

Kan Shida<sup>a, b</sup>  
Kumiko Makino<sup>b</sup>  
Aki Morishita<sup>b</sup>  
Kotaro Takamizawa<sup>b</sup>  
Satoshi Hachimura<sup>a</sup>  
Akio Ametani<sup>a</sup>  
Takehito Sato<sup>c</sup>  
Yoshihiro Kumagai<sup>d</sup>  
Sonoko Habu<sup>c</sup>  
Shuichi Kaminogawa<sup>a</sup>

<sup>a</sup> Department of Applied Biological  
Chemistry, University of Tokyo,

<sup>b</sup> Yakult Central Institute for Microbiological  
Research, Kunitachi,

<sup>c</sup> Department of Immunology, Tokai  
University School of Medicine, Isehara, and

<sup>d</sup> Precursory Research for Embryonic Science  
and Technology, Research Development  
Corporation of Japan, Yokohama, Japan

## Key Words

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*Lactobacillus casei*

Th1/Th2

# *Lactobacillus casei* Inhibits Antigen-Induced IgE Secretion through Regulation of Cytokine Production in Murine Splenocyte Cultures

## Abstract

**Background:** *Lactobacillus casei* is a nonpathogenic gram-positive bacterium widely used in dairy products and has been shown to enhance the cellular immunity of the host. **Methods:** To examine the inhibitory effect of *L. casei* on IgE production, splenocytes obtained from ovalbumin (OVA)-primed BALB/c mice were restimulated in vitro with the same antigen in the presence of heat-killed *L. casei*. The effect of this bacterium on T helper (Th) phenotype development was also examined with naive T cells from OVA-specific T cell receptor-transgenic mice. **Results:** *L. casei* induced IFN- $\gamma$ , but inhibited IL-4 and IL-5 secretion, and markedly suppressed total and antigen-specific IgE secretion by OVA-stimulated splenocytes. The inhibitory effect of *L. casei* on IgE, IL-4, and IL-5 production was partially abrogated by addition of neutralizing antibody to IFN- $\gamma$ . Augmented IL-12 production was also observed in the cell cultures containing *L. casei*, and anti-IL-12 monoclonal antibody completely restored the IgE, IL-4, and IL-5 production to the control levels. The IL-12 augmentation by *L. casei* was macrophage-dependent. The Th cell development assay showed the ability of *L. casei* to induce Th1 development preferentially. This effect was also completely blocked by anti-IL-12 antibody. **Conclusions:** This is the first demonstration that a nonpathogenic microorganism, *L. casei*, can inhibit antigen-induced IgE production through induction of IL-12 secretion by macrophages. The findings suggest a potential use of this organism in preventing IgE-mediated allergy.

## Introduction

Because allergen-specific IgE is directly involved in mediating many allergic reactions, development of a method for inhibiting IgE production is a useful approach for preventing allergic diseases. Cytokines derived from T helper (Th) cells are major modulators of IgE synthesis. IL-4 induces IgE isotype class switching [1] and plays a cru-

cial role in IgE production. IL-5 [2] and IL-6 [3] enhance IL-4-dependent IgE production. All of these cytokines are produced by Th2 cells. On the other hand, IFN- $\gamma$  produced by Th1 cells suppresses IgE production both by interfering with the IL-4-derived isotype switching [4] and by inhibiting the proliferation of Th2 cells [5]. The balance between Th1 and Th2 cytokines is considered to be critical for IgE production. Therefore, enhancing the Th1-type immune re-

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Correspondence to: Dr. Kan Shida  
Department of Applied Biological Chemistry  
University of Tokyo, Bunkyo-ku  
Tokyo 113 (Japan)  
Tel. +81 3 3812 2111, Fax +81 3 5802 3316

sponse can be of clinical importance with respect to IgE suppression [6].

Lactic acid bacteria and their products have been reported to have beneficial effects on host homeostasis, including activation of the immune system [reviewed in 7, 8]. The effects of *Lactobacillus casei*, a member of the lactic acid bacteria, have been studied intensively with regard to therapeutic aspects. *L. casei* exhibits remarkable antitumor activity against syngeneic and allogenic tumors [9, 10] and anti-infectious activity against various pathogens such as *Pseudomonas aeruginosa* and *Listeria monocytogenes* [11, 12]. These activities are mediated by the activated host immune system, mainly by activated macrophages [11, 13]. NK cells [14] and cytotoxic T cells [15] are also stimulated by *L. casei* and can contribute to these activities.

These findings show that *L. casei* enhances cellular immunity, in which Th1 cells may play an important role. In fact, some lactic acid bacteria including *L. casei* are known to enhance levels of production of a typical Th1 cytokine, IFN- $\gamma$  [16]. To date, although the potentiation of cellular immunity by *L. casei* has been well documented from the standpoint of host resistance to tumors and infections, as described above, the effect of *L. casei* on IgE production has not been clarified.

In the present study, we examined the effect of *L. casei* on allergen-induced IgE production by murine splenocytes, since augmentation of the Th1 response by this bacterium leading to high levels of IFN- $\gamma$  is likely to suppress IgE production. The data presented show that *L. casei* markedly inhibits IgE production through inducing a dominant Th1-type cytokine profile mediated by IL-12. The findings suggest a potential use of this organism in preventing IgE-mediated allergy.

## Materials and Methods

### Mice

Female BALB/c mice (6–8 weeks old) purchased from Japan SLC (Hamamatsu, Japan) were used for the ovalbumin (OVA)-induced IgE production system. Offsprings (male, 15–20 weeks old) of OVA23–3 mice [17] transgenic for OVA<sub>323–339</sub>-specific and I-A<sup>d</sup>-restricted T cell receptor (TCR)- $\alpha\beta$ , with a BALB/c genetic background were used for the Th cell development assay.

