

Antigen-specific secretory IgA antibodies in the gut are decreased in a mouse model of food allergy

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Background: A large body of evidence implicates IgA antibodies in the immune response to pathogens present in the gut. Whether IgA antibodies play a similar role in food allergy remains to be determined.

Objective: We sought to characterize β -lactoglobulin (BLG)-specific serum and secretory IgA antibody production in the gut and to define the role of antigen-induced cytokines in IgA production in a murine model of food allergy.

Methods: BLG-specific IgA antibodies were measured in the sera and feces of mice anaphylactic or tolerant to BLG. The number of antibody-secreting cells in the spleen and Peyer's patches was determined by means of ELISPOT. Mesenteric lymph node cells and Peyer's patch T cells were transferred to naive mice, and antibody production in the sera and feces in recipient mice, as well as antibody-secreting cell numbers, were measured.

Results: Serum IgA antibody titers were strongly increased in anaphylactic mice. In contrast, BLG-specific IgA antibody titers were increased in feces but not in sera from tolerant mice. These results were correlated with an increased number of BLG-specific IgA-secreting cells in Peyer's patches from tolerant mice. The adoptive transfer of Peyer's patch CD3⁺ cells from tolerant mice induced an increased number of IgA-secreting cells preferentially in the Peyer's patches of naive recipient mice. Furthermore, an increase of BLG-induced IL-10 and TGF- β levels was found at IgA production sites.

Conclusions: These results suggest a role for secretory IgA in tolerance mechanisms to foods. Peyer's patch CD3⁺ cells are primarily involved by favoring IgA production through the release of IL-10 and TGF- β . (*J Allergy Clin Immunol* 2004;114:377-82.)

Key words: Food hypersensitivity, IgA antibody, anaphylaxis, tolerance, T lymphocytes, β -lactoglobulin, IL-10, TGF- β

IgE-mediated food allergy is a disease most prevalent in young children, with a high risk of potentially life-threatening anaphylactic reactions. A better understanding of the disease and active research for efficient prevention

Abbreviations used

BLG: β -Lactoglobulin
CTX: Cholera toxin
MNC: Mesenteric lymph node cell
PPC: Peyer's patch cell

or treatment is important. Using a well-defined murine model of food allergy,¹⁻³ we recently showed that Peyer's patch T cells were implicated in tolerance mechanisms, mostly through increased IL-10 secretion.⁴

Secretory IgA is an important player in the immune homeostasis of the gut. Peyer's patches are the site at which IgA-producing B cells develop.^{5,6} T cell-dependent synthesis of IgA is initially driven by TGF- β ,⁷⁻¹⁰ and IL-10 is instrumental in the differentiation phase.^{11,12} A large body of evidence suggests a protective role of secretory IgA against pathogens in the gut.¹³ Furthermore, several reports suggest a role for IgA in the pathogenesis of food allergy.¹⁴ We hypothesized that a specific pattern of IgA secretion might explain the phenomena of tolerance or allergy to foods by involving antigen-driven TGF- β and IL-10 secretion by T cells. Using a well-defined murine model of food-induced anaphylaxis, we studied antigen-specific IgA antibody production in the serum and feces, as well as antibody-producing cells in lymphoid organs. To study the capacity of T cells to induce production of IgA, we transferred T cells from tolerant and anaphylactic mice to naive recipient mice. Finally, we determined antigen-induced cytokine production in T cells to identify T cells that might regulate the IgA response.

METHODS

Oral sensitization and active tolerization to β -lactoglobulin

Four- to 5-week-old female C3H/HeOuJ mice purchased from Charles River (L'Arbresle, France) were immunized at days 0, 7, 14, and 21 by means of intragastric gavage with 20 mg of β -lactoglobulin (BLG; Sigma, Buchs, Switzerland) and 10 μ g of cholera toxin (CTX; List Biological Labs, Campbell, Calif; sensitized mice). Mice in the group exposed to antigen but without active sensitization with CTX (tolerized mice) received 0.8 mg/mL BLG in their drinking water ad libitum for 4 weeks. On day 28, all mice were challenged by means of intragastric gavage with 100 mg of BLG. Anaphylaxis was recorded and graded by using a reaction score (0, no reaction; 3, severe reaction or death) and correlated with the body temperature measured by

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means of infrared scanning at the ear (before challenge and 30 minutes after gavage) and the plasma histamine level measured by means of a commercial ELISA kit (Immunotech, Marseille, France).^{1,4} The study has been examined and approved by the institutional review board for animal experiments.

