glucan pretreatment group on day 16 after intravenous inoculation with 2.5×10^7 promastigotes. The group injected with the viable parasite after glucan pretreatment (Fig. 1) and the group injected with the parasite and dextrose (Fig. 2) both developed granulomas in the liver. The macrophages in the infected glucan-treated hamsters appeared to be activated and contained numerous glucan vacuoles and an occasional parasite (Fig. 1). It was not difficult to locate macrophages containing glucan vacuoles in the livers of glucan–parasite-injected animals. Macrophages containing parasites however, occurred with low frequency in the glucan-treated group. The number of parasites seen in any glucan-stimulated liver macrophage was never more than three.

Macrophages in the parasite-infected control animals also appeared to be activated; they contained well-developed arrays of rough endoplasmic reticulum and were frequently engorged with parasites (Fig. 2). As many as nine profiles of *L. donovani* were seen within single macrophages in ultrathin sections of liver from parasite-infected control animals.

In vitro macrophage-*L. donovani* interaction. At 5 h after inoculating cultures of adherent peritoneal cells with promastigotes, the proportion of infected cells and numbers of parasites per cell did not vary significantly between macrophages harvested from control dextrose-pretreated hamsters and glucan-pretreated ham-
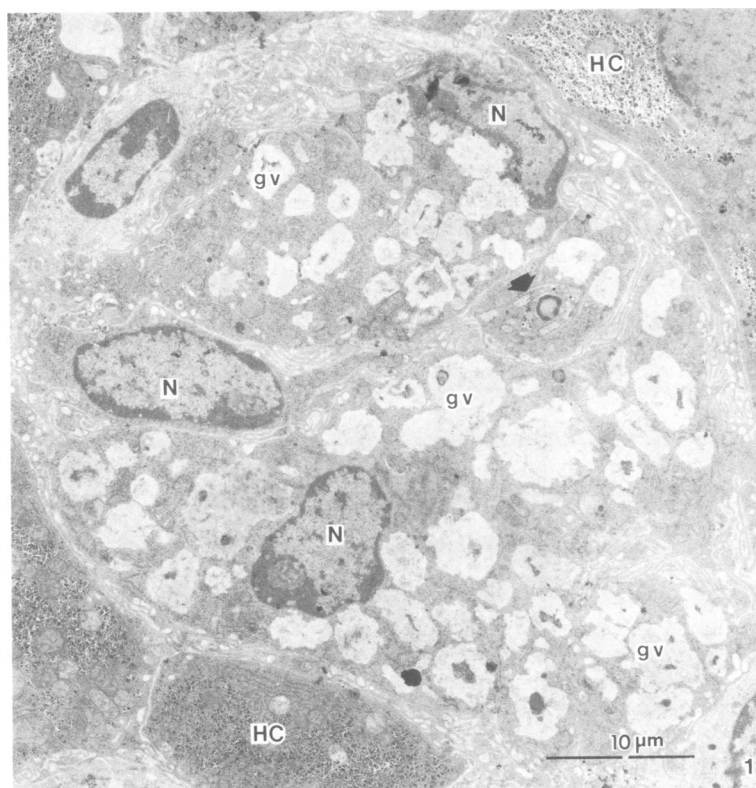
FIG. 1. Typical granuloma in a glucan-pretreated animal 16 days after challenge with virulent promastigotes. The granuloma occupies a liver sinusoid and is composed mainly of hypertropic macrophages (nuclei labeled N) engorged with glucan vacuoles (gv). One macrophage contains both glucan vacuoles and a single profile of *L. donovani* (arrow). Other macrophages do not exhibit parasite profiles (HC, hepatic cells).

sters (Table 3). At 24 hours, however, only 14.5 ± 3.1% of cells from glucan-treated animals were infected as compared to 53.9 ± 6.5% of cells from control animals ($P < 0.001$); the mean number of parasites per cell was 1.4 ± 0.1 and 2.0 ± 0.1 ($P < 0.001$) for those groups, respectively. On day 3 postinoculation, the percentage of infected cells was 31.8 ± 8.3% with 1.5 ± 0.1 parasites per cell for cells from the glucan-treated group, as opposed to 79.0 ± 5.3% with 3.42 ± 0.6 parasites per cell for cells from the control group ($P < 0.001$).

