

Antiallergic Effects of *Lactobacillus pentosus* Strain S-PT84 Mediated by Modulation of Th1/Th2 Immunobalance and Induction of IL-10 Production

Yuji Nonaka^a Takayuki Izumo^a Fumi Izumi^a Toshihiro Maekawa^a
Hiroshi Shibata^a Aoi Nakano^b Atsuko Kishi^b Kaoru Akatani^b Yoshinobu Kiso^a

^aInstitute for Health Care Science, Suntory Ltd., Osaka, and ^bLouis Pasteur Center for Medical Research, Kyoto, Japan

Key Words

Lactobacillus pentosus strain S-PT84 • Lactic acid bacteria • Heat-killed • Interleukin-12 • Interleukin-10 • Immunoglobulin E • T helper type 1 • T helper type 2 • Natural killer cell • Active cutaneous anaphylaxis reaction

Abstract

Background: Many types of fermented food are consumed in Japan. Although some are produced by plant-origin lactic acid bacteria (LAB) fermentation, the physiological functions of such bacteria remain unclear. We therefore isolated LAB of plant origin from Kyoto pickles and determined the immunological activity of heat-killed preparations of plant-origin LAB. **Methods:** The *Lactobacillus pentosus* strain S-PT84 was selected from among 16 LAB of plant origin as the strongest interleukin (IL)-12-inducing strain. IL-12- and IL-10-inducing activities were determined with macrophages from BALB/c mice. The in vivo immunomodulating effect of S-PT84 was determined with BALB/c mice fed S-PT84. The antiallergic activity of S-PT84 was examined in ovalbumin (OVA)/alum-administered BALB/c mice. **Results:** The *L. pentosus* strain S-PT84 induced production of both IL-12 and IL-10 in vitro. S-PT84 enhanced splenic natural-killer activity and modulated the T helper (Th) type 1/type 2 balance to-

ward a Th1-dominant state. In the OVA-induced allergy model, orally administered S-PT84 lowered serum IgE levels and suppressed active cutaneous anaphylaxis reaction and splenic IL-4 production. IL-10 production from splenocytes of OVA-immunized mice was upregulated by feeding S-PT84. **Conclusions:** Despite heat-killing, S-PT84 exhibited antiallergic effects by modulating the Th1/Th2 balance and inducing regulatory T cells. The *L. pentosus* strain S-PT84, which is of plant origin and isolated from a traditional Japanese food, is expected to be useful for treatment of many immune diseases including allergies, tumors, infectious diseases and auto-immune diseases.

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Introduction

Allergic conditions such as atopic disease and hay fever are increasingly common chronic disorders, especially in developed countries, and are a grave social problem [1]. Allergy depends on T helper type 2 (Th2)-derived immune responses characterized by the production of cytokines interleukin (IL)-4, -5, and -13, which promote the production of immunoglobulin (Ig)E and other chemical mediators [2, 3]. Th1 cytokines inhibit the proliferation

of Th2 cells and suppress the switching of B cells. IL-12 is a Th1 maturation factor that stimulates natural killer (NK) cells and Th1 cells to produce interferon (IFN)- γ [4] and prevents the development of Th2 responses independently of IFN- γ [5]. Modulation of the levels of Th1 cytokines is thus expected to be beneficial in preventing and treating allergic diseases.

Numerous studies have indicated that some lactic acid bacteria (LAB) can modulate the Th1/Th2 balance toward a Th1-dominant state. LAB which live in the gastrointestinal tract can alter the microbiota and affect bacterial immunogenicity [6–8]. Ishida et al. [9] demonstrated that the live *Lactobacillus acidophilus* strain L-92 improved the symptoms of Japanese cedar pollinosis in a randomized, placebo-controlled clinical study. Perinatal administration of a strain of probiotic bacteria, live *L. rhamnosus* GG, significantly reduced the development of eczema during the first 2–4 years of life in children with a family history of atopic disease [10–12]. On the other hand, some studies have suggested that nonviable LAB can also show immunomodulatory activity, including induction of IL-12 from macrophages [13, 14], inhibition of IgE production in an ovalbumin (OVA)-induced food allergy model [15, 16], and increasing fecal antigen-specific IgA [17]. However, it is still unclear whether LAB must be alive to exhibit immunomodulatory activity.

Many types of fermented foods are consumed in Japan, such as pickles, soy sauce and miso (bean paste). These are produced by plant-origin LAB-mediated fermentation. LAB of plant origin exhibit broader hydrocarbon-assimilating activity than the more common LAB that live in the gastrointestinal tract and are used to make yogurt and cheese [18], though their physiological functions remain unclear. We have isolated 16 types of LAB of plant origin from Kyoto pickles. In this study, we selected *L. pentosus* strain S-PT84, the strongest IL-12 inducer among them, and examined its immunomodulatory activity (modulation of Th1/Th2 balance, NK activity, and IL-10-inducing activity) and antiallergic activity in OVA-immunized BALB/c mice using heat-killed cell preparations to determine whether LAB must be alive to exhibit immunomodulatory activity.