Mesenteric lymph node and Peyer's patch cell isolation and preparation

The procedure has been described in detail previously.⁴ In brief, Peyer's patches were excised from the gut and incubated for 30 minutes in HBSS medium supplemented with 5 mmol/L EDTA (Life Technologies, Paisley, Scotland), and similarly to the spleens and mesenteric lymph nodes, they were gently crushed and filtered through a 70- μ m nylon filter. CD3⁺ cells were selected by means of incubation on a nylon wool column at 37°C for 1 hour, followed by anti-MHC class II antibodies (m5/114.15.2; ATCC, Rockville, Md) and rabbit low-tox M complement (Cedarlane, Hornby, Ontario, Canada). The purity of the cell population measured by means of flow cytometry was greater than 97%.

Enzyme-linked immunoassays for BLG-specific serum and feces antibodies

Sera were obtained from tail bleedings for determination of BLG-specific antibody levels at various times during and after sensitization or tolerization. Feces obtained at the same times were resuspended in PBS plus 1% FCS (Life technologies) plus pepstatin 1:1000 (Fluka, Buchs, 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. 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 serially diluted sera (1:6, 1:18, and 1:54) or feces (1:2, 1:6, and 1:18) in PBS plus 10% horse serum was added in duplicates. Either 0.5 μ g/mL goat anti-mouse IgA or rat anti-mouse IgG1 peroxidase-labeled antibodies (Southern Biotechnologies, Birmingham, Ala) were incubated for 2 hours. 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

ELISPOT plates 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 (Amersham, Zurich, Switzerland) were resuspended as 2×10^6 in Iscove's modified Dulbecco's medium supplemented with penicillin, streptomycin, L-glutamine, 100 μ g/mL gentamicin, polymyxin B, and 5% FCS for 24 hours at 37°C, followed by overnight incubation at 4°C with anti-IgA and anti-IgG1 antibodies (Southern Biotechnology). 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 per 10^6 cells.

For assessment of IL-10 production, ELISPOT plates (Millipore, Bedford, Mass) were coated with mAbs against IL-10 (clone JES5-2A5; BD Pharmingen, Heidelberg, Germany; 1 μ g/mL) overnight at 4°C, followed by blocking with cell culture medium for 3 hours. Cells were added as 4×10^5 CD3⁺ cells/well and 6×10^5 irradiated total cells as antigen-presenting cells in the presence of 2.5×10^{-5} mol/L BLG in DMEM supplemented with penicillin, streptomycin, non-essential amino acids, L-glutamine, 100 μ g/mL gentamicin, polymyxin B, sodium pyruvate, and 10% FCS. The plates were incubated

for 4 days at 37°C. Biotin-labeled antibodies against IL-10 (clone SXC-1; BD Pharmingen) at 0.1 μ g/well were added for overnight incubation at 4°C, followed by streptavidin-horseradish peroxidase at 1:1000 for 2 hours.

TGF- β measured by means of cytotoxic cell assay

Four hundred thousand CD3⁺ cells/well and 6×10^5 irradiated total cells as antigen-presenting cells were incubated for 3 days at 37°C in flat-bottom, 96-well plates in the presence of 2.5×10^{-5} mol/L BLG in DMEM plus 10% serum replacement 2 (Sigma). After irradiation of the plate, 5000 Mv1Lu cells (mink lung epithelial cells¹⁵) were added for 66 hours at 37°C, followed by 1 μ Ci/well tritiated thymidine (Amersham, Zurich, Switzerland) for 6 hours, and then harvested. The standard curve was determined with TGF- β 1 purchased from R & D systems (Minneapolis, Minn).

Adoptive transfers

After isolation of CD3⁺ cells from the mesenteric lymph nodes, the spleen, or the Peyer's patches, the cells were resuspended in autologous mouse serum and PBS (1:1) at a concentration of 50×10^6 cells/mL. Ten million cells were injected through the tail vein to 4-week-old naive C3H/HeOuJ mice. Forty-eight hours after the cell transfer, the mice were boosted by means of an intragastric gavage with 20 mg of BLG.

