

***Lactobacillus casei* strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model**

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Summary

Background Our previous study using allergen-sensitized murine splenocyte cultures has shown that *Lactobacillus casei* strain Shirota (LcS), a lactic acid bacterium widely used as a starter for fermented milk products, suppresses IgE production through promoting a dominant Th1-type response mediated by IL-12 induction.

Objective We tried to evaluate the ability of LcS to suppress both IgE response and allergic reactions *in vivo* using a food allergy model with ovalbumin-specific T cell receptor transgenic (OVA-TCR-Tg) mice.

Methods The ability of heat-killed LcS to induce IL-12 in serum was tested. OVA-TCR-Tg mice were fed a diet containing OVA for 4 weeks and injected with LcS intraperitoneally three times in the first week of this period. Cytokine and antibody secretion by splenocytes, and serum IgE and IgG1 responses were examined. The inhibitory effect of LcS on systemic anaphylaxis induced by intravenous challenge of OVA-fed OVA-TCR-Tg mice with OVA was also tested.

Results Intraperitoneal injection of LcS induced an IL-12 response in the serum of OVA-TCR-Tg mice. In the food allergy model, LcS administration skewed the pattern of cytokine production by splenocytes toward Th1 dominance, and suppressed IgE and IgG1 secretion by splenocytes. The ability of LcS to modulate cytokine production was blocked by anti-IL-12 antibody treatment. LcS also inhibited serum OVA-specific IgE and IgG1 responses and diminished systemic anaphylaxis.

Conclusion LcS administration suppresses IgE and IgG1 responses and systemic allergic reactions in a food allergy model, suggesting a possible use of this lactic acid bacterium in preventing food allergy.

Keywords lactic acid bacterium, *Lactobacillus casei* strain Shirota, food allergy, TCR transgenic mouse, ovalbumin, IgE, IgG1, anaphylaxis, IL-12, Th1, Th2

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Introduction

IgE is involved in mediating many allergic diseases, including food allergy. The production of IgE is promoted by Th2 cells and their cytokines such as IL-4 and IL-5, while IFN- γ secreted by Th1 cells can inhibit IgE production [1]. The balance of responding Th1 and Th2 cells is critical for regulating IgE production. Several studies have shown that a polarized Th2 response is often observed in patients with allergy [2]. Thus, enhancing a Th1-type immune response is expected to be beneficial for the treatment of allergic diseases [3].

Lactobacillus casei strain Shirota (LcS) was originally isolated from the faeces of healthy humans and is widely used as a starter for fermented milk products. It has been well established that LcS can activate cellular immunity of the host, and LcS has been studied intensively with regard to its anti-tumour and

anti-infectious activities [4–6]. Our recent paper [7] showed a novel ability of LcS to suppress IgE secretion from antigen-stimulated splenocytes, and suggested a possible use of LcS in preventing IgE-mediated allergy. In an *in vitro* study, we have shown that heat-killed LcS stimulates macrophages to secrete IL-12, shifts the cytokine production pattern from Th2 to Th1 predominance, and then suppresses IgE production. In addition, Matsuzaki et al. [8] have revealed that LcS can also inhibit the serum IgE response elicited by intraperitoneal immunization of BALB/c mice with ovalbumin (OVA) adsorbed onto alum adjuvant. However, whether LcS can inhibit the serum IgE response elicited by a naturally fed food allergen and whether suppression of the IgE response by LcS can lead to a reduction of allergic reactions has not been elucidated. With the final goal of preventing IgE-mediated allergy in humans, further basic studies on the anti-allergic activity of LcS in a suitable experimental animal model are required to examine these problems.

Recently, we developed a novel IgE response model of food allergy in allergen-specific T cell receptor (TCR) transgenic mice

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[9]. In this food allergy model, feeding a diet containing OVA to transgenic mice expressing TCR specific for a dominant T cell determinant peptide of OVA (OVA-TCR-Tg mice) induces T cells that secrete a high level of IL-4 and can promote IgE production by B cells *in vitro*, and elicits OVA-specific IgE and IgG1 responses in serum. Subsequent intravenous challenge of OVA-fed OVA-TCR-Tg mice with OVA results in systemic anaphylaxis. This model is suitable for examining the anti-allergic effect of an agent, considering the T cell and IgE responses to orally ingested antigens.

In the present study, we used this food allergy model to evaluate the ability of LcS to regulate both the cytokine response of splenic T cells, and the IgE response in serum and systemic allergic reactions. The data presented clearly indicate the anti-allergic activity of this bacterium.

Materials and methods

Animals

Male 8–10 week-old OVA23-3 mice [10] transgenic for OVA₃₂₃₋₃₃₉-specific and I-A^d restricted TCR- $\alpha\beta$ (V α 3/V β 15), with a BALB/c genetic background, were used in the experiments. The percentage of transgene-expressing cells among peripheral T cells was greater than 90%. All experiments were performed in accordance with the guidelines for the care and use of laboratory animals of the Yakult Central Institute for Microbiological Research.