### Microorganisms

*Lactobacillus casei* strain Shirota was originally isolated from human feces at Yakult Central Institute for Microbiological Research (Tokyo, Japan) and *Lactobacillus johnsonii* JCM 0212, 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 a preliminary study examining the IFN- $\gamma$ -inducing activity of several type strains of *Lactobacilli* had shown that

*L. johnsonii* and some other strains scarcely induced IFN- $\gamma$  production in cultures of BALB/c splenocytes (unpubl. observation). These organisms were cultured for 20 h at 37°C in Lactobacilli-MRS broth (DIFCO, Detroit, Mich., USA), collected by centrifugation, and washed several times with sterile distilled water. The organisms were killed by heating at 100°C for 30 min and lyophilized. The lyophilized preparations were suspended in phosphate-buffered saline and autoclaved (121°C, 20 min) before addition to cell cultures.

### Treatment of *L. casei* under Simulated Gastric Conditions

Heat-killed *L. casei* cells were suspended in distilled water, and the pH was adjusted to 2.5 with 1 N HCl. Pepsin (pepsin A from porcine stomach mucosa; Sigma, St. Louis, Mo., USA) at 0.5 mg/ml was added to the suspension containing the bacterium at 5 mg/ml. The treatment was performed at 37°C for 3 h. After adjustment of the pH to 7.2 with 0.2 N NaOH, the bacterial cells were extensively washed by centrifugation with phosphate-buffered saline.

### Cell Lines and Antigen

The murine macrophage cell line, J774.1, was purchased from Riken Gene Bank (Tsukuba, Japan) and maintained with RPMI-1640 medium (Nikken Bio Medical Lab., Kuze, Japan) supplemented with 10% heat-inactivated fetal calf serum (FCS; Moregate, Melbourne, Australia). The murine B lymphoma cell line, A20.2J, was kindly supplied by Dr. Sercarz (University of California at Los Angeles) and maintained with DMEM medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% heat-inactivated FCS. The antigen of OVA (2  $\times$  crystallized) was purchased from Seikagaku Kogyo (Tokyo, Japan).

### Cell Cultures for OVA-Induced IgE and Cytokine Production

The effect of *L. casei* on OVA-induced production of IgE and cytokines was investigated using a culture system with OVA-primed BALB/c splenocytes as described by Marcelletti and Katz [18] with some modifications. BALB/c mice were intraperitoneally immunized with 50  $\mu$ g of OVA adsorbed onto 1 mg of alum in 0.1 ml sterile saline. After 10 days, splenocytes were isolated as a single-cell suspension from the sensitized mice. After depletion of erythrocytes, the cells ( $2.5 \times 10^6$ /ml) were cultured with OVA (100  $\mu$ g/ml) in the absence or presence of lactic acid bacteria (0.01–1  $\mu$ g/ml) in 0.2 ml of RPMI-1640 supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin in a 96-well culture plate (Nunc, Roskilde, Denmark). Supernatants were collected on day 7 for cytokine determination and on day 14 for IgE determination, unless indicated otherwise, and stored at –80°C for further analysis. In the neutralization study with rat antimouse IFN- $\gamma$  (Pharmingen, San Diego, Calif., USA; clone XMG1.2) or rat antimouse IL-12 (Pharmingen; clone C17.8) monoclonal antibody, the antibody was added at indicated concentrations with *L. casei* (0.5  $\mu$ g/ml) at the beginning of culture. In this system, splenocytes stimulated with the sensitizing antigen produced IgE, whereas splenocytes cultured with irrelevant antigens did not.

To monitor OVA-specific IgE production, we modified the above culture system slightly. Splenocytes were cultured in 1 ml of medium with OVA in the presence or absence of *L. casei* for 3 days in a 48-well plate. The cultured cells were harvested, washed to remove OVA, and cultured without OVA for a further 7 days. Supernatants were collected for determination of OVA-specific IgE by ELISA.

### Th Cell Development Assay

The effect of *L. casei* on Th phenotype development was studied with naive splenic T cells from OVA-specific TCR transgenic mice by the method of Hsieh et al. [19]. Splenocytes were prepared from non-immunized TCR transgenic mice and depleted of erythrocytes. After depletion of plastic-adherent cells by incubation in a plastic dish for 2 h, T cells were isolated using a nylon wool column (Wako Pure Chemical, Osaka, Japan) and the cells obtained were more than 90% Thy1+ as indicated by flow cytometry. The T cells ( $2.5 \times 10^5$ /ml) were primarily cultured in 2 ml of RPMI-1640 supplemented with 5% heat-inactivated FCS, 0.05 mM 2-mercaptoethanol, 100 U/ml penicillin, and 100 µg/ml streptomycin in a 24-well plate (Costar, Cambridge, Mass., USA) with OVA (100 µg/ml) plus irradiated BALB/c splenocytes (3,000 rad,  $2.5 \times 10^6$ /ml) as antigen-presenting cells in the absence or presence of IL-4 (10 ng/ml; Pepro Tech, Rocky Hill, N.J., USA), IL-12 (7–700 pg/ml; Genzyme, Cambridge, Mass., USA), or *L. casei* (0.01–1 µg/ml). Supernatants (0.3 ml) were collected at 48 h after primary stimulation for determination of cytokines by ELISA, and cells were expanded 2-fold into fresh medium on day 3. Cultured T cells were harvested on day 7, washed, and restimulated at  $2.5 \times 10^5$ /ml in 1 ml in a 48-well plate (Costar) with OVA (100 µg/ml) plus irradiated A20.2J B lymphoma cells (10,000 rad,  $1.25 \times 10^5$ /ml) as antigen-presenting cells in order to determine the resulting Th phenotype. Supernatants were collected at 48 h for determination of cytokines by ELISA. In the neutralization study with monoclonal rat antimouse IL-12 antibody, the antibody was added at indicated concentrations to the primary cultures containing *L. casei* (1 µg/ml). Irradiation had no discernible effects on macrophage function as previously reported [19].

### IL-12 Producing Cells Reacting to *L. casei*

BALB/c splenocytes ( $5 \times 10^6$ ), splenic plastic-adherent cells (included in  $2 \times 10^7$  whole splenocytes), J774.1 macrophage cells ( $2 \times 10^4$ ), and A20.2J lymphoma cells ( $2 \times 10^5$ ) were plated and cultured in 2 ml of RPMI-1640 supplemented with 5% heat-inactivated FCS, 2-mercaptoethanol, penicillin, and streptomycin in a 24-well plate without antigen in the absence or presence of *L. casei* (1 µg/ml). Supernatants were collected at 48 h for determination of IL-12 by ELISA. The plastic-adherent cell preparation was obtained by 2 h incubation of BALB/c splenocytes in the culture plate.

