

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 IgG₁ 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 113:1000-1008, 2004.)

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, *Lactobacilli reuteri* and *Lactobacilli 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 al¹⁷ 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

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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

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 response, inducing an immune response suggesting oral tolerance.

METHODS**Preparation of the microorganisms**

L. lactis wild-type and LL-rmIL10¹⁷ 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 Na₂HPO₄, 3 g KH₂PO₄, 1 g NH₄Cl, 0.5 g NaCl, 2 mmol MgSO₄, 0.1 mmol CaCl₂, 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 NaHCO₃. Mice were infected by intragastric gavage with 10⁹ 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'Arbresle, France) were immunized at days 0, 7, 14, and 21 by intragastric gavage with 20 mg β -lactoglobulin (BLG; Sigma, Buchs, Switzerland) and 10 μ g CTX (List Biological Laboratories, Campbell, Calif) in 0.2 mol/L NaHCO₃. 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 μ L 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, IgG₁, 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 μ L of a solution of polyvinylpyrrolidone 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 μ g/mL goat anti-mouse IgA, rat antimouse IgG₁, 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- μ m nylon filter. Spleen cells were previously incubated for 5 minutes in TRIS-buffered NH₄Cl to remove red blood cells. Lymphoblasts were isolate on a Percoll 60%/66% gradient (Amersham, Zurich, Switzerland).

For the measurement of BLG-specific IgG₁, IgG_{2a}, and IgA antibodies, ELISpot plates (Millipore, Bedford, Mass) were coated with streptavidin overnight at 37°C, followed by addition of 1 μ g 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, polymyxin B, and 5% FCS for 24 hours at 37°C, followed by overnight incubation at 4°C with anti-IgA, anti-IgG₁, 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 μ g/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 μ g/well was added for overnight incubation at 4°C followed by streptavidin horseradish peroxidase at 1:1000 for 2 hours. Amino-ethyl-carbazole, 100 μ L/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 10⁶ 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 μ g/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 μ g/mL was added for 1 hour. Optical densities were read after adding streptavidin horseradish peroxidase 1:10,000 for 30 minutes and o-phenyldiamine. Titers were calculated by comparing the results to a standard curve.

TABLE I. Anaphylactic reactions after oral antigen challenge assessed by symptom scores and modification of body temperature

Groups of mice	Anaphylaxis scores at 30 min (no. of mice)						Temperature drop at 30 min (°C ± SD)
	Score 0 (nonreactors)	Score 1 (nonreactors)	Total nonreactors (score 0 & 1)	Score 2 (reactors)	Score 3 (reactors)	Total reactors (score 2 & 3)	
Control mice	1	1	2	4	6	10	2.6 ± 1.4**
LLwt pretreated	0	3	3	5	4	9	2.7 ± 1.6**
LL-mIL10 pretreated	4	4	8*	1	3	4	1.9 ± 1.9

* $P < .05$ compared with the group of control mice.

**Drop of body temperature at 30 minutes compared with the temperature before challenge; $P < .05$.

Antigen-induced T-cell proliferation

PPC and mesenteric lymph node cells were obtained as described. Cells at 1.5×10^5 cells/well were then activated with antigen or concavalin A in complete Dulbecco modified Eagle medium (Life Technologies) with 10% nonessential amino acids, 100 U/mL penicillin, 100 μ g/mL streptomycin, 2 mmol L-glutamine, 100 μ g/mL gentamicin, 10 nmol sodium pyruvate, 2×10^{-5} mol 2-mercaptoethanol, and 1 μ g/mL polymyxin B (all from Sigma) plus 10% FCS (Life Technologies). β -Lactoglobulin was added at different concentrations (2.5×10^{-5} mol, 2.5×10^{-6} mol, 2.5×10^{-7} mol). To assess the proliferate capacity, the cells were pulsed on day 5 of culture with 1 μ Ci/well ^{3} H-labeled thymidine (Amersham) for 18 hours and harvested.

Statistical analysis

Results are presented as mean values $\pm$ SDs. Values between groups were analyzed by the Wilcoxon signed-rank test for nonparametric unpaired data. Anaphylaxis scores (reactors vs nonreactors) were analyzed by Fisher exact test.