Statistical analysis

Values between groups were analyzed by using the Wilcoxon signed-rank test for nonparametric unpaired data.

RESULTS

Tolerance is characterized by low serum antigen-specific IgA titers but increased gut secretory IgA antibodies

BLG-specific IgA serum and feces antibody titers were obtained before each sensitization and before the food challenge in both sensitized and tolerized animals. IgA titers remained close to presensitization values in both the sera and the feces in the control group (Fig 1). In the tolerized group exclusively, a significant increase in BLG-specific IgA antibody titer was observed in the feces ($P < .05$), whereas sera levels remained low. In contrast, increased titers of BLG-specific IgA were observed in the sera but not feces of BLG-sensitized mice ($P < .05$).

Peyer's patches in tolerant mice contain increased numbers of antigen-specific IgA-producing cells

In the next set of experiments, we explored the location of BLG-specific IgA-producing cells determined by means of ELISPOT. The spleen and Peyer's patches contained low numbers of BLG-specific IgA-producing cells in the sensitized group and in the CTX group (Fig 2). However, a strong increase in the number of antigen-specific IgA-producing cells was seen in Peyer's patches but not in spleen cells from the tolerized group ($P < .05$).

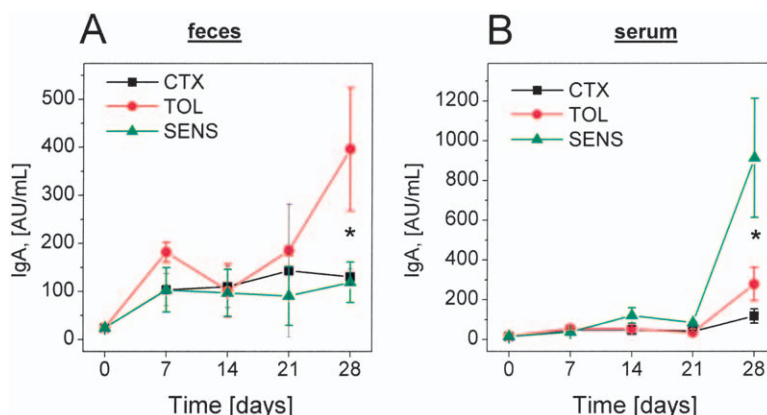


FIG 1. BLG-specific serum IgA antibody titers in the feces (A) and the serum (B) from control mice (CTX), tolerized mice (TOL), and sensitized mice (SENS). The results are pooled from at least 3 different experiments. * $P < .05$ SENS vs TOL; otherwise, $P =$ not significant.

Both Peyer's patch cells and mesenteric lymph node cells can induce antigen-specific serum and secretory IgA in naive mice by means of adoptive transfer

By means of adoptive transfer, we further explored whether the localization of the cells or the immune status of mice influence T cells that stimulate IgA production. Both mesenteric lymph node cells (MNCs) and Peyer's patch cells (PPCs) from tolerant mice induced BLG-specific fecal IgA in recipient mice, whereas MNCs and PPCs from sensitized mice induced BLG-specific serum IgA (Fig 3). These results show that both MNCs and PPCs can promote the generation of IgA-producing cells but that T cells from sensitized mice induce serum IgA, whereas T cells from tolerant mice induce fecal IgA.

Transfer of Peyer's patch T cells from the tolerized group generate an increased number of BLG-specific IgA-producing cells

After adoptive transfer of CD3⁺ cells and collection of sera and feces, the mice were killed, and spleen cells and PPCs were collected for ELISPOT to count the number of BLG-specific IgA-producing cells. The transfer of mesenteric lymph node T cells from the tolerized group increased the number of IgA-producing cells in Peyer's patches but not in the spleen (Fig 4, A). When Peyer's patch T cells were transferred, only cells from the tolerized group generated a significant number of BLG-specific IgA-producing cells in both the spleen and the Peyer's patches ($P < .05$ when compared with cells from the sensitized group; Fig 4, B). The transfer of mesenteric lymph node T cells from sensitized mice increased IgA-producing cells in the Peyer's patches but not in the spleen (Fig 4, A), whereas Peyer's patch T cells did not increase IgA-producing cells in the spleen or Peyer's patches (Fig 4, B).