### ELISA for Total and OVA-Specific IgE

Determination of total IgE was carried out by the sandwich ELISA. Rat antimouse IgE monoclonal antibody (Pharmingen; clone R35–92) was coated onto a Maxisorp immunoplate (Nunc). After blocking unoccupied plastic sites with bovine serum albumin (Seikagaku Kogyo), samples and standard mouse IgE (Pharmingen) were added. Subsequently, bound IgE was detected by sequential incubations with biotinylated rat antimouse IgE (Serotec, Oxford, UK; clone LO-ME-2), streptavidin-alkaline phosphatase conjugates (Genzyme), and an enzyme substrate.

To assay for OVA-specific IgE titers, biotinylated OVA (5 µg/ml) was used instead of the second antibody of biotinylated rat antimouse IgE antibody. OVA-specific IgE titers were estimated by comparison with hyperimmunized mouse serum. The hyperimmunized mouse serum was obtained from BALB/c mice sensitized by three intraperitoneal injections of OVA adsorbed onto alum, and the OVA-specific IgE titer of this control serum was arbitrarily taken to be 1,000 units/ml. Biotinylated OVA was prepared by ECL protein biotinylation module (Amersham, Buckinghamshire, UK).

### ELISA for Cytokines

Determination of IFN-γ, IL-4, IL-5, and IL-12 was done by the sandwich ELISA. IFN-γ and IL-4 were measured using DuoSet mouse IFN-γ and IL-4 ELISA kits (Genzyme), respectively, according to the recommended protocol. For IL-5 and IL-12, rat antimouse IL-5 (Pharmingen; clone TRFK5) and rat antimouse IL-12 (Genzyme; clone C15.6) monoclonal antibodies were used as the capture antibody, with biotinylated rat antimouse IL-5 (Pharmingen; clone TRFK4) and biotinylated rat antimouse IL-12 (Genzyme; clone C17.8) monoclonal antibodies as the detection antibody, respectively. Standard recombinant mouse IL-5 and IL-12 were purchased from Pharmingen and Genzyme, respectively.

## Results

### *L. casei* Induces IFN-γ and Inhibits IL-4, IL-5, and Total IgE Secretion in OVA-Stimulated BALB/c Splenocyte Cultures

Splenocytes from OVA-sensitized BALB/c mice were cultured with the same antigen in the absence or presence of heat-killed lactic acid bacteria, and the levels of total IgE and cytokines in supernatants were measured. As shown in figure 1, *L. casei* induced IFN-γ, but inhibited IL-4 and IL-5 secretion, and extensively suppressed IgE production in a dose-dependent manner. On the other hand, another strain of lactic acid bacteria, *L. johnsonii*, scarcely enhanced IFN-γ production and had no effects on either IgE, IL-4, or IL-5 production. The inhibition of IgE and Th2-type cytokine production seemed to be associated with the *L. casei*-induced IFN-γ. Results of kinetics studies are shown in figure 2. IFN-γ production was satisfactorily induced by *L. casei* after 3 days of culture.

### *L. casei* Inhibits OVA-Specific IgE Secretion in OVA-Stimulated BALB/c Splenocyte Cultures

We tried to ascertain whether OVA-specific IgE production was also inhibited by *L. casei*. In our culture system OVA-specific IgE could not be detected even in the supernatants of control cultures without *L. casei*, probably because OVA present in the supernatant interfered with measurement of OVA-specific IgE by the ELISA employed. After stimulation of splenocytes with OVA for 3 days, the antigen was removed from the cultures and the splenocytes were cultured without OVA for a further 7 days. In this modified system OVA-specific IgE could be detected in the supernatants of control cultures, and it was evident that *L. casei* inhibited OVA-specific IgE production as well as total IgE production (fig. 3). The extent of inhibition of OVA-specific IgE was similar to that of total IgE.

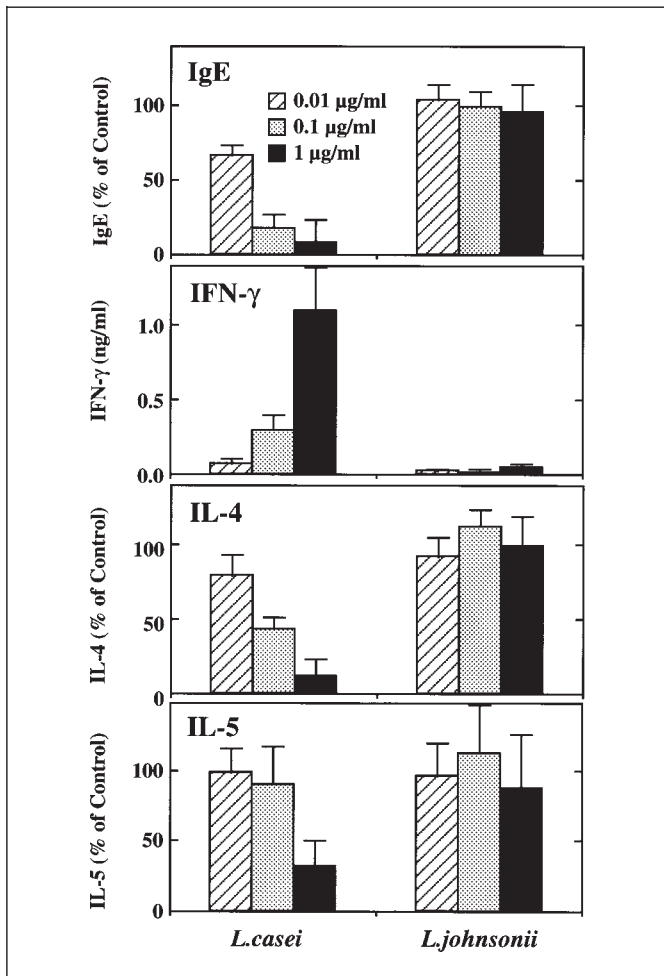


**Fig. 1.** Effect of *L. casei* and *L. johnsonii* on OVA-induced total IgE, IFN- $\gamma$ , IL-4, and IL-5 production by BALB/c splenocytes. Splenocytes from OVA-primed BALB/c mice were cultured with OVA in the absence (control) or presence of *L. casei* or *L. johnsonii* (0.01–1  $\mu$ g/ml). The levels of total IgE on day 14 and cytokines on day 7 in supernatants were determined by ELISA. Results are means  $\pm$  SD of three independent experiments expressed as the relative values to control experiments, except IFN- $\gamma$ . The values of control experiments are shown in figure 2.

