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DEPARTMENT OF MEMBRANE RESEARCH מחלקה לחקר ממברנות

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Glucan and Tuftsin– Potential Antileishmanial Agents

Summarized in the following are the methods used and the results obtained in the first 14 months of our studies in the framework of the grant. Experiments that did not yield conclusive results are not presented.

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Experimental

Parasites: Two isolates of *L. major* were used; Fredlin' (World Health Organization designation, MHOM/ IL/ 80/ Fredlin) and WR309 (MHOM/ IL/ 79/ Perlstein).

Virulent parasites were maintained in BALB/c mice. Fresh isolates were made by needle aspiration and maintained as promastigotes in axenic culture for not more than five to eight passages. The culture medium consisted of Schneider's Drosophila medium containing 200 mM glutamine, 20% heat inactivated fetal calf serum and antibiotics (Penicillin 100 U/ml, streptomycin 100 μg/ml). Stationary phase promastigotes were washed three times in Dulbecco's phosphate-buffered saline and finally resuspended in sterile, nonpyrogenic, 0.9 % NaCl . The promastigotes were injected in 20 to 30 μl , intradermal into one footpad of 6 to 8 week old BALB/c male mice (Olac, England). The basal footpad thickness was 1.5 mm. Glucan and control solutions (nonpyrogenic saline or 5% glucose) were injected i.p. or i.v. at the specified times and amounts. Three hundred μl were injected i.p. and 100 μl were injected i.v. For injection, glucan preparations were suspended or dissolved in the respective solutions (5% glucose or saline), and sterilized by γ-irradiation (2 Mrad, ^{60}Co, Gamma Cell 220, Atomic Energy of Canada). Footpad thickness was measured weekly with a caliper.

Glucan: Baker's yeast (*Saccharomyces cerevisiae*) were digested sequentially with boiling NaOH and HCl, and extracted with isopropyl alcohol. The glucan particles (glucan$_P$) were further treated to obtain nonparticulate material. Incubating glucan$_P$ with 1 M KOH for 20 min at 60 °C resulted in the solubilization of 50-70 % of the glucan (estimated on the basis of glucose determination). The alkali soluble glucan (glucan$_{as}$) was precipitated by cold (4°C) 70 % ethanol. The precipitate was collected by centrifugation, resuspended in water and alcohol precipitation repeated. The pellet was resuspended in warm water and gave homogeneous turbid solutions. To obtain low molecular weight glucan polymers, glucan$_P$ was degraded by treatment with 90% formic acid at 95°C for 20 min . A fraction containing polymers with average chain length of 25 monomers was used as soluble glucan in the study. A gel forming glucan (glucan$_g$) secreted from *Sclerotium rolfsii* was a gift of Dr. I Chet. Particulate rice starch (starch$_P$) was obtained

from BDH (Poole,England).

Results

The conditions for optimal glucan mediated suppression of *L. major* development were examined. Glucan^{P} (0.5 mg) were injected i.v. at 7 day intervals (up to 4 injections) starting 7 days after parasite challenge. Mice receiving only parasites or receiving injections of a glucose solution at concurrent times served as controls. In addition several groups of animals received starch particles in a regime which paralleled the glucan^{P} treatment. Starch was used as a control particulate polysaccharide material since it has a different backbone structure from glucan. Figure 1 shows the progression/regression of *L. major* lesions in susceptible BALB/c mice. When examined at 17, 21 and 28 weeks after parasite injection both the control mice and mice receiving starch particles showed a progressive increase in footpad thickness and developed lesions. Since advanced lesions were often covered with scar tissue and reached sizes >4 mm for footpad thickness, such a value (>4 mm) was arbitrarily assigned to all these lesions. Thus, all points above the dividing line in the figures (at >4) represent mice with big lesions. Two to four injections of glucan^{P} suppressed lesion development. Four injections gave a more rapid and a higher extent of suppression than 1, 2 or 3 injections. Several of the mice which received glucan^{P} developed relatively big lesions (up to 0.5 mm bigger then the controls) at early stages following parasite injection (i.e. at 9 weeks). However,these lesions healed leaving no visible scar and hair formation resumed normal growth.

Mice were sensitive to the dose of glucan^{P} used for i.v. injection. At a higher dose of particles (1 mg/mouse) many mice died within hours following the i.v. injection.

Injection of glucan^{P} i.p. did not prevent the onset of cutaneous disease.

In a preliminary experiment the alkali soluble glucan (glucan^{as}) was injected two or four times i.p. into two groups of mice. Five hundred μg of glucan^{as} suppressed lesion formation in both groups, a four injection regime resulted in a higher suppressive effect than that of two injections when measured at 12 weeks after challenge (Figure 2).

In another experiment the effects of 4 injections i.p. of 500 μg of glucan^{as} , a gel forming glucan synthesized and secreted by *Sclerotium rolfsii* ,

and a low molecular weight glucan solubilized by formic acid treatment were compared (Figure 3). While the glucan^{as} and the gel-forming glucan from *Sclerotium rolfsii* exerted a suppressive effect on lesion formation, the soluble small molecular weight glucan had no suppressive effect.