Preparation of heat-killed bacteria

Lactobacillus casei strain Shirota (LcS) was originally isolated from human faeces at the Yakult Central Institute for Microbiological Research (Tokyo, Japan), and *Lactobacillus johnsonii* JCM 2012, which is the type strain of this species, was obtained from the Japan Collection of Microorganisms (Wako, Japan). *L. johnsonii* was used as a negative control in this study, since it had shown no suppressive effect on IgE production in our previous study using murine splenocyte cultures [7]. Preparation of heat-killed bacteria has been described elsewhere [7]. In brief, these bacteria were cultured at 37 °C for 20 h in Lactobacilli-MRS broth (DIFCO, Detroit, MI, USA), washed with sterile distilled water, heated at 100 °C for 30 min, and then lyophilized.

IL-12-inducing activity

To test the IL-12-inducing activity of bacteria, OVA-TCR-Tg mice were intraperitoneally administered various doses (12.5–800 μ g) of heat-killed bacteria suspended in 0.1 mL sterile saline and bled 6 h after LcS injection. For the kinetics study, OVA-TCR-Tg mice were intraperitoneally administered 200 μ g LcS and bled 3, 6, 12, 24 and 48 h after LcS injection. IL-12 level in serum was measured by ELISA.

Food allergy model with OVA-TCR-Tg mice

The experimental procedure of the food allergy model is summarized in Fig. 1. OVA-TCR-Tg mice were fed a pelleted diet containing egg-white protein at 20% weight ratio (11% as OVA) (OVA diet; Funabashi Farm, Funabashi, Japan) for 4 weeks and injected with 200 μ g heat-killed bacteria intraperitoneally on days 1, 3 and 5 in the first week of this period. The mice

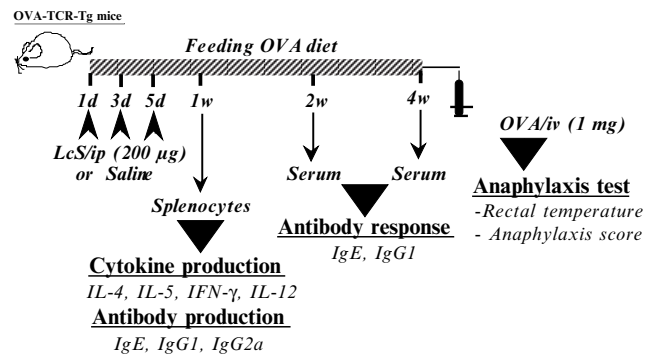


Fig. 1. Experimental procedure of food allergy model.

consumed 250 mg OVA per day on average. Splenocytes were prepared from mice fed the diet for 1 week, to study cytokine and antibody production. The mice were bled on weeks 2 and 4 to examine the antibody response. After 4 weeks of the OVA diet, the mice were fed an OVA-free diet (F-2 diet, Funabashi Farm) for 2 days, and then systemic anaphylaxis was induced.

Treatment with anti-IL-12 antibody

OVA-TCR-Tg mice were fed an OVA diet and intraperitoneally administered 100 μ g anti-IL-12 monoclonal antibody (PharMingen, San Diego, CA, USA; clone C17.8) immediately after injection of 200 μ g LcS on days 1, 3 and 5. The dose of anti-IL-12 antibody administration was determined to be 100 μ g, since our preliminary study had shown that this dose of anti-IL-12 antibody could reduce the serum level of IL-12 6 h after LcS administration from 16.26 ± 5.44 to 1.85 ± 0.20 ng/mL. Splenocytes were prepared on week 1, and cytokine production was examined.

Induction of anaphylaxis

Systemic anaphylaxis was induced by intravenous challenge of OVA-fed OVA-TCR-Tg mice with OVA, as described previously [9]. One milligram of OVA dissolved in 0.2 mL sterile saline was injected into the tail vein. The mice were observed for a 30-min period following OVA injection, and the anaphylactic reaction was rated according to the following four scores as described by Poulsen et al. [11]: –, no reaction observed; +, mice are slow but mobile if provoked; ++, mice are stationary even if provoked; +++ ; mice lie down and suffer severe anaphylactic shock. The anaphylaxis score was determined by a person who did not know whether each mouse belonged to the control or LcS group. In addition, rectal temperature was measured 15 and 30 min after OVA challenge, since systemic anaphylaxis results in a decrease of body temperature [12].

Cell cultures for cytokine and antibody production

Splenocytes were isolated as a single-cell-suspension from OVA-TCR-Tg mice fed an OVA diet for 1 week. After depletion of erythrocytes, the cells (2.5×10^6 /mL) were cultured with OVA (10 μ g/mL) in 1 mL RPMI-1640 medium (Sigma, St Louis, MO, USA) supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin and 100 μ g/mL streptomycin in a 48-well culture plate (Costar, Cambridge, MA, USA). Supernatants were collected at 48 h or day 7 for determination of cytokine or antibody levels, respectively.

