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Perinatal and Postweaning Exposure to Galactooligosaccharides/Inulin Prebiotics Induced Biomarkers Linked to Tolerance Mechanism in a Mouse Model of Strong Allergic Sensitization

Pascal Gourbeyre,[†] Nicolas Desbuards,[‡] Guilaine Grémy,[†] Olivier Tranquet,[†] Martine Champ,[‡] Sandra Denery-Papini,[†] and Marie Bodinier*[,†]

[†]INRA, UR 1268 BIA, équipe Allergie, rue de la Géraudière, B.P. 71627, F-44316 Nantes Cedex 03, France

[‡]INRA, UMR 1280 PhAN, CRNH, CHU Hôtel-Dieu, Bât. HNB1, 1 place Alexis Ricordeau, F-44093 Nantes Cedex 1, France

ABSTRACT: Food allergies are increasing, and no treatment exists, thus enhancing interest in prebiotic strategies. This study aimed to analyze the preventive effects of prebiotic feeding during perinatal and postweaning periods in a mouse model of allergy by studying biomarkers related to tolerance (IgG2a, IgA, IFN-γ, TGF-β, and IL-10), to allergy (IgE, IgG1, IL-4, IL-17, symptoms), and to microbiota (propionate and MyD88). Balb/c mice, both dams and their pups, were fed a diet supplemented with (+Prb) or without (−Prb) GOS/inulin prebiotics. Mice were then sensitized with allergens. Regardless of diet, sensitized mice exhibited similar levels of IgE, IgG1, CD-23, IL-4, IL-17, and symptoms. However, in comparison to −Prb-sensitized mice, +Prb-sensitized mice displayed higher concentrations of total IgG2a (6669 ± 1788 vs 3696 ± 1326 fluorescence units, $p < 0.005$), specific IgA (285 ± 26 vs 156 ± 9 fluorescence units, $p < 0.01$), IFN-γ (3194 ± 424 vs 1853 ± 434 pg/mL, $p < 0.01$), IL-10 (777 ± 87 vs 95 ± 136 pg/mL, $p < 0.005$), TGF-β (4853 ± 1959 vs 243 ± 444 pg/mL, $p < 0.01$), MyD88 (0.033 ± 0.019 vs 0.009 ± 0.004 relative expression, $p < 0.01$), and propionate (4.15 ± 0.8 vs 2.9 ± 1.15 μmol, $p < 0.05$). In a mouse model of allergy, prebiotic exposure during perinatal and postweaning periods induced the highest expression of biomarkers related to tolerance without affecting biomarkers related to allergy.

KEYWORDS: *allergy, gut, immunity, Treg, Th2, wheat*

INTRODUCTION

Allergic reactions are commonly known to be triggered by Th2 cells that secrete IL-4 and are able to activate IgE production (IgG1 and IgE in mice).[1] The low-affinity IgE receptor FcεRII (CD23), expressed on enterocytes, has been implicated in the bidirectional transport of allergen−IgE complexes from the lumen to the serosa in the intestine.[2] This mechanism enhances the triggering phase of food allergic reactions during which mast cells degranulate, releasing allergic mediators such as histamine that are involved in clinical manifestations of allergy. The major role of the Th2 response during allergy establishment may be completed by that of a novel Th17 subset recently identified. Th17 cells and IL-17 secretion have been involved in allergy mechanisms, in both animal models[3−7] and humans, notably in allergic asthma[8] and in food allergies.[6,9] To avoid allergic pathologies, organisms have developed the complex and not yet fully understood mechanism of oral tolerance. Oral tolerance appears to be dependent on different immunological processes: Th1/Th2 balance regulation, cell anergy, cell deletion, or cell suppression induced by regulatory T (Treg) cell activation.[10] The Th1 response consists of an inflammatory pathway induced by bacterial antigens in which Th1 cells secrete IFN-γ and are able to induce IgG2a production by B cells (in mice).[11] The Treg response, an anti-inflammatory pathway, involves cells that secrete TGF-β and IL-10 and are able to promote IgA production.[12]