Materials and Methods

Preparation of Heat-Killed LAB

The bacterial strains used in this study were isolated from Kyoto pickles and kept at the Louis Pasteur Center for Medical Research. These bacterial strains were cultured in MRS broth (Difco Laboratories, Detroit, Mich., USA) at 35°C for 48 h. Cul-

tured bacteria were collected by centrifugation, washed twice with sterile saline, and heat-killed at 95°C for 5 min. Heat-killed bacteria were washed with distilled water, freeze-dried, and subjected to the following experiments. *L. pentosus* strain S-PT84, which has the strongest IL-12-inducing activity among LAB used in this study, was deposited at the National Institute of Advanced Industrial Plant Organism Depository, Patent Microorganisms Depository (Tsukuba, Japan) as FERM ABP-1002.

Animals

Male BALB/c mice (7 weeks of age) were purchased from Japan SLC Inc. (Shizuoka, Japan). These animals were housed with a commercial diet (CE-2) and tap water ad libitum for 1 week at 25 $\pm$ 1°C and 60 $\pm$ 5% humidity under a 12-h light-dark cycle before experimentation. Experiments were performed according to 'The Guidelines for the Care and Use of Experimental Animals of the Japanese Association for Laboratory Animals.'

IL-12-Inducing Activity in vivo

BALB/c mice were injected intraperitoneally with 0.5 mg of each heat-killed bacterial strain in 0.2 ml sterile saline. Blood was collected 6 h after injection and serum was prepared by centrifugation. IL-12 (p70) levels in serum were measured by ELISA as described below.

IL-12- and IL-10-Inducing Activities in vitro

BALB/c mice were injected intraperitoneally with 2 ml of 4.05% thioglycolate medium (Difco). Peritoneal macrophages were harvested 4 days after injection of the thioglycolate medium by lavage of the peritoneal cavity with 8 ml of ice-cold phosphate buffered saline (PBS). The peritoneal exudate cells were centrifuged and suspended in RPMI 1640 medium (Nacalai Tesque, Kyoto, Japan) with 10% FBS at a density of 2×10^6 cells/ml. The cells were precultured on 24-well plastic plates (BD Bioscience, Franklin Lakes, N.J., USA) at 37°C in 5% CO₂. After 2 h, the non-adherent cells were removed and the adherent cells were cultured in RPMI 1640 medium with 0.1–100 μ g/ml of each bacterial strain for 48 h. The supernatant of the cells was collected for measurement of IL-12 and IL-10 by ELISA as described below.

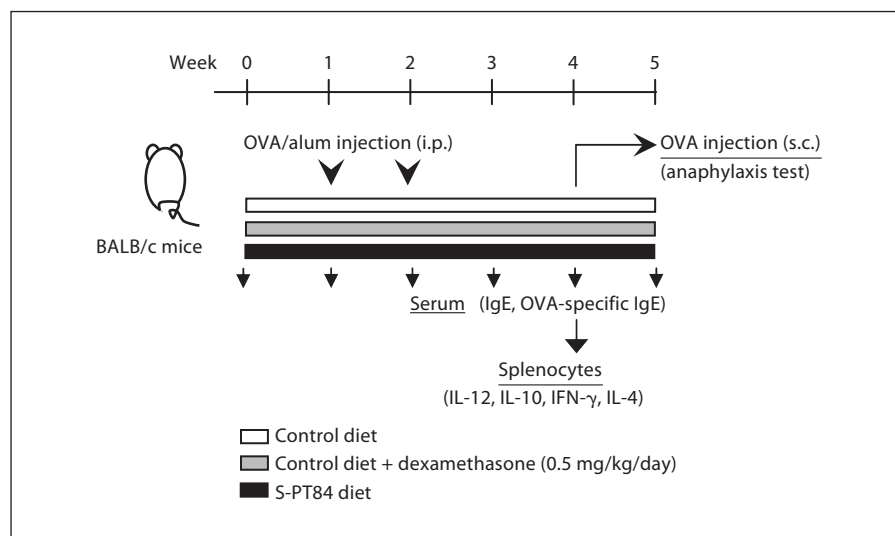
Feeding Procedure

To determine the immunomodulating effects of free feeding of S-PT84, S-PT84 (final dose: 0.075%) was added to an AIN-93M diet (maintenance formulation) (Oriental Yeast Co. Ltd., Tokyo, Japan) and given to BALB/c mice. The control group was given normal AIN-93M. Mice were fed AIN-93M with or without 0.075% S-PT84 for 1 week ad libitum, and ex vivo IFN- γ - and IL-4-inducing activities, NK activity, and antiallergic activity were then measured.