#### *Anti-IFN- $\gamma$ Antibody Only Partially Restores the L. casei-Suppressed Production of IgE, IL-4, and IL-5 in OVA-Stimulated BALB/c Splenocyte Cultures*

Since IFN- $\gamma$  is known to inhibit IgE production and Th2 cell proliferation, we tried to evaluate the role of *L. casei*-induced IFN- $\gamma$  in the IgE suppression by means of a neutralization study using a monoclonal antibody. Addition of anti-IFN- $\gamma$  antibody even at 10  $\mu$ g/ml to the cultures containing *L. casei* partially abrogated the IgE suppression (fig. 4). The suppressed production of IL-4 and IL-5 was also only partially restored. In the presence of anti-IFN- $\gamma$  antibody at

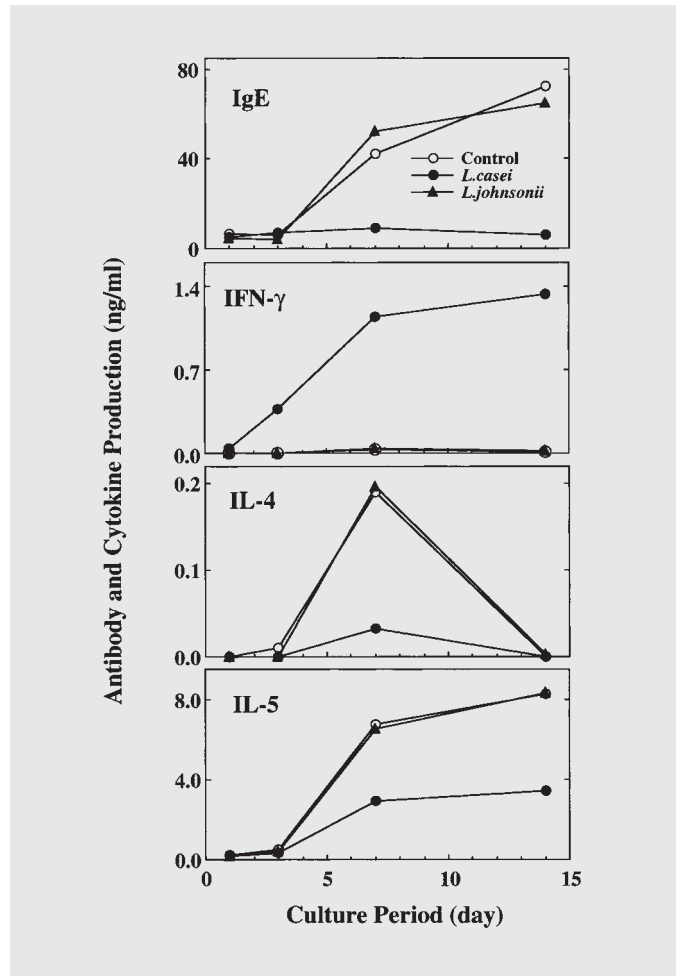


**Fig. 2.** Kinetics of total IgE, IFN- $\gamma$ , IL-4, and IL-5 production by BALB/c splenocytes cultured with or without *L. casei* or *L. johnsonii*. Splenocytes from OVA-primed BALB/c mice were cultured with OVA in the absence (control) or presence of *L. casei* or *L. johnsonii* (1  $\mu$ g/ml). The levels of total IgE and cytokines in supernatants were determined by ELISA after the indicated time periods. Data shown are representative of three independent experiments with similar results.

30  $\mu$ g/ml, the degree of restoration of IgE and Th2-type cytokine production was similar to that observed at 10  $\mu$ g/ml (data not shown).

#### *L. casei Induces IL-12 Production, and Anti-IL-12 Antibody Completely Restores the L. casei-Suppressed Production of IgE, IL-4, and IL-5 in OVA-Stimulated BALB/c Splenocyte Cultures*

The results of neutralization study with anti-IFN- $\gamma$  antibody suggested that the induced IFN- $\gamma$  plays a role in the suppression of IgE, while some other factors that can inhibit

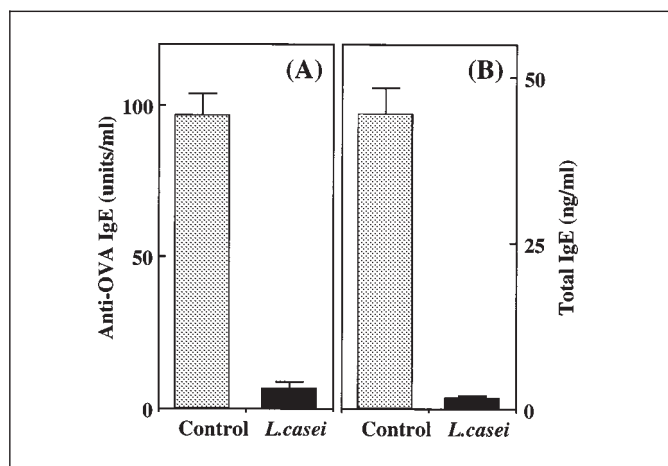


**Fig. 3.** Effect of *L. casei* on OVA-specific IgE production by BALB/c splenocytes. Splenocytes from OVA-primed BALB/c mice were cultured with OVA in the absence (control) or presence of *L. casei* (1 µg/ml) for 3 days. After removal of OVA from the cultures, splenocytes were cultured without OVA for a further 7 days, and supernatants were collected. The levels of total (B) and OVA-specific (A) IgE in supernatants were determined by ELISA. Results are means ± SD of three independent cultures.