RESULTS

To test the effect of the LL-mIL10 on IgE sensitization to foods, we orally pretreated mice with LL-mIL10 for 3 days before each of 4 oral sensitizations with BLG in presence of CTX. In addition, we constituted a nonpretreated group but sensitized with BLG and CTX (control group), and a group orally pretreated with *Lactococcus lactis* wild type (LLwt). Oral BLG challenges showed significantly more mice having antigen-induced anaphylaxis in the nonpretreated group than in the group pretreated with LL-mIL10 (Table I). These observations correlated with a significant decrease in body temperature in the 2 groups with anaphylaxis (drop in temperature 30 minutes after challenge in the nonpretreated group and in the LLwt pretreated group but not in the LL-mIL10 pretreated group). In addition, anaphylactic mice had marked increased vascular permeability with marked Evan's blue extravasation visible on the snouts and the footpaths, correlating with anaphylaxis scores and body temperature. No visible signs of intestinal symptoms such as diarrhea could be observed, but macroscopic examination of the gut revealed a manifest edema in the control group, markedly decreased in the LL-mIL10 group (Fig 1). Sensitized mice from the control group had a strong increase in BLG-

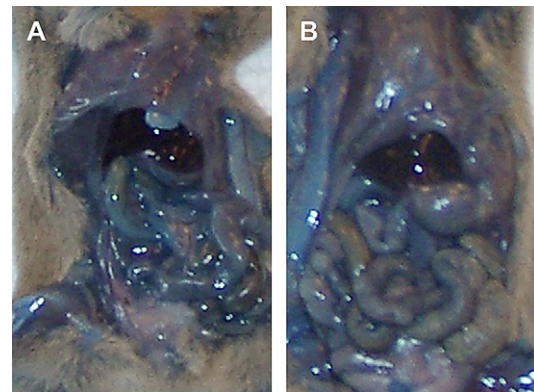


FIG 1. Macroscopic examination of the gut after intravenous injection of Evan's blue dye and antigen challenge, with no edema in a mouse pretreated with LL-mIL10 with mild anaphylaxis (A) and marked edema in a control mouse with generalized symptoms of anaphylaxis (B).

specific serum IgG₁ and IgE during the oral sensitization phase associated with a moderate IgG_{2a} and IgA response. Oral administration of LLwt before each sensitization partially inhibited the IgE responses (42.9 ± 5.1 arbitrary units [AU]/mL vs 62.9 ± 4.1 AU/mL; $P < .05$) in the serum of treated animals (Fig 2, A). The inhibition of IgE antibody production was much more important when LL-mIL10 was given (9.6 ± 0.5 AU/mL vs 62.9 ± 4.1 AU/mL in the control group; $P < .005$). A similar trend was observed for BLG-specific IgG₁ titers (control group, 1312 ± 430 AU/mL; LLwt, 879 ± 200 AU/mL; $P < .05$; LL-mIL10, 334 ± 140 AU/mL; $P < .005$; Fig 2, B). LL-mIL10 did not modify BLG-specific IgG_{2a} production compared with the group of nontreated animals (Fig 2, C). Furthermore, we detected a significant increase of BLG-specific IgA titers in the serum after pretreatment with LLwt exclusively (control group, 2457 ± 1011 AU/mL; LLwt, 5137 ± 1819 AU/mL; $P < .05$; Fig 2, D).

In the next experiments, we measured by ELISpot the frequency of antibody-producing spleen cells. The frequency of BLG-specific IgG₁ Ab-secreting cells from the spleen significantly decreased in both groups of mice treated with *Lactococcus* (LLwt, 26.5 ± 2.1 CFU; LL-mIL10, 13.3 ± 4 CFU; $P < .005$) compared with the control group (85.7 ± 13.1 CFU; Fig 3, A). IgG_{2a}-producing