Increased antigen-specific secretory IgA titer is related to increased TGF- β and IL-10 levels

Cytokines known to be instrumental in the class-switch recombination and differentiation of IgA production in the

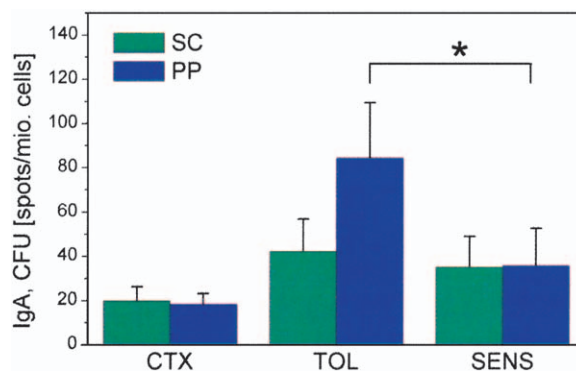


FIG 2. Numbers of BLG-specific IgA antibody-producing cells counted by means of ELISPOT in the spleen (SC) or in Peyer's patches (PP) from control mice (CTX), tolerized mice (TOL), and sensitized mice (SENS). The results are pooled from at least 3 different experiments. CFU, Cell-forming units. * $P < .05$.

gut were measured in MNCs and PPCs. In tolerized mice antigen-induced TGF- β levels were greater in PPCs than in MNCs, whereas in sensitized mice TGF- β levels were similarly increased in Peyer's patches and in mesenteric lymph nodes. Furthermore, TGF- β levels were lower in Peyer's patches from sensitized mice than in tolerized mice ($P < .05$; Fig 5, A and B). BLG-induced IL-10-producing cells were identified in MNCs only after sensitization, whereas Peyer's patches had IL-10-producing cells both after sensitization and tolerization (Fig 5, C and D). However, the number of IL-10-producing cells was significantly higher in Peyer's patches after tolerization than after sensitization ($P < .05$).

DISCUSSION

The secretory form of IgA antibodies is unique in its protective function in mucosal organs. We explored the pattern of antigen-specific IgA secretion in a murine model of food-induced anaphylaxis to provide a better

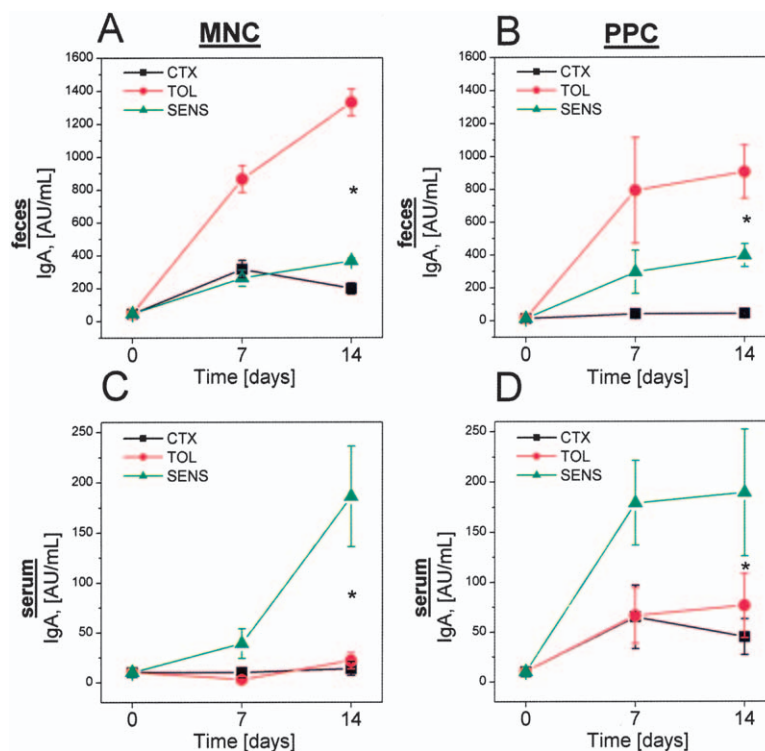


FIG 3. BLG-specific serum IgA antibody titers after adoptive transfer of CD3⁺ cells. IgA in the feces after adoptive transfer of MNCs (A) and after transfer of PPCs (B) is shown, as is IgA in the serum after adoptive transfer of MNCs (C) and after transfer of PPCs (D). The results are pooled from at least 3 different experiments. * $P < .05$ SENS vs TOL.

