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Biological activity of *Pityrosporum*. I. Enhancement of resistance in mice stimulated by *Pityrosporum* against *Salmonella typhimurium*

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Summary. The effect of administration with *Pityrosporum (P. orbiculare, P. ovale, P. pachydermatis* and *Pityrosporum* sp.) on susceptibility of mice to *Salmonella typhimurium* infection was studied. Pretreatment of mice with 50 mg (wet weight) of killed *Pityrosporum* 4 days prior to the intraperitoneal (i.p.) challenge of 4×10^5 (10 LD_{50}) *S. typhimurium* elicited resistance comparable to that induced by 500 μg (dry weight) of killed *Propionibacterium acnes* and over 30% of the infected mice survived. Among the species tested, *P. pachydermatis* was slightly less effective. The challenged organisms were not detected from the blood of mice treated with *Pityrosporum* but were present in the liver and spleen in approximately level amounts (10^4–10^5/organ) during the course of testing. These results suggest that the increased resistance in mice is the result of stimulation of the reticuloendothelial system by *Pityrosporum*.

INTRODUCTION

Propionibacterium (Marples, 1969), *Staphylococcus* (Kloos & Musseluhite, 1975) and *Pityrosporum* (Noble & Somerville, 1974; Sloof, 1970) are resident organisms on normal human skin. *Pityrosporum orbiculare* and *Pityrosporum ovale* are not only saprophytic organisms but also the probable aetiologic agents of tinea versicolor (Faergemann, 1979) and seborrheic dermatitis (McGinley *et al.*, 1975). Among these organisms, *Propionibacterium* is a well-known potent stimulator of the reticuloendothelial system and administration of the organism into experimental animals results in an enhancement of the host resistance to certain bacteria (Miyata, Nomoto & Takeya, 1980) and viral infection (Glasgow *et al.*, 1977) and transplantation tumour cells (Halpern, 1975). Thus far, the cultivation of *Pityrosporum*, particularly *P. orbiculare*, has been regarded as difficult due to lack of an appropriate culture medium. Recently, however, we developed an appropriate enrichment medium (Ushijima, Takahashi & Ozaki, 1981) and used this medium to biochemically and serologically characterize the organisms (Takahashi, Ushijima & Ozaki, 1981).

We have already reported that *Pityrosporum* cells activate complement through an alternative pathway and in doing so generate macrophage chemotactic factors (Takahashi, Ushijima & Ozaki, 1984). We now report the effect of treatment of mice with *Pityrosporum* cells on the susceptibility to *Salmonella typhimurium* infection.

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MATERIALS AND METHODS

Mice

Outbred ICR mice of both sexes at 5–6 weeks of age were used.

Pityrosporum

P. orbiculare (KMCH 1226), *P. ovale* (KMCH 1166), *P. pachydermatis* (KMCH 1189) and *Pityrosporum* sp. (KMCH 1224) kindly provided by Dr Soh, Kobe Municipal Central Hospital, Japan were maintained on the preservation medium (Ushijima *et al.*, 1981). The fresh cultured cells were collected in 0·1% Tween 80 solution, washed several times with distilled water and killed by heating at 60° for 15 min. In some cases, the cells were lyophilized and stored in a dessicator until use.

Bacterium

The *Salmonella typhimurium* LT-2 used was originally obtained from the Department of Microbiology, School of Medicine, Keio University, Japan and maintained in our laboratory for 7 years by growing on heart infusion broth (Nissui). The fresh cultures prepared by growing in the same medium at 37° for 24 hr were used for the present experiment. The number of viable cells were counted by plating samples onto heart infusion agar. The 50% lethal dose (LD_{50}) for mice determined by i.p. route of infection was approximately 4×10^4 viable cells.

Procedures for the treatment by Pityrosporum to mice

(a) To examine the dose-response effect, mice were injected with 0·5 ml of suspension of *P. ovale* containing 2, 10, 20 and 100 mg (wet weight) of cells/ml 4 days prior to the challenge of *S. typhimurium*. (b) To determine the time factor for pre-treatment, mice were given i.p. 5 mg (wet weight) of *P. ovale*, 14, 10, 7, 4 and 1 days prior to the challenge. (c) To compare the protective effect among various species of *Pityrosporum*, mice were given 5 mg (wet weight) of *P. orbiculare, P. ovale, P. pachydermatis*, or *Pityrosporum* sp. 4 days prior to the challenge. Dose of *S. typhimurium* used for challenge to mice were 10 LD_{50} ($4–5 \times 10^5$ cells). In all tests, ten to thirty mice per group were used for the calculation of survival rate. In these tests, 1 ml of 10% proteose peptone (Daigo), 0·1% glycogen (Oyster), 3% thioglycollate medium (BBL), phosphate buffered saline (PBS) at pH 7·4 or 500 μg (dry weight) of *Propionibacterium acnes* were used as negative or positive controls for pre-treatment.

