

Heat-killed *Lactobacillus plantarum* L-137 suppresses naturally fed antigen-specific IgE production by stimulation of IL-12 production in mice

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Background: Food allergy is caused by production of IgE against dietary antigen induced by T_{H2} response. IL-12 inhibits T_{H2} responses and strongly suppresses IgE production. We have recently established a murine model for IgE production with a predominant T_{H2} response induced by feeding antigen. **Objective:** We here show a suppressive effect of heat-killed *Lactobacillus plantarum* L-137, a potent inducer of IL-12, on IgE production against naturally fed antigen in a murine model. **Methods:** The ability of *L. plantarum* L-137 to induce IL-12 production was examined in vitro and in vivo. DBA/2 mice were fed a casein diet and injected intraperitoneally with *L. plantarum* L-137 from the beginning of feeding or 2 weeks later. Recombinant mouse IL-12 was also injected 2 weeks after the start of feeding. Casein-specific IgE and IgG1 in plasma were determined by ELISA.

Results: *L. plantarum* L-137 directly induced IL-12 production by the peritoneal macrophages and also stimulated spleen cells to produce both IL-12 and interferon- γ in vitro. In vivo treatment of *L. plantarum* L-137 also increased the plasma level of IL-12 in mice. Plasma anti-casein IgG1 and IgE levels were gradually elevated in DBA/2 mice fed a casein diet. Administration of *L. plantarum* L-137 from the beginning of feeding suppressed the elevation of anti-casein IgE levels, whereas the levels of anti-casein IgG1 were rather augmented by *L. plantarum* L-137. IL-12 production of the peritoneal macrophages was enhanced, but IL-4 production of concanavalin A (Con A)-stimulated spleen cells was suppressed in the *L. plantarum* L-137-treated mice compared with control mice fed a casein diet. When *L. plantarum* L-137 was given from 2 weeks after the start of feeding, anti-casein IgE levels were also significantly suppressed, which was similar to the result found in mice treated with IL-12.

Conclusion: Our results suggest that *L. plantarum* L-137, a

potent IL-12 inducer, is useful for prevention and treatment of food allergy. (J Allergy Clin Immunol 1998;102:57-64.)

Key words: IgE, IL-12, food allergy, *Lactobacillus plantarum*

The production of IgE specific for dietary protein plays an important role in the mediation of food allergy.¹ Allergic disease is thought to be caused by the inappropriate generation and activation of T_{H2} cells.^{2,3} T_{H2}-type cytokine IL-4 not only induces switching of B cells to IgE-producing cells but also inhibits the production of T_{H1}-type cytokine interferon (IFN)- γ .⁴⁻⁶ Conversely, IFN- γ inhibits the proliferation of T_{H2} cells and suppresses the switching of B cells. IL-12, formerly known as natural killer cell stimulatory factor and cytotoxic lymphocyte maturation factor, has an ability to stimulate natural killer and T_{H1} cells to produce IFN- γ , resulting in inhibition of T_{H2}-type immune responses.⁷⁻⁹ Moreover, IL-12 prevents the development of the T_{H2} response independently of IFN- γ .¹⁰ In fact, IL-12 has been reported to strongly suppress the production of IgE in vitro.¹⁰ However, in vivo effects of IL-12 on production of IgE are controversial.¹¹⁻¹⁴

There are several lines of evidence that IL-12 production is induced by infection with various pathogens. Hsieh et al.¹⁵ demonstrated *Listeria monocytogenes*-induced development of T_{H1} cells through production of IL-12. A high level of IL-12 was produced by monocytes infected with *Mycobacterium tuberculosis* in vitro¹⁶ and macrophages from mice infected with *Leishmania major*.¹⁷ Cleveland et al.¹⁸ reported that lipoteichoic acid, a predominant surface glycolipid of gram-positive bacteria, induced IL-12 in monocytes through a CD14-mediated pathway. Thus these bacteria may act as potent IL-12 inducers to activate natural killer cells and promote the development of T_{H1}-type immune responses. Indeed, some gram-positive *Lactobacillus* species are reported to potentiate IFN- γ production and enhancement of natural killer activity.¹⁹ The effect of *Lactobacillus* species on IL-12 induction may be applied not only for promotion of T_{H1}-type immune response but also for inhibition of T_{H2}-type immune response.

Animal models of allergen-induced IgE production without adjuvant have recently been developed. Renz et

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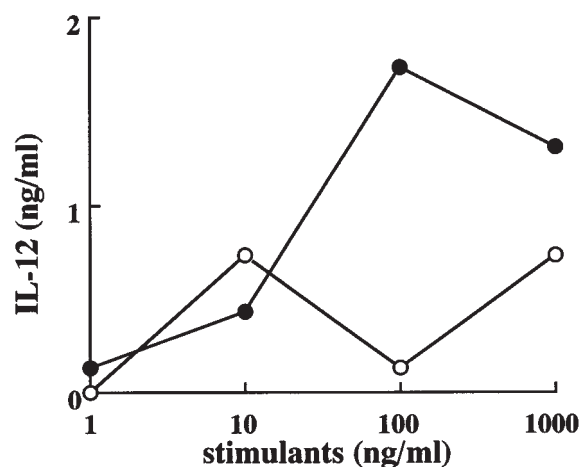


FIG. 1. IL-12 production of macrophages stimulated with *L. plantarum* L-137 in vitro. Peritoneal macrophages were pooled from three normal mice, and cells (1×10^5 /well) were cultured for 15 hours with LPS (open circles) or heat-killed *L. plantarum* L-137 (closed circles) at various concentrations. Amounts of IL-12 or TNF- α in supernatants were assayed by ELISA. Each data point is mean of triplicate cultures. Results show one representative experiment from two independent experiments.