ELISA for antibodies

Determination of total and OVA-specific IgE levels was carried out by sandwich ELISA as described previously [9] with slight modifications. To determine total IgE level, the wells of a Maxi-sorp immunoplate (Nunc, Roskilde, Denmark) were coated with rat anti-mouse IgE monoclonal antibody (PharMingen, clone R35-92). After blocking the unoccupied sites on the plastic with bovine serum albumin, the test samples and standard mouse IgE (PharMingen) were added. Subsequently, bound IgE was detected by sequential incubation with biotinylated rat anti-mouse IgE monoclonal antibody (Serotec, Oxford, UK; clone LO-ME-2), streptavidin-peroxidase conjugate (Genzyme, Cambridge, MA, USA), and TMB substrate (Vector Laboratory, Burlingame, CA, USA). For the assay of OVA-specific IgE, biotinylated OVA (5 µg/mL) was used instead of biotinylated rat anti-mouse IgE antibody. The level of OVA-specific IgE was estimated by comparison with hyperimmunized mouse serum. Hyperimmunized mouse serum was obtained from OVA-TCR-Tg mice sensitized by intraperitoneal administration of three doses of OVA adsorbed onto alum, and the OVA-specific IgE level of this standard serum was taken to be 1000 units/mL. One unit/mL of OVA-specific antibody gave an optical density of approximately 1 at 450 nm in our ELISA system.

To assay OVA-specific IgG1 level, the wells of Maxi-sorp immunoplates were coated with OVA (5 µg/well). After blocking the unoccupied sites on the plastic, the test samples were added. Subsequently, bound antibodies were detected by means of peroxidase-conjugated anti-mouse IgG1 antibody (Zymed, South San Francisco, CA, USA). The OVA-specific IgG1 level was estimated by comparison with hyperimmunized mouse serum. The hyperimmunized mouse serum was the same as that for IgE, and the OVA-specific IgG1 level of the standard serum was taken to be 50 000 000 units/mL.

Determination of total IgG1 and IgG2a levels was performed by sandwich ELISA. Rabbit anti-mouse IgG antibody (Zymed) was used as the capture antibody for both IgG1 and IgG2a, with peroxidase-conjugated anti-mouse IgG1 and IgG2a antibodies (Zymed), respectively, as the detection antibody. Standard myeloma mouse IgG1 and IgG2a were obtained from Cappel (West Chester, PA, USA).

ELISA for cytokines

Determination of IFN- γ , IL-4, IL-5 and IL-12 was performed by sandwich ELISA, as previously described [9]. IFN- γ and IL-4 were measured with Duo Set mouse IFN- γ and IL-4 ELISA kits (Genzyme), respectively. For IL-5 and IL-12, rat anti-mouse IL-5 (clone TRFK5) and IL-12 (clone C15.6) monoclonal antibodies were used as the capture antibody, and biotinylated rat anti-mouse IL-5 (clone TRFK4) and IL-12 (clone C17.8) monoclonal antibodies, respectively, were used as the detection antibody. These antibodies and standard recombinant mouse IL-5 and IL-12 were all obtained from PharMingen.

Statistical analysis

Differences in cytokine and antibody levels in splenocyte cultures, between non-treated, control, LcS and *L. johnsonii* groups, were analysed by Tukey or Tukey-Kramer multiple comparison test. Differences in both antibody levels in serum and anaphylaxis score, between control and LcS groups, were analysed by Mann-Whitney *U*-test. Student's *t*-test was used to

compare the rectal temperature between the two groups. A *P*-value less than 0.05 was considered significant.

Results

Increase of serum IL-12 level by LcS administration

First, we examined the IL-12-inducing activity of LcS in OVA-TCR-Tg mice, since IL-12 is a key cytokine mediating the ability of LcS to suppress IgE production in an *in vitro* culture system [7]. As shown in Fig. 2(a), single intraperitoneal administration of heat-killed LcS at doses more than 200 µg to the mice resulted in a substantial increase of serum IL-12 level. The increase of IL-12 level was transient with a peak at 6 h after 200 µg LcS administration (Fig. 2b). The transient increase of IL-12 level induced by LcS administration was also observed in non-transgenic BALB/c mice (data not shown). On the other hand, *L. johnsonii* JCM2012, which had shown no suppressive effect on IgE production in our previous *in vitro* study [7], scarcely induced an IL-12 response in the serum of OVA-TCR-Tg mice (Fig. 2a).

Shift of cytokine production pattern of splenocytes toward Th1 dominance by LcS administration

We evaluated the anti-allergic activity of LcS using a food allergy model with OVA-TCR-Tg mice. The experimental procedure is summarized in Fig. 1.