Legends to figures

Fig. 1 Effect of i.v. injection of particulate glucan (glucan$_P$) on the progression of *L. major* footpad lesions. Groups of mice that did not receive any treatment (x0), that received 2 and 4 times (x2 and x4) a 5% glucose solution, and that received 1 to 4 times particulate starch (Starch$_P$,1 mg/mouse each time) were included as controls. Glucan$_P$ was given 1 to 4 times, 0.5 mg/mouse each time. *L. major* (WR 309, 10^7 promastigotes/footpad) was injected at day zero. Agent injection started 7 days later and was given at weekly intervals. Footpad thickness was measured each week. Representative measurements at weeks 17, 21 and 28 are given. Each point represents one mouse.

Fig.2 The effect of 2 and 4 i.p. injections of alkali solubilized glucan (glucan^{as}) on *L. major* lesion progression. *L. major* (Fredlin, 10^7 promastigotes/footpad) was injected at day zero. Seven days later and one (x2) or three (x4) more times at weekly intervals, mice received 0.5 mg glucan^{as}. Results represent footpad thickness measured at 12 weeks after parasite injection. The control group received saline injections.

Fig. 3 The effect of i.p. injections of glucan preparations on *L. major* (Fredlin) lesion progression. Groups of mice were injected with parasites ($2x10^6$ promastigotes/ mouse). Seven days later and three more times at weekly intervals, mice received injections of either; soluble glucan (glucan^s), a gel forming glucan from *Sclerotium rolfsii* (glucan$_g$) or alkali solubilized glucan (glucan^{as}) (0.5 mg glucan in 300 µl of saline) . Results represent footpad thickness measured at 7 weeks after parasite injection The control group received saline injections.

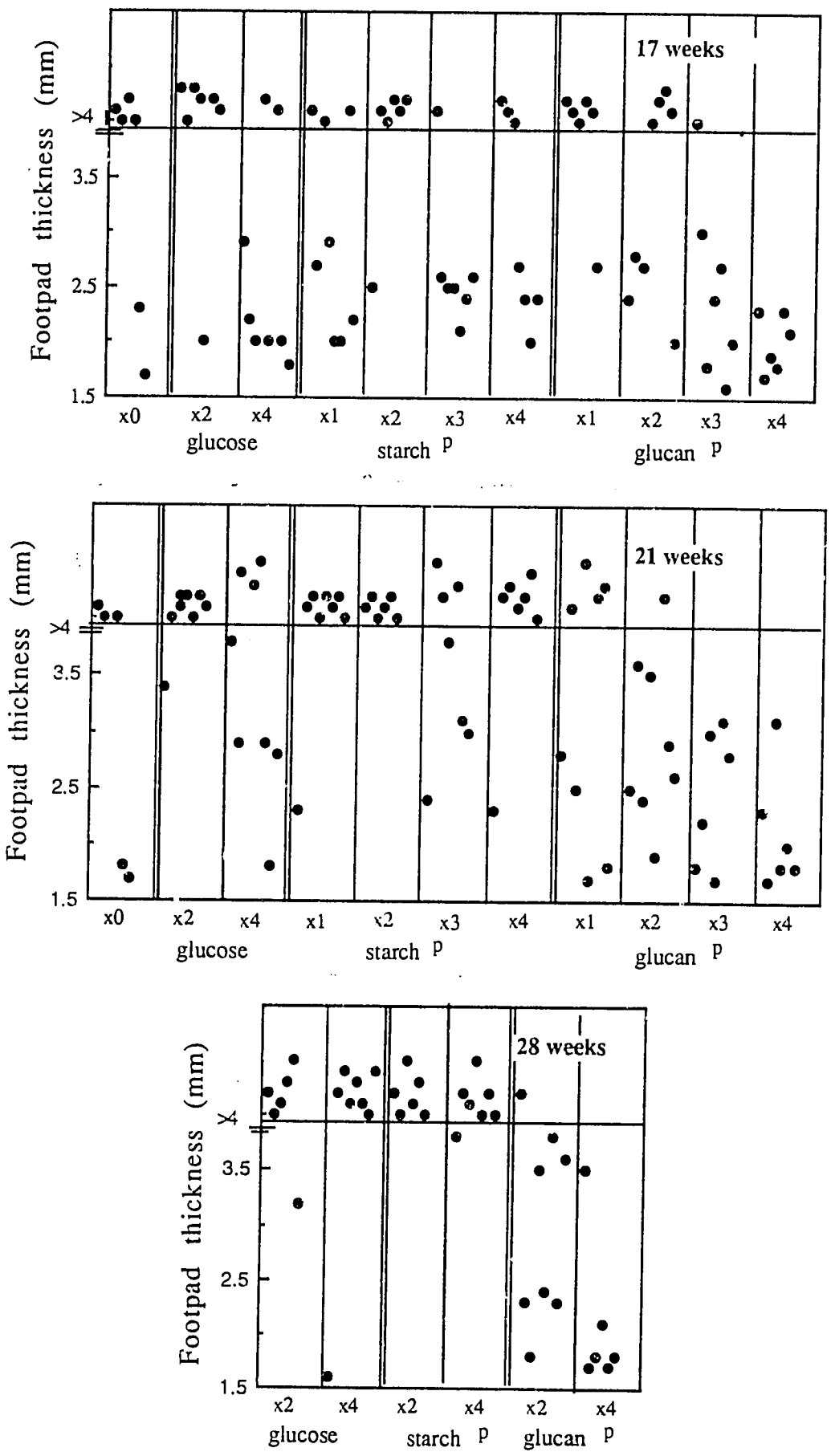
17 weeks
Footpad thickness (mm)
>4
3.5
2.5
1.5
x0
x2
x4
x1
x2
x3
x4
x1
x2
x3
x4
glucose
starch
p
glucan
p
21 weeks
Footpad thickness (mm)
>4
3.5
2.5
1.5
x0
x2
x4
x1
x2
x3
x4
x1
x2
x3
x4
glucose
starch
p
glucan
p
28 weeks
Footpad thickness (mm)
>4
3.5
2.5
1.5
x2
x4
x2
x4
x2
x4
glucose
starch
p
glucan
p

