# Oral administration of an IL-10–secreting *Lactococcus lactis* strain prevents food-induced IgE sensitization

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**Background: Because tolerance to food is potentially modulated by IL-10, strategies to prevent food allergy should favor an increased delivery of IL-10 to the gut.**

**Objectives: We hypothesized that administration of a *Lactococcus lactis* transfected to secrete murine IL-10 could prevent sensitization in a mouse model of food allergy.**

**Methods: Before each oral sensitization with β-lactoglobulin in the presence of cholera toxin, young mice were administered the transfected *Lactococcus lactis*. Antigen-induced anaphylaxis after oral challenge assessed clinical protection achieved by the pretreatment. Serum and feces antigen-specific antibody concentrations were sequentially measured. Antibody titers were correlated with antibody and IL-10–secreting cell numbers in the spleen and in Peyer patches.**

**Results: Pretreatment with transfected *Lactococcus lactis* contributed to diminish anaphylaxis significantly, and inhibit antigen-specific serum IgE and $IgG_1$ production strongly. In addition, transfected *Lactococcus lactis* increased the production of antigen-specific IgA in the gut. Variations of antibody levels in the serum and the gut correlated with the numbers of antibody-producing cells. In addition, the presence of exogenous IL-10 in the gut by transfected *Lactococcus lactis* induced IL-10 secretion by Peyer patches cells. Increased IL-10 titers were also measured in the plasma.**

**Conclusion: These results suggest that a microorganism bioengineered to deliver IL-10 in the gut can decrease food-induced anaphylaxis and provide an option to prevent IgE-type sensitization to common food allergens.**

**Clinical implications: Nonpathogenic IL-10–producing microorganisms in the gut could have a potential to prevent systemic food-induced anaphylaxis. (J Allergy Clin Immunol 2007;119:952-9.)**



***Key words:*** *Food hypersensitivity, anaphylaxis, tolerance, T-lymphocytes, β-lactoglobulin, IL-10*

Food allergy affects 5% to 8% of young children and is a disease most prevalent among those with an atopic predisposition. Unfortunately, the pathogenesis of the disease is incompletely understood, and no effective treatment is available to cure or actively prevent food allergy.[1,2] To explore the mechanisms of food allergy involving the gut-associated immune system, several investigators have developed an animal model of food-induced anaphylaxis consisting of oral sensitization with common food allergens in presence of the mucosal adjuvant cholera toxin (CTX).[3-6]

It is so far hypothesized that most immediate clinical reactions of food allergy are mediated by antigen-specific IgE antibodies and occur as a result of a breach in oral tolerance.[7] It has also been found in mice that early feedings of high doses tend to induce oral tolerance.[8] In a previously published study, we characterized the immune response in the gut in a group of tolerized mice and compared it with mice with food antigen-induced anaphylaxis.[9] In tolerized mice, Peyer patches T lymphocytes produced significantly more IL-10 and TGF-β after antigen activation than allergic mice. These findings prompted us to explore various strategies for inducing IL-10 production in the gut to favor tolerance induction.

Allergy in general could result from a modification of the infectious environment including the gut microflora.[10-13] Present in the gut, lactobacilli are common nonpathogenic microorganisms of the gut shown to induce IL-10.[14-16] Among them, *Lactocobacilli reuteri* and *Lactocobacilli casei* have been found to prime human dendritic cells to drive the development of IL-10–secreting T-regulatory cells.[14] To enhance IL-10 production by *Lactococcus lactis*, Steidler et al[17] transfected the bacteria with a gene coding for murine IL-10. In these experiments, mice with chronic dextran sulfate sodium–induced colitis were fed with the transfected bacteria, resulting in local production of IL-10 and resolution of the colitis. In a similar way, IL-10–deficient mice were protected from the development of inflammatory colitis.

The study we report here aimed to promote induction of oral tolerance by administering IL-10–inducing microorganisms before sensitization to a common food allergen. We found that the *L lactis* transfected with the gene for recombinant murine IL-10 (LL-mIL10) prevented the pretreated mice from anaphylaxis after oral antigen challenge, and almost completely abrogated the $T_H2$-type

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*Abbreviations used*
AU: Arbitrary units
BLG: β-Lactoglobulin
CFU: Cell-forming units
CTX: Cholera toxin
LL-mIL10: *Lactococcus lactis* transfected with the gene for recombinant murine IL-10
LLwt: *Lactococcus lactis* wild type

response, inducing an immune response suggesting oral tolerance.

## METHODS

### Preparation of the microorganisms

*L lactis* wild-type and LL-rmIL10[17] were routinely grown in liquid M17 medium (Difco, Detroit, Mich) supplemented with 0.5% glucose. Five micrograms per milliliter erythromycin was added to LL-mIL10 cultures to select transfected bacteria. The GM9E growth medium (Difco) containing 6 g $Na_2HPO_4$, 3 g $KH_2PO_4$, 1 g $NH_4Cl$, 0.5 g NaCl, 2 mmol $MgSO_4$, 0.1 mmol $CaCl_2$, 5 g glucose, 5 g peptone, and 5 mg erythromycin per liter was used in experiments measuring IL-10 production. After the expansion phase overnight at 37°C in M17 medium, cultures were centrifuged and resuspended in 0.2 mol/L $NaHCO_3$. Mice were infected by intragastric gavage with $10^9$ plaque-forming units LL-mIL10 in a single dose every day during 3 days before the sensitization.