Glucan-dead parasite immunization against *L. donovani*. To test the effectiveness of glucan as an adjuvant, studies were conducted in which hamsters were inoculated with Formalin-killed promastigotes, with or without glucan (Fig. 1). At 60 days after the final immunization, each animal was challenged by intravenous injection of 6.40 × 10⁶ amastigotes, and the subsequent amounts of hepatic and splenic parasites were monitored. At 1 h after injection of the amastigotes, hepatic parasite counts were not significantly different for all groups (Fig. 3). Hamsters

immunized only with Formalin-killed parasites exhibited some increased resistance to infection, as indicated by the reduced number of hepatic parasites compared with untreated control animals on day 10 (6.94 × 10⁷ ± 0.37 × 10⁷ versus 1.34 × 10⁸ ± 0.13 × 10⁸, $P < 0.05$), and on day 35 (9.61 × 10⁷ ± 0.97 × 10⁷ versus 3.38 × 10⁸ ± 0.24 × 10⁸, $P < 0.05$). Groups receiving glucan alone exhibited sustained nonspecific resistance to infection, and parasite numbers were significantly reduced ($P < 0.005$) on day 10 (3.71 × 10⁷ ± 0.25 × 10⁷), day 21 (6.38 × 10⁷ ± 0.61 × 10⁷), and day 35 (3.15 × 10⁷ ± 0.39 × 10⁷), relative to both the untreated group and the group treated with killed parasites alone. The values found for groups treated with glucan and killed parasites were not significantly different from those found for the glucan-treated group on day 10 (2.48 × 10⁷ ± 1.4 × 10⁷), day 21 (4.0 × 10⁷ ± 2.3 × 10⁷), and day 35 (2.99 × 10⁷ ± 0.46 × 10⁷).

At 1 h after challenge with amastigotes, splenic parasites were too few to enumerate in each group (Fig. 4). By day 10, splenic parasites in the untreated group had increased to 2.98 × 10⁶ ±
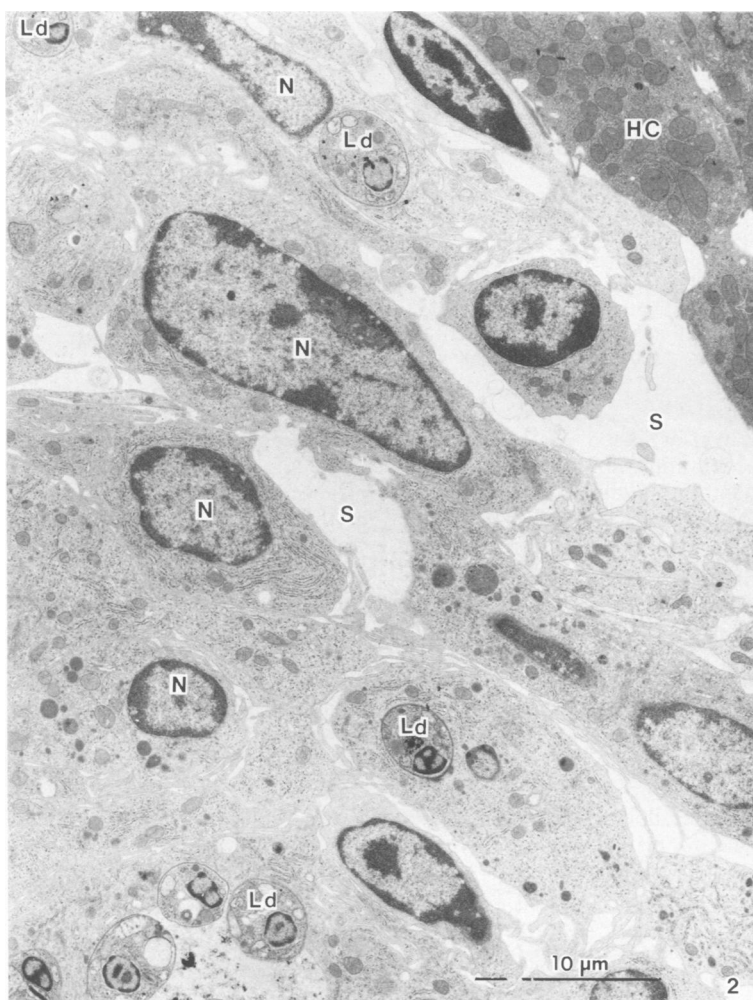
FIG. 2. A portion of a granuloma in a liver sinusoid (S) of a dextrose-pretreated control animal 16 days after challenge with virulent promastigotes. The granuloma is composed mainly of hypertrophied macrophages (nuclei labeled N), several of which contain profiles of *L. donovani* (Ld) (HC, hepatic cell).