IL-4 and IL-5 production also mediate the inhibitory effect of *L. casei* on IgE secretion. It is well-known that IL-12 can induce a dominant Th1-type cytokine profile [reviewed in 20, 21]. Therefore, IL-12 production in the OVA-stimulated splenocyte cultures containing *L. casei* was studied. *L. casei* enhanced IL-12 production in a dose-dependent manner, whereas *L. johnsonii*, which had no IgE suppressive effect, induced only a very low level of IL-12 (fig. 5A). Induction of IL-12 production by *L. casei* was observed on day 1 (fig. 5B), followed by IFN-γ secretion (fig. 2).

We further examined the effect of anti-IL-12 antibody on inhibition of IgE secretion by *L. casei*. Addition of anti-IL-12 monoclonal antibody to the cultures containing *L. casei* completely restored the IgE, IL-4, and IL-5 production to the control levels (fig. 6).

#### *The Inhibitory Activity of L. casei on IgE Production Survives Simulated Intra-gastric Conditions*

*L. casei* is a bacterium commonly used in dairy products and can be administered orally. We examined whether the ability of *L. casei* to inhibit IgE production is retained after exposure of this bacterium to intra-gastric conditions. After treatment with pepsin at a low pH for 3 h, *L. casei* still enhanced IL-12 production, promoted the development of a dominant Th1-type cytokine profile, and inhibited IgE production as observed in the case of untreated *L. casei* (fig. 7).

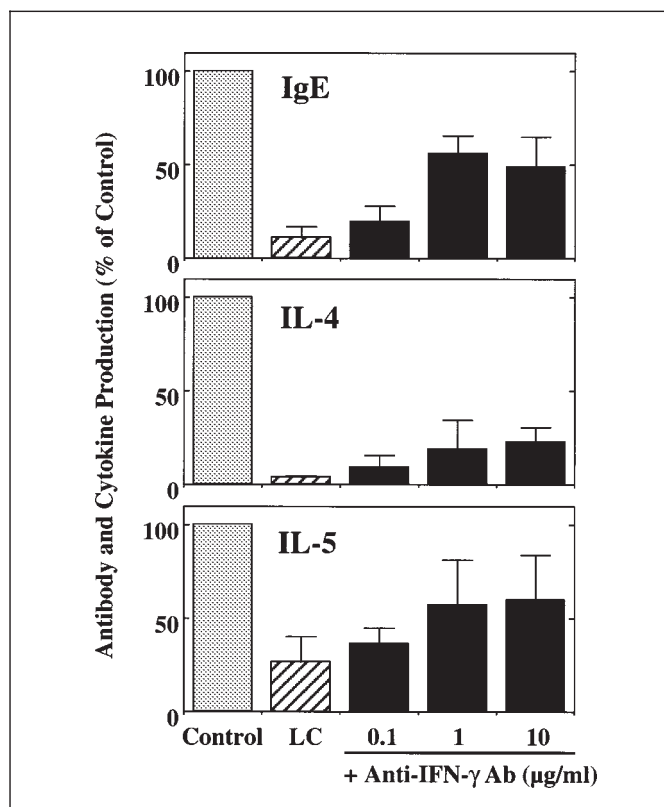


**Fig. 4.** Effect of anti-IFN-γ antibody on the *L. casei*-mediated suppression of IgE, IL-4, and IL-5 production in OVA-stimulated BALB/c splenocytes cultures. Splenocytes from OVA-primed BALB/c mice were cultured with OVA in the presence of *L. casei* (LC; 0.5 µg/ml). Anti-IFN-γ antibody (0.1–10 µg/ml) was added at the beginning of the culture period. Control cultures contained the antigen only. The levels of total IgE on day 14 and cytokines on day 7 in supernatants were determined by ELISA. Results are means ± SD of three independent experiments expressed as the relative values to control experiments. The values of control experiments are shown in figure 2.

#### *L. casei Induces IL-12 Production by BALB/c Splenic Plastic-Adherent Cells and J774.1 Macrophages*

To examine what type of cells secreted IL-12 in response to stimulation by *L. casei*, BALB/c splenocytes, splenic plastic-adherent cells, the J774.1 macrophage cell line, and the A20.2J B cell line were stimulated with *L. casei* and IL-12 secretion in cultures was measured. As shown in table 1, *L. casei* induced IL-12 production from both splenic plastic-adherent cells and J774.1 cells, indicating that the cells producing IL-12 upon stimulation with *L. casei* are macrophages.

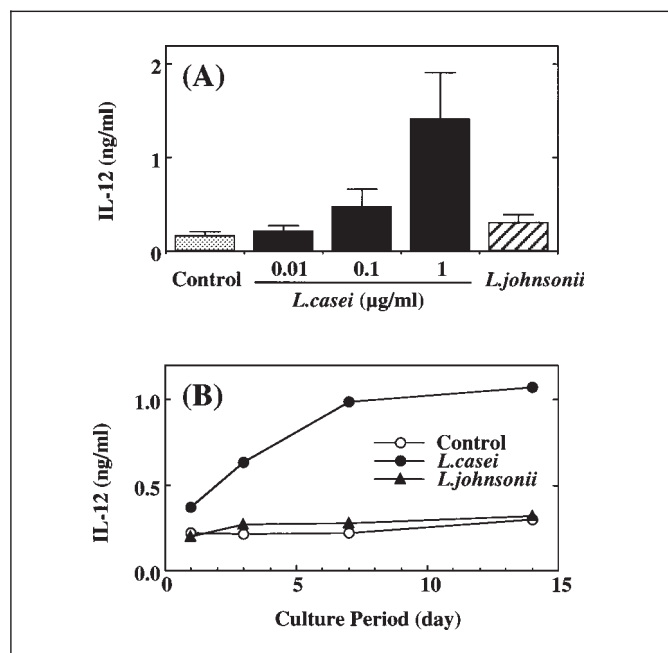


**Fig. 5.** Induction of IL-12 production by *L. casei* in OVA-stimulated BALB/c splenocyte cultures. Splenocytes from OVA-primed BALB/c mice were cultured with OVA in the absence (control) or presence of *L. casei* (0.01–1 µg/ml for **A**, 1 µg/ml for **B**) or *L. johnsonii* (1 µg/ml for **A** and **B**). The levels of IL-12 in supernatants were determined by ELISA on day 7 (**A**) or after the indicated time periods (**B**). **A** Results are expressed as means ± SD of three independent experiments.

