

Effect of glucan on *Leishmania major* infection in BALB/c mice

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Summary. The effect of glucan on *Leishmania major* infection was studied in BALB/c mice, which are highly susceptible to leishmania infection. Glucan (0.45 mg), or isovolumetric dextrose, was administered intraperitoneally 7, 5, 3 and 1 day before infection with *L. major* promastigotes. At 3, 5, 6, 8 and 10 weeks after infection, animals were killed; the liver and spleen of each animal were weighed and the parasite burden was calculated. A significant ($p < 0.01$) reduction in amastigote proliferation in liver and spleen of animals pretreated with glucan was demonstrated 4, 6 and 8 weeks after infection.

Introduction

Leishmania are protozoan parasites that infect mammalian macrophages. *Leishmania tropica*, *L. major* and *L. mexicana* cause a localised or diffuse skin ulcer whereas *L. donovani* and *L. infantum* cause visceral infection of the reticulo-endothelial system (Manson-Bahr, 1961; Zuckerman and Lainson, 1977). Glucan, a β -1–3 polyglucose derived from the cell wall of *Saccharomyces cerevisiae* is an immunomodifier that causes a dose-dependent hypertrophy and hyperplasia of reticulo-endothelial macrophages (Di Luzio, 1967; Burgaleta and Golde, 1978). Glucan-activated macrophages kill micro-organisms *in vitro* and *in vivo*. Moreover, glucan significantly inhibits mortality and other sequelae of infection in mice challenged with *Staphylococcus aureus* (Di Luzio and Williams, 1978), *Mycobacterium leprae* (Delville and Jacques, 1977), *Candida albicans* (Williams *et al.*, 1978), hepatitis viruses (Williams and Di Luzio, 1980), and *Plasmodium berghei* (Holbrook *et al.*, 1981). Glucan can also enhance the non-specific and specific resistance of mice against systemic infection with *L. donovani* (Cook *et al.*, 1980 and 1982). However, Avila *et al.* (1982) reported that glucan failed to modify non-specific immunity to cutaneous infections with *L. mexicana*, *L. braziliensis* and *L. garnhami*, suggesting possible species differences in response.

We have studied the effect of glucan administration on visceralisation of *L. major* in spleen and liver of highly susceptible BALB/c mice, after subcutaneous inoculation of promastigotes.

Materials and methods

Parasite and host

L. major strain HOM/SA/1983/SAYED was originally isolated from a patient at King Khalid University

Hospital, Riyadh, Saudi Arabia, and maintained *in vitro* and *in vivo* in this laboratory as described earlier (Mahmoud *et al.*, 1985). Inbred BALB/c male mice weighing 20–25 g were kept in air-conditioned quarters with food and water provided *ad libitum*. At the start of each infection the mice were 6–8 weeks old.

Treatment and infection

Glucan, kindly provided by N. R. Di Luzio (Tulane University), was prepared by a modification of the method of Hassid *et al.* (1941) described by Di Luzio *et al.* (1979). Each mouse was given intraperitoneal injections of 0.45 mg of glucan 7, 5, 3 and 1 day before parasitic infection. Control groups received isovolumetric injections of sterile dextrose 5% solution.

Promastigotes of *L. major* cultured in Novy, MacNeal and Nicolle's (NNN) medium as described by Mahmoud *et al.* (1985) were harvested, concentrated by centrifugation at 900 rpm for 30 min, suspended in 15 ml of the fluid phase of the medium and counted by microscopy in a haemocytometer. Each mouse treated with glucan or dextrose was given a subcutaneous injection of 5×10^7 promastigotes. Five mice from each group were killed at 3, 5, 6, 8 and 10 weeks after infection. Spleen and liver weights were recorded at each interval. Liver and spleen impression slides were fixed with methanol and stained with Giemsa's stain. The parasite burden was estimated by the method of Stauber (1955); the number of amastigotes per 500 cell nuclei in each organ was counted and the total parasite burden (TPO) was calculated according to the following formula:

$$\text{TPO} = \frac{P}{N} \times \text{OW} \times 2 \times 10^5,$$

where P is the number of amastigotes, N is the number of cell nuclei and OW is the organ weight in mg.

Statistical analysis

Student's *t* test was used to compare the results in mice treated with glucan or dextrose.