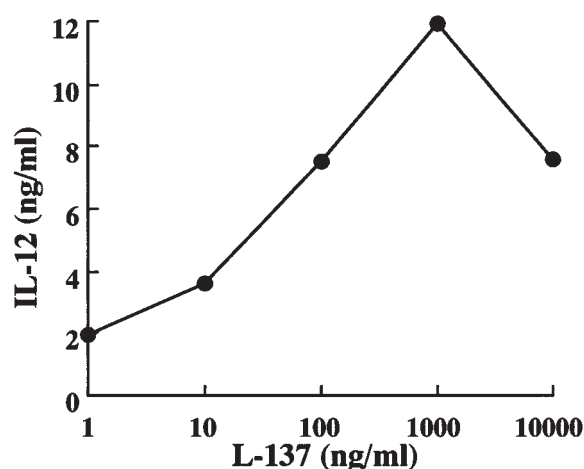


FIG. 2. IFN- γ and IL-12 production of spleen cells stimulated with *L. plantarum* L-137 in vitro. Spleen cells (5×10^5 /well) from normal mouse were cultured for 96 hours with heat-killed *L. plantarum* L-137 at concentrations indicated. Amounts of IL-12 or IFN- γ in supernatants were assayed by ELISA. Each data point is mean of triplicate cultures. Results show one representative experiment from two independent experiments.

Abbreviations used

ANOVA:	Analysis of variance
BB:	Borate buffer
Con A:	Concanavalin A
IFN- γ :	Interferon- γ
LPS:	Lipopolysaccharide
rmIL-12:	Recombinant mouse IL-12
TCR:	T-cell receptor
TNF- α :	Tumor necrosis factor- α

al.²⁰ reported that aerosolized antigen exposure caused increased IgE production in mice in a model of bronchial asthma. Wang et al.²¹ found that epicutaneous exposure to protein antigen induced a predominant T_{H2} -like response with high IgE production in mice as a model of atopic dermatitis. We have recently developed a murine model of IgE

production characterized by elevation of IgE levels specific for casein in association with predominant T_{H2} -like responses in DBA/2 mice fed a casein diet.²² This model for IgE production against food antigen provides an insight into the therapeutic approach to controlling food allergy.

To develop reagent for controlling food allergy, we examined the effects of *L. plantarum* L-137 on IgE production in our murine model. *L. plantarum* L-137 directly induced IL-12 production by the peritoneal macrophages and spleen cells of naive mice in vitro. Administration of *L. plantarum* L-137 from the beginning of feeding of a casein diet to DBA/2 mice suppressed the elevation of anti-casein IgE levels. Consistent with the in vitro finding, IL-12 production of the peritoneal macrophages from *L. plantarum* L-137-treated mice was enhanced, but IL-4 production of the spleen cells in response to con-

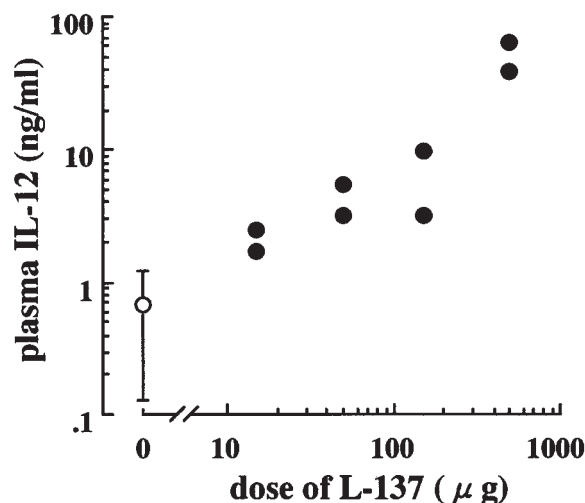


FIG. 3. Increase in plasma IL-12 level by injection of *L. plantarum* L-137. *L. plantarum* L-137 was injected intraperitoneally at doses indicated. Plasma IL-12 levels 4 hours after injection were assayed by ELISA. Data from control mice are shown as mean $\pm$ SEM ($n = 5$, open circles). All data are given for *L. plantarum* L-137-injected mice ($n = 2$ for dose, closed circles).

canavalin A (Con A) was suppressed in these mice. Administration of *L. plantarum* L-137 or recombinant mouse IL-12 (rmIL-12) from 2 weeks after the start of feeding also significantly suppressed the elevation of anti-casein IgE levels. Secretion of IL-12 induced by *L. plantarum* L-137 may be related to suppression of IgE production.