#### *L. casei* Induces Th1 Phenotype Development in TCR-Transgenic T cells through IL-12 Production

Using a Th cell development assay with naive T cells from OVA-specific TCR-transgenic mice, we examined whether *L. casei* promotes Th1 phenotype development through IL-12 induction. Freshly isolated TCR-transgenic T cells, to be developed into Th1/Th2 cells, were primarily cultured with OVA plus irradiated BALB/c splenocytes as antigen-presenting cells in the absence or presence of IL-4, IL-12, or *L. casei*. Culture supernatants were assayed for cytokines (fig. 8A). Then, to determine which type of Th cells were promoted to develop by these additives, the resulting Th cells were restimulated without the additives and the cytokines in supernatants were assayed (fig. 8B).

Addition of IL-4 to the primary cultures resulted in the development of Th2 cells with enhanced IL-4 and suppressed IFN-γ production in the secondary cultures, whereas IL-12 induced the development of Th1 cells as previously described [22, 23]. *L. casei* enhanced IL-12 production in primary cultures and promoted the development of Th1

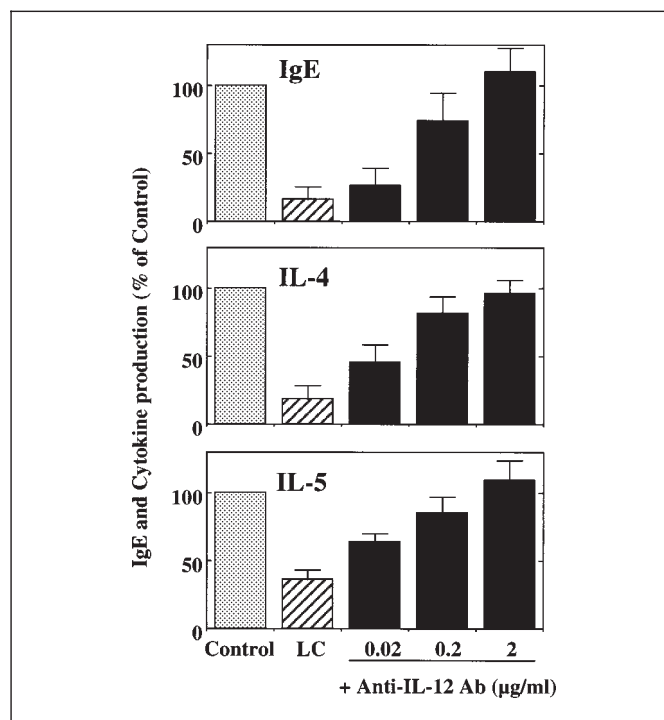


**Fig. 6.** Effect of anti-IL-12 antibody on the *L. casei*-mediated suppression of IgE, IL-4, and IL-5 production in OVA-stimulated BALB/c splenocyte cultures. Splenocytes from OVA-primed BALB/c mice were cultured with OVA in the presence of *L. casei* (LC; 0.5 µg/ml). Anti-IL-12 antibody (0.02–2 µg/ml) was added at the beginning of the culture period. Control cultures contained the antigen only. The levels of total IgE on day 14 and cytokines on day 7 in supernatants were determined by ELISA. Results are means ± SD of three independent experiments expressed as the relative values to control experiments. The values of control experiments are shown in figure 2.

**Table 1.** *L. casei*-induced IL-12 production by macrophages

| Cells                  | IL-12 production, pg/ml |                 |
|------------------------|-------------------------|-----------------|
|                        | Medium                  | <i>L. casei</i> |
| Splenocytes            | 74                      | 2,119           |
| Plastic adherent cells | <22                     | 550             |
| J774.1 (macrophage)    | <22                     | 6,860           |
| A20.2J (B cell)        | <22                     | <22             |

Whole splenocytes from nonimmunized BALB/c mice, plastic-adherent cells, J774.1 macrophage cells, and A20.2J B lymphoma cells were cultured with *L. casei* (1 µg/ml), and the levels of IL-12 in supernatants at 48 h were determined by ELISA.