As of yet, there is no effective treatment that can cure food allergies. One promising approach to prevent or reduce these diseases might be the modulation of the microbiota, as suggested by the hygiene hypothesis concept.[13,14] Indeed, numerous data link commensal microorganisms to oral tolerance acquisition.[15−17] In fact, it has been shown that some autochthonous bacteria can play a key role in the modulation of immune parameters (IL-10 secreting Treg cells, Th1 and Th17 lymphocytes, and IgA production).[18,19] The microbiota can modulate the immune system through different mechanisms: by direct fixation on cellular receptors or indirectly by the fermentation of carbohydrates by bacteria composing this intestinal ecosystem. Indeed, immune cells possess many receptors specialized in the recognition of microorganism-associated molecular patterns such as pattern-recognition receptors (PRR), including Toll-like receptors (TLR).[20] The activation of some TLR induces MyD88-dependent pathways, leading to cytokine secretion by immune cells. Moreover, certain autochthonous bacteria can indirectly modulate the expression of various genes related to the immune system[21] via short-chain fatty acid (SCFA) (i.e., acetate, propionate, and butyrate) production after carbohydrate fermentation.[22,23] Thus, a strategy to prevent or reduce allergies would consist of modulating the host microbiota using either allochthonous bacteria (probiotics) or non-digestible food ingredients that can regulate the autochthonous microbiota (composition and metabolism), such as prebiotics.[21]

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In the context of allergy prevention, two human studies have tested the antiallergic effects of a prebiotic galactooligosaccharides (GOS)/inulin mix with[24] or without[25,26] pectin-derived acidic oligosaccharides during the lactation period. In the studies of Moro et al., an Italian infant cohort demonstrated that the incidence of allergic diseases was significantly reduced at both 6 months[26] and 2 years[25] in children that received a prebiotic-enriched formula. This effect was confirmed during the multicenter study of Gruber et al.[24] In animal models, four studies using early-weaned[27] or adult mice[28−30] demonstrated that prebiotics (GOS and/or inulin or other fructans called fructooligosaccharides (FOS)) were able to alleviate allergic manifestations,[27,29,30] such as a lower rate of edema formation in the duodenum.[28] Indeed, a nutritional intervention with GOS/inulin appears to effectively induce a protective effect against allergy. However, in both humans and mice, the period during which these food supplements must be ingested for optimal results remains unclear.

In an earlier study,[31] we showed that prebiotics exerted a marked effect on the immune system orientation when mice were exposed during both perinatal and postweaning periods. Under these conditions, several biomarkers related to Th1 and Treg responses, which are potentially involved in tolerance mechanisms, were highly expressed in mice fed a prebiotic-supplemented diet. Therefore, these encouraging results have motivated our choice to expose mice to prebiotics for a long period comprising both the entire perinatal period and the postweaning period (from conception to 12 weeks of age) and to evaluate their effect on allergic parameters.

In the current study, we thus set forth to analyze the preventive effects of this long exposure period to prebiotics in a robust mouse model of allergy. We chose a model obtained by intraperitoneal sensitizations with modified gliadins that was already well characterized and validated.[32,33] We first evaluated the effects of prebiotic exposure during perinatal and postweaning periods on tolerance-related biomarkers (IgG2a, IgA, IFN-γ, TGF-β ,and IL-10), allergic sensitization biomarkers (IgG1, IgE, and CD23 expression), and/or allergy-triggering biomarkers (clinical symptoms, gut morphological alterations, and histamine release). The effects of prebiotic feeding on the microbiota after allergen sensitization was also analyzed by prebiotic fermentation via SCFA production (acetate, propionate, and butyrate) and the ability to induce TLR pathways via MyD88 expression.

MATERIALS AND METHODS

Chemicals. The oligosaccharides used in this study were short-chain GOSs extracted from Vivinal GOS syrup (Friesland Food Domo, Amersfoort, The Netherlands) spray-dried by la Laiterie de Montaigu (France) and inulin (Orafti-Beneo, Tienen, Belgium), respectively, mixed in a 9:1 ratio. Crude gliadins were extracted from wheat flour (Hardi cultivar), and chemical deamidation was carried out as previously described.[32,33]

Animals. *Mice and Housing Conditions.* BALB/c mice and their progeny were obtained from the Centre d'Elevage René Janvier (France). The mice were housed in a ventilated cage system (IVC Racks, Allentown, NJ, USA) under controlled temperature and humidity conditions with a 12 h light−dark cycle. Mice were acclimatized to these conditions for 2 weeks prior to mating. Pups were weaned at 3 weeks. The experimental protocol was approved by the Ethics Committee in Animal Experimentation of Pays de la Loire (accreditation no. C44502).