0.37×10^6, the glucan-pretreated group had increased to $1.6 \times 10^6 \pm 0.28 \times 10^6$, and the killed parasite-pretreated group had increased to $2.01 \times 10^6 \pm 0.17 \times 10^6$ (Fig. 4). The number of splenic parasites in hamsters immunized with glucan and killed parasites was $4.4 \times 10^5 \pm 0.54 \times 10^5$ and was significantly less than in groups receiving no treatment ($P < 0.025$), glucan alone ($P < 0.005$), or killed parasites alone ($P < 0.005$). Similar results were observed on day 21 after challenge. The number of splenic parasites in the untreated group was $4.47 \times 10^7 \pm 0.49 \times 10^7$; the numbers of splenic parasites in the glucan-pretreated and killed parasite-treated group were $1.60 \times 10^7 \pm 0.16 \times 10^7$ and $1.60 \times 10^7 \pm 0.12 \times 10^7$, respectively. The splenic parasite burden in glucan–killed parasite-pretreated hamsters was $5.45 \times 10^6 \pm 0.72 \times 10^6$, which was significantly lower ($P < 0.01$) than that of the control groups. By day 31 however, the splenic parasite burden of the immunized group was not significantly different than that of hamsters pretreated with either killed parasites or glucan alone.

The mean survival time (Table 4) of animals that died from the glucan–killed parasite-pretreated group (101 ± 12 days) was significantly ($P < 0.05$) greater than that for untreated hamsters that died (66 ± 4 days). The survival times of hamsters treated with only killed parasites (76 ± 7 days) or glucan (73 ± 3) did not vary significantly from the untreated group. All survivors (two from the glucan-treated group and one each from the glucan–killed parasite-pretreated group and the untreated group) were sacrificed on day 168 after inoculation. No parasites were

TABLE 3. In vitro infection by *L. donovani* of adherent peritoneal cells from hamsters

Time after infection	Treatment group[a]			
	G		D	
	% cells infected[b]	Parasites per cell[b]	% cells infected[b]	Parasites per cell[b]
5 h	51.7 ± 12.9	2.0 ± 0.2	69.1 ± 7.1	2.5 ± 0.6
1 day	14.5 ± 3.1[c]	1.4 ± 0.1[c]	53.9 ± 6.5	2.9 ± 0.1
3 days	31.8 ± 8.3[c]	1.5 ± 0.1[c]	79.0 ± 5.3	3.4 ± 0.6

[a] G, Adherent peritoneal cells harvested 4 days after receiving intraperitoneal glucan (3 mg); D, adherent peritoneal cells from dextrose-treated control animals. Cultures contained 1.4×10^6 cells per ml and were inoculated with 4 promastigotes per cell.
[b] Values are expressed as the mean ± standard error of the mean ($N = 3$ per group).
[c] Significant value ($P < 0.001$) versus that for group D.

seen on slides of liver or spleen tissue from these hamsters. However, minced spleen inoculated into culture medium resulted in the growth of promastigotes for one hamster in each group. A culture prepared from spleen tissue from one of the two surviving glucan-treated hamsters, however, remained negative for parasites.