### Oral sensitization and test challenge to β-lactoglobulin

Female C3H/HeOuJ mice 4 to 5 weeks old (Charles River, L'Arbesle, France) were immunized at days 0, 7, 14, and 21 by intragastric gavage with 20 mg β-lactoglobulin (BLG; Sigma, Buchs, Switzerland) and 10 μg CTX (List Biological Laboratories, Campbell, Calif) in 0.2 mol/L $NaHCO_3$. On day 28, all mice were challenged by intragastric gavage with 100 mg BLG. Anaphylaxis was recorded and graded using a reaction score (0, no reaction; 1, mild reactions including decreased activity, random scratching, myocloni; 2, moderate symptoms including marked decreased reactivity, continuous scratching, abnormal breathing; 3, severe reaction including low reactivity or absence of reactivity, abnormal breathing, no reversibility of symptoms within 15 minutes or death), and correlated with the body temperature measured by infrared at the ear (before challenge and 30 minutes after gavage) as previously described.[9] In addition, 100 μL 2% Evan's blue dye was injected into the tail vein 30 minutes before oral challenge to assess anaphylaxis-induced vascular leakage. All experimental procedures involving animals were approved by the Ethics Committee for Animal Studies at the University of Geneva School of Medicine and performed in accordance with their guidelines.

### ELISAs for BLG-specific serum and feces antibodies

Sera were obtained from tail bleedings before each sensitization phase and 1 week after the last procedure. Feces were obtained at the same times and resuspended in PBS plus 1% FCS (Life Technologies, Paisley, Scotland) supplemented with pepstatin 1:1000 (Fluka, Switzerland) at 0.1 mg/mL. The samples were mechanically disaggregated and vortexed for 2 minutes, followed by 2 centrifugations at 4°C for 20 minutes at 14,000 rpm.

Sera and feces were assayed for BLG-specific IgE, $IgG_1$, $IgG_{2a}$, and/or IgA antibody levels by a method adapted from Adel-Patient et al.[18] In brief, MaxiSorp microtiter plates (Nunc, Roskilde, Denmark) were coated for 18 hours at room temperature with 250 ng/well streptavidin (Fluka), followed by 300 μL of a solution of polyvinylpyrrolidon K25 (Fluka) overnight. One microgram of biotinylated BLG was incubated for 3 hours, and sera dilutions in PBS plus 10% horse serum, determined by the optimal point on a titration curve, were added in duplicates in presence of 0.5 μg/mL goat antimouse IgA, rat antimouse $IgG_1$, or anti-mouse $IgG_{2a}$ peroxidase-labeled antibodies (Southern Biotechnologies, Birmingham, Ala) for 2 hours. For IgE measurement, a monoclonal rat antimouse IgE Ab (clone R35-72; BD Pharmingen, Heidelberg, Germany) was added, followed by peroxidase-coupled antirat Ab (Caltag, San Francisco, Calif). Optical density was measured at 490 nm. Results were expressed as arbitrary units, with pooled sera from BLG plus alum-immunized mice used as a reference serum.

### Antigen-specific antibody and cytokine production measured by means of ELISpot

Peyer patches were excised mechanically from the gut and incubated for 30 minutes in HBSS medium supplemented with 5 mmol EDTA (Life Technologies). Peyer patches and spleens were gently crushed and filtered through a 70-μm nylon filter. Spleen cells were previously incubated for 5 minutes in TRIS-buffered $NH_4Cl$ to remove red blood cells. Lymphoblasts were isolate on a Percoll 60%/66% gradient (Amersham, Zurich, Switzerland).

For the measurement of BLG-specific $IgG_1$, $IgG_{2a}$, and IgA antibodies, ELISpot plates (Millipore, Bedford, Mass) were coated with streptavidin overnight at 37°C, followed by addition of 1 μg of biotinylated BLG for 3 hours. Lymphoblasts isolated on a Percoll 60%/66% gradient from spleen or Peyer patches cells (PPC) were resuspended at 2 different concentrations, 1 and $2 \times 10^6$ in Iscove's modified Dulbecco medium supplemented with penicillin, streptomycin, L-glutamine, gentamicin, polymixin B, and 5% FCS for 24 hours at 37°C, followed by overnight incubation at 4°C with anti-IgA, anti-$IgG_1$, and anti-$IgG_{2a}$ antibodies (Southern Biotechnologies). For assessment of cytokine production, ELISpot plates were coated with a mAb against IL-10 (clone JES5-2A5; 1 μg/mL; BD Pharmingen) overnight at 4°C, followed by blocking with cell culture medium for 3 hours. Cells were added as $4 \times 10^5$ cells/well. The plates were incubated for 3 days at 37°C. A biotin-labeled antibody against IL-10 (clone SXC-1; BD Pharmingen) at 0.1 μg/well was added for overnight incubation at 4°C followed by streptavidin horseradish peroxidase at 1:1000 for 2 hours. Amino-ethyl-carbazole, 100 μL/well, was added for 10 minutes, and the spots were automatically counted by using the KS ELISpot 4.2.1 Software (Zeiss, Halbermoos, Germany) and expressed as cell-forming units (CFU) per $10^6$ cells.

### IL-10 measurement in the plasma by ELISA

Plasma samples were collected 2, 4, 6, 12, 18, and 24 hours after administration of LL-rmIL10 to the mice. Plates were coated overnight with 4 μg/mL of a mAb against IL-10 (clone JES5-2A5), blocked with PBS + 10% horse serum for 2 hours. Plasma was then added at various concentrations (1/3 and 1/10) overnight, and a biotinylated antibody against IL-10 (clone SXC-1) at 0.1 μg/mL was added for 1 hour. Optical densities were read after adding streptavidin horseradish peroxidase 1:10,000 for 30 minutes and o-phenylendiamine. Titers were calculated by comparing the results to a standard curve.

### Antigen-induced T-cell proliferation

PPC and mesenteric lymph node cells were obtained as described. Cells at $1.5 \times 10^5$ cells/well were then activated with antigen or concavalin A in complete Dulbecco modified Eagle medium (Life