Diets. Progeny were fed either a semisynthetic control diet (−Prb purified diet 210, SAFE, Augy, France) or a diet supplemented with 4% prebiotics (+Prb purified diet modified 210 with 4% of the GOS/inulin mixture).[29,31] Dams received the same diets during gestation and lactation as their pups, which were fed from weaning to the end of the experimental protocol with each diet (−Prb, n = 31; +Prb, n = 20).

Mouse Sensitization. The experimental protocol was similar to that described by Gourbeyre et al.[32,33] Freeze-dried deamidated gliadins were solubilized in 70% ethanol at 5 mg/mL and then diluted to 0.1 mg/mL in sterile PBS. Approximately half of each mouse group (+Prb, n = 16; −Prb, n = 10) was sensitized with four intraperitoneal (ip) injections of 10 μg of deamidated gliadins adsorbed on aluminum hydroxide (Alum; Alhydrogel, Sigma-Aldrich, Saint Quentin Fallavier, France). The other half of each group of mice (+Prb, n = 15; −Prb, n = 10) received an intraperitoneal injection of Alum diluted with PBS. Intraperitoneal sensitizations were conducted on days 0, 10, 20, and 30.

Characterization of Immune Responses. *Immunoglobulin Assays.* Blood and feces samples were collected on the 37th day (after four ip injections). Blood samples were drawn from the retro-orbital venous plexus of mice. The blood samples were incubated for 20 min at 37 °C and then centrifuged for 20 min at 2000g. The supernatant was removed to assay the immunoglobulins in the serum. Mouse total IgA, IgE, IgG1, and IgG2a were assayed by F-ELISA as previously described.[31,32] Mouse IgE, IgG1, and IgG2a specific to deamidated gliadins were assayed as previously described.[32,33] Different serum dilutions were used to assay the immunoglobulins: 1:50 for IgE, 1:500 for IgG2a and IgG1 from unsensitized mice, and 1:10^6 for IgG1 from sensitized mice. Mouse IgA was extracted from pooled feces samples according to the method described by Chatel et al.[34] The protein contents of each extracted pool were determined in triplicate with a colorimetric assay (Bio-Rad Laboratories, Munich, Germany) using bovine serum albumin as the protein standard. These samples were diluted 1:30 for specific IgA assays and 1:50 for total IgA assays. Specific IgA was assayed with an indirect ELISA in which deamidated gliadins were coated on the plate, and IgA was detected using a detection goat antibody directed against mouse IgA conjugated to alkaline phosphatase (Southern Biotechnology Associates, ref 1040-04, Birmingham, AL, USA) diluted 1:3000 in PBS with 0.1% Tween-20 and 0.5% gelatin. Fluorescence intensity (at 440 nm after excitation at 360 nm) was measured after incubation with an alkaline phosphatase substrate (4-methylumbeliferin, Sigma-Aldrich). Total IgA was assayed as previously described.[31] All IgA values were expressed as a ratio of fluorescence intensity/milligrams of proteins × 100.

Allergic Challenge. On the 43rd day, all mice were challenged with an ip administration of deamidated gliadins (1 mg) solubilized in 10 mM acetic acid at pH 5. Mouse symptoms were observed 30 min after the injection, and scores were assigned according to the scale of Li et al.[35] One hour after challenge, blood samples were collected for histamine detection. Histamine was assayed using the Histamine Research ELISA kit (LDN 10-2100, Labor Diagnostika Nord, Nordhorn, Germany) following the manufacturer's recommendations.

Allergen-Specific and Nonspecific Activation of Mouse Spleen Cells and Cytokine Assays. Bled mice were euthanized by vertebral dislocation, and spleen cells were harvested from each group. Single spleen cell cultures were activated in the presence of either PBS (negative control), concanavalin A (1 μg/mL), or allergen (deamidated gliadins, 20 μg/mL in PBS) as previously described.[33] The supernatants were then collected and stored at −20 °C until further assay. IL-4, IL-10, IL-17A, INF-γ, and TGF-β were assayed using the following kits: mouse IL-4, Il-10, and INF-γ CytoSet kits (CMC0043, CMC0103, and CMC4033 from Invitrogen, Paisley, UK) and mouse IL-17A and TGF-β ELISA Ready-Set-Go! Kits (88-7371-88 and 88-7344-88, from eBioscience, Paris, France) following the manufacturer's instructions.