Ex vivo Assessment of IFN- γ and IL-4

BALB/c mice were fed AIN-93M with or without 0.075% S-PT84 for 1 week and splenocytes were prepared. The cells were suspended in RPMI 1640 with 10% FBS at a density of 5×10^6 cells/ml and stimulated with 2.5 μ g/ml concanavalin A (Con A) (Wako Pure Chemical Industries, Osaka, Japan) in 24-well plastic plates (BD Bioscience). After incubation for 24 h at 37°C, the culture supernatant was collected and levels of IFN- γ and IL-4 were measured by ELISA as described below.

Fig. 1. Experimental procedure of OVA-induced allergy model.



Measurement of NK Activity (PINK Method)

Yac-1 target cells were suspended in PBS at a density of 1×10^6 cells/ml and stained with 40 $\mu\text{g/ml}$ of 3,3'-diiododipropylcarbocyanine perchlorate (DiO) (Sigma, St. Louis, Mo., USA) for 10 min at 37°C. The stained cells were washed once with PBS and resuspended in RPMI 1640 with 10% FBS at a density of 5×10^4 cells/ml. Then 100 μl of the DiO-stained target cell suspensions and 100 μl suspensions of various numbers of effector cells and 25 $\mu\text{g/ml}$ of propidium iodide (PI) (Molecular Probes, Eugene, Oreg., USA) were added to 96-well U-shaped microplates (BD Bioscience). The plates were centrifuged at 200 g and incubated at 37°C in 5% CO₂ for 4 h. Spontaneous cell death was determined by incubating target cells without effector cells. After incubation, DiO⁺PI⁺ cells were analyzed using an Epics XL (Beckman Coulter). NK activity was calculated according to the formula:

$$\text{NK activity (\%)} = \frac{(\text{DiO}^+\text{PI}^+)}{(\text{DiO}^+\text{PI}^- + \text{DiO}^+\text{PI}^+)} \times 100 - \text{spontaneous lysis (\%)}$$

OVA-Induced Allergy Model

The experimental procedure used to produce the OVA-induced allergy model is summarized in figure 1. BALB/c mice were fed AIN-93M with or without 0.075% S-PT84 for 5 weeks, and were injected intraperitoneally with 20 μg of OVA (Grade V, Sigma) and 2 mg of Al(OH)₃ in a total volume of 0.2 ml at weeks 1 and 2. The positive control group was fed an AIN-93M diet and given drinking water containing 3.125 $\mu\text{g/ml}$ dexamethasone (Nacalai Tesque) (equivalent to 0.5 mg/kg/day) from 1 week before the first OVA administration during the experimental period. Serum was collected from the base of the tail every week, and total IgE and OVA-specific IgE were measured by ELISA. Splenocytes from 4 mice of each group were collected on week 4, and treated with OVA (100 $\mu\text{g/ml}$). Four days after treatment, supernatants were collected to measure IL-12, IL-10, IFN- γ and IL-4 levels. The active cutaneous anaphylaxis (ACA) experiment was performed using mice of each group at week 4 as described below.

Active Cutaneous Anaphylaxis

ACA was induced by subcutaneous challenge of OVA-immunized BALB/c mice with OVA. An intravenous injection of 0.05 ml of 1% Evans Blue (Nacalai Tesque) was performed, and then ACA was elicited in the skin of the belly after subcutaneous injection of OVA (150 μg in 0.05 ml sterile saline). Thirty minutes after OVA challenge, mice were killed, and a piece of skin shaved from each was incubated in 1-N KOH (0.7 ml) at 37°C overnight. Then, 9.3 ml acetone containing 28% 0.6-N H₃PO₄ was added and shaken vigorously. The amount of extravasated dye was determined by measuring absorbance at 630 nm.

ELISA

Measurements of IL-12, IFN- γ , IL-4, IL-10 and total IgE was carried out by sandwich ELISA using an OptEIA ELISA set (BD Pharmingen, San Diego, Calif., USA). Measurement of OVA-specific IgE was carried out by sandwich ELISA using OptEIA ELISA with slight modifications. Briefly, the wells of a Maxisorp immunoplate (Nunc, Roskilde, Denmark) were coated with 0.2 mg/ml OVA (Sigma) in 0.1 M carbonate buffer (pH 9.5) overnight at 4°C. After blocking the unoccupied sites on the well with assay diluent (BD Bioscience), the test samples were added. Subsequently, OVA-bound IgE was detected by biotinylated anti-mouse IgE monoclonal antibody and avidin-horseradish peroxidase conjugate (BD Bioscience). The level of OVA-specific IgE was estimated by comparison with hyperimmunized mouse serum collected from BALB/c mice sensitized by intraperitoneal administration of 2 doses of OVA adsorbed in Al(OH)₃. The OVA-specific IgE level of this standard serum was taken to be 1,000 U/ml.

Statistical Analysis

The significance of differences in values was tested by one-way ANOVA, followed by Fisher's PLSD as a post-hoc test for more than 3 groups, while Student's t test was used for 2-group comparisons.