Results

There were no significant differences in liver or spleen weights of mice pretreated with glucan or dextrose up to 6 weeks after challenge with *L. major*. However, a significant difference in spleen weights between the two groups was observed 8 weeks after infection ($p < 0.001$) and in the weights of livers 10 weeks after infection ($p < 0.01$).

The parasite burdens of the glucan and dextrose treated mice differed 4, 6 and 8 weeks after infection (table). Whereas amastigotes were seen in spleens of the control (dextrose treated) mice during the fourth week, they were not seen in spleens of mice receiving glucan until the sixth week after infection. The parasite burden in the spleens of the control group increased sharply during the fourth week to a peak of $(33.22 \pm 9.3) \times 10^6$ amastigotes per organ, after which it gradually declined to $(6.8 \pm 15.3) \times 10^6$ amastigotes by the end of week 10. The splenic parasite count in mice treated with glucan was significantly lower than in control mice 6 and 8 weeks after infection ($p < 0.01$ and $p < 0.001$ respectively), but not at week 10. The mean parasite count in the liver showed a similar pattern; there was a significantly lower ($p < 0.01$) parasite count in glucan treated mice 4, 6 and 8 weeks after infection, but no significant difference at the tenth week.

Discussion

L. major, which causes human cutaneous leishmaniasis in Saudi Arabia, usually proliferates in the spleen and liver when injected into BALB/c mice (Nasseri and Modabber, 1979; Leclerc *et al.*, 1981 and 1982). The finding that glucan initially suppressed proliferation of *L. major* in the spleen and liver of mice is in agreement with the results obtained by Cook *et al.* (1980 and 1982) with *L. donovani*. They suggested that non-specific activation of fixed macrophage populations in spleen and liver enhances killing or inhibits multiplication of the parasites. Failure to detect inhibition 10 weeks after infection may be due to a loss of glucan potency (Di Luzio, 1976). The lack of protection by glucan against other species of *Leishmania* that cause infection in man, e.g., *L. braziliensis* may be related to the restricted localisation of the parasites in cutaneous infection (Avila *et al.*, 1982).

Lysozyme and peroxidase may play an important role in the mechanism of killing of *Leishmania* spp. (Bray, 1982; Alexander, 1981) and *Trypanosoma cruzi* within phagosomes (Nathan *et al.*, 1979). Immunostimulants such as glucan markedly increase lysozyme levels in experimental animals (Kokoshis and Di Luzio, 1979) and we have shown that leishmania infection causes a significant decrease in serum lysozyme (Al Mofleh *et al.*, 1986). The reduction in *L. major* amastigote proliferation by glucan could therefore be due, in part, to the release of increased amounts of intracellular lysozyme.

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Table . Organ weight and parasite burden in BALB/c mice infected with *L. major* after treatment with glucan

Weeks after infection	Number of mice	Weight and parasite burden of spleen after treatment with				Weight and parasite burden of liver after treatment with			
		dextrose		glucan		dextrose		glucan	
		weight (mg)	TPO $\times 10^6$	weight (mg)	TPO $\times 10^6$	weight (mg)	TPO $\times 10^6$	weight (mg)	TPO $\times 10^6$
3	2	134.0 \pm 95.3	ND	120.0 \pm 31.3	ND	1.736 \pm 0.36	ND	1.509 \pm 0.28	ND
4	5	192.0 \pm 93.0	33.22 \pm 9.3	162.4 \pm 47.5	ND	1.398 \pm 0.71	31.10 \pm 27.1	1.476 \pm 0.32	7.14 \pm 8.3†
6	5	128.6 \pm 35.7	17.80 \pm 4.60	127.6 \pm 8.20	1.58 \pm 2.4*	1.257 \pm 0.14	38.32 \pm 20.2	1.488 \pm 0.33	4.70 \pm 6.8†
8	5	159.8 \pm 20.0	10.32 \pm 2.28	125.0 \pm 17.0*	2.50 \pm 5.0†	1.466 \pm 0.38	19.40 \pm 3.6	1.442 \pm 0.23	4.57 \pm 5.6†
10	5	161.6 \pm 20.0	6.80 \pm 15.3	161.0 \pm 24.0	10.0 \pm 3.1	1.333 \pm 0.19	10.32 \pm 2.1	1.784 \pm 0.14*	11.18 \pm 1.5

ND = not detected. TPO = total parasite burden per organ.

* $p < 0.001$.

† $p < 0.01$.

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