Figure 1

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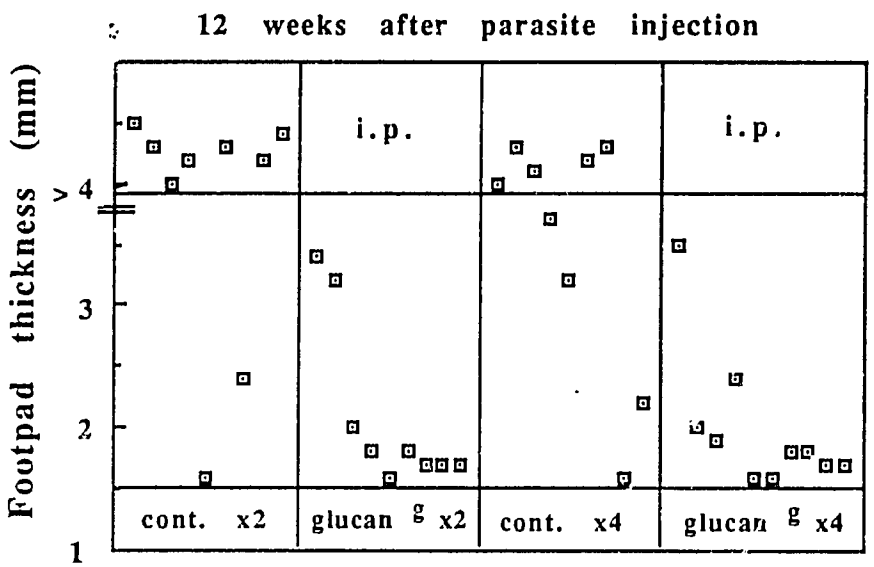
12 weeks after parasite injection
Footpad thickness (mm)
> 4
3
2
1
i.p.
i.p.
cont. x2
glucan g x2
cont. x4
glucan g x4

Fig. 2

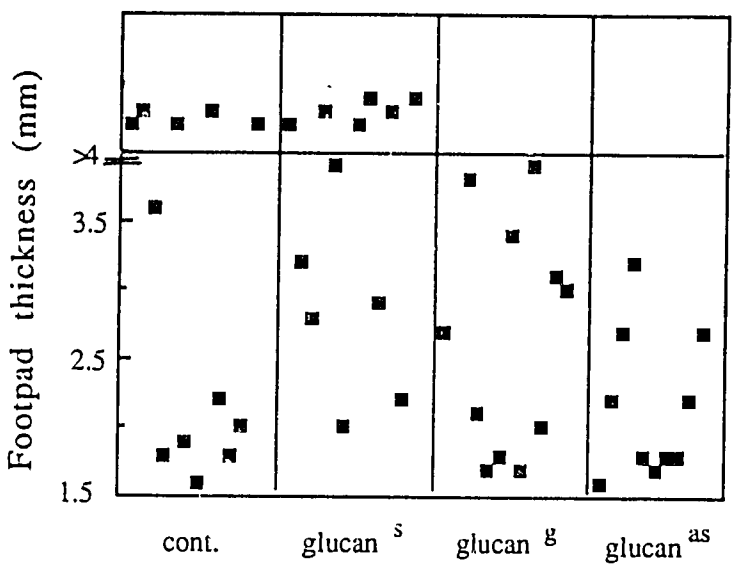
Footpad thickness (mm)
>4
3.5
2.5
1.5
cont.
glucan s
glucan g
glucan as

Figure 3