Orally Administered *Lactobacillus gasseri* TMC0356 Inhibits Growth of Tumors Transplanted into Mice

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*Lactobacillus gasseri* TMC0356 (TMC0356), a probiotic strain originally isolated from the human intestine, was tested for its anti-tumor activities in vivo in murine tumor models. Oral administration of TMC0356 characteristically inhibited the growth of sarcoma S-180 (S-180) and hepatoma H22 (H22) cells, which had been implanted in mice. Serum gamma interferon was significantly enhanced during TMC0356 administration of 1,000 mg/kg in S-180-bearing mice (p<0.01). Serum levels of tumor necrosis factor-α and interleukin-18 were also altered, though these changes were not statistically significant. These results indicate that the anti-tumor effects of TMC0356 might, at least in part, arise from the impact of this bacterium on cell-mediated immunity in host animals.

Key words: *Lactobacillus*; sarcoma; pro-inflammatory cytokines

Cancer is a leading cause of death worldwide. It accounted for 4.9 million deaths (around 13% of all deaths) in 2007, thus ranking it the second deadliest disease after heart disease (20). Chemotherapy, the use of medications to treat cancer, currently plays a major role in cancer treatment. Recently, many findings in clinical nutrition have indicated that nutrition could be used as an adjunct to cancer treatment, because many food components have the potential to enhance host immunity and improve the physical function.

Fuller (3) defined the term probiotic as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.” The definition of probiotic has broadened over the years as knowledge on the symbiotic interaction between microbes and host animals has increased (4, 18, 19). At present, the most appropriate definition, as published by an Expert Consultation Committee at a meeting convened by the Food and Agriculture Organization (FAO)/WHO in 2001, is as follows: “Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host.” Most current probiotics are lactic acid bacteria. Emerging evidence from recent clinical and animal studies supports the notion that lactobacilli, especially some selected strains, can modify host immune responses and protect against cancer (1, 10, 17).

In this study, *Lactobacillus gasseri* TMC0356 (TMC0356) was tested for its potent suppressive effect on the proliferation of sarcoma S-180 (S-180) and hepatoma H22 (H22) cells implanted in mice. Serum levels of pro-inflammatory cytokines were analyzed to obtain additional information on the mechanisms underlying the anti-tumor effects exhibited by TMC0356.

Six-week-old female and male ICR mice, weighing 18 ± 2 g, were purchased from the Changchun Institute of Biological Products (Changchun, China). The mice were housed under normal laboratory conditions (24 ± 2 °C, 50 ± 20%, 12/12-hr light/dark cycle) and with free access to standard diet (J12003-0008, Changchun Institute of Biological Products, Changchun, China) and water. This study was conducted based on the Guidelines for Use and Care of Laboratory Animals (The Prime Minister’s Office announcement No. 6, Japan, 1980).

TMC0356 was originally isolated from a healthy adult intestine and has been stored at the Technical Research Laboratory of Takanashi Milk Products Co., Ltd (Yokohama, Japan). TMC0356 was routinely cultured at 37°C for 18 hr in MRS broth (Becton, Dickinson and Company, MD, USA). After incubation, the bacteria were collected by centrifugation (7,000 × g) and washed three times with sterilized 0.85% NaCl. The washed bacteria were lyophilized, and kept at −80°C until use [available bacterial count: >1.0 × 10¹¹ colony-forming units (CFU)/g].

S-180 and H22 cell lines were purchased from the Jilin Institute of Tumor Research (Changchung, China). S-180 and H22 cells were implanted according to the methods...
described by Li et al. (11). Briefly, sarcoma cells (1.0 × 10^8 cells/ml) were diluted to 4-fold with 0.9% NaCl. The diluted solution was inoculated subcutaneously in the right armpit of the mice (0.2 ml per mouse). These tumor-bearing mice were divided into five groups (n=10 per group, female or male, 6 weeks old) control group (normal saline), positive control group [30 mg/kg cyclophosphamide (CTX)], and three groups of TMC0356 administration at dosages of 1,000, 500, or 250 mg/kg/day. The mice were administered intragastrically the tested bacteria and CTX once a day for 10 days. They were sacrificed on day 11, and sera, tumors, spleen, and thymus were collected. The inhibition rate of tumors was calculated using the following equation:

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\text{Inhibition rate (％)} = \left( \frac{(C-T)}{C} \right) \times 100, \quad \text{where} \quad C \text{ is the average weight of tumor in the control group and} \quad T \text{ is the average weight of tumor in the treatment group.}
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The organ index was calculated as organ weight (mg) divided by body weight (g). Each experiment was repeated three times.

Serum was obtained after incubation at room temperature and centrifugation at 10,000 × g for 15 min. Serum gamma interferon (IFN-γ) was tested with a commercial ELISA kit (GE Healthcare, UK) and tumor necrosis factor α (TNF-α) and interleukin-18 (IL-18) were also examined with a commercial ELISA kit (Biowen, Canada).

Data were expressed as mean ± standard deviation (SD), and the values were compared between the treatment and control groups using Student’s t-test. The level of significance was set at \( p<0.05 \)–0.01 (Student’s t-test).