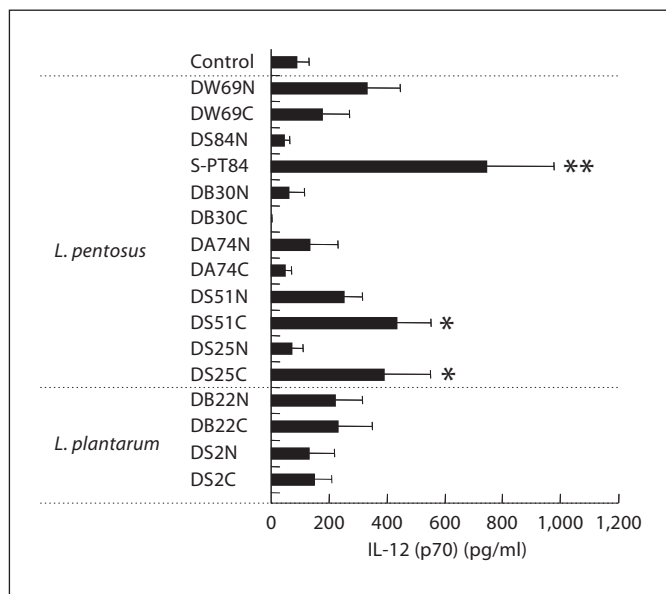


Fig. 2. Effects of various LAB strains of plant origin on IL-12 (p70) production in vivo. Heat-killed LAB suspended in sterile saline (0.5 mg/0.2 ml) were administered intraperitoneally to BALB/c mice, and IL-12 concentrations in serum 6 h after administration of LAB were measured by ELISA. Values are the means $\pm$ SE of 5 mice. Asterisks indicate significant differences from the control group (* $p < 0.05$, ** $p < 0.01$).

Results

Cytokine-Inducing Activities of Various LAB of Plant Origin from Kyoto Pickles in vivo

To determine the immunomodulatory activities of various LAB strains of plant origin (12 types of *L. pentosus* and 4 types of *L. plantarum*), heat-killed LAB preparations were administered intraperitoneally to BALB/c mice, and serum IL-12 levels at 6 h after administration were measured (fig. 2). *L. pentosus* strains S-PT84, DS51C, and DS25C exhibited strong IL-12-inducing activity in vivo. S-PT84 possessed the strongest induction of IL-12, a key cytokine inducing Th1-dominant immunity, of all the LAB of plant origin in this study.

IL-12- and IL-10-Inducing Activities of *L. pentosus* Strain S-PT84

We examined IL-12- (p70) and IL-10-inducing activities using peritoneal macrophages derived from BALB/c mice. The cells were cultured with a range of doses of S-PT84 (0.1, 0.3, 1, 3, 10, 30, 100 $\mu\text{g/ml}$) for 48 h, and concentrations of IL-12 and IL-10 were measured (fig. 3a, b).

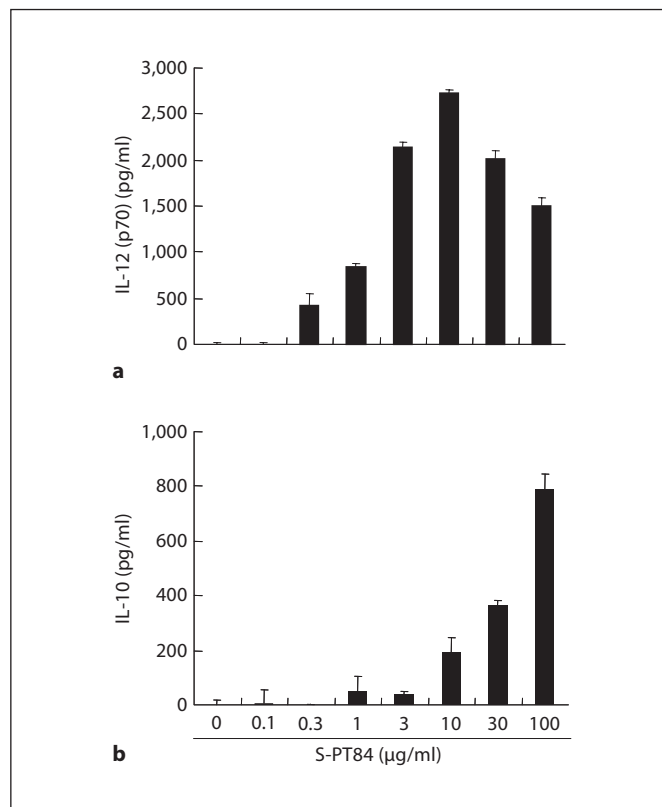


Fig. 3. Production of IL-12 and IL-10 by peritoneal macrophages after exposure to *L. pentosus* strain S-PT84. Peritoneal macrophages were prepared from BALB/c mice. The cells were cultured in the presence of 0.1–100 $\mu\text{g/ml}$ of heat-killed *L. pentosus* strain S-PT84 for 48 h. Culture supernatant was collected and measured for IL-12 (p70) (a) and IL-10 (b) concentration by ELISA as described in Materials and Methods. Values are means $\pm$ SE (n = 3).