DISCUSSION

It is particularly noteworthy that glucan is effective in enhancing host resistance against *L. donovani* infection in hamsters. Unlike mice, which respond spontaneously and generate a degree of specific immunity when infected with *L. donovani*, parasites injected systemically in hamsters continue to proliferate in liver and
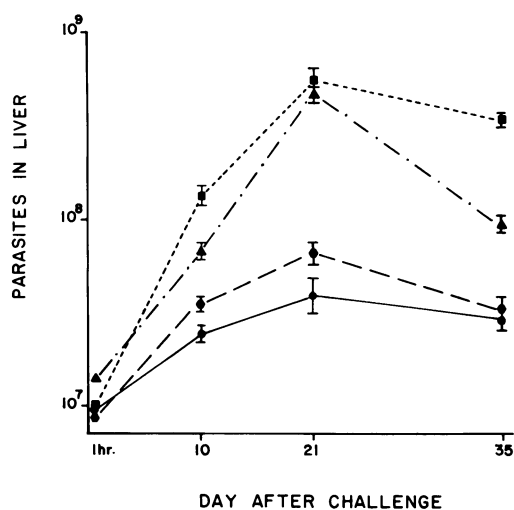

DAY AFTER CHALLENGE

FIG. 3. Proliferation of hepatic parasites on designated days after challenge after an intravenous inoculation of viable amastigotes into untreated control animals (■), killed-parasite treated animals (▲), glucan treated animals (●), and glucan–killed parasite-treated animals (●). Values are expressed as the mean ± standard error of the mean.

spleen cells, and these animals usually succumb to the infection (25). Previous attempts at immunization against different forms of leishmaniasis in this highly susceptible animal model have yielded mostly marginal or negative results. Injection of small numbers of virulent *L. donovani* elicits a relative degree of resistance to subsequent challenge with larger numbers of parasites in hamsters (8). Similar results were obtained in hamsters infected with virulent *L. tropica*, although progressive development of infection occurs in stimulated but unchallenged hamsters (23). Additionally Lainson and Bray (16) found that immunization of hamsters with Formalin-killed *L. mexicana* amastigotes with Freund's adjuvant fails to elicit resistance against challenge with that parasite.

Our experiments provide the first evidence of enhanced nonspecific resistance of hamsters against visceral leishmaniasis. Glucan administration markedly attenuated the course of *L. donovani* infection, as evidenced by reduced proliferation of amastigotes in both liver and

TABLE 4. Mortality and survival time of immunized and control hamsters against *L. donovani* infections

Group[a]	Survival until day 100 postinfection (dead/total)	Mean survival time of hamsters that died (days)[b]
GP ($N = 9$)	4/9[c]	101 ± 12[d]
P ($N = 6$)	6/6	76 ± 7
G ($N = 7$)	5/7	73 ± 3
NT ($N = 10$)	9/10	66 ± 4

[a] Groups were inoculated with 6.4×10^6 amastigotes in glucan–killed parasite (GP), killed parasite (P), or glucan (G) or received no treatment (NT).
[b] Values are expressed as the mean ± standard error of the mean.
[c] A significant value ($P < 0.05$) versus the P and NT groups.
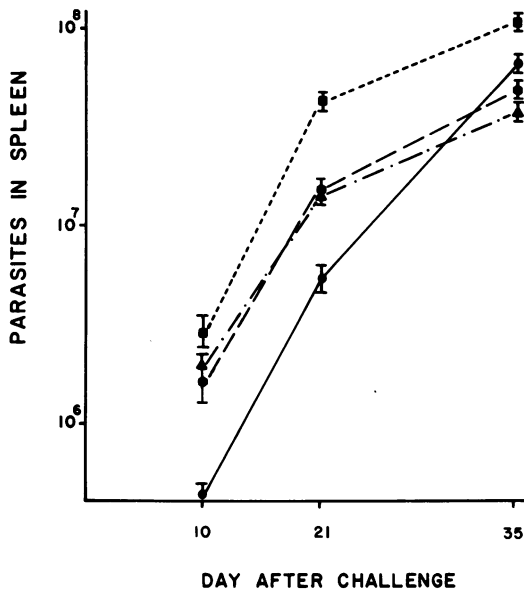[d] A significant value ($P < 0.05$) versus the NT group.