METHODS

Animals

Female 5-week-old DBA/2 or B10.A mice were obtained from Charles River Japan (Atsugi, Japan) or Japan SLC (Hamamatsu, Japan). The mice were maintained in our facility and fed a commercial nonpurified diet (CE-2; CLEA Japan, Tokyo, Japan) and tap water for 1 or 2 weeks before experiments were begun. A notice of the Prime Minister's Office of Japan (no. 6 of March 27, 1980) for the care and use of laboratory animals was followed.

Preparation of *L. plantarum* L-137

Heat-killed *L. plantarum* L-137 was used in this study. *L. plantarum* L-137 was incubated in glucose=yeast extract=peptone (GYP) broth (starch was substituted for glucose) at 32°C for 24 hours, harvested by centrifugation at 4200 g for 35 minutes at 4°C, washed three times with saline, and suspended in distilled water. The bacteria were heated at 70°C for 10 minutes and lyophilized. The preparation was suspended in saline and sonicated before use in experiments.

In vitro stimulation of *L. plantarum* L-137

Mice were killed by decapitation and injected intraperitoneally with 5 ml Hank's balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.) supplemented with HEPES, 5 U/ml heparin, and 5% NuSerum (Becton Dickinson Labware, Bedford, Mass.), and the ascites fluid was collected. The peritoneal cells were washed and suspended in RPMI 1640 (GIBCO) supplemented with HEPES, L-glutamine, penicillin, streptomycin, 2-mercap-

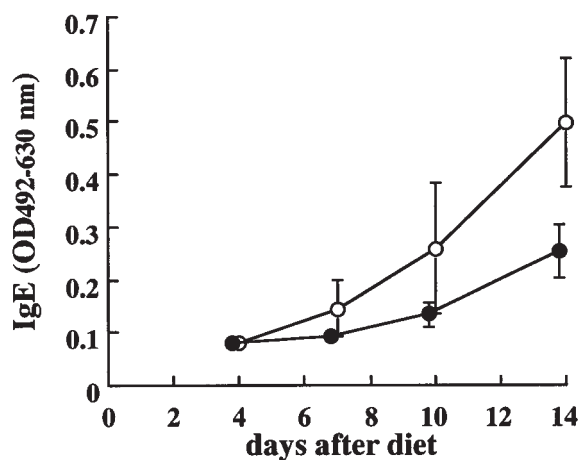
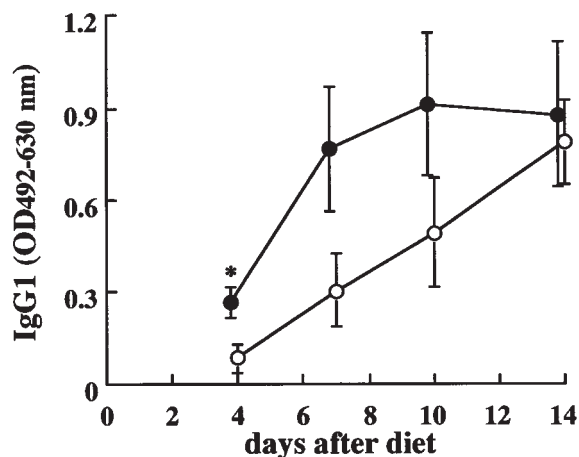


FIG. 4. Prevention of naturally fed antigen-specific IgE production by *L. plantarum* L-137. DBA/2 mice were fed casein diet from day 0 and were injected intraperitoneally with 50 μ g of *L. plantarum* L-137 (closed circles) or saline (open circles) on days 0, 4, 7, and 10. Plasma levels of casein-specific IgG1 or IgE were determined by ELISA. Data are shown as means $\pm$ SEM ($n = 5$ or 6). The upward curves (from day 4 to 14) of IgG1 and IgE were significantly ($p < 0.05$) different between groups as determined by two-way ANOVA. Asterisks indicate values significantly different from control ($p < 0.05$) each day.

toethanol, and 10% NuSerum at a concentration of 1×10^6 large size cells/ml determined by an automatic blood cell counter (F-800; Sysmex, Kobe, Japan). Aliquots of the cell suspension (100 μ l) were poured into the wells of a 96-well, flat-bottom plate (Nippon InterMed, Tokyo, Japan), and the plate was incubated for 2 hours at 37°C in a humidified CO₂ incubator. The wells were then washed three times with warmed saline, and the adherent peritoneal cells were used as macrophages. The macrophages were stimulated by 0.001 to 1 μ g/ml *L. plantarum* L-137 or by 0.001 to 1 μ g/ml lipopolysaccharide (LPS) (*Escherichia coli* 0127: B8; Difco Laboratories, Detroit, Mich.) for 15 hours at 37°C in a humidified CO₂ incubator. Culture supernatants were then harvested for determination of cytokine production. Alternatively, spleens were removed aseptically and single-cell suspensions were prepared by gently squeezing them between two glass slides in Hank's balanced salt solution. The cells were washed and suspended in RPMI 1640. Cultures of 200 μ l of 2.5×10^6 cells/ml