S-PT84 (0.3–10 $\mu\text{g/ml}$) induced production of IL-12 in a dose-dependent fashion, though in 30 or 100 $\mu\text{g/ml}$ S-PT84-treated samples the IL-12 level was less than that with 10 $\mu\text{g/ml}$ S-PT84. Concentrations above 10 $\mu\text{g/ml}$ of S-PT84 induced production of IL-10 in a dose-dependent fashion.

Effect of Oral Feeding of *L. pentosus* Strain S-PT84 on Th1 and Th2 Cytokine Production and NK Activity

BALB/c mice were fed an AIN-93M diet with or without 0.075% S-PT84 for 1 week, and IFN- γ and IL-4 production by Con A-stimulated splenocytes from the mice was measured. IFN- γ -inducing activity was not changed, although IL-4-inducing activity was decreased by S-PT84 feeding, with a significant shift in the IFN- γ /IL-4 ratio toward a Th1-dominant state (fig. 4a–c). The NK activity

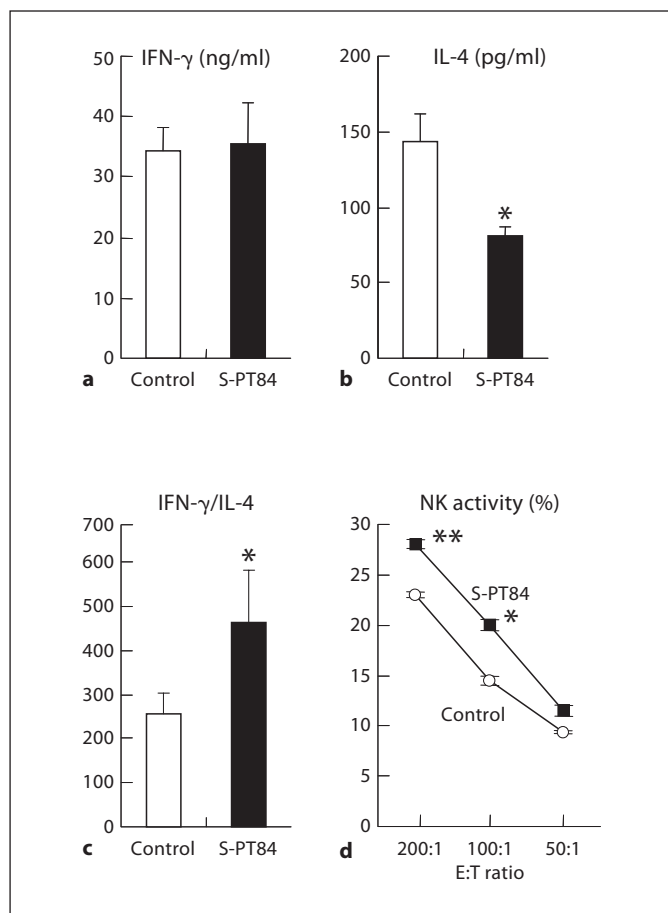


Fig. 4. Effect of oral feeding of *L. pentosus* strain S-PT84 on Th1 and Th2 cytokine production and NK activity. Mice were fed an AIN-93M diet with or without S-PT84 for 1 week, and their splenocytes were obtained. The cells were treated with Con A (2.5 μ g/ml) for 24 h. IFN- γ (a) and IL-4 (b) concentrations in culture supernatants were measured by ELISA, and the IFN- γ /IL-4 ratio was calculated (c). d NK activity of splenocytes from S-PT84-fed mice. The cells were cocultured with DiO-stained Yac-1 cells at various effector-to-target ratios for 4 h, and their cytotoxic activity was determined by the PINK method as described in Materials and Methods. Values are means $\pm$ SE of three mice. Asterisks indicate significant differences from the control group (* $p < 0.05$, ** $p < 0.01$).

of the splenocytes was also increased by S-PT84 feeding (fig. 4d).

Antiallergic Activity of *L. pentosus* S-PT84 in OVA-Induced Allergy Model

BALB/c mice were fed an AIN-93M diet with or without S-PT84 for 5 weeks. After 1 and 2 weeks of S-PT84 feeding, OVA and Al(OH)₃ were injected intraperitone-

ally into the mice. Serum was collected weekly during the experiment, and total IgE and OVA-specific IgE was measured (fig. 5). The increases in both total IgE and OVA-specific IgE induced by OVA were significantly suppressed by S-PT84 feeding. The suppressive effect of S-PT84 on the IgE level was the same as or tended to be slightly stronger than that of dexamethasone.

Three weeks after the first OVA injection, we examined by ACA reaction whether S-PT84 could suppress allergic inflammatory response in this model. ACA reaction was increased by OVA immunization, but was significantly suppressed by S-PT84 feeding (fig. 6). The suppressive effect observed was the same as with dexamethasone.