Enumeration of bacteria in infected mice

Mice were inoculated i.p. with 4×10^4 cells (1 LD_{50}) of *S. typhimurium* 4 days after pre-treatment with 5 mg (wet weight) of *P. ovale*, 500 μg (dry weight) of *P. acnes*, 1 ml of 0·1% of glycogen or 0·5 ml of distilled water. At various intervals, the liver and spleen were removed aseptically from each group of mice, homogenized in saline, and the homogenate was used for enumeration of bacteria. Ascitic fluid was obtained by washing the peritoneal cavity with saline. The blood sample was taken from the inferior vena cava and immediately diluted with saline. These test samples were appropriately diluted and then placed onto BTB agar (Nissui). After the incubation at 37° for 24 hr, the number of colonies produced by *S. typhimurium* was counted.

RESULTS

Dose–response effect of *Pityrosporum* on the mortality of mice

As described in the section of methods, various amounts of *P. ovale* were inoculated into mice 4 days prior to the challenge of *S. typhimurium* and the mortality rate recorded for 10 days. The mortality rate of mice treated with *Pityrosporum* was constantly lower than that of control mice. Although the survival rate reduced with decreases in the dose, 45% of mice treated with 5 or 10 mg of the organism were alive on day 10 after infection. Even a dose of 1 mg had a slight effect on the elongation of death time, but the survival rate on day 10 after infection was the same as that in control groups (Fig. 1).

Effect of interval between pre-treatment and challenge on the mortality of mice

To elicit the suitable interval from pre-treatment to challenge, mice were challenged with *S. typhimurium* at various intervals after the pre-treatment by *P. ovale* and the survival rate scored for 10 days after infection. The most effective protection was obtained in the group treated 4 days prior to the challenge. Even treatment given in 1 day prior to the challenge induced a protective effect, although the effect reduced as compared with the case of pre-treatment 4 days prior to the challenge.

In contrast, when the pre-treatment was given 7, 10 or 14 days prior to the challenge, the protective effect was not found (Fig. 2).

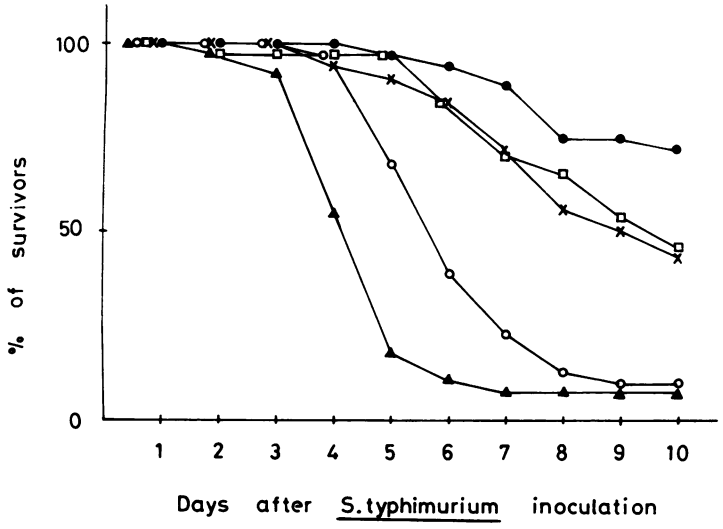

Figure 1. Effect of doses in *Pityrosporum* pre-treatment on the mortality of mice. Mice were given i.p. 50 mg (wet weight) (●), 10 mg (×), 5 mg (□), 1 mg (○) of *P. ovale*, or 0·5 ml of distilled water (▲), 4 days prior to the challenge i.p. with 4×10^5 (10 LD_{50}) *S. typhimurium*.

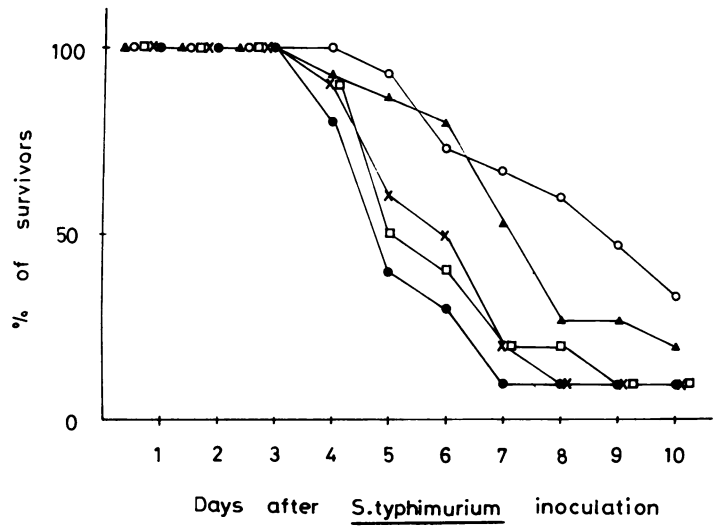

Figure 2. Effect of intervals of *Pityrosporum* pre-treatment on the mortality of mice. Mice were given i.p. 5 mg (wet weight) of *P. ovale* 14 (●), 10 (×), 7 (□), 4 (○) or 1 day (▲) prior to the challenge i.p. with 4×10^5 (10 LD_{50}) *S. typhimurium*.