FIG. 4. Proliferation of splenic parasites on designated days after challenge after an intravenous inoculation of viable amastigotes into untreated control animals (■), killed-parasite treated animals (▲), glucan-treated animals (◆), glucan–killed parasite-treated animals (●). Values are expressed as the mean ± standard error of the mean.

spleen tissue. Peritoneal macrophages obtained from glucan-treated hamsters also exhibited reduced numbers of intracellular parasites in vitro. We have previously reported similar results with peritoneal macrophages from glucan-treated mice (3). The mechanism whereby glucan treatment reduces the number of intracellular parasites in vivo and in vitro remains to be delineated. Recent observations, have implicated peroxidase-mediated formation of reactive oxygen metabolites in the in vitro leishmanicidal activity of mouse peritoneal macrophages against *L. enriettii* (1). It is pertinent that Kupffer cells in glucan-treated rats (5; C. F. Way, Ph.D. thesis, Medical University of South Carolina, Charleston, 1981) and mice (C. F. Way, Ph.D. thesis, Medical University of South Carolina, Charleston, 1981) exhibit increased rough endoplasmic reticulum activity with endogenous peroxidase activity. Glucan could have other salutary actions against visceral leishmaniasis. Purified granulocyte-macrophage colony-stimulating factor potentiates in vitro killing of *L. tropica* by mouse peritoneal macrophages (11), and treatment of mice with glucan augments release of such factors from macrophages (2). Haidaris and Bonventre (10) recently observed that elimination of *L. donovani* amastigotes by activated mouse macrophages is lymphokine dependent. Macrophages activated by

either *Corynebacterium parvum* or *Mycobacterium tuberculosis* are ineffective for killing amastigotes unless the activated state is maintained by daily addition of lymphokine to infected monolayers (2). Possible lymphokine involvement in glucan-induced leishmanicidal activity merits special future consideration, particularly in mice which exhibit prolonged immunity to the parasite after conjoint glucan-killed parasite treatment (13).

The response of the hamster to glucan was unique with regard to the absence of measurable glucan-induced splenomegaly or hepatomegaly when compared with reactions of mice or rats given similar glucan doses (6; C. F. Way, Ph.D. thesis, Medical University of South Carolina, Charleston, 1981). These findings suggest that hamsters may have a relatively reduced capacity to recruit monocytes in response to inflammatory stimuli, which may be relevant to their increased vulnerability to leishmanial infections. Nevertheless, granulomatous foci and hypertrophic Kupffer cells with recognizable glucan vacuoles were present in the livers of glucan-treated hamsters, and such a response was associated with reduced numbers of intracellular parasites.

Our results with glucan as an adjuvant in combination with killed promastigotes do not allow us to determine whether the increased resistance was due to glucan potentiation of specific immunity against *L. donovani*. Such an effect may have been partially masked by the unanticipated degree of protection afforded by glucan alone at challenge 60 days after immunization. Relative to the glucan-treated control group, hamsters receiving glucan–killed promastigote inoculations exhibited highly significant reduction of splenic parasite burdens on days 10 and 21 postinfection. Likewise, this was the only group which exhibited significantly prolonged mean survival time relative to animals not treated before infection. By day 35, however, amastigotes in splenic tissue of the immunized group did not vary significantly from the glucan-treated or parasite-treated control group. These observations suggest that before day 10 postinfection, either fewer parasites failed to initiate infection after challenge of the immunized group or there was enhanced killing or suppression of amastigote proliferation during the early course of the infection. We are presently directing attention to in vitro measures of specific immune responsiveness to clarify these possibilities.

Clinical management of mucocutaneous and visceral leishmaniasis presently involves chemotherapy. Several drugs are active against leishmaniasis, but these compounds have serious disadvantages with regard to toxicity and treatment failure (28). A potential alternative approach which has received limited attention is

immunoprophylaxis. It seems clear, however, that such an approach would likely necessitate the use of appropriate adjuvants, since killed or attenuated parasite preparations alone provide limited or no measurable protection in experimental hosts (4, 16, 17, 20, 21).

