Homeostasis as Regulated by Activated Macrophage. VI. Protective Effect of LPSw (a Lipopolysaccharide from Wheat Flour) against Acute Infection by *Toxoplasma gondii* in Mice

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An oral administration of partially purified LPSw, a lipopolysaccharide (LPS) from wheat flour, at a concentration of 20 ng/ml in drinking water beginning 1 d after infection significantly decreased mouse mortality and prevented animal weight loss in acute infection with *Toxoplasma gondii*. Whereas 71% (5/7) of mice in a control group that did not receive LPSw died of toxoplasmosis, only 14% (1/7) of mice treated with LPSw died ($p<0.05$). The administration of LPS purified from *Bordetella pertussis* also significantly decreased the mortality of infected mice. LPS from *Escherichia coli* and synthetic lipid A (LA-15-PP(506)), however, did not show a significant decrease in mortality.

Keywords *Toxoplasma gondii*; macrophage; lipopolysaccharide (LPS); LPSw; toxoplasmosis; homeostasis

Introduction

Toxoplasma gondii is an obligate intracellular parasite and an important pathogen in man and animals. Cell-mediated immunity is considered to be the major mechanism of resistance against *Toxoplasma gondii*.[1)] We reported that interferon gamma (IFN-γ) is the major mediator of cell mediated immune responses against toxoplasmosis.[1a,2)] IFN-γ can activate macrophages to effectively phagocytize and kill tachyzoites.[3)] It was also reported that IFN-γ can inhibit proliferation of the organisms in human fibroblasts.[4)] These facts suggest that immunomodulators which induce or increase IFN-γ production could be beneficial in the treatment of acute toxoplasmosis through macrophage activation.

We have been studying the reproduction of "ontogenic inflammation" in adults,[5)] by activating macrophages to make it ready for endogenous production of tumor necrosis factor (TNF) by IFN-γ,[6)] bacterial lipopolysaccharide (LPS)[7)] and other agents.[6a)] As described in previous reports of this series,[7,8)] LPSw (a LPS from wheat flour) can activate macrophages without harm by oral or percutaneous administration,[7)] offering a high protective effect against the incidence of ulcer[8a)] and diabetes.[8b)]

These facts led us to examine whether the oral administration of LPSw can protect against acute toxoplasmosis, using a murine model. Our results revealed that the oral administration of LPSw to mice decreased mortality on infection.

Materials and Methods

LPS LPS from *Escherichia coli* (*E. coli*) 0127:B8 was purchased from Difco Lab. (Detroit, U.S.A.). The LPS from *Bordetella pertussis* (*B. pertussis*) was purified by the conventional method of Westphal *et al.*[9)] The method of LPS preparation from wheat flour was described in detail previously.[7)] Briefly, low molecular fractions (<5 kDa (kilodalton)) were excluded from water extracts of wheat flour by ultrafiltration. This preparation used in this experiment contained 0.01—0.1% of LPSw. The dose of LPSw in this experiment were estimated by Limulus reaction (Toxicolor; Seikagaku Co., Tokyo, Japan).

LPSw was solubilized in distilled water and diluted LPSw solutions (20 or 200 ng/ml) were administered to BALB/c mice in drinking water beginning 1 d after challenge infection. A solution containing 20 ng/ml of LPS of either *E. coli*, or *B. pertussis*, or synthetic lipid A (LA-15-pp(506); Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) was also administered to mice in the same manner as LPSw. Control mice received distilled water for drinking water.

Animals and Toxoplasma Female BALB/c and ddY mice obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan) were 8 weeks of age when used. Each experimental group contained 7 or 9 mice.

Tachyzoites of the virulent C56 strain (kindly supplied by Dr. Jack S. Remington, Stanford Univ.) were used for challenge infection. They were obtained from the peritoneal fluid of ddY mice that had been inoculated 7 d earlier with brain tissue from ddY mice chronically infected with the C56 strain as described previously.[1b)] BALB/c mice were inoculated intraperitoneally with 5×10^5 or 1×10^5 tachyzoites.

Statistical Analysis Levels of significance of observed differences between groups of mice were determined using χ^2-test for mortality and Student's *t*-test for body weight.

Results

Protective Effect of LPSw against Toxoplasma We have reported that orally administered LPSw can induce primed macrophages ready for TNF-production.[7)] Therefore, we tested the protective effect of LPSw against toxoplasma by the oral route, using its crude sample.