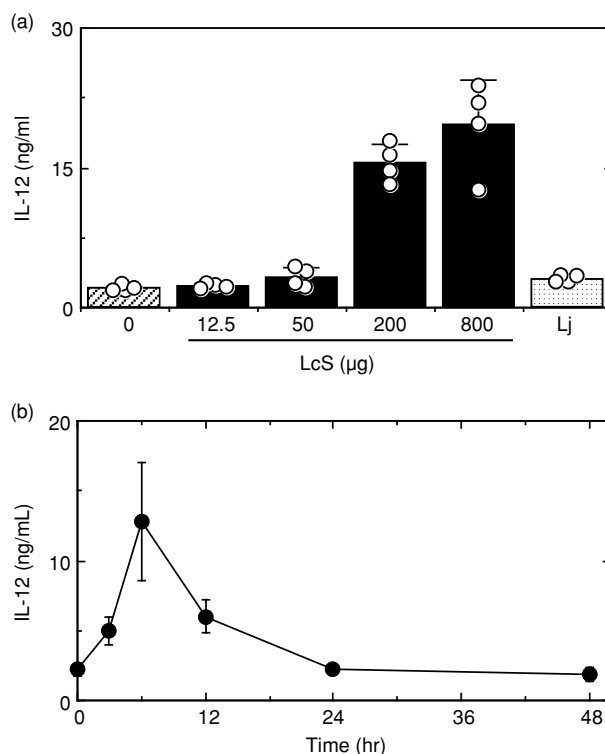


Fig. 2. Induction of IL-12 in serum by intraperitoneal administration of LcS. For the dose-response study (a), heat-killed LcS (0–800 µg) or *L. johnsonii* (Lj, 800 µg) suspended in 0.1 mL sterile saline was administered intraperitoneally to OVA-TCR-Tg mice, and the serum level of IL-12 6 h after administration of bacteria was determined by ELISA. Data are expressed as mean $\pm$ SD with individual values of four mice. For the kinetics study (b), 200 µg LcS was administered intraperitoneally to OVA-TCR-Tg mice, and the serum level of IL-12 was determined by ELISA. Data are expressed as mean $\pm$ SD of four mice.

Splenocytes from OVA-TCR-Tg mice fed an OVA diet for 1 week with or without bacteria (LcS or *L. johnsonii*) treatment were cultured with OVA for 2 days, and the secretion of cytokines was examined. Cytokine secretion by splenocytes from the mice not fed OVA (NT group) was also tested. As shown in Fig. 3, feeding OVA to OVA-TCR-Tg mice enhanced IL-4 and IL-5 secretion by splenocytes (control group), compared with the NT group. Compared with the control group, LcS administration suppressed the enhanced secretion of IL-4 and IL-5, whereas IL-12 and IFN- γ secretion was enhanced by LcS. The levels of IL-4 and IL-5 in the LcS groups were similar to those in

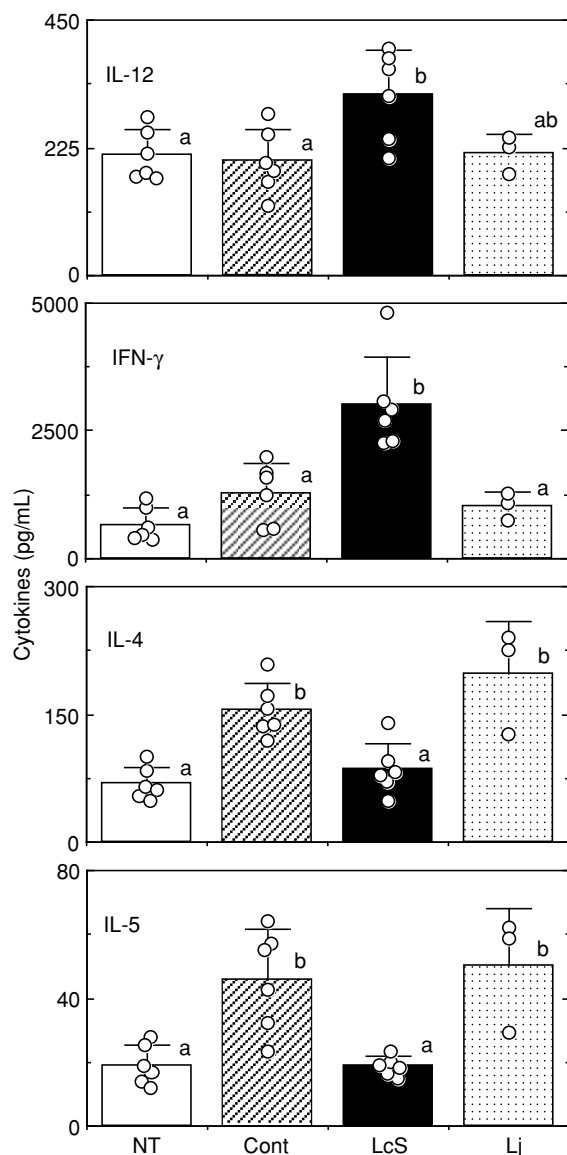


Fig. 3. Effects of LcS administration on cytokine production by splenocytes. OVA-TCR-Tg mice were fed an OVA diet for 1 week, and 200 μ g LcS, 200 μ g *L. johnsonii* or saline (Cont) was administered intraperitoneally three times (on days 1, 3 and 5). Splenocytes were cultured with OVA (10 μ g/mL) for 48 h, and the levels of cytokines in culture supernatants were determined by ELISA. NT is data for OVA-TCR-Tg mice not fed the OVA diet. Data are expressed as mean $\pm$ SD with individual values of 6 or 3 independent spleens. Bars not sharing a common letter are significantly different, analysed by Tukey-Kramer multiple comparison test.

the NT group, while those of IL-12 and IFN- γ were higher than those in the NT and control groups. These results indicate that LcS can skew the cytokine production toward a Th1 predominant pattern. *L. johnsonii* had no effect on cytokine production.

Effect of IL-12 antibody treatment on cytokine production modulated by LcS administration

Whether IL-12 induced by LcS administration led to the shift of cytokine production pattern toward Th1 dominance was examined. As shown in Fig. 4, treatment with neutralizing antibody to IL-12 immediately after LcS injection reduced the enhanced secretion of IL-12 and IFN- γ , while restored the IL-4 and IL-5 secretion to the control levels. The results suggest that the promotion of predominant Th1-type cytokine production by LcS administration is IL-12 dependent.

Suppression of antibody secretion in splenocyte cultures by LcS administration

Total antibody levels on day 7 in the cultures of splenocytes from OVA-TCR-Tg mice fed an OVA diet for 1 week with or without LcS treatment were determined. As shown in Fig. 5, the secretion of IgE and IgG1 by splenocytes was enhanced by OVA feeding (control group), compared with the NT group. LcS administration markedly inhibited the secretion of IgE and IgG1, while IgG2a secretion by splenocytes was not inhibited.

Suppression of antibody responses in serum by LcS administration

As shown in Fig. 6, feeding an OVA diet elicited total IgE, OVA-specific IgE and IgG1 responses in the serum of OVA-TCR-Tg mice (control group). LcS administration significantly suppressed the increase of all these antibody levels 2 weeks after the start of feeding the diet. At week 4, OVA-specific IgG1 level in the LcS group was significantly lower than that in the control group. Total and OVA-specific IgE levels in the LcS group at week 4 were also lower than those in the control group, although the inhibition was not significant ($P=0.088$ for total IgE, $P=0.076$ for OVA-specific IgE). OVA-specific IgG2a was detected only in 2/11 mice in the control group, and in 1/11 mice in the LcS group. The IgG2a response induced was not sufficient to analyse the difference between the groups.

Suppression of systemic anaphylaxis by LcS administration

Whether LcS administration can suppress allergic reactions in this food allergy model was examined. Intravenous injection of OVA to control OVA-TCR-Tg mice fed OVA for 4 weeks led to a decrease of rectal temperature from 37.6 ± 0.5 to 33.7 ± 1.1 (30 min after OVA injection), and resulted in a reduction in their mobility. LcS administration significantly inhibited the decrease of rectal temperature (Fig. 7a), and also reduced the anaphylaxis score rated from their mobility (Fig. 7b). The results indicated the inhibitory activity of LcS on systemic allergic reactions.

Discussion

We have previously revealed using an *in vitro* culture system with OVA-sensitized BALB/c mouse splenocytes that heat-killed LcS can inhibit IgE production through promoting

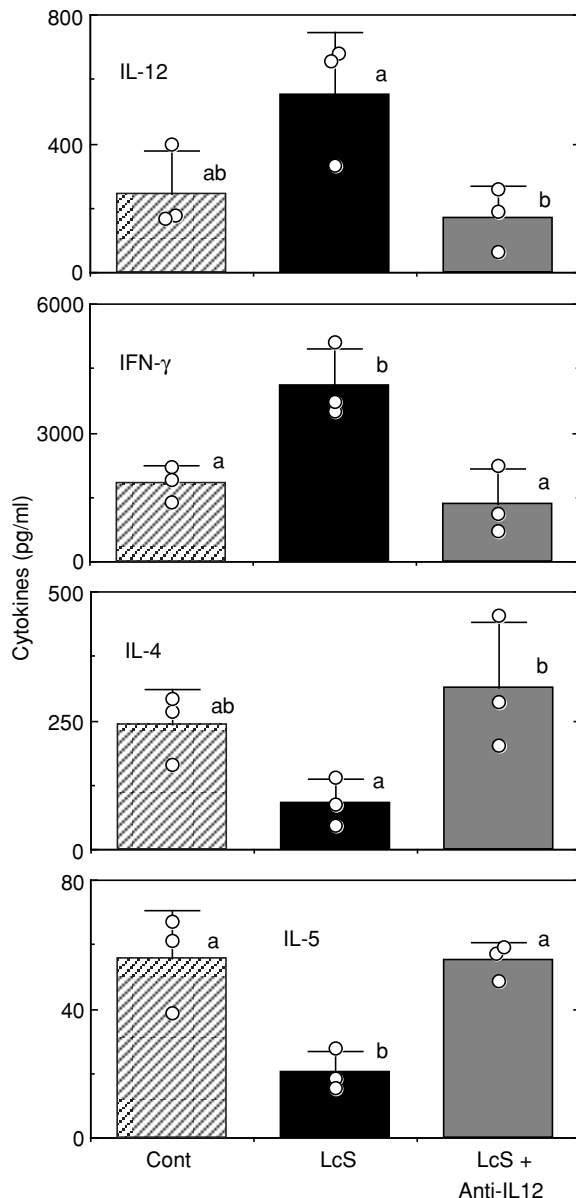


Fig. 4. Effects of anti-IL-12 antibody treatment on cytokine production modulated by LcS administration. OVA-TCR-Tg mice were fed an OVA diet for 1 week, and 200 µg LcS with 100 µg anti-IL-12 monoclonal antibody (LcS + Anti-IL-12) or saline (LcS) was administered intraperitoneally three times (on days 1, 3 and 5). Splenocytes were cultured with OVA (10 µg/mL) for 48 h, and the levels of cytokines in culture supernatants were determined by ELISA. Cont is data for OVA-TCR-Tg mice fed the OVA diet without LcS nor anti-IL-12 treatment. Data are expressed as mean $\pm$ SD with individual values of three independent spleens. Bars not sharing a common letter are significantly different, analysed by Tukey multiple comparison test.

predominant Th1-type cytokine secretion [7]. The regulation of cytokine production could be mediated by enhanced production of IL-12. Thus, in the present study, we first examined whether the *in vivo* response of a key cytokine, IL-12, is induced by administration of LcS. Intraperitoneal administration of heat-killed LcS, but not *L. johnsonii*, to OVA-TCR-Tg mice led to a transient increase of serum IL-12 level. Recent reports have shown that some non-pathogenic lactic acid bacteria other than LcS can also promote IL-12 production *in vitro* and *ex vivo*

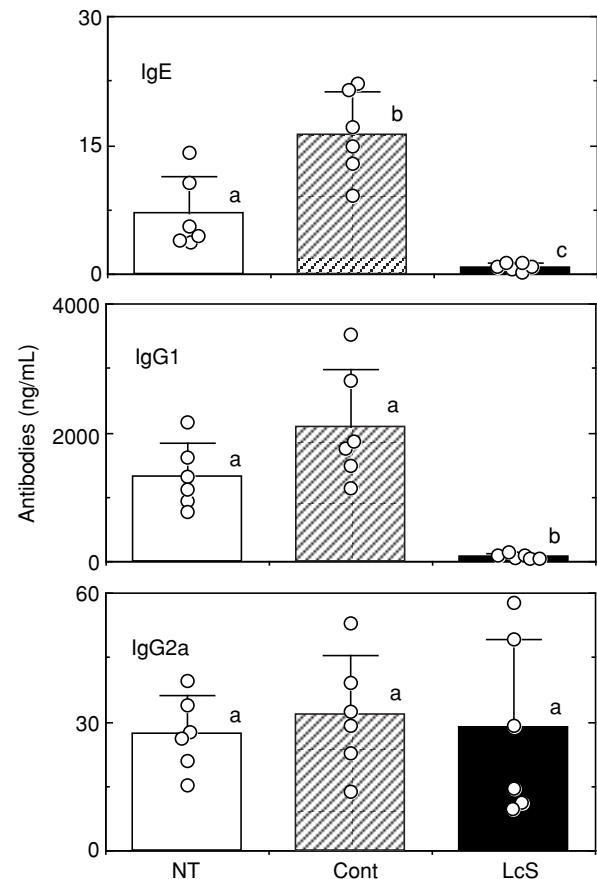


Fig. 5. Effects of LcS administration on antibody secretion by splenocytes. OVA-TCR-Tg mice were fed an OVA diet for 1 week, and 200 µg LcS or saline (Cont) was administered intraperitoneally three times (on days 1, 3 and 5). Splenocytes were cultured with OVA (10 µg/mL) for 7 days, and the levels of antibodies in culture supernatants were determined by ELISA. NT is data for OVA-TCR-Tg mice not fed the OVA-diet. Data are expressed as mean $\pm$ SD with individual values of six independent spleens. Bars not sharing a common letter are significantly different, analysed by Tukey multiple comparison test.

[13,14], although it is well known that many pathogenic infections induce IL-12 production [15]. Since IL-12 can enhance the Th1-type immune response and prevents allergic reactions *in vivo* as well as *in vitro* [16,17], IL-12 potentiators are expected to be anti-allergic agents.

We next tried to evaluate the inhibitory activity of LcS on IgE production in a food allergy model that we have developed recently [9]. In the food allergy model, feeding a diet containing OVA without any adjuvant to OVA-TCR-Tg mice elicits a serum antibody response, including OVA-specific IgE response, after feeding the diet for 2 weeks. T cells that secrete a high level of IL-4 and a moderate level of IL-5 and have helper activity for IgE production appear in the spleen 1 week after the start of feeding the diet. The helper activity of splenic T cells diminishes after 2 weeks of OVA feeding. Thus, the high IL-4-producing Th2-like cells appearing at week 1 are considered to be important in the subsequent increase of serum OVA-specific IgE level in this model. We attempted to modulate the cytokine production of splenic T cells at week 1 by LcS administration, in order to inhibit the IgE response in serum. Three intraperitoneal injections of LcS inhibited IL-4 and IL-5 and enhanced IFN-γ

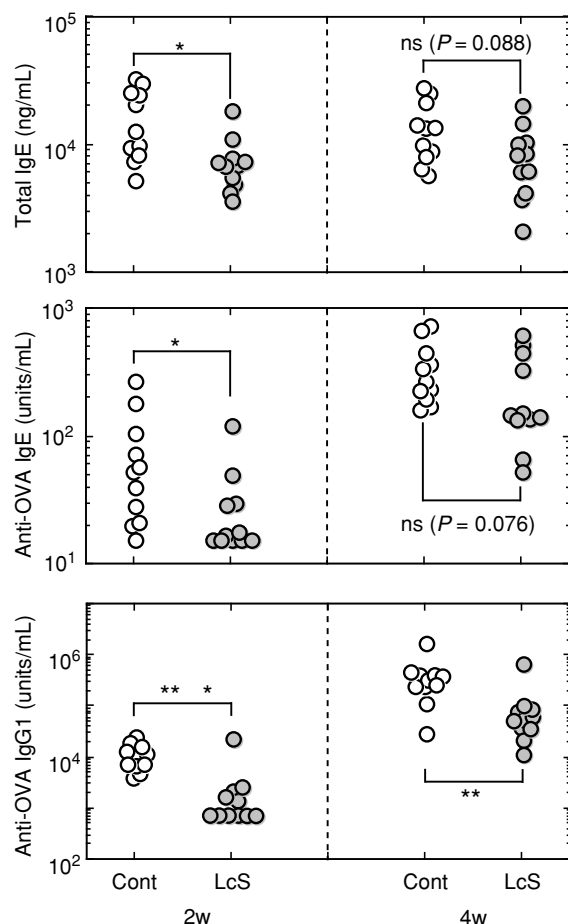


Fig. 6. Inhibition of serum antibody responses by LcS administration. OVA-TCR-Tg mice were fed an OVA diet for 4 weeks, and 200 μ g LcS or saline (Cont) was administered intraperitoneally three times (on days 1, 3 and 5). The serum levels of total IgE, OVA-specific IgE and OVA-specific IgG1 at week 2 and week 4 were determined by ELISA. The values for individual mice ($n = 11$) are expressed. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, calculated by Mann-Whitney U-test.

and IL-12 secretion by splenocytes from OVA-TCR-Tg mice fed OVA for 1 week. These results clearly indicate that LcS can shift the Th2 pattern of cytokine production by splenocytes to a Th1-type pattern. The study with neutralizing antibody to IL-12 suggests that the modulatory effect of LcS on cytokine production is mediated by IL-12 induction. In the cultures of splenocytes, secretion of IgE and IgG1, which is highly dependent on Th2-type cytokines, was markedly inhibited by intraperitoneal administration of LcS, whereas IgG2a secretion was not inhibited. The results agree well with the cytokine levels in the cultures. Furthermore, LcS administration could also suppress serum OVA-specific IgE and IgG1 responses elicited by OVA feeding. These data have confirmed our previous findings obtained in the *in vitro* study [7] and suggest that intraperitoneal administration of LcS induces IL-12 in serum, and skews the pattern of cytokine production by splenocytes toward Th1 dominance, and that these Th1-type reactions inhibit serum IgE and IgG1 responses.

We have shown here that LcS administration can suppress systemic anaphylaxis induced by intravenous challenge of OVA-fed OVA-TCR-Tg mice with OVA, in addition to the inhibition of serum IgE and IgG1 responses. The reduction of systemic anaphylaxis is considered to result from the inhibition of OVA-specific antibody responses by LcS. However, it could not be determined whether the inhibition of IgE or IgG1 production was critical for the suppression of anaphylaxis. It is well known that IgE mediates immediate-type allergic reactions including systemic anaphylaxis [12]. On the other hand, the roles of IgG1 in allergic reactions have not been well established. It has been reported that allergen-specific IgG can act as a blocking antibody and inhibit IgE-mediated allergic reactions by interfering with the binding of an allergen to IgE bound on mast cells [18]. However, advanced studies with gene-targeted mice have shown that IgG1 as well as IgE can induce systemic anaphylaxis [19,20]. In our food allergy model, we have previously revealed a correlation of the severity of anaphylaxis with the serum level of OVA-specific IgG1 as well as OVA-specific IgE [9]. Thus, inhibition of the IgG1 response, in

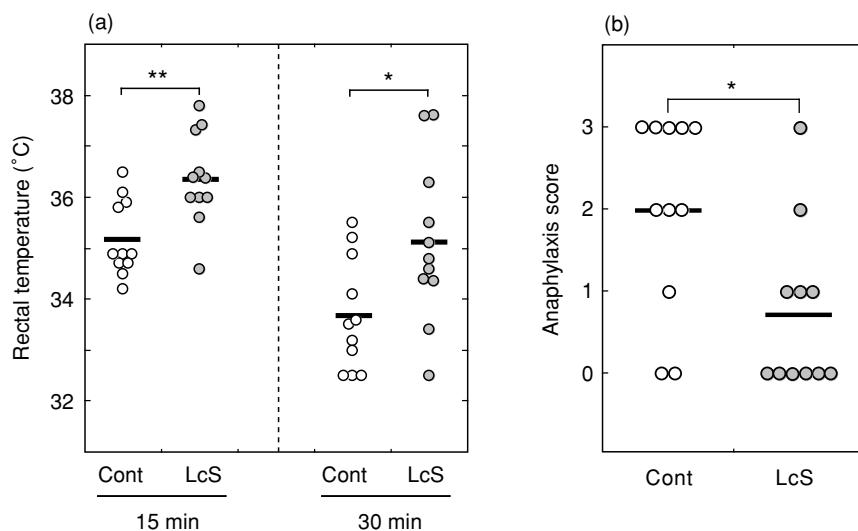


Fig. 7. Inhibition of systemic anaphylaxis by LcS administration. OVA-TCR-Tg mice were fed an OVA diet, and 200 μ g LcS or saline (Cont) was administered intraperitoneally three times (on days 1, 3 and 5). After 4-week feeding of the OVA diet, systemic anaphylaxis was induced by intravenous challenge with OVA (1 mg). (a) Rectal temperature was measured 15 and 30 min after OVA challenge. The values for individual mice ($n = 11$) are plotted, and bars indicate mean values. * $P < 0.05$; ** $P < 0.01$, calculated by Student's *t*-test. (b) The anaphylaxis score was rated from the mobility according to four scores as described in Materials and Methods. The scores for individual mice ($n = 11$) are plotted, and bars indicate mean values. * $P < 0.05$, calculated by Mann-Whitney U-test.

addition to inhibition of the IgE response, may contribute to the reduction of systemic anaphylaxis.

All these findings clearly indicate the anti-allergic activity of LcS. Similar activity of LcS was also observed in *Lactobacillus plantarum* L-137, which belongs to the same lactobacillus genus as LcS. Murosaki et al. [21] have shown in a model of DBA/2 mice fed a diet containing milk casein that intraperitoneal administration of *L. plantarum* increased the plasma level of IL-12, inhibited IL-4 secretion by splenocytes, and suppressed plasma IgE response. In their DBA/2 mouse model, cytokine secretion by splenocytes was induced by antigen-non-specific stimulation with Con A, and *L. plantarum* could not enhance IFN- γ secretion. In our OVA-TCR-Tg mouse model, we examined the antigen-specific cytokine response of splenocytes and clearly revealed the ability of LcS to skew the pattern of cytokine secretion from Th2 dominance to Th1 dominance. In addition, *L. plantarum*, in contrast to LcS, did not inhibit plasma IgG1 response. We cannot specify whether the different effects of these two bacteria on cytokine production and IgG1 response were due to either the different nature of the bacteria or the different assay systems. The critical difference between the studies of Murosaki et al. and our group is that we have shown a reduction of allergic reactions by LcS, while they did not.

The prevalence of atopic disorders is increasing in developed countries, and that is thought to be due, at least in part, to the decline of infectious diseases [22]. Indeed, epidemiological studies have demonstrated an inverse relationship between atopy and infections with measles, hepatitis A or mycobacteria [23–25]. This relationship could lead to the hypothesis that infections with microorganisms that have the ability to induce a dominant Th1 response are of benefit in preventing allergy. In fact, it has been revealed that experimental *Mycobacterium bovis* BCG infection promotes a dominant Th1 response and can inhibit both IgE response and bronchial allergic reactions in a murine model of allergic asthma [26]. However, it is too risky to use pathogenic microorganisms in humans to prevent allergic diseases. On the contrary, the use of non-pathogenic lactic acid bacteria for this purpose is thought to be safe, since humans consume lactic acid bacteria in dairy and other food products. Isolauri et al. [27] demonstrated that oral administration of *Lactobacillus* strain GG or *Bifidobacterium lactis* Bb-12 to infants with atopic eczema improved the clinical score of atopic dermatitis. In addition, epidemiological surveys in Estonia and Sweden have shown that allergic children were less often colonized with lactobacilli, as compared with non-allergic children [28]. The mechanisms of the anti-allergic activity of lactic acid bacteria have not yet been elucidated in the case of human allergic patients. We believe that the findings obtained in this study with an experimental animal model can give useful information for understanding the anti-allergic mechanisms of lactic acid bacteria.

LcS can be expected to act as an anti-allergic agent in humans, since previous studies have demonstrated that oral administration of LcS to humans successfully modulated the immune system. Daily intake of fermented milk containing LcS for 3 weeks potentiated NK activity in peripheral blood mononuclear cells [29]. A double-blind clinical trial revealed that oral administration of LcS was effective for preventing recurrence of superficial bladder cancer [30]. These findings indicate that oral LcS administration in humans can modulate the host immune

system, namely leading to enhanced cellular immunity, which in turn may prevent allergy. Human studies examining the anti-allergic activity of LcS remain to be done.

In conclusion, the data presented in this paper clearly indicate that LcS, an IL-12 potentiator, can act as an anti-allergic agent in a murine food allergy model. This lactic acid bacterium may be beneficial for the prevention of allergic disorders.

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