Evaluation of Intestinal Biomarkers. *Intestinal Morphological Analysis.* Jejunum samples from each group of challenged mice were taken after washing and immediately immersed in paraformaldehyde (4% in PBS) for 3 h at room temperature, followed by standard procedure for paraffin embedding. Serial 3 mm sections were cut for each jejunal sample and stained with hematoxylin−eosin and periodic acid Schiff. The sections were observed under a photomicroscope.

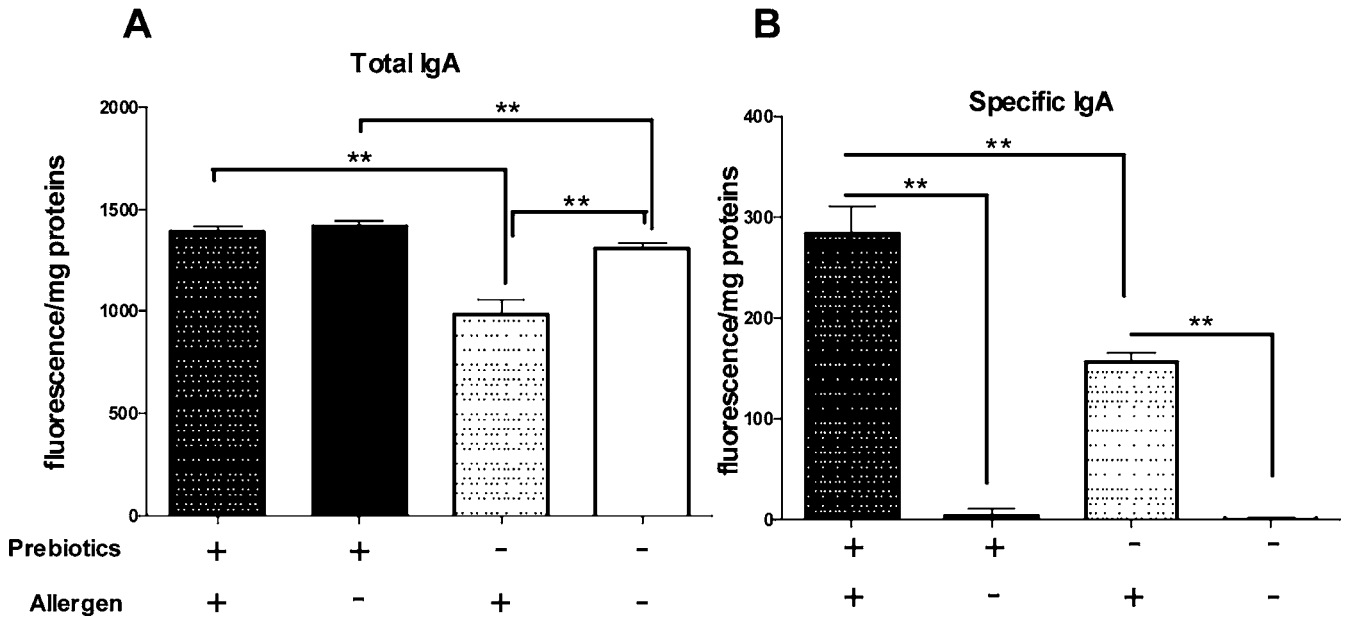

Figure 1. Effects of perinatal consumption of prebiotics and sensitization to wheat allergens on IgA excretion in mice. Total (A) and specific (B) IgA were assayed in pooled fecal samples. Mice were fed either a prebiotic-supplemented diet (black bar) or a control diet during perinatal life (white bar). Immunoglobulins were detected in 12-week-old mice that had received four intraperitoneal injections of either allergen (bar with pattern) or vehicle (bar without pattern). The data represent the mean ± SD; **, $p < 0.01$; ***, $p < 0.005$.

Histological analysis was performed in a blinded fashion. Morphological alterations were assessed by considering the various parameters scored on a 0−3 scale as follows: state of epithelium and glands; inflammatory cell infiltration; edema; atrophia.[36] The alteration scores of individual mice represent the sum of the subscores of the different histological parameters. The results have been summarized as the mean ± SD for the mean of the sums of all the animals' subscores (minimum possible result is 0 and maximum possible result is 12).