Glucan as an adjuvant may be more advantageous than certain bacterial adjuvants such as BCG, *C. parvum*, or *Bordetella pertussis*. The microbial agents have several disadvantages relating to their bacterial nature, antigenic qualities, difficulty of standardization, possible infectious complications, unknown metabolites, and the fact that the immunostimulant is, in most cases, an unknown entity (6). In comparison, glucan is nonviable and chemically defined and in limited clinical trials appears to be relatively nontoxic (6, 18).

In addition to enhancing nonspecific resistance of hamsters and mice to *L. donovani* infections (3), glucan increases host resistance to a variety of other infectious agents, including viruses (22, 30), bacteria (15), fungi (29), and protozoa (14). When used as an adjuvant with killed promastigotes, glucan potentiated immunity of mice against *L. donovani* infections when challenged several weeks after immunization (13). Glucan was likewise effective in enhancing the immunogenicity of killed erythrocytic-stage *Plasmodium berghei*, as determined by an increased proportion of mice surviving after infection with this protozoan (14). In addition, Reynolds et al. (22) demonstrated that glucan was more effective than Fruend's complete adjuvant in eliciting resistance of mice against Venezuelan equine encephalitis virus (22). Similarly, injection of the vaccine for that virus in combination with glucan produced a greater antibody response in monkeys than did immunization with the vaccine alone (22).

These observations, coupled with these studies, which demonstrate that glucan alone or with antigen enhanced resistance against *L. donovani* in the highly vulnerable hamster, make glucan particularly attractive as an immunotherapeutic agent and as a potential adjuvant in immunoprophylactic trials. The mechanism whereby glucan enhances host resistance to visceral leishmaniasis and the potential use of glucan immunotherapy against severe infectious diseases, including severe forms of leishmaniasis, merits further evaluation.