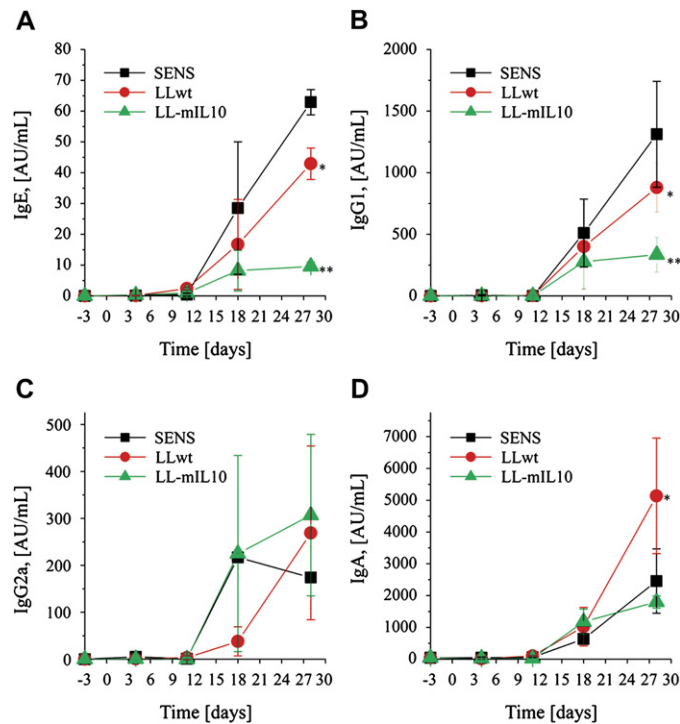


FIG 2. Serum antibody concentrations measured by ELISA after pretreatment with LLwt and LL-mIL10, and compared with nonpretreated mice (SENS). BLG-specific IgE antibodies (A), BLG-specific IgG₁ antibodies (B), BLG-specific IgG_{2a} antibodies (C), BLG-specific IgA antibodies (D). The results are pooled from at least 2 separate experiments. The results are presented as mean values $\pm$ SDs. * P < .05, ** P < .005 when compared with the SENS group.

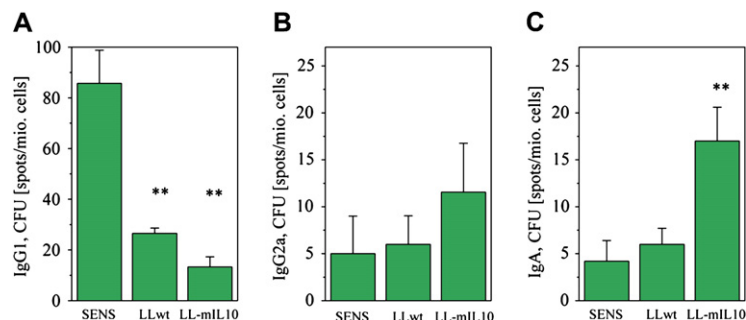


FIG 3. Antibody-secreting spleen cell numbers per million (mio.) cells counted by ELISpot after pretreatment with LLwt and LL-mIL10 and compared with nonpretreated mice (SENS). (A) IgG₁, (B) IgG_{2a}, and IgA-secreting cells (C). The results are pooled from at least 2 separate experiments. The results are presented as mean values $\pm$ SDs. ** P < .005 compared with the SENS group.

cells were found in similar numbers in all 3 groups of mice (Fig 3, B). In contrast with IgG₁, the spleen of LL-mIL10-treated mice contained an increased number of BLG-specific IgA-producing cells (17 ± 3.6 CFU) compared with controls (4.2 ± 2.2 CFU; P < .005; Fig 3, C). ELISpot experiments correspond to the BLG-specific serum antibody titer levels found after various interventions with LLwt and LL-mIL10. IgE-secreting cells could not be counted because of the low number of cells.

This set of experiments showed that *Lactococcus* and local intestinal IL-10 production decreased the frequency

and titers of IgG₁ and IgE in the systemic compartment of animals treated with the bacteria, suggesting an inhibition of the T_H2 response. Moreover, induction of IL-10 is associated with a switch to IgA isotype.

Further, we were interested in quantifying the antibody response in the gut after pretreatment. Feces were harvested at various times during and after the sensitization phase, diluted in PBS, and the suspension cleared of fecal residues after homogenization. BLG-specific IgG₁ and IgA antibodies were then measured by ELISA following the same method used for antibodies in the serum. IgG₁ titers

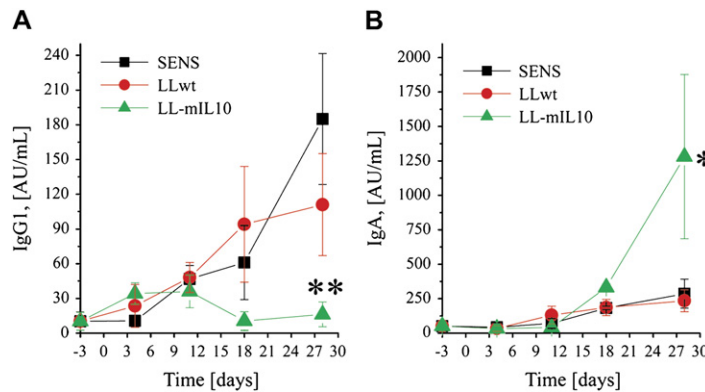


FIG 4. Antibody concentrations in the feces measured by ELISA after pretreatment with LLwt and LL-mIL10, and compared with nonpretreated mice (SENS). BLG-specific IgG₁ antibodies (A) and BLG-specific IgA antibodies (B). The results are pooled from at least 2 separate experiments. The results are presented as mean values $\pm$ SDs. * $P < .05$, ** $P < .005$ compared with the SENS group.