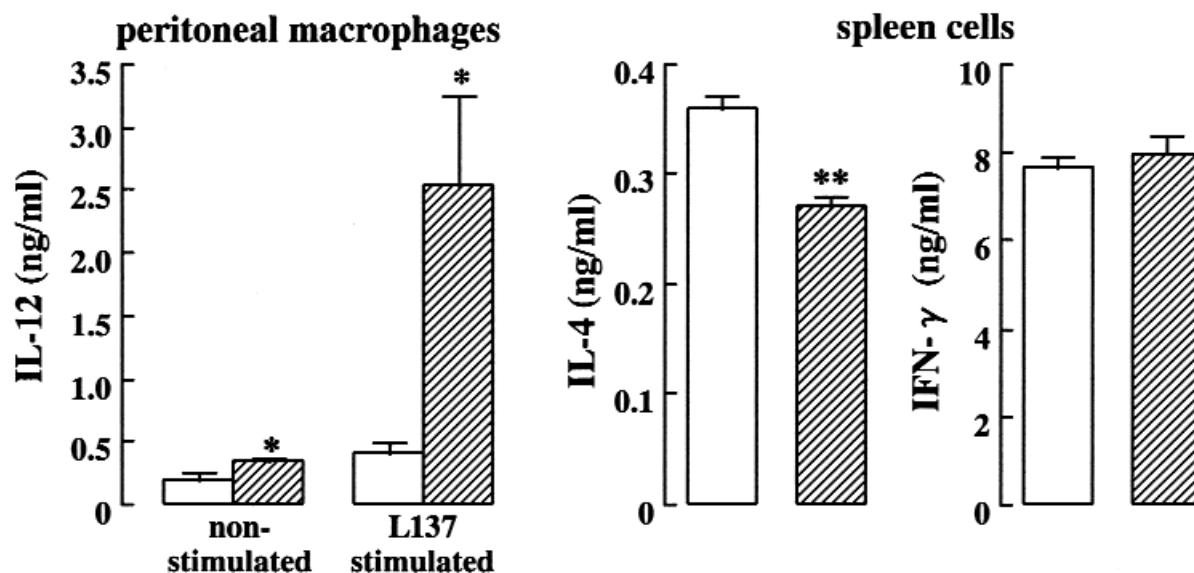


FIG. 5. Change in cytokine production after in vivo treatment with *L. plantarum* L-137. Cells from mice fed casein diet for 14 days and injected intraperitoneally with 50 μ g of *L. plantarum* L-137 (hatched bars) or saline (open bars) on days 0, 4, 7, and 10 were cultured. Peritoneal macrophages were cultured for 15 hours in presence or absence of 1 μ g of *L. plantarum* L-137, or spleen cells were cultured for 96 hours in presence of 2 μ g of Con A. The amounts of IL-12 or IL-4 in supernatants were assayed by ELISA. Data are shown as means $\pm$ SEM ($n = 5$ or 6). Asterisks indicate values significantly different from controls (* $p < 0.05$, ** $p < 0.01$).

were prepared in 96-well, flat-bottom plates. Cultured cells were stimulated by 0.001 to 10 μ g/ml *L. plantarum* L-137 for 96 hours at 37°C in a humidified CO₂ incubator. Culture supernatants were then harvested for determination of cytokine production.

In vivo stimulation of *L. plantarum* L-137

Mice were injected intraperitoneally with 15 to 500 μ g *L. plantarum* L-137. Plasma from the mice before and 4 hours after the injection was collected and analyzed for IL-12 levels with an ELISA. Results were shown by subtracting plasma IL-12 concentration before the injection from that obtained 4 hours after the injection.

Determination of cytokines

The concentrations of IL-12, IFN- γ , IL-4, and tumor necrosis factor (TNF)- α in the cultured supernatants were respectively examined by sandwich ELISA. Capture antibodies used were monoclonal rat anti-mouse IL-12 (Clone C17.8; Genzyme, Cambridge, Mass.), monoclonal hamster anti-murine IFN- γ (Genzyme), rat anti-murine IL-4 monoclonal (Clone BVD4-1D11; Endogen, Cambridge, Mass.), and monoclonal hamster anti-murine TNF- α (Genzyme). Detection antibodies used were monoclonal rat anti-mouse IL-12 (Clone C15.6, Genzyme), monoclonal immunoglobulin anti-mouse IFN- γ , rat IgG1 (Lee Biomolecular, San Diego, Calif.), rat anti-murine IL-4 monoclonal (Clone BVD6-24G2, Endogen), and polyclonal rabbit anti-mouse TNF- α (Genzyme). Standard cytokines used were rmIL-12 (Genzyme), recombinant mouse IFN- γ (Genzyme), recombinant mouse IL-4 (Genzyme), and recombinant mouse TNF- α (Genzyme). Each well of the 96-well, flatbottom plates was coated with 100 μ l of capture antibody (6 μ g/ml) diluted with borate buffer (BB) (pH 8.5). After overnight incubation at room temperature, the wells were blocked with 1% bovine serum albumin in BB for 30 minutes at room temperature. The wells were washed three times with BB containing 0.05% Tween 20 (BBT), and then culture supernatants or the serial diluted standard cytokine were incubated for