Oral administration of 20 ng/ml of LPSw (as estimated by Limulus reaction) in drinking water *ad libitum* significantly decreased mouse mortality in acute infection with 1×10^5 tachyzoites of the C56 strain (Fig. 1). Seventy-

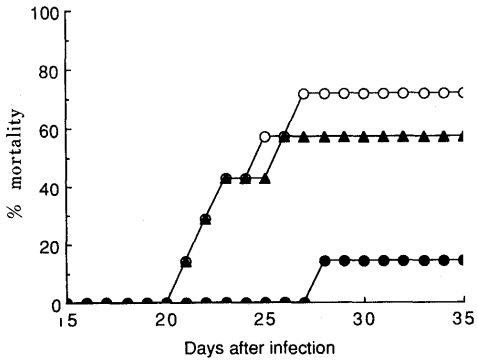

Fig. 1. Effect of Oral Administration of LPSw on Acute Toxoplasmosis in Mice

BALB/c mice were orally administered with solutions of LPSw at concentrations of either 200 (▲) or 20 ng/ml (●) in drinking water from 1 d after infection. Control mice (○) received distilled water for drinking. A challenge infection was performed by intraperitoneal injection of 1×10^5 tachyzoites of the C56 strain of *Toxoplasma gondii*.

TABLE I. Effects of Oral Administration of Various LPS against Acute Toxoplasmosis in Mice

Drinking water[a]	% mortality (number of mice dead/number of mice tested)
Distilled water	44 (4/9)
LPSw	0 (0/7)[b]
LPS (*Bordetella pertussis*)	0 (0/7)[b]
LPS (*Escherichia coli*)	14 (1/7)
Lipid A	14 (1/7)

a) A 20 ng/ml solution of each LPS was administered orally to BALB/c mice in drinking water from 1 d after infection. Infection was achieved by an intraperitoneal injection of 1×10^5 tachyzoites of the C56 strain. *b*) Significantly different from control mice that received distilled water at $p < 0.05$ (χ^2-test).

one percent (5/7) of the control mice died of toxoplasmosis after challenge infection, whereas only 14% (1/7) of those treated with 20 ng/ml of LPSw died ($p < 0.05$). In contrast to mice treated with 20 ng/ml of LPSw, 57% (4/7) of those treated with 200 ng/ml of LPSw died after infection (Fig. 1). There was no difference in time to death between the control and the experimental group treated with 200 ng/ml of LPSw.

In challenge infection with 5×10^5 tachyzoites, all mice died of toxoplasmosis regardless of whether 20 or 200 ng/ml of LPSw was administered. There was no difference as to time to death of mice between the control and experimental groups at this high dose of infection (data not shown).

Comparison of Protective Effect of Other LPSs The effect of LPSw against acute toxoplasmosis was compared with that of other LPSs purified from bacteria, *E. coli* and *B. pertussis* and synthetic lipid A which is the active site of endotoxic activity of LPS. A solution of 20 ng/ml of each compound was orally administered in drinking water beginning 1 d after infection with 1×10^5 tachyzoites. Four of 9 mice in the control group died of toxoplasmosis between days 23 and 27 after infection (Table I). The administration of either LPSw or LPS from *B. pertussis* prevented the death of infected animals ($p < 0.05$), whereas LPS from *E. coli* and lipid A did not show any significant protective effect (Table I). In addition to preventing death, treatment with LPSw significantly prevented the weight loss of mice during acute infection. On day 30 of infection, the average body weight of mice treated with LPSw was 17.1 ± 1.74 g (mean $\pm$ SD), whereas that of control mice was 12.3 ± 0.86 g ($p < 0.001$). It is to be noticed that administration of LPS from *B. pertussis* did not show any significant effect in preventing weight loss in infection (data not shown) although it significantly decreased mouse mortality (Table I).

Discussion

In the present communication we have shown that LPSw can protect mice from toxoplasma infection when administered orally *ad libitum*, dissolved in drinking water. This finding suggests that similar protection can be expected from infections due to either parasitic protozoa or other organisms causing opportunistic infections.

Furthermore, oral administration in drinking water using only a small amount of LPSw (about 20 ng/ml) is successful. When the effect of LPSw is compared with that of other LPSs derived from *E. coli* (5—50 kDa) and *B. pertussis* (4 kDa), LPSw is most efficacious for the least weight loss and superior to LPS of *E. coli* (Table I) which cannot be given orally. Consumption of large amounts of wheat flour or bread may unexpectedly provide protection against infection or unnoticed invasion of foreign agents because LPSw is contained in these foodstuffs.

The crude LPSw preparation used in this experiment contained an LPSw concentration 20 to 200 fold that present in wheat flour and included a large amount of contaminant composed of polyxylan and proteins. This contaminant is not active, since wheat flour which contained the same amount of the contaminant as this crude preparation is inactive (data not shown). Comparable amounts of LPS purified from *B. pertussis* showed an effect similar to LPSw.

The optimal dose of LPSw in mice was extremely low (20 ng/ml) while the high dose (200 ng/ml) was even toxic in this experiment. This observation is consistent with the fact that an effect of recombinant TNF in mice against acute toxoplasmosis is contraversial,[10] though the real cause remains to be ascertained.

We do not yet know the precise mechanism of this protective effect, however, a weak inflammation induced by LPS may be responsible, since we have found that LPSw can reproduce "ontogenic inflammation"[5] in adults even when administered orally.[7] The inflammation produced may contain IFN-γ, TNF or other cytokines, especially activated macrophages at primed stage ready for endogenous TNF production, thus regulating the homeostasis of the living body.

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