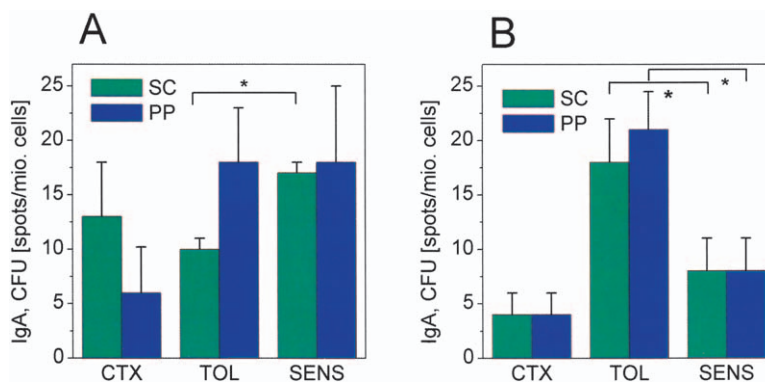


FIG 4. Numbers of BLG-specific IgA antibody-producing cells counted by means of ELISPOT in the spleen (SC) or in Peyer's patches (PP) after adoptive transfer of MNCs (A) or PPCs (B) from control mice (CTX), tolerized mice (TOL), and sensitized mice (SENS). The results are pooled from at least 3 different experiments. CFU, Cell-forming units. * $P < .05$.

understanding of the implication of IgA antibodies in the protection against detrimental reactions to food antigens. Previous experiments revealed that food-sensitive mice have a definite T_H2 pattern, with increased antigen-specific IgE and IgG1 antibodies, and that T cells generate IL-4 after antigen-induced activation.⁴ It was also well described that mice orally sensitized to a food antigen in the presence of CTX have an increased food antigen-specific IgA antibody titer in the serum.³ In the

present study we showed that BLG-anaphylactic mice had also increased BLG-specific serum IgA titers but low BLG-specific secretory IgA in the feces. Mice tolerant to BLG have low or no detectable antigen-specific IgE and IgG1 but increased IL-10 secreted by BLG-activated Peyer's patch T cells.⁴ These food-tolerant animals have a specific IgA antibody profile with low BLG-specific serum IgA titers and high titers of BLG-specific secretory IgA antibodies in their feces.