90 minutes at room temperature. The wells were washed with BBT before adding diluted detection antibody. After incubation and a wash with BBT, diluted peroxidase-monoclonal mouse anti-rat IgG1 (Zymed Laboratories, San Francisco, Calif.) or diluted peroxidase-monoclonal rat anti-rabbit IgG (Zymed Laboratories) was added to the wells for detection of IL-12, IFN- γ , and IL-4 or TNF- α . After incubation for 90 minutes, the wells were washed with BBT, and o-phenylenediamine (1 mg/ml) in phosphate buffer (pH 7.0, 0.2 μ l/ml H₂O₂) was applied. Reactions were stopped with 1.5 N H₂SO₄ after incubation for 40 minutes. OD492-630 values were measured with a microplate reader MTP-32 (Corona Electric, Ibaragi, Japan).

L. plantarum L-137 treatments in mice fed casein

Systemic humoral responses against casein were induced in the mice by feeding them a 25% casein pellet diet for 2 or 5 weeks. The diet was a standard purified diet purchased from Oriental Yeast (Tokyo, Japan). The composition of the diet was (in weight percent): starch, 48%; casein, 25%; cellulose, 8%; soybean oil, 6%; sucrose, 5%; mineral mixture, 6%; and vitamin mixture, 2%. The mice were injected intraperitoneally with 50 μ g *L. plantarum* L-137 on days 0, 4, 7, and 10 after the start of casein feeding. Control mice were injected with saline intraperitoneally with the same schedule. After 2 weeks of feeding, spleen cells or peritoneal macrophages from these mice were stimulated with Con A (Type IV; Sigma Chemical, St. Louis, Mo.) or *L. plantarum* L-137 in vitro. In the 5-week feeding experiment, the mice were injected intraperitoneally with 500 μ g *L. plantarum* L-137 or 0.25 μ g rmIL-12 (Genzyme) on days 14, 17, 20, and 24 after the start of feeding.

Determination of anti-casein antibodies

Casein-specific IgE and IgG1 antibodies in the plasma were measured by ELISA. Each well of the 96-well, flat-bottom plates was coated with 100 μ l of casein diluted with BB (5 μ g/ml). After overnight incubation at room temperature, the wells were blocked

with 1% bovine serum albumin in BB for 30 minutes at room temperature. The wells were washed three times with BBT, and 1:10 diluted plasma samples were incubated for 90 minutes at room temperature. The wells were washed with BBT, and diluted peroxidase-conjugated goat-anti-mouse IgE (FC) antibody (Nordic Immunological Laboratories, Netherlands) or peroxidase-conjugated rabbit anti-mouse IgG1 antibody (Zymed) was added to the wells for the detection. After incubation for 90 minutes, the wells were washed with BBT, and *o*-phenylenediamine (1 mg/ml) in phosphate buffer (pH 7.0, 0.2 µl/ml H₂O₂) was applied. Reactions were stopped with 1.5 N H₂SO₄ after incubation for 20 minutes. OD492-630 values were measured with a microplate reader MTP-32.

Statistical analysis

Results of anti-casein IgE and IgG1 antibodies were analyzed by two-way analysis of variance (ANOVA) for main effects (time and treatment) and interaction (time × treatment) followed by Student's *t* test between two groups or by Duncan's multiple range test²³ among three groups. Significance was assessed at *p* values less than 0.05.

RESULTS

L. plantarum L-137 is a potent inducer of IL-12 production

To elucidate whether *L. plantarum* L-137 induces the production of IL-12, we added heat-killed *L. plantarum* L-137 to the culture of the peritoneal adherent cells of normal mice. After 15 hours of culture, with an optimum concentration of 0.1 µg/ml of *L. plantarum* L-137, the peritoneal macrophages remarkably produced IL-12 (Fig. 1). Although the ability of *L. plantarum* L-137 to induce the production of IL-12 was much higher than that of LPS, the level of TNF-α induced by *L. plantarum* L-137 was lower than that induced by LPS (Fig. 1). To confirm that *L. plantarum* L-137 has an ability to enhance IFN-γ production, we examined the levels of both IL-12 and IFN-γ in supernatants after culture of spleen cells of normal mice. *L. plantarum* L-137 also induced the production of IL-12 from spleen cells with an optimum concentration at 1 µg/ml (Fig. 2). In correlation with IL-12 production, IFN-γ was produced from spleen cells in response to *L. plantarum* L-137. Induction of both IL-12 and IFN-γ showed the same dose-dependency to the concentration of *L. plantarum* L-137 (Fig. 2).