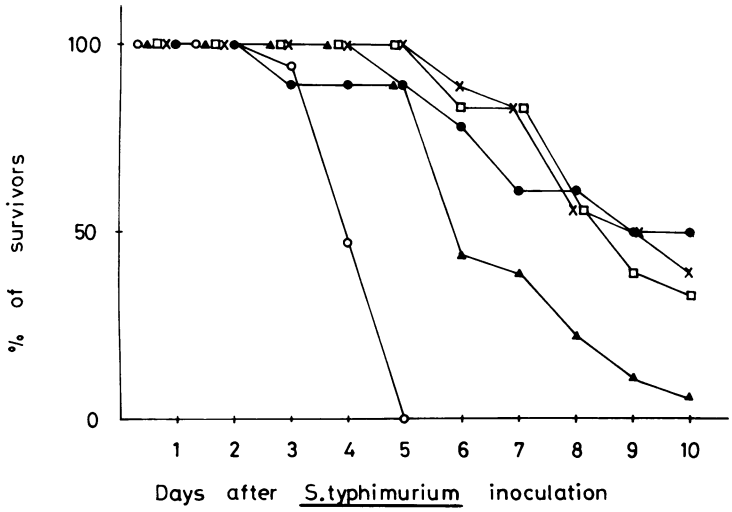

Figure 3. Comparison of the protective effect in mice among species of *Pityrosporum*. Mice were given i.p. with 5 mg (wet weight) of *P. orbiculare* (●), *P. ovale* (□), *P. pachydermatis* (▲), *Pityrosporum* sp. (×) or 0·5 ml of distilled water (○) 4 days prior to the challenge i.p. with 4×10^5 ($10\ LD_{50}$) *S. typhimurium*. The mortality in each group of mice was scored daily × 10.

Comparison of the protective effect on mice among species of *Pityrosporum*

The protective effect of each species was compared among *P. orbiculare, P. ovale, P. pachydermatis* and *Pityrosporum* sp. The experiments were carried out according to the procedures described in the preceding section. As shown in Fig. 3, in mice treated with *P. orbiculare, P. ovale* and *Pityrosporum* sp. the survival rates were virtually the same, but *P. pachydermatis* was comparatively less effective.

No enhanced protection was observed in groups of mice treated with proteose peptone, glycogen, or thioglycollate medium, and all mice died within 12 days after infection. All mice treated with *P. acnes* survived. Pre-treatment with *P. orbiculare* was slightly

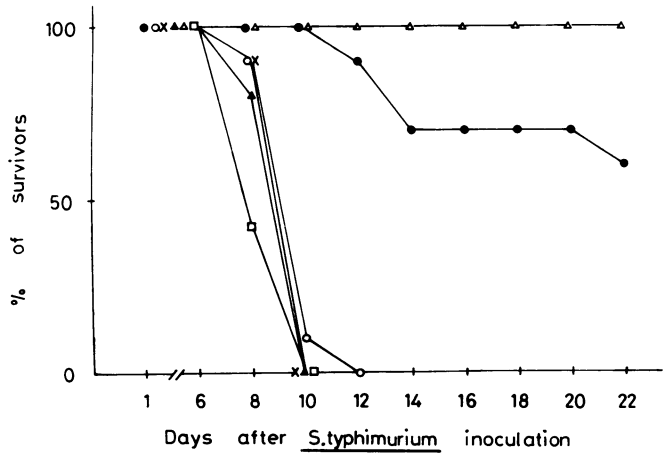

Figure 4. Comparison of the protective effect in mice among macrophage-stimulating agents. Mice were given i.p. 5 mg (dry weight) of *P. orbiculare* (●), 500 μg (dry weight) of *P. acnes* (△), 1 ml of 10% proteose peptone (○), 1 ml of 0·1% glycogen (×), 1 ml of 3% thioglycollate medium (▲), or 1 ml of PBS (□) 4 days prior to the challenge i.p. with 4×10^5 ($10\ LD_{50}$) *S. typhimurium*. The mortality in each group was scored daily × 22.

less effective as compared with *P. acnes*. These results are shown in Fig. 4.