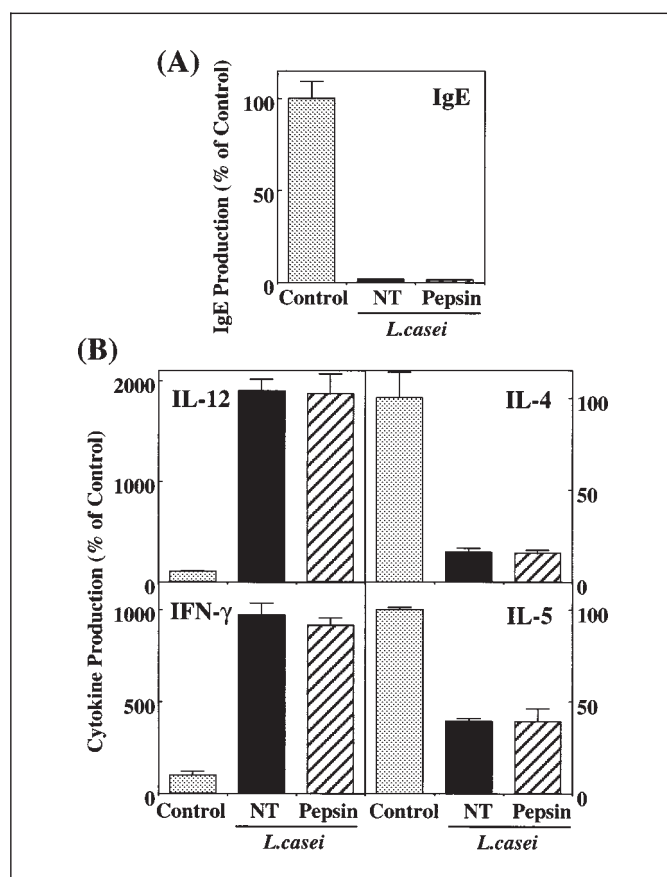


**Fig. 7.** Effect of pepsin treatment on the activities of *L. casei*. Splenocytes from OVA-primed BALB/c mice were cultured with OVA in the absence (control) or presence of pepsin-treated (pepsin) or untreated (NT) *L. casei* (1  $\mu$ m/ml). The levels of total IgE (**A**) on day 14 and cytokines (**B**) on day 7 in supernatants were determined by ELISA. Results are means  $\pm$  SD of three independent cultures expressed as the relative values to control experiments.

cells with enhanced IFN- $\gamma$  and suppressed IL-4 production. The effect of *L. casei* to promote Th1 development was completely blocked by addition of anti-IL-12 antibody to the primary cultures (fig. 9).

## Discussion

It is generally accepted that the balance between Th1 and Th2 cytokines is critical for IgE production. Since an augmented Th2-type immune response with enhanced IL-4 production appears to favor IgE production, potentiation of the Th1-type immune response should be beneficial in sup-

pressing IgE. Several previous studies [11, 13–15] have shown that *L. casei*, a lactic acid bacterium widely used in dairy products and other foods, can enhance the cellular immunity of the host. These findings led us to hypothesize that *L. casei* may inhibit IgE production through promoting the Th1-type immune response, because Th1 cells and their cytokines are thought to play a major role in enhancing cellular immunity. We, therefore, tried to confirm this hypothesis.

In this report, we clearly demonstrate that heat-killed *L. casei* markedly inhibits total IgE production by OVA-stimulated murine splenocytes through promoting a dominant Th1-type cytokine profile with enhanced IFN- $\gamma$  and diminished IL-4 and IL-5 production. The inhibitory effect of *L. casei* on IgE production specific for nominal antigen was also observed. The results of the IFN- $\gamma$  neutralization study demonstrate that the inhibitory effect of *L. casei* on IgE, IL-4, and IL-5 production was evoked through both IFN- $\gamma$ -dependent and IFN- $\gamma$ -independent mechanisms. The IFN- $\gamma$  independent inhibition was likely due to promotion of the development of uncommitted cells into Th1 cells by *L. casei*. We found that the regulatory effect of *L. casei* on IgE and cytokine production is dependent on levels of IL-12 and the cells producing IL-12 in response to stimulation by *L. casei* were revealed to be macrophages. Furthermore, the Th cell development assay with naive T cells from OVA-specific TCR-transgenic mice clearly showed the potential of *L. casei* to direct Th1 phenotype development through induction of IL-12 secretion, as previously shown by Hsieh et al. [23] in the case of *L. monocytogenes*.

Recent reports have shown that IL-12 inhibits IgE production in both IL-4-stimulated human peripheral blood mononuclear cells [24] and mice treated with goat anti-mouse IgD [25]. In our culture system of murine splenocytes primed by antigen immunization, IgE was secreted in response to antigen, which can be considered to reflect the physiological state of IgE production rather than other culture systems. Our findings revealed that an IL-12 potentiator, *L. casei*, is effective in inhibiting IgE production in this system. In addition, diminished production of IL-4 and IL-5, which can promote the proliferation of mast cells and eosinophils, respectively [6], was also observed in response to treatment with *L. casei*. Mast cells and eosinophils as well as allergen-specific IgE antibody play an important role in allergic reactions. Thus, the findings obtained from this in vitro study suggest that *L. casei* has the potential to suppress symptoms of allergy. Since *L. casei* can be recognized as a safe bacterium for humans, oral drugs from this bacterium or its components may be developed for suppressing IgE-mediated allergy. We found that the activity of *L. casei* can

**Fig. 8.** Induction of Th1 phenotype development by *L. casei* in OVA-specific TCR-transgenic T cells. TCR-transgenic T cells were stimulated with OVA plus irradiated BALB/c splenocytes in the absence (control) or presence of IL-4 (10 ng/ml), IL-12 (7–700 ng/ml), or *L. casei* (0.01–1 µg/ml). Supernatants were collected at 48 h after the primary stimulation for determination of primary IL-12, IFN-γ, and IL-4 production, and cells were expanded 2-fold into fresh medium on day 3. Cultured T cells were harvested on day 7, washed, and restimulated with OVA plus irradiated A20.2J B lymphoma cells as antigen-presenting cells without the additives. The levels of IFN-γ and IL-4 at 48 h in supernatants of secondary cultures were measured by ELISA in order to determine the resulting Th phenotype. Results are for cytokine production in primary (A) or secondary (B) cultures. Data shown are representative of two independent experiments with similar results. ND = Not determined.