We next examined the effects of *L. plantarum* L-137 on IL-12 production in vivo. *L. plantarum* L-137 was injected intraperitoneally into normal mice, and plasma IL-12 levels were determined. As shown in Fig. 3, in vivo administration of *L. plantarum* L-137 increased plasma IL-12 levels in a dose-dependent manner: slight increases at doses of 15, 50, and 150 µg and a steep increase at 500 µg.

Administration of *L. plantarum* L-137 to DBA/2 mice from the beginning of casein feeding inhibits IgE production against casein

We have recently developed a murine model of IgE production by feeding antigen. DBA/2 mice fed a casein diet without any adjuvant produced anti-casein IgE anti-

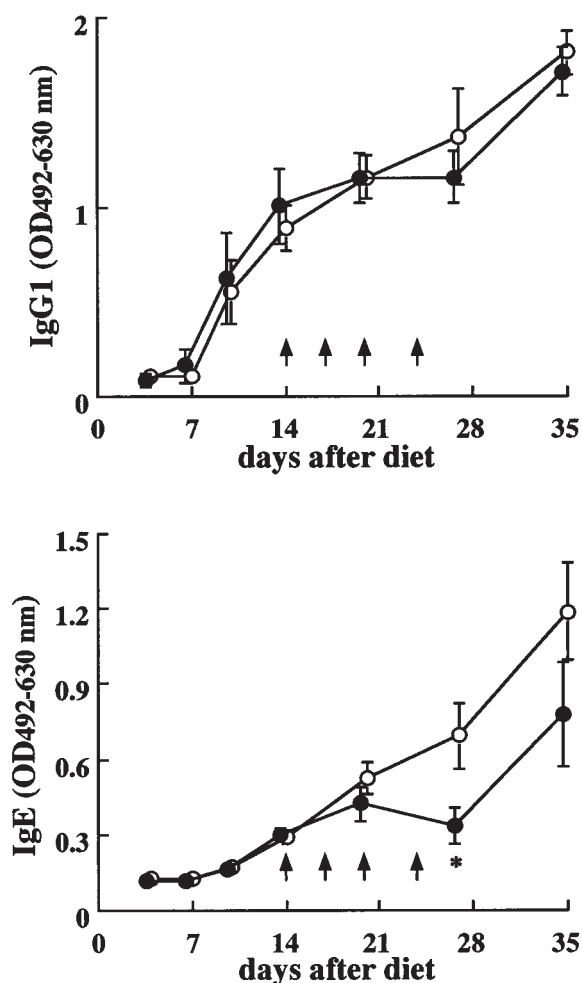


FIG. 6. Decrease of naturally fed antigen-specific IgE production by *L. plantarum* L-137. DBA/2 mice were fed casein diet from day 0 and were injected intraperitoneally with 500 µg of *L. plantarum* L-137 (closed circles) or saline (open circles) on days 14, 17, 20, and 24. Plasma levels of casein-specific IgG1 or IgE were determined by ELISA. Data are shown as means ± SEM (*n* = 5). Upward curves (from day 20 to 35) of IgE were significantly (*p* < 0.05) different between groups as determined by two-way ANOVA. Asterisks indicate values significantly different from control (*p* < 0.05) each

bodies with a predominant T_{H2}-like response.²² To investigate whether the IgE response against food antigen could be regulated by *L. plantarum* L-137, *L. plantarum* L-137 was injected intraperitoneally into DBA/2 mice at a dose of 50 µg on days 0, 4, 7, and 10 after the start of casein feeding, and plasma casein-specific IgG1 and IgE levels were measured. Plasma casein-specific IgG1 levels gradually elevated after 7 days on the diet in control mice. The level of anti-casein IgG1 antibodies was higher in mice treated with *L. plantarum* L-137 on day 4 after the start of feeding, and thereafter those levels were further increased (Fig. 4). On the other hand, plasma casein-specific IgE levels were lower in mice treated with *L. plantarum* L-137 in contrast with the response of IgG1 (Fig. 4). The upward curves from day