Measurement of Markers Related to Allergy and Tolerance by Western Blot. Four mice per group were euthanized before challenge. Jejunum samples were homogenized in a solution containing TRIzol (50−100 mg of tissue in 1 mL) for 5 min at room temperature. The homogenate was suspended in chloroform, vigorously vortexed for 15 s, and incubated for 2−3 min at room temperature. The solution was then centrifuged at 12000*g* and 4 °C for 15 min. The organic phase was washed twice with acetone at 4 °C, dried, and resuspended in lysis buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and a protease inhibitor cocktail). The proteins were determined in duplicate with a colorimetric assay (Bio-Rad Laboratories, Munich, Germany) using bovine serum albumin as the protein standard. Electrophoresis was performed using a 12% SDS−polyacrylamide gel. The separate proteins were electrophoretically transferred onto nitrocellulose membranes. Before immunostaining, nonspecific binding sites were blocked with 5% cow's milk in Tris-buffered saline (TBS) containing 0.1% Tween-20. The membranes were incubated for 1 h with detection antibodies: CD23 (sc-19631, Santa Cruz Biotechnology, Santa Cruz, CA, USA), MyD88 (rabbit AB-16527, Millipore, Bedford, MA, USA), and β-actin (C4, sc-47778, Santa Cruz Biotechnology), which were all diluted 1:2000. Following several washes in TBS 0.1% Tween-20, the membranes were incubated with appropriate secondary antibodies (goat anti-rabbit IgG or goat anti-mouse IgG; Jackson, West Grove, PA, USA) diluted 1:5000. A chemiluminescent substrate (SuperSignal West Dura Extended Duration Substrate, Pierce, Rockford, IL, USA) was added, and the luminescence was measured with a CCD camera (Luminescent Image Analyzer LAS 3000, Fujifilm, Tokyo, Japan).

Short-Chain Fatty Acid Analysis by Capillary Gas−Liquid Chromatography. The contents of the ceca were collected from the challenged 12-week-old mice for SCFA analysis. Approximately 1 g of the cecal content was homogenized in 4 volumes of sterile water. After centrifugation of the thawed samples (10000*g* for 10 min), 0.1 mL of the supernatant was supplemented with 0.9 mL of oxalic acid (0.5 M). SCFA contents were analyzed by capillary gas−liquid chromatography (SGE BP21 capillary column, 25 m × 0.53 mm; nitrogen as carrier gas, 17 mL/min). The injector and detector temperatures were maintained at 250 and 200 °C, respectively. The oven temperature was maintained at 90 °C. The samples were introduced by splitless injection with a split flow of 50 mL/min starting 1 min after injection. The concentration of each SCFA was determined in comparison to a known concentration of acetate, propionate, and butyrate solutions (from 0.2 to 2 mM), which were analyzed under the same chromatographic conditions.

Statistical Analysis. Values are expressed as the mean ± SD. GraphPad Prism version 5.02 for Windows software (GraphPad Software, San Diego, CA, USA; www.graphpad.com) was used to perform the statistical analysis. A nonparametric Mann−Whitney test was used to compare total IgE, IgG1, and IgG2a production between the groups of mice. When samples were pooled (to determine fecal IgA and plasma cytokines), Student's *t* test was used. Any differences with p values strictly < 0.05 were considered to be statistically significant.

RESULTS

Effects of a Prebiotic-Enriched Diet on Different Immune Markers after Allergen Sensitization. *Analysis of Fecal IgA Excretion (Figure 1).* Without sensitization, +Prb mice exhibited higher levels of total IgA than −Prb mice ($p < 0.01$; Figure 1A). After sensitization, +Prb animals displayed higher levels of both total and specific IgA than −Prb animals ($p < 0.01$; Figure 1A,B). For mice given the control diet, sensitized animals exhibited a weaker level of total IgA than unsensitized animals ($p < 0.01$; Figure 1A). Nevertheless, in the +Prb group, all mice either sensitized with wheat gluten allergens or not sensitized showed the same levels of total fecal IgA (Figure 1A). Therefore, ip sensitizations to wheat gluten allergens decreased IgA excretion, but prebiotic feeding appeared to maintain IgA levels, even after mouse sensitization.