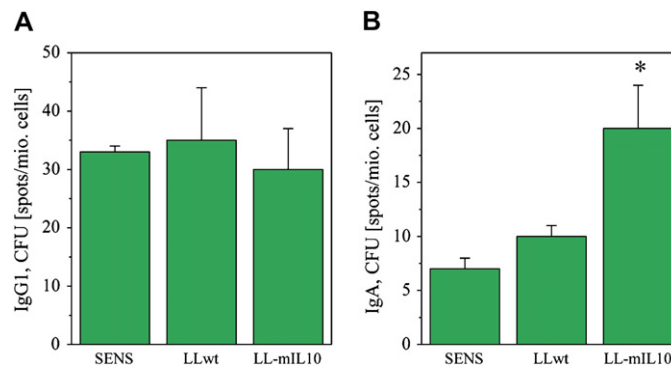


FIG 5. Antibody-secreting PPC numbers per million (mio.) cells counted by ELISpot after pretreatment with LLwt and LL-mIL10, and compared with nonpretreated mice (SENS). IgG₁ (A) and IgA-secreting cells (B). The results are pooled from at least 2 separate experiments. The results are presented as mean values $\pm$ SDs. * $P < .05$ compared with the SENS group.

remained similar in both control (185 ± 56.6 AU/mL) and LLwt (111 ± 44.1 AU/mL) groups. However, in the LL-mIL10 group exclusively, a significant decrease in BLG-specific IgG₁ titer (16.2 ± 10.7 AU/mL) was observed in the feces ($P < .05$; Fig 4, A). Opposite, BLG-specific IgA antibody titers strongly increased exclusively in the LL-mIL10-treated group (1281 ± 596.1 AU/mL; $P < .05$) compared with the control group (286 ± 105.6 AU/mL; Fig 4, B). These results show that oral administration of LL-mIL10 increases the number of IgA-secreting cells in Peyer patches in correlation with an increased titer of antigen-specific IgA in the feces of LL-mIL10-treated mice.

We then wanted to correlate these findings with the local secretion of antibody in Peyer patches by measuring antibody-secreting cell numbers by ELISpot. Interestingly, the frequency of IgG₁-secreting cells in the Peyer patches was not modified in LLwt-treated mice (30 ± 7 CFU) compared with control mice (33 ± 1 CFU; Fig 5, A), suggesting a passive secretion of serum antibodies into the gut. In contrast, the frequency of IgA Ab-secreting cells from Peyer patches was higher in

the LL-mIL10 group (20 ± 4 CFU) than in control mice (7 ± 1 CFU; $P < .05$; Fig 5, B). These data correlate with the increase of IgA titers in the feces of these animals and suggest secretion of locally produced IgA into the gut.

Next, we wanted to demonstrate that the regulating effect on IgE and IgG₁ production observed predominantly in the LL-mIL10 group was a result of live microorganisms that were locally secreting murine IL-10. To abrogate active IL-10 secretion by living microorganisms, LL-mIL10 was heat-killed at 100°C for 30 minutes. We found that heat-killed bacteria lost their capacity to proliferate and to secrete IL-10. The inhibiting effect of LL-mIL10 on IgE and IgG₁ antibody production was suppressed after treatment by heat, because levels of BLG-specific IgE and IgG₁ antibodies in the heat-killed LL-mIL10 group were similar to levels in the LLwt pretreated mice (results not shown).

This set of experiments clearly shows that the strong inhibition of the T_H2 response by LL-mIL10 requires live, IL-10-secreting bacteria, suggesting a role for local IL-10 in allergy protecting mechanisms.