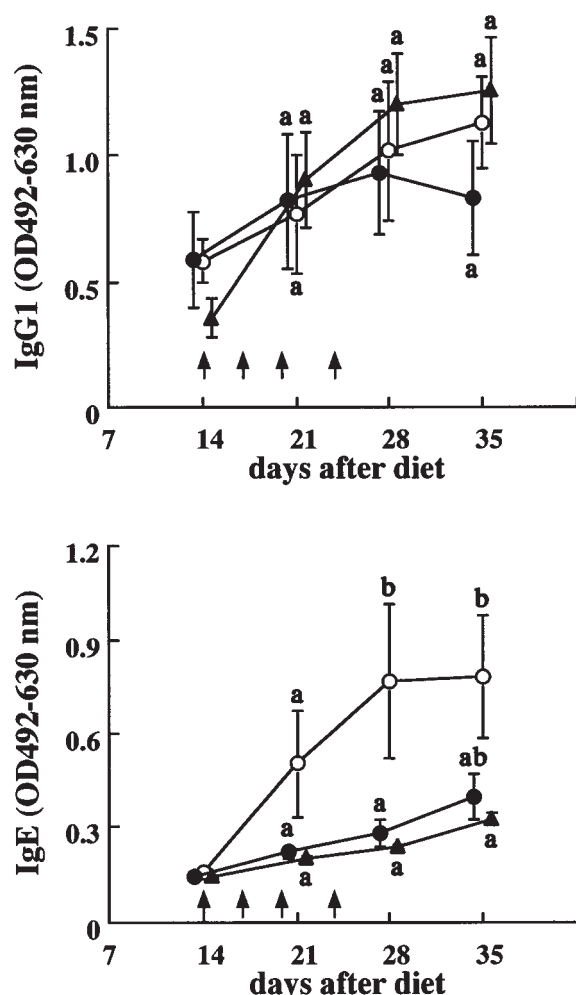


FIG. 7. Prevention of naturally fed antigen-specific IgE production by IL-12. DBA/2 mice were fed casein diet from day 0 and were injected intraperitoneally with 0.25 μ g rmIL-12 (closed triangles) or 500 μ g of *L. plantarum* L-137 (closed circles) or saline (open circles) on days 14, 17, 20, and 24. Plasma levels of casein-specific IgG1 or IgE were determined by ELISA. Data are shown as means $\pm$ SEM ($n = 5$). Upward curves (from day 21 to day 35) of IgE were significantly ($p < 0.01$) different among three groups as determined by two-way ANOVA. Differences among three groups for each day were analyzed by Duncan's multiple range test: points without common letters differ significantly ($p < 0.05$).

4 to 14 or the levels on day 14 of casein-specific IgE were significantly, or tended to be, different ($p < 0.05$ or $p = 0.079$) between the two groups.

Cytokine production in mice treated with *L. plantarum* L-137

The production of IgE depends on IL-4 synthesized by T_{H2} cells and is strongly suppressed by IL-12. To confirm the in vivo effect of *L. plantarum* L-137 on IL-12 production, we measured the level of IL-12 after 15 hours of culture of the peritoneal macrophages from mice injected with *L. plantarum* L-137. The level of IL-12 production in mice treated with *L. plantarum* L-137 was signif-

icantly high compared with control mice irrespective of in vitro restimulation with *L. plantarum* L-137 (Fig. 5). We have previously reported that Con A-stimulated spleen cells from mice fed casein produced a high level of IL-4. Therefore we next examined the IL-4 production in Con A-stimulated spleen cells from mice treated with *L. plantarum* L-137. After culture for 72 hours under stimulation of Con A, the levels of IL-4 in the culture supernatants were significantly lower in mice treated with *L. plantarum* L-137 than in nontreated mice (Fig. 5). Thus these results suggest that in vivo treatment with *L. plantarum* L-137 induces IL-12 production and consequently suppresses T_{H2} -like responses in casein-fed mice.

Administration of *L. plantarum* L-137 to DBA/2 partially sensitized with casein

To investigate whether ongoing IgE responses by feeding antigens could be suppressed by *L. plantarum* L-137, we injected *L. plantarum* L-137 to DBA/2 mice 2 weeks after the start of feeding. After feeding for 2 weeks, we divided the mice into two groups as each group had the same plasma levels of casein-specific IgG1 and IgE, and injected *L. plantarum* L-137 into the mice at a dose of 500 μ g on days 14, 17, 20, and 24. Although plasma casein-specific IgE levels in control mice continued to rise until day 35, the elevation of plasma casein-specific IgE levels was suppressed by treatment with *L. plantarum* L-137 (Fig. 6). The casein-specific IgE levels in mice treated with *L. plantarum* L-137 were significantly lower than those in control mice on day 28. However, the suppressive effects of *L. plantarum* L-137 were abolished on day 35, 11 days after the last injection (Fig. 6). Although the injection of *L. plantarum* L-137 from day 0 enhanced the elevation of plasma casein-specific IgG1 levels (Fig. 4), the injection from day 14 had little effect on plasma casein-specific IgG1 levels (Fig. 6).

Administration of rmIL-12 to DBA/2 mice partially sensitized with casein

To examine whether *L. plantarum* L-137-induced IL-12 is effective in suppressing IgE production in this murine model, we injected rmIL-12 into DBA/2 mice 2 weeks after the start of feeding. After feeding for 2 weeks, we divided the mice into three groups as each group had the same plasma levels of casein-specific IgE, and injected 0.25 μ g of rmIL-12 or 500 μ g of *L. plantarum* L-137 to the mice on days 14, 17, 20, and 24. Although plasma casein-specific IgE levels in control mice were fully elevated until day 28, the elevation of plasma casein-specific IgE levels was suppressed by treatment with either rmIL-12 or *L. plantarum* L-137 (Fig. 7). The casein-specific IgE levels in mice treated with either rmIL-12 or *L. plantarum* L-137 were significantly lower than those in control mice on day 28. In degree of suppression of IgE, *L. plantarum* L-137 treatment was much the same as rmIL-12 treatment. Thus these results suggest that IL-12 induced by in vivo treatment with *L. plantarum* L-137 contributes to the suppression of IgE production in casein-fed mice.