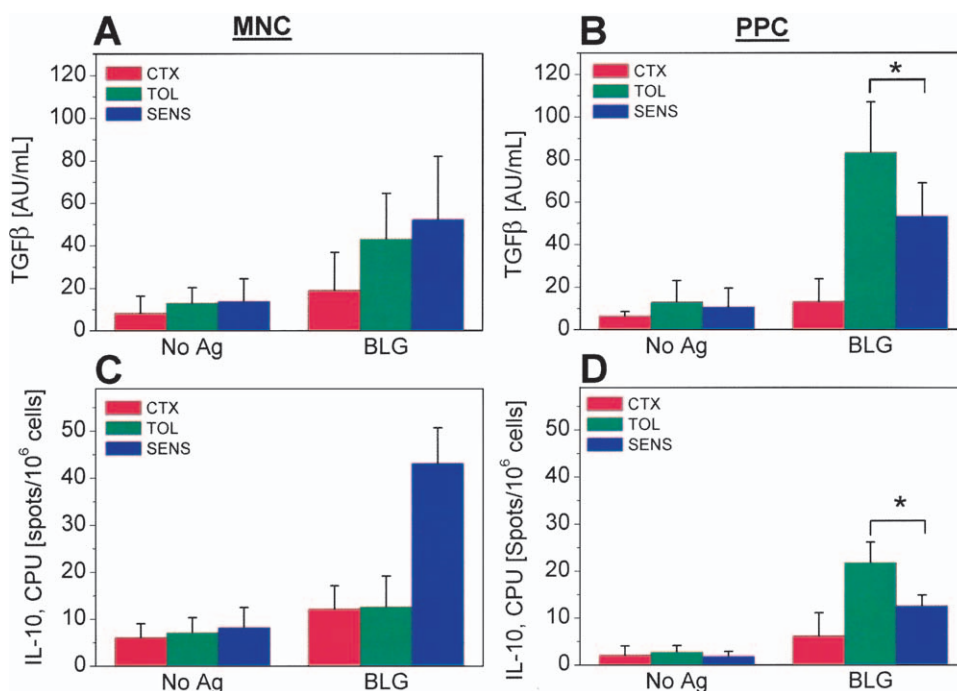


FIG 5. TGF- β titers measured in the supernatant of unstimulated and BLG-activated cells from MNCs (A) or PPCs (B) isolated from control mice (CTX), tolerized mice (TOL), and sensitized mice (SENS) is shown, as are the numbers of unstimulated and BLG-specific IL-10-producing cells in MNCs (C) or PPCs (D) counted by means of ELISPOT. The results are pooled from 3 different experiments. Ag, Antigen. * $P < .05$.

Although the protective effect of IgA antibodies against infectious antigens present in the gut is well established in human and animal models, only little is known on the role of IgA in food allergy. The group of Lee et al¹⁶ showed in a model of food allergy similar to ours that mice sensitized by a food antigen in the presence of CTX have antigen-specific secretory IgA in the feces. However, they did not have a study group similar to our group of tolerized mice, thus not allowing comparison between anaphylactic and allergen-exposed but clinically tolerant mice. Healthy infants have cow's milk-specific secretory IgA in their saliva and feces within the first weeks of life.¹⁷ In children with non-IgE-mediated cow's milk allergy, Hidvegi et al¹⁸ found increased serum IgA specific to BSA. However, cow's milk-specific IgA levels were also increased in children sensitized to food allergens.^{19,20} In the study by Bottcher et al,²⁰ secretory IgA levels were measured in the saliva and were found to be increased in sensitized but clinically tolerant infants. These studies in human subjects clearly show a protective role of antigen-specific secretory IgA antibodies.

Although BLG-specific IgA-producing cells were distributed in the spleen and in Peyer's patches in anaphylactic mice, they were predominantly found in Peyer's patches in BLG-tolerant mice. One might speculate that cells located in Peyer's patches are mostly producing BLG-specific IgA of the secretory type and account for the high titer of secretory IgA in the feces, whereas cells in the spleen mostly produce BLG-specific

serum IgA antibodies. With adoptive transfer of CD3⁺ cells, we further explored whether the anatomic origin of T cells (Peyer's patches or mesenteric lymph nodes) influence IgA production in recipient mice. Overall, the transfer of CD3⁺ cells of both origins induced the production of BLG-specific IgA. These results suggest a recirculation of BLG-specific T cells in adjacent lymphoid structures. However, T cells from tolerized mice induced secretory IgA in the feces, whereas T cells from sensitized mice induced BLG-specific serum IgA. Furthermore, these experiments showed that there are mostly CD3⁺ cells from tolerized mice located in Peyer's patches that can induce large numbers of BLG-specific IgA secretory cells, suggesting a preferential homing to Peyer's patches. Interestingly, this selective localization of BLG-specific IgA-producing cells did not influence the BLG-specific IgA titers observed in the serum. The observation that IgA antibody-producing cell clones can be isolated from Peyer's patches on administration of a dietary antigen is in line with the results of previous studies.²¹

TGF- β and IL-10 are 2 cytokines involved in mechanisms of allergy regulation and IgA synthesis, and we previously showed that tolerant mice are characterized by increased production of IL-10 in PPCs.⁴ Thus we were interested to further characterize TGF- β secretion by BLG-activated cells. TGF- β secretion in PPCs was similar to what we found with IL-10 (ie, a selective increase of TGF- β in PPCs from tolerant mice). These findings in

Peyer's patches suggest that the effect of CTX to induce IgA switching in the spleen through the action of TGF- β 1 does not affect PPCs and that the increased titers of TGF- β after BLG feeding found in the tolerized animals are independent of the action of CTX (not used in this group).²²

Taken together, our results suggest the following scenario in allergy to a common food antigen. Continuous exposure to BLG induces antigen-specific T cells preferentially located in Peyer's patches to secrete TGF- β and IL-10, 2 cytokines involved in regulatory mechanisms and synthesis of secretory IgA. In contrast, in sensitized animals antigen-responsive T cells producing TGF- β and IL-10 are located in the mesenteric lymph nodes, causing antigen-specific serum IgA increase. In Peyer's patches, however, T cells exerting a regulatory function through IL-10 and TGF- β are deficient in food-anaphylactic mice.

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