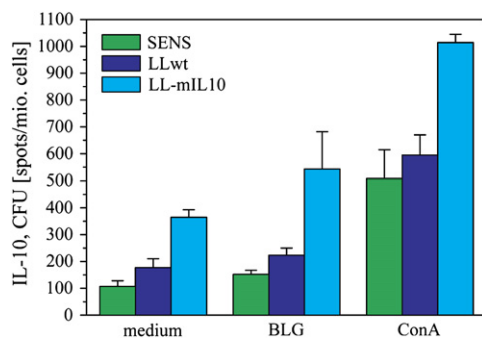


FIG 6. IL-10-secreting PPC numbers per million (mio.) cells after activation with antigen or concavalin A counted by ELISpot. Cells obtained in mice sensitized after pretreatment with LLwt and LL-mIL10, and compared with nonpretreated mice (SENS). Results shown from a representative experiment. The results are presented as mean values $\pm$ SDs.

Further, we wanted to explore whether pretreatment with *L lactis* was acting locally by increasing the number of IL-10-producing cells in the Peyer patches and acting systemically by rising IL-10 plasma levels. We found that without any previous stimulation, the number of IL-10-secreting cells in the Peyer patches was partially increasing in mice having received LLwt, and highly increasing in mice having received LL-mIL10 (no pretreatment, 107 ± 21 CFU; LLwt pretreatment, 177 ± 33 CFU; LL-mIL10 pretreatment, 365 ± 28 CFU; Fig 6). Cells activated in presence of BLG were found in a much higher number in LL-mIL10 pretreated mice (543 ± 139 CFU) than in LLwt pretreated mice (223 ± 27 CFU). Similarly, nonantigen-specific activation with concavalin A induced much higher numbers in LL-mIL10 pretreated mice (1014 ± 31 CFU) than in LLwt pretreated (595 ± 75 CFU) or in nonpretreated mice (508 ± 107 CFU). Plasmatic IL-10 levels were measured by ELISA before and at various times after oral gavage with LL-mIL10. Levels were below detection before gavage, and clearly increased in most mice starting at 4 hours after gavage (249 ± 226 pg/mL) until 18 hours (221 ± 313 pg/mL), returning to undetectable levels at 24 hours after gavage (Fig 7).

Taken together, these results suggest that active secretion of IL-10 in the gut by LL-mIL10 up acts locally by up-regulating IL-10-secreting cells. In addition, LL-mIL10 has a potential systemic effect by increasing levels of plasmatic IL-10 secreted by LL-mIL10 or by T cells.

DISCUSSION

Tolerance to foods is regulated in the gut-related immune system to a large extent by IL-10, and modulated in part by local secretion of IgA antibodies. In this study, we demonstrated in a mouse model of food-induced IgE-type sensitization the capacity of a *L lactis* strain transfected with an IL-10-producing gene to induce immune tolerance, assessed by decreased severity of food-induced anaphylaxis, inhibition of antigen-specific IgE, and

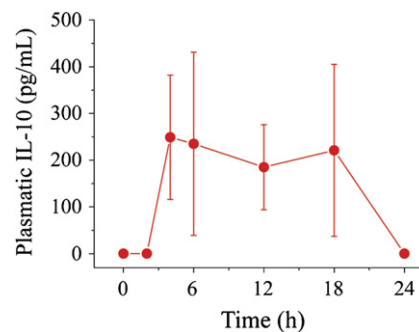


FIG 7. IL-10 levels in the plasma at various times after gavage of LL-mIL10. Control mice had undetectable levels. The results are presented as mean values $\pm$ SDs.

induction of IL-10 and antigen-specific IgA in Peyer patches.

In this mouse model, animals are sensitized with the milk antigen BLG in presence of CTX, a T_H2 -inducing mucosal adjuvant. The following characteristics demonstrate that we use here a mouse model of predominantly food-induced systemic anaphylaxis. Sensitized mice have strongly increased serum titers of antigen-specific IgE and IgG₁, they have multiorgan symptoms of anaphylaxis after antigen challenge, and after intravenous injection of anti-IgE and anti-Fc γ receptor antibodies, as also investigated by others,¹⁹ they have severe multiorgan anaphylaxis. The gut is also involved in food-induced anaphylaxis with increased edema, but in absence of diarrhea. We previously showed that Peyer patches T cells play a major role for sensitization to the food, because adoptive transfer of these cells can induce the local gut response in sensitized mice (increased antigen-specific IgG₁ in the feces), but also a systemic response (increased antigen-specific IgG₁ in the serum).⁹

In previous studies, we have shown that immunologic tolerance could be achieved by continuous feedings with BLG.⁹ Furthermore, anaphylaxis after subsequent immunization could be prevented by an early tolerization procedure, indicating active and persisting protection. These studies have also highlighted upregulation of IL-10 and TGF- β secretion by Peyer patches CD4⁺ cells in tolerant animals. These results prompted us to explore a strategy targeting increased IL-10 levels in the gut in presence of the antigen for active tolerance induction.