The cytokine-inducing activities of the splenocytes from the mice in each group were measured as described in 'Materials and Methods.' As shown in figure 7a–d, Th1 cytokine (IL-12, IFN- γ) levels were decreased and Th2 cytokine (IL-4 and IL-10) levels were increased significantly by OVA immunization. Although Th1 cytokine levels were not changed, IL-4 levels were decreased and IL-10 levels were increased by S-PT84 feeding.

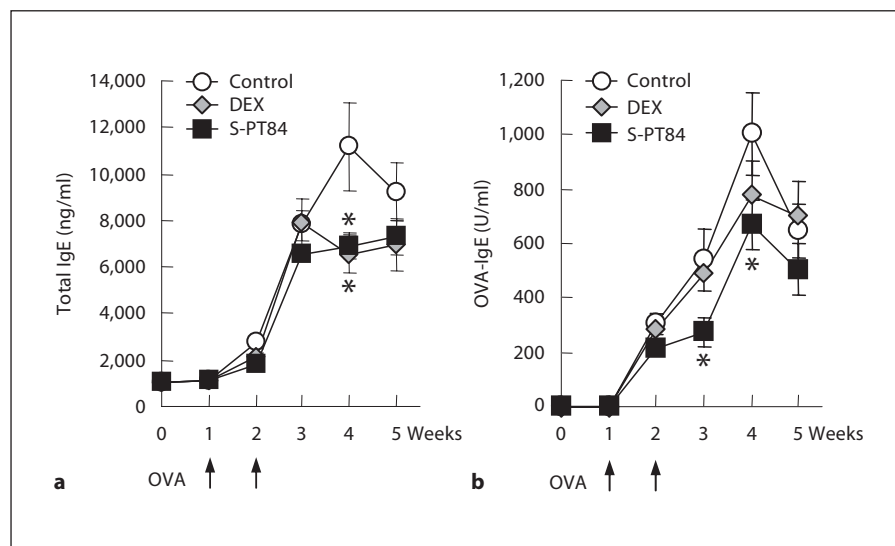
Discussion

The immunological effects of LAB, such as protective effects against infection [19], anticancer effects [20], and antiallergic effects [15, 16], have been well studied. However, most LAB examined have been of animal origin. Many types of fermented foods are consumed in Japan, such as pickles, soy sauce and miso (bean paste). These foods are produced by fermentation with LAB of plant origin [18]. However, the effects of such LAB on human health have been not well studied. The purpose of this study was to discover new LAB of plant origin which exhibit immunomodulatory and antiallergic activity despite use in heat-killed preparations.

We selected the *L. pentosus* strain S-PT84, which had the strongest IL-12-inducing activity among 16 types of LAB of plant origin isolated from Kyoto pickles (fig. 2). S-PT84 modulates the Th1/Th2 balance toward a Th1-dominant state (fig. 4a–c), activates NK cells (fig. 4d), and suppresses serum IgE levels (fig. 5) and the ACA reaction induced by OVA (fig. 6). All of these effects were induced by heat-killed S-PT84 preparations.

It has been believed that the immunogenicity of LAB depends on survival of the bacteria in the gastrointestinal tract, resistance to gastric acid, and ability to adhere to the mucosal surface [6–8]. However, some studies have sug-

Fig. 5. Effects of oral feeding of *L. pentosus* strain S-PT84 on IgE production in OVA-immunized mice. Mice were fed an AIN-93M diet with or without S-PT84 from week 0 for 5 weeks, and were intraperitoneally injected with 20 μ g OVA and 2 mg Al(OH)₃ at weeks 1 and 2 in a total volume of 0.2 ml. The positive control group was given drinking water containing dexamethasone (DEX) (0.5 mg/kg/day) from 1 week before the first OVA administration (week 0) during the experimental period. Serum was collected from each mouse, and total IgE and OVA-specific IgE were measured by ELISA as described in Materials and Methods. Values are means $\pm$ SE of 10–11 mice. Asterisks indicate significant differences from the control group (* $p < 0.05$).



gested that nonviable LAB can also show immunomodulatory activity, and induce IL-12 production by macrophages [13, 14], inhibit IgE production in an ovalbumin (OVA)-induced food allergy model [15, 16], and increase fecal antigen-specific IgA [17]. These previous findings and those of the present study indicate that LAB do not have to be alive to exert immunomodulatory effects such as antiallergic activity. It is possible that killed LAB preparations exhibit such activity because the components of LAB function as pattern ligands and trigger immunostimulation.