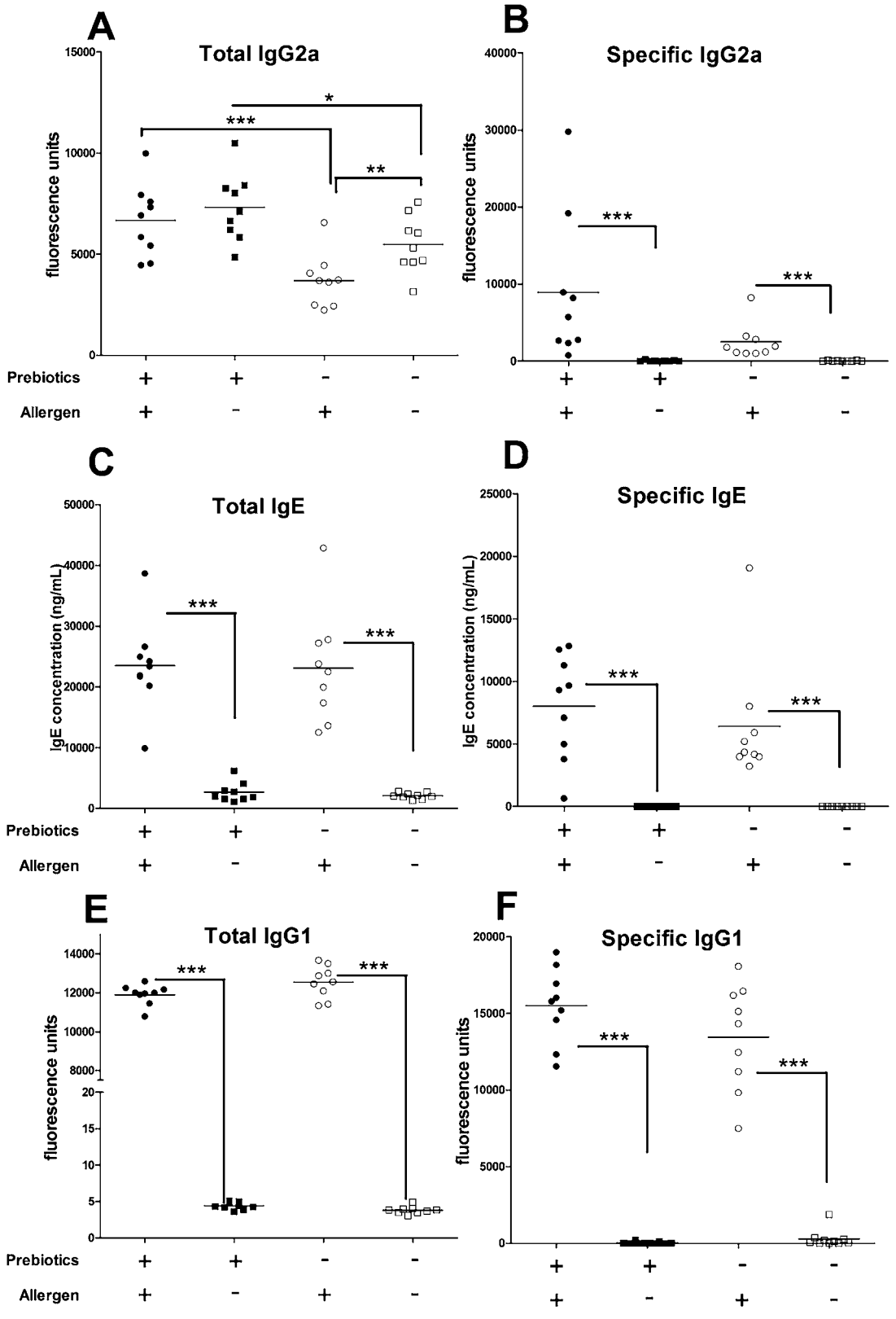

Figure 2. Effects of perinatal consumption of prebiotics and sensitization to wheat allergens on allergy-related immunoglobulins in mice. Total (A) and specific (B) IgG2a, total (C) and specific (D) IgE, and total (E) and specific (F) IgG1 were assayed in serum from each mouse. Mice were fed either a prebiotic-supplemented diet (black shapes) or a control diet during perinatal life (white shapes). All immunoglobulins were detected in 12-week-old mice that had received four intraperitoneal injections of either allergen (circles) or vehicle (squares). The data represent the mean ± SD; ∗, $p < 0.05$; ∗∗, $p < 0.01$; ∗∗∗, $p < 0.005$.