Local delivery of IL-10 in the gut can be achieved by using nonpathogenic transfected microorganisms. Therefore, we chose to use the *L lactis* transfected with a gene coding for murine IL-10 developed by Steidler et al.¹⁷ This microorganism has been shown to secrete significant amounts of IL-10 in the gut capable of reversing dextran sulfate sodium-induced colitis in wild-type mice and preventing inflammatory colitis in IL-10-deficient mice. By using this microorganism in our studies, we showed that administration of LL-mIL10 before oral sensitization could significantly diminish food-induced anaphylaxis and almost completely abrogate induction of BLG-specific IgE and IgG₁ antibodies. Interestingly,

part of this effect is attributable to the microorganism itself, because the wild-type *L. lactis* contributed to only approximately half of this diminution. In addition, we have shown that there is an increased number of cells secreting IL-10 in Peyer patches, potentially enhancing the effect of endogenous IL-10 secreted by LL-mIL10. Besides this specific effect of LL-mIL10 on the gut, our studies indicate that LL-mIL10 has a systemic effect. Food-induced anaphylaxis strongly decreased in treated mice, serum antigen-specific IgE and IgG₁ antibodies significantly decreased, and serum IL-10 could be measured as long as 18 hours after administration of LL-mIL10.

Other investigators have explored various strategies involving microorganisms to prevent IgE-type sensitization in an animal model of food-induced anaphylaxis. Adel-Patient et al²⁰ have recently reported a protective effect in mice of a recombinant BLG-expressing *L. lactis* gavaged before intraperitoneal sensitization. In this report, the *L. lactis* strain engineered to produce high amounts of BLG diminished IgE production by 50%, similar to BLG in the presence of *L. lactis*, or BLG alone. Interestingly, *L. lactis* alone did not affect BLG-specific IgE production. It must be stressed that the sensitization procedure used by these authors (intraperitoneal BLG in incomplete Freund adjuvant) was different from the one used in our studies (BLG with CTX by gavage). In a mouse similar to the one used by others and us, Li et al²¹ have explored the efficacy of subcutaneous desensitization with recombinant peanut antigens together with heat-killed *Listeria monocytogenes* in mice already orally sensitized to peanuts. They could demonstrate a significant reduction in the severity of anaphylaxis, as well as a reduction in peanut-specific IgE levels. In a similar experimental protocol, the same authors could demonstrate an effect of intrarectally administered heat-killed *Escherichia coli* engineered to express recombinant peanut proteins.²²

In the study we report here, mice having received LL-mIL10 had significantly increased levels of IgA antibodies in their feces. We previously demonstrated that tolerized mice had similarly increased levels of secretory, antigen-specific IgA antibody titers in the feces. T-cell-dependent secretion of secretory IgA antibodies by B cells is mostly located in Peyer patches and is largely induced by an IL-10 and TGF- β -driven antigenic stimulus.²³⁻²⁶ Our findings suggest that pretreatment with LL-mIL10 not only provides the local immune environment with increased IL-10 levels but could also be an active promoter of development of secretory IgA. Interestingly, mice pretreated with LLwt had no change of IgA titers compared with the control group. This finding goes along with the previously discussed study by Adel-Patient et al²⁰ in which *L. lactis* did not induce any secretory IgA. Thus, increased BLG-specific secretory IgA exclusively in the LL-mIL10 pretreated mice indirectly suggests a prime role for IL-10 in the induction of IgA secretion.

In conclusion, we showed in these studies that by preventively administering LL-mIL10, a nonpathogenic microorganism of the gut flora bioengineered for local production of IL-10, we could prevent in the majority of

the treated mice antigen-induced anaphylaxis, and strongly inhibit IgE-type sensitization in mice through an increased IL-10 secretion in the gut. Moreover, these microorganisms were able to induce a strong antigen-specific IgA response in the gut similarly to titers found in food-tolerant mice. These findings open interesting potential options in human beings for the prevention of allergies elicited through sensitization in the gut.

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