It has become evident that the recognition of specific microbial/pathogen-associated patterns is mediated by pattern recognition receptors that signal the presence of specific microorganisms to the host. The toll-like receptor (TLR) family is the best characterized class of pattern recognition receptors in mammalian species. Some studies have reported that LAB modulate host immune reactions via TLR-2 [16, 21, 22] or TLR-9 [23] on antigen-presenting cells. TLR-2 is mainly involved in responses to cell wall components such as lipoteichoic acid from Gram-positive bacteria. Grangette et al. [24] reported the importance of lipoteichoic acid composition in determining the ability of *L. plantarum*, a near relation of *L. pentosus*, to differentially impact the host immune system. TLR-2 is expressed on the surface of macrophages and dendritic cells isolated from Peyer's patches, and the number of TLR-2-positive cells in Peyer's patches was increased after LAB administration [22]. It is likely that S-PT84 modulates host immunological function via TLR-2 on the surface of antigen-presenting cells. On the other

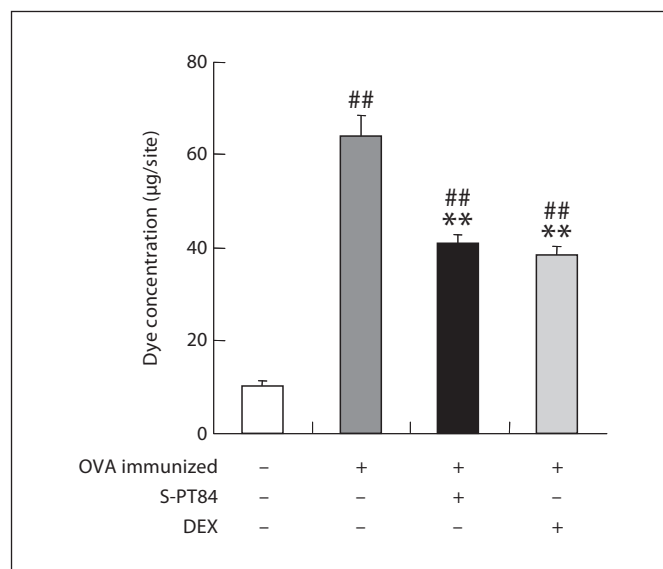
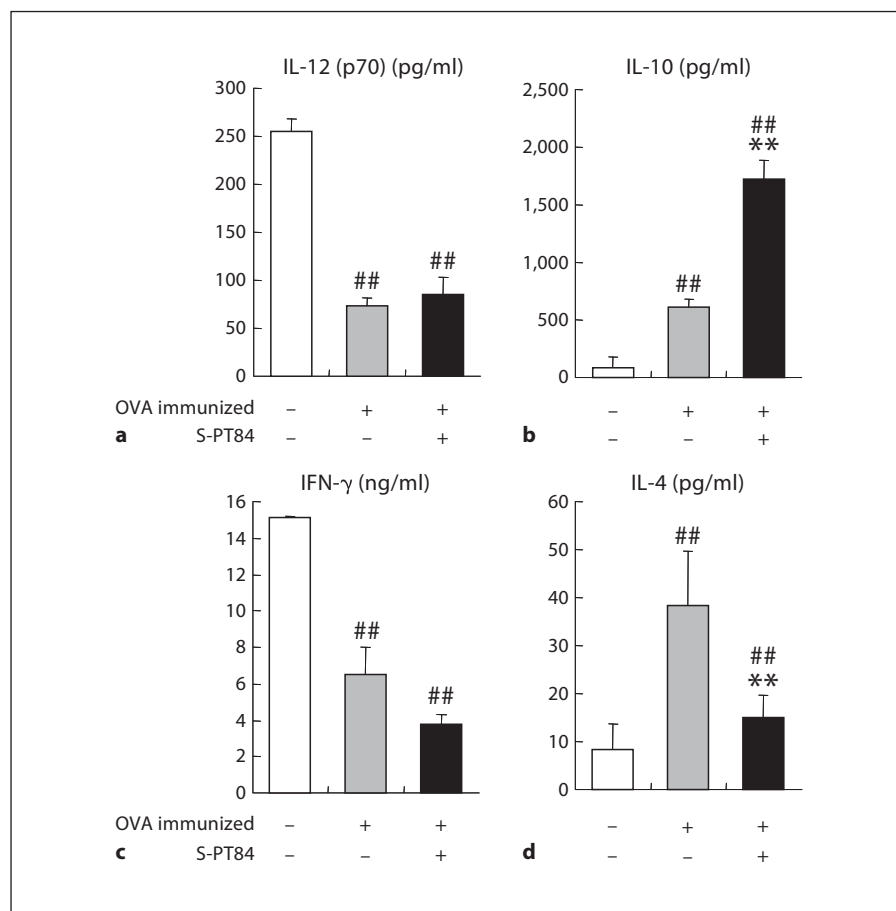


Fig. 6. Effects of oral feeding of *L. pentosus* strain S-PT84 on ACA in ovalbumin (OVA)-immunized mice. Mice were fed an AIN-93M diet with or without S-PT84 from week 0 for 4 weeks, and were intraperitoneally injected with 20 μ g of OVA and 2 mg of Al(OH)₃ at weeks 1 and 2 in a total volume of 0.2 ml. The positive control group was given drinking water containing dexamethasone (DEX) (0.5 mg/kg/day) from 1 week before the first OVA administration (week 0) during the experimental period. On week 4, ACA was induced by subcutaneous challenge with OVA (150 μ g) as described in Materials and Methods. Values are the mean $\pm$ S.E. of 8–10 mice. Asterisks indicate significant differences from the OVA-immunized group (second column; ** $p < 0.01$), and hash marks indicate significant differences from the control group (first column; ## $p < 0.01$).