Analysis of Serum IgG2a, IgG1, and IgE Production (Figure 2). (1) IgG2a. Without sensitization, +Prb mice exhibited higher levels of total IgG2a than −Prb mice ($p < 0.05$; Figure 2A). After sensitization, +Prb animals displayed

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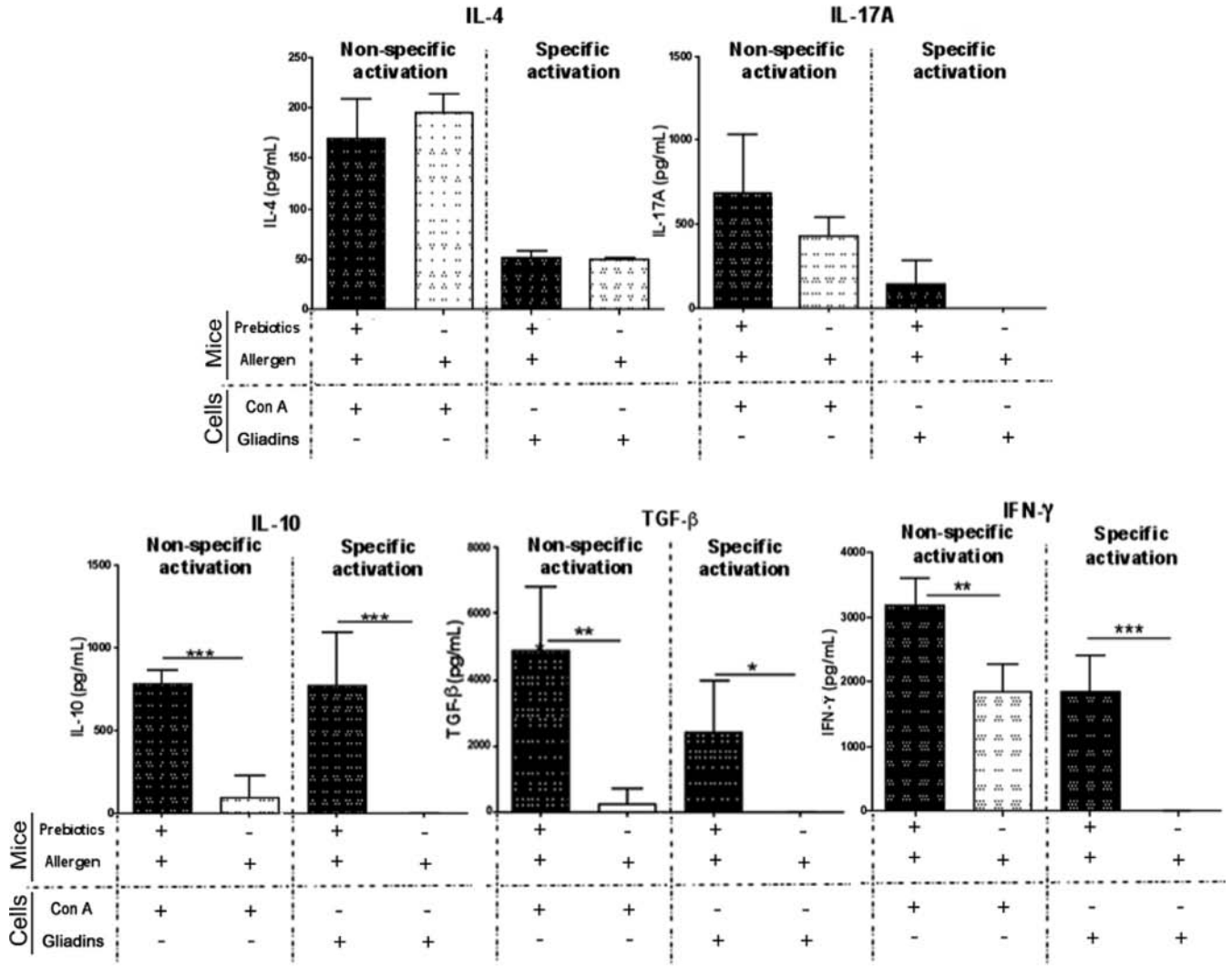