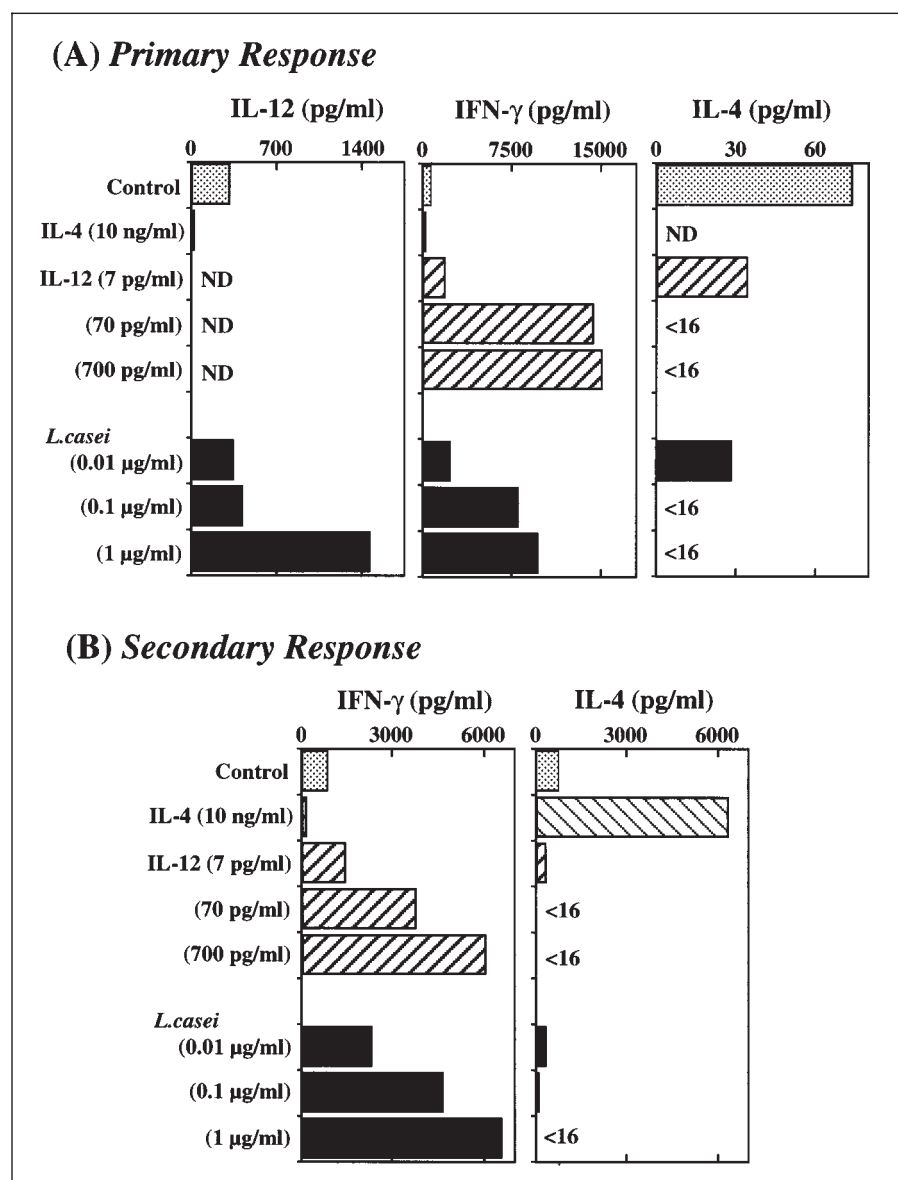
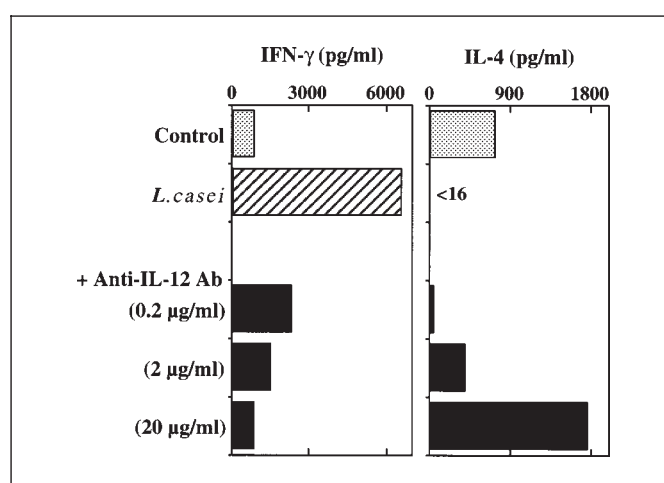


**Fig. 9.** Effect of anti-IL-12 antibody on *L. casei*-induced Th1 development in OVA-specific TCR-transgenic T cells. TCR-transgenic T cells were stimulated with OVA plus irradiated BALB/c splenocytes in the presence of *L. casei* (1 µg/ml). Anti-IL-12 antibody (0.2–20 µg/ml) was added at the beginning of the period of primary culture. Control cultures contained the antigen only. The cells were expanded 2-fold into fresh medium on day 3, harvested on day 7, washed, and restimulated with OVA plus irradiated A20.2J B lymphoma cells without the additives. The levels of IFN-γ and IL-4 at 48 h in supernatants of secondary cultures were measured by ELISA in order to determine the resulting Th phenotype. Data shown are representative of two independent experiments with similar results.



resist the gastric barrier. Then, it should be worth while testing an effect of orally administered *L. casei* on suppressing IgE production. We are now trying to evaluate the inhibitory effect of this bacterium on allergic reactions in in vivo model systems, considering the systemic and mucosal immune systems.

It is well known that IL-12 plays a major role in enhancing cellular immunity, resulting in prevention of allergic diseases [26], augmentation of antitumor immunity [27], and potentiation of host resistance to infection by various pathogens [28] including human immunodeficiency virus [29]. Due to the pivotal multifunctionality of this cytokine, much attention has been paid to searching for an efficient IL-12 potentiator. Several intracellular pathogens such as *L. monocytogenes*, *Toxoplasma gondii*, and *Leishmania major* are known to have a remarkable IL-12-potentiating activity [21]. Lipopolysaccharide [30] from gram-negative bacteria and lipoteichoic acid [31] from pathogenic extracellular gram-positive bacteria such as *Staphylococcus aureus* are also known to induce IL-12 production. *L. casei*, a novel IL-12 potentiator, is a nonpathogenic bacterium and, indeed, is considered to be beneficial to the health of the

host, whereas the above-mentioned IL-12 potentiators are either pathogenic organisms, their components, or toxic lipopolysaccharides.

In conclusion, the present study has clearly revealed that *L. casei*, a beneficial lactic acid bacterium, can inhibit allergen-induced IgE production by murine splenocytes and suggests a possible use of this microorganism in preventing IgE-mediated allergy. This organism is expected to act as an antiallergy agent in vivo, because many reports have demonstrated that *L. casei* administered not only by intraperitoneal [9–11] and intravenous [12, 14] routes, but also by the oral [32] route, displays its biological activities through the potentiation of cellular immunity, which may results from its induction of IL-12 production.

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