Fig. 7. Effects of oral feeding of *L. pentosus* strain S-PT84 on cytokine production in OVA-immunized mice. Mice were fed an AIN-93M diet with or without S-PT84 from week 0 for 4 weeks, and were injected intraperitoneally with 20 μ g of OVA and 2 mg of Al(OH)₃ at weeks 1 and 2 in a total volume of 0.2 ml. Splenocytes were obtained from the mice on week 4, and treated with OVA (100 μ g/ml) for 4 days. Culture supernatants were collected and measured for IL-12 (p70) (a), IL-10 (b), IFN- γ (c), and IL-4 (d) concentrations by ELISA as described in Materials and Methods. Values are means $\pm$ SE of 4 mice. Asterisks indicate significant differences from the OVA-immunized group (second columns; ** $p < 0.01$), and hash marks indicate significant differences from the control group (first columns; ## $p < 0.01$).



hand, Forsythe et al. reported that *L. reuteri* decreased allergen-induced airway hyperresponsiveness in TLR-9-dependent fashion [25]. TLR 9 recognizes CpG oligodeoxynucleotides. It was recently reported that AT oligodeoxynucleotides, a new group of TLR-9 ligands, were identified from LAB [23]. These findings indicate that LAB exhibit immunomodulatory activity via not only TLR-2 but also TLR-9. However, since little is known concerning the mechanisms of immunomodulation by LAB of plant origin, further studies of them are required for application to new food products.

Previous studies have demonstrated that LAB exhibit antiallergic activity by modulating the Th1/Th2 balance toward a Th1 dominant state. Although these LAB exhibit IL-12-inducing activity in vitro, the patterns of Th1/Th2 cytokine production by antigen-immunized animals differ. For example, the *L. casei* strain Shirota increases the production of Th1 cytokines, IL-12, and IFN- γ , and decreases that of the Th2 cytokines IL-4 and IL-5, in OVA-immunized mice [15]. The *L. paracasei*

strain KW3110 increases IL-12, but does not affect IFN- γ or IL-4 production [16]. The *L. plantarum* strain L137 increases IL-12, does not affect IFN- γ production and decreases IL-4 production [14]. S-PT84 does not affect IL-12 or IFN- γ production, decreases IL-4 production, and increases that of IL-10. IL-10 is a regulatory cytokine in OVA-immunized mice (fig. 7). Thus, the mechanisms of the antiallergic effects of LAB differ depending on the strain. It is thus necessary to study the antiallergic mechanisms of LAB at the strain level.

It is known that excessive Th1 causes Th1-type diseases such as rheumatic arthritis [26, 27], type 1 diabetes [28, 29] and hepatopathy [30, 31]. There is a risk of side effects of excessive LAB-mediated Th1 immunity. In this study, we confirmed that intraperitoneally administered S-PT84 induced production of both IL-12 (fig. 2) and IL-10 (data not shown) in an in vivo study. S-PT84 induced production of IL-12 in a dose-dependent fashion up to 10 μ g/ml, though production of IL-12 was downregulated by high doses of S-PT84 (fig. 3a). Interestingly, IL-10 lev-

els increased in the same experiment (fig. 3b) and levels of Th2 cytokines, such as IL-4 and IL-5, were unchanged (data not shown). In splenocytes isolated from OVA-immunized mice, IL-10 was produced, although production of IL-4 was downregulated (fig. 7b, d). IL-10 is able to induce the development of regulatory T cells [32, 33] and inhibit the production of IL-12 and IL-4. These findings suggest that S-PT84 can suppress immune responses by regulatory T cells when Th1 or Th2 responses are excessive. These findings agree with previous observations that LAB induce IL-10 production in human peritoneal blood mononuclear cells [34] and cord cells [35] and induce IL-10-producing regulatory T cells [36]. In this study, S-PT84 and dexamethasone exhibited the same degrees of antiallergic effect (fig. 5, 6), although it appears

that the mechanism of the antiallergic effects of S-PT84 differs from those of antiallergy drugs such as dexamethasone, which depress host immune function including production of IL-10 [37].

In conclusion, the *L. pentosus* strain S-PT84 of plant origin, an IL-12 potentiator, can enhance NK activity, modulate Th1/Th2 balance and IL-10 production, and act as an antiallergic agent in vivo. Since LAB of plant origin can ferment many materials of plant origin, applications in new food products will be widespread. Further studies, such as applications in food, determination of their active components and their mechanisms of action, their antiinfective activity, and their clinical application are needed.

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