The growth of S-180 tumors in mice treated orally with TMC0356 was significantly suppressed compared to those treated with saline (\( p<0.01 \)) (Table 1). The inhibition rates of S-180 tumors were 34.88%, 36.44%, and 23.21% at TMC0356 doses of 1,000, 500, and 250 mg/kg, respectively. TMC0356 at a dose of 1,000 mg/kg also significantly inhibited the growth of H22 (\( p<0.05 \)) (Table 2) at an inhibition rate of 26.54%. Oral administration of TMC0356 did not significantly alter the spleen indexes of S-180 and H22 bearing mice and the thymus indexes of S-180 and H22 bearing mice (Figs. 1 and 2). The thymus index was significantly increased in H22 bearing mice exposed to TMC0356 (Fig. 2). However, CTX treatment significantly decreased all of these indexes in both S-180 and H22 bearing mice as compared to the control group (\( p<0.05 – 0.01 \)) (Figs. 1 and 2).

The concentration of serum IFN-γ was significantly greater in mice fed with TMC0356 at a dose of 1,000 mg/kg than in the other groups (\( p<0.001 \)) (Fig. 3). However,
no significant differences in serum IFN-γ were observed between the H22 bearing mice treated with TMC0356 and those treated with CTX (data not shown).

The production of serum TNF-α and IL-18 in TMC0356 and CTX groups was also analyzed and compared with the control group. However, no significant differences were found between the tested groups (data not shown).

TMC0356 was originally isolated from the intestine of a healthy human adult (5). This bacterium activates macrophages in the host to produce the pro-inflammatory cytokine IL-12, which promotes development of Th1 immunity, characterized by elevated production of IFN-γ (13). In several animal and human studies, TMC0356 significantly improved allergic symptoms in patients with Japanese cedar pollinosis and down-regulated IgE metabolism in OVA-immunized mice and allergic persons with high serum IgE levels (8, 9, 14). These anti-allergic effects of TMC0356 are believed to partly result from its IFN-γ-dependent immunomodulatory effects in host animals, because IFN-γ is a cytokine that plays a physiologically important role in promoting innate and adaptive immune responses.
adaptive immune responses. Recently, an IFN-γ role in preventing the development of primary and transplanted tumors was documented, though the mechanism by which this cytokine achieves its effects remains unclear (6). In light of these observations, we hypothesize that TMC0356 might exert anti-tumor effects by promoting the cytolytic activity of natural killer (NK) cells and cytolytic T lymphocytes following TMC0356 stimulation of IFN-γ production.

In the present study, oral administration of TMC0356 significantly inhibited the growth of S-180 and H22 tumors in tumor-bearing mice, although observed anti-tumor activity of TMC0356 was less than that of CTX, a commonly used anti-tumor agent in various pre-clinical and clinical studies. Several studies have been published in which lactobacilli were found to have anti-tumor effects against the tumor sarcoma-180 in vivo (7, 12), but these lactobacilli were injected intralesionally. To our knowledge, the present study is the first to indicate that orally administered selected lactobacillus strains, such as TMC0356, can exhibit anti-tumor effects against the growth of tumors implanted in mice.

The spleen and thymus indexes of normal healthy mice were 5.16 ± 0.96 and 3.24 ±1.07, respectively. These indexes were slightly increased after tumor transplantation which could be the result of one kind of immunological self defence response to the transplanted tumor. Interestingly, the spleen and thymus indexes were significantly suppressed in the CTX-treated mice, whereas oral administration of TMC0356 did not significantly alter them. CTX is a well known chemotherapy drug that is given as a treatment for many types of cancer to stop cell growth. On the other hand, CTX is also an immunosuppressive medication that can decrease the immune system’s response to various diseases. The decreased spleen and thymus indexes caused by CTX in the present study may be an aspect of the negative impact of CTX on host immunity. Degeneration and atrophy of immune organs influences the normal functioning of the entire immune system. For example, the spleen serves as a reservoir for blood, and filters and purifies the blood and lymph fluid that flows through it. When it is damaged or removed, susceptibility to infection increases (21). The present results indicate that the mechanisms by which TMC0356 and CTX exert their anti-tumor effects might be different. The negative effect of TMC0356 on immune organs in the host animal seems to be less than that of CTX in terms of spleen and thymus sizes.

In the present study, serum IFN-γ significantly increased in tumor-bearing mice after oral administration of TMC0356 at a dose of 1,000 mg/kg, as it did in our previous studies (13, 14). Therefore, the anti-tumor effects of TMC0356 might be partly due to production of IFN-γ being enhanced by TMC0356, at least in the tested mice fed 1,000 mg/kg of TMC0356, which in turn could enhance the cytolytic activity of NK cells and cytolytic T lymphocytes following TMC0356 stimulation of IFN-γ production.

IL-18 (IFN-γ-inducing factor) is a novel pro-inflammatory cytokine that augments NK activity in spleen cells (15). This cytokine can induce IFN-γ production by T lymphocytes. The combined activities of IL-18 and IL-12 have been shown to inhibit IL-4-dependent IgE and IgG1 production, and enhance IgG2a production by B cells. In the present study, no significant
differences in serum IL-18 were found among the test groups (data not shown). TNF-α can induce apoptotic cell death and inflammation, and inhibits tumorigenesis and viral replication in a manner different from that of IFN-γ (16). A slight increase in serum TNF-α was found in some tumor-bearing mice after treatment with TMC0356, but these changes were not significant (data not shown). Therefore, the immunomodulatory effects of TMC0356 still remains unclear and further studies should be conducted to clarify the mechanisms of TMC0356 effects on host immunity. Additional clinical evidence should be obtained to support its anti-tumor effects observed in animal studies.

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