LITERATURE CITED

1. Buchmuller, Y., and J. Mauel. 1981. Studies on the mechanism of macrophage activation: possible involvement of oxygen metabolites in killing of *Leishmania enriettii* by activated macrophages. RES J. Reticuloendothel. Soc. 29:181–192.
2. Burgalata, C., and D. W. Golde. 1977. The effect of glucan on granulopoiesis and macrophage genesis in mice. Cancer Res. 37:1739–1742.
3. Cook, J. A., T. W. Holbrook, and B. W. Parker. 1980. Visceral leishmaniasis in mice: protective effect of glucan. RES J. Reticuloendothel. Soc. 27:567–573.
4. Coutinho, J. O. 1954. Preventive vaccination with dead leptomonads against leishmaniasis of guinea pig due to *Leishmania enriettii*. Folia Clin. Biol. 21:321–326.
5. Deimann, W., and H. D. Fahimi. 1979. The appearance of transition forms between monocytes and Kupffer cells in the liver of rats treated with glucan. J. Exp. Med. 149:883–897.
6. Di Luzio, N. R. 1976. Pharmacology of the reticuloendothelial system: accent on glucan, p. 412–421. In S. Richard, M. Escobar, and H. Friedman (ed.), The reticuloendothelial system in health and disease. Functions and characteristics. Plenum Publishing Corp., New York.
7. Di Luzio, N. R., D. L. Williams, R. B. McNamee, B. F. Edwards, and A. Kitahama. 1979. Comparative tumor-inhibitory and anti-bacterial activity of soluble and particulate glucan. Int. J. Cancer 24:773–779.
8. Farrell, J. P. 1976. *Leishmania donovani*: acquired resistance to visceral infection in the golden hamster. Exp. Parasitol. 40:89–94.
9. Greenblatt, C. L. 1980. The present and future of vaccination for cutaneous leishmaniasis, p. 259–285. In New developments with human and veterinary vaccines. Alan R. Liss, Inc., New York.
10. Haidaris, C. G., and P. F. Bonventre. 1981. Elimination of *Leishmania donovani* amastigotes by activated macrophages. Infect. Immun. 33:918–926.
11. Handman, E., and A. W. Burgess. 1979. Stimulation by granulocyte-macrophage colony stimulating factor of *Leishmania tropica* killing by macrophages. J. Immunol. 122:1134–1137.
12. Hassid, W. Z., M. A. Joslyn, and R. M. McCready. 1941. The molecular constitution of an insoluble polysaccharide from yeast, *Saccharomyces cerevisiae*. J. Am. Chem. Soc. 63:295–298.
13. Holbrook, T. W., J. A. Cook, and B. W. Parker. 1981. Immunization against *Leishmania donovani*. Glucan as an adjuvant with killed promastigotes. Am. J. Trop. Med. Hyg. 30:762–768.
14. Holbrook, T. W., J. A. Cook, and B. W. Parker. 1981. Glucan-enhanced immunogenicity of killed erythrocytic stages of *Plasmodium berghei*. Infect. Immun. 32:542–546.
15. Kokoshis, P. L., D. L. Williams, J. A. Cook, and N. R. Di Luzio. 1978. Increased resistance to *Staphylococcus aureus* infection and enhancement in serum lysozyme activity by glucan. Science 199:1340–1342.
16. Lainson, R., and R. S. Bray. 1964. *Leishmania mexicana*. Trans. R. Soc. Trop. Med. Hyg. 58:94.
17. Lemma, A., and L. Cole. 1974. *Leishmania enriettii*: radiation effects and evaluation of radioattenuated organisms for vaccination. Exp. Parasitol. 35:161–169.
18. Mansell, P. W. A., G. Rowden, and C. Hammer. 1978. Clinical experiences with the use of glucan, p. 255–280. In M. A. Chirigos (ed.), Immune modulation and control of neoplasia by adjuvant therapy. Raven Press, New York.
19. Manson-Bahr, P. E. C. 1961. Immunity in kala-azar. Trans. R. Soc. Trop. Med. Hyg. 55:550–555.
20. Marva, E., and C. L. Greenblatt. 1979. Vaccination of mice with chemically attenuated *Leishmania tropica*. J. Protozool. 26:49a.
21. Preston, P. M., and D. C. Dumonde. 1976. Immunology of clinical and experimental leishmaniasis, p. 167–202. In S. Cohen and E. Sadun, (ed.), Immunology of parasitic infections. Blackwell Scientific Publications, Oxford.
22. Reynolds, J. A., M. D. Kastello, D. G. Harrington, C. L. Crabbs, C. J. Peters, J. V. Jemski, G. H. Scott, and N. R.

Di Luzio. 1980. Glucan-induced enhancement of host resistance to selected infectious diseases. Infect. Immun. **30**:51–57.

23. **Schur, L. F.** Preliminary report on an attempt to vaccinate Syrian hamsters against leishmaniasis using a live vaccine at low concentration. Ann. Trop. Med. Parasitol. **70(3)**:371–373.

24. **Spurr, A. R.** 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastructure Res. **26**:31–43.

25. **Stauber, L. A.** 1955. Leishmaniasis in the hamster, p. 76–90. *In* W. H. Cole (ed.), Some physiological aspects and consequences of parasitism. Rutgers University Press, New Brunswick, New Jersey.

26. **Stauber, L. A.** 1958. Host resistance to the Khartoum strain of *Leishmania donovani.* Rice Inst. Pam. **445**:80–96.

27. **Stauber, L. A.** 1966. Characterization of strains of *Leishmania donovani.* Exp. Parasitol. **18**:1–11.

28. **Steck, E. A.** 1974. The leishmaniases, p. 289–351. *In* E. Jucker (ed.), Progress in drug research, vol. 8. Birkhauser Verlag, Basel.

29. **Williams, D. L., J. A. Cook, E. O. Hoffman, and N. R. Di Luzio.** 1978. Protective effect of glucan in experimentally induced candidiasis. RES J. Reticuloendothel. Soc. **23**:479–490.

30. **Williams, D. L., and N. R. Di Luzio.** 1980. Glucan-induced modification of murine viral hepatitis. Science **208**:67–69.

31. **Zuckerman, A., and R. Lainson.** 1977. Leishmania, p. 57–133. *In* J. P. Kreier (ed.), Parasitic Protozoa, vol. 1. Academic Press, Inc., New York.