Inhibition of the bacterial growth in mice treated with *Pityrosporum*

The preceding experiments demonstrated that pre-treatment of mice with *Pityrosporum* enhanced markedly their resistance against infection with *S. typhimurium*. The next experiment was designed to examine the effect of pre-treatment on the growth of challenged organisms in mice. The experimental procedures are outlined in the preceding section. In this series of experiments, *P. acnes*, glycogen and distilled water were used as the positive and negative control stimulators. The number of bacteria in each sample from mice treated with glycogen and distilled water increase up to 6 days after infection and then decreased gradually, whereas no significant change in the number of bacteria was observed in mice treated with *P. ovale* or *P. acnes* during the course of infection (Fig. 5). Despite the occurrence of a high level of bacteremia in mice treated with glycogen or distilled water, this bacteremia was completely inhibited by treatment with *P. ovale* or *P. acnes*.

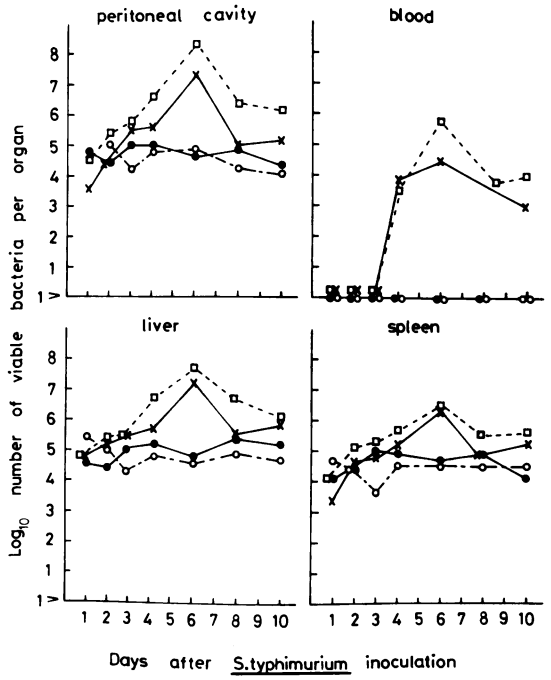

Figure 5. Effect of macrophage-stimulating agents on the growth of *S. typhimurium* in mice. Mice were given i.p. 5 mg (wet weight) of *P. ovale* (●), 500 μg (dry weight) of *P. acnes* (○), 1 ml of glycogen (×), or 0·5 ml of distilled water (□) 4 days prior to the challenge i.p. with 4×10^4 (1 LD_{50}) *S. typhimurium*. The number of bacteria in the liver, spleen, blood and peritoneal cavity was checked daily × 10.

DISCUSSION

The present experiment showed that mice given i.p. killed *Pityrosporum* acquire a high-level resistance against *S. typhimurium* infection. Since *S. typhimurium* is a facultative intracellular parasite, activation of macrophage seems to be essential for development of resistance on mice against infection of the organism (Collins, 1974).

In a preceding paper, we found in *in-vitro* experiments that treatment of the serum with killed *Pityrosporum* resulted in activation of complement, through an alternative pathway and chemotactic factors for macrophages were generated (Takahashi *et al.*, 1984). In addition, the injection of killed *Pityrosporum* into mice gave rise to a remarkable increase in peritoneal exudate cells. On day 4 after the injection, 70% of the cells were not only macrophages but also had a high bactericidal activity as compared with those stimulated by thioglycollate medium or glycogen, compounds which in themselves are stimulatory for peritoneal macrophage (Ögmundsdottir & Weir, 1980).

We conclude that the increased resistance of mice injected with *Pityrosporum* resulted mainly from the activation of both fixed and free macrophages, cells considered to be main protective ones in the early phase of infection (Mitsuyama *et al.*, 1978) as in cases of immunization of mice with *P. acnes* (Miyata *et al.*, 1980). Although the survival rate of mice treated with *Pityrosporum* was slightly lower than that of mice treated with *P. acnes*, the clearance of bacteria from the blood and the inhibition of bacterial growth in the liver and spleen occurred at almost the same levels in mice treated with *Pityrosporum* and *P. acnes*. Since *P. acnes* is a good stimulator and activator of the reticuloendothelial system (Halpern, 1975), these results strongly suggest that *Pityrosporum* also has excellent stimulating and activating function.

P. orbiculare, together with *P. acnes*, is a most dominant organism on normal human skin. This coincides with data that indicates that antibodies to *Pityrosporum* are widely distributed in humans (Furukawa *et al.*, 1981). Thus, as in the case of *P. acnes*, *Pityrosporum* may also facilitate certain general resistance mechanisms in humans.

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