Figure 3. Effects of perinatal consumption of prebiotics in mice sensitized to wheat allergens on cytokine production by activated spleen cells. IFN-γ, IL-10, TGF-β, IL-4, and IL-17A levels were measured in the supernatants of pooled spleen cells from sensitized mice. Mice were fed either a prebiotic-supplemented diet (+Prb, black bar) or a control diet during perinatal life (−Prb, white bar). All spleens were collected from 12-week-old mice that had received four intraperitoneal injections of either allergen (bar with pattern) or vehicle (bar without pattern). Spleen cells were either specifically activated with wheat allergens or nonspecifically activated with concanavalin A. The data represent the mean ± SD; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$.

higher levels of total IgG2a than −Prb animals ($p < 0.005$; Figure 2A) and a tendency to increase their levels of gliadin-specific IgG2a (Figure 2B). For mice under the −Prb diet, sensitized animals exhibited a weaker level of total IgG2a than unsensitized animals ($p < 0.01$; Figure 2A). Nevertheless, in the +Prb group all mice, either sensitized with wheat gluten allergens or not, showed the same levels of total serum IgG2a (Figure 2A). Thus, intraperitoneal sensitizations to wheat gluten allergens decreased IgG2a secretion, but prebiotic feeding appears to maintain IgG2a levels even after mouse sensitization.

(2) IgG1 and IgE. Without sensitization, both total IgG1 and IgE levels were the same, regardless of mouse diet (Figure 2C,E). All sensitized mice also showed the same levels of both total and specific IgE (Figure 2C,D) and IgG1 (Figure 2E,F), independent of diet. Sensitized mice with both diets displayed stronger total and specific IgE (Figure 2C,D) and IgG1 (Figure 2E,F) levels than unsensitized mice ($p < 0.005$). Prebiotics did not appear to modulate IgE and IgG1.

Analysis of Cytokine Secretion by Spleen Cells from Sensitized Mice after Specific and Nonspecific Activation (Figure 3). Regardless of diet, spleen cells secreted the same amounts of both IL-4 and IL-17A after either nonspecific (Con A) or specific (gliadin) activation with allergen. Spleen cells from +Prb mice secreted higher levels of both INF-γ ($p < 0.005$ for nonspecific activation and $p < 0.01$ for specific activation), IL-10 ($p < 0.005$), and TGF-β ($p < 0.01$ for nonspecific activation and $p < 0.05$ for specific activation) than cells from −Prb mice after either nonspecific or specific activation. Therefore, prebiotic feeding had no impact on allergy-related cytokines (both IL-4 and IL-17A secretion), whereas prebiotics appeared to increase the secretion of tolerance-related cytokines (INF-γ, IL-10, and TGF-β).

Analysis of CD23 Expression in the Jejunum (Figure 4). Without sensitization, CD23 levels were the same regardless of diet. All sensitized mice also showed the same levels of CD23. For animals given the −Prb diet, sensitized mice displayed stronger CD23 levels than unsensitized mice ($p < 0.005$). For animals given the +Prb diet, the same upward trend ($p = 0.059$)

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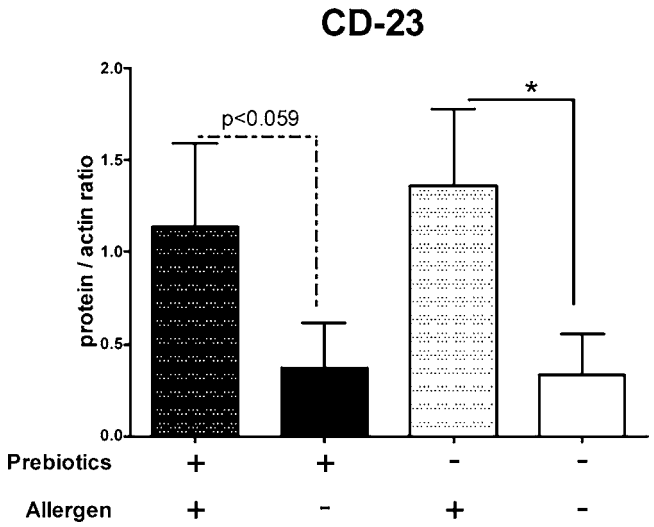

Figure 4. Effects of