DISCUSSION

We demonstrated here that plasma casein-specific IgE levels in DBA/2 mice fed a casein diet are significantly suppressed by injection of *L. plantarum* L-137, which can induce IL-12 production from macrophages. Injection of *L. plantarum* L-137 from the beginning of feeding inhibited the production of casein-specific IgE accompanied by the enhancement of casein-specific IgG1 levels. Injection of *L. plantarum* L-137 from 2 weeks after the start of feeding also inhibited the production of casein-specific IgE but had no influence on the production of casein-specific IgG1. Our results suggest that potent inducers of IL-12, such as *L. plantarum* L-137, are useful as therapeutic or prophylactic agents for controlling food allergy accompanying the elevation of IgE against food antigens.

We have recently established a murine model of IgE production induced by feeding protein antigen as a constituent of the diet.²² DBA/2 mice fed a casein diet without any adjuvant produced plasma casein-specific IgE antibodies. In DBA/2 mice fed the casein diet, T_{H2}-like immune responses were enhanced as represented by the high levels of IL-4 produced upon T-cell receptor (TCR) triggering in mesenteric lymph node, spleen, and liver cells. The fact that spleen cells in DBA/2 mice fed casein proliferated in response to casein and produced detectable levels of IL-4 suggested an important role for the spleen in casein-specific T_{H2} responses in this murine model of IgE production. Furthermore, the increased number of TCR α β bearing intraepithelial lymphocytes and TCR^{intermediate} IL-2 receptor- β ⁺ liver T cells suggested that these cells contribute to the initiation of T_{H2} response for IgE production through IL-4 production. The fact that the casein-fed DBA/2 mice treated with *L. plantarum* L-137 produced lower levels of casein-specific IgE antibodies accompanied by a decreased level of IL-4 production in mitogen-stimulated spleen T cells also supports an important role for the spleen in T_{H2} responses for IgE production in this murine model.

IL-12 is a heterodimeric cytokine composed of a constitutively expressed 35 kd (p35) subunit and inducible 40 kd (p40) subunit.²⁴ Although the p40 subunit can be produced as monomer or homodimer in the absence of p35, only the heterodimer is biologically active.²⁵ Because both the capture antibodies and detection antibodies used in this study for the determination of IL-12 recognize the p40 subunit, it is possible that *L. plantarum* L-137 induced the production of monomer or homodimer of the p40 subunit instead of the bioactive IL-12 heterodimer. However, we also found that *L. plantarum* L-137 induces the production of IFN- γ from spleen cells in a dose-dependent manner similar to induction of IL-12, indicating the ability of *L. plantarum* L-137 to induce bioactive IL-12 heterodimer. Our finding that *L. plantarum* L-137 stimulated macrophages to produce IL-12 in vitro and in vivo suggested that the IL-12 released from macrophages is the primary cause of the well-known biologic effects of lactobacilli on IFN- γ produc-

tion and augmentation of natural killer activity.^{19,26} We have not characterized which components of *L. plantarum* L-137 are important for IL-12 induction. Recently, Cleveland et al.¹⁸ reported that lipoteichoic acid, a predominant surface glycolipid of gram-positive bacteria, induced IL-12 production from monocytes through a CD14-mediated pathway similar to IL-12 induction by gram-negative LPS. Thus it is possible that lipoteichoic acid of *L. plantarum* L-137 induces IL-12 through a CD14-mediated pathway.

Exogenous IL-12 has been shown to inhibit IL-4-induced IgE synthesis in vitro¹⁰ and suppress anti-IgD-induced IgE production in vivo.¹¹ Endogenous IL-12 has also been shown to inhibit parasite antigen-induced IgE production.²⁷ The presence of IL-12 during initial antigen presentation has been shown to inhibit T_{H2} responses, whereas delayed treatment with IL-12 showed limited inhibitory effects on T_{H2} responses.^{28,29} This was recently confirmed by Kips et al.¹⁴ in a murine in vivo model of allergic airway diseases. They showed that administration of IL-12 during initial antigen presentation suppressed allergen-specific IgE production, but administration of IL-12 during subsequent antigen exposure did not. We demonstrated in this study, however, that induction of endogenous IL-12 at not only the initial antigen exposure but also the later stage of antigen exposure suppressed the casein-specific IgE production. The antigen-priming phase was clear when mice were sensitized by one shot of intraperitoneal injection in the allergic airway disease model but not clear by continued antigen feeding. Therefore it is possible that the IL-12 induced endogenously 2 weeks after antigen feeding acts on the T cells primarily stimulated with feeding antigens. To clarify the mechanism of suppression of IgE by *L. plantarum* L-137, one must define the direct roles of IL-12 in T_{H2} immune response for IgE production.

In conclusion, we showed that the production of casein-specific IgE antibodies in DBA/2 mice fed casein were suppressed by heat-killed *L. plantarum* L-137 in correlation with an increase in IL-12 production. Our results imply that IL-12 inducers, such as *L. plantarum* L-137, may be beneficial in the treatment of allergic diseases in which allergen-specific T_{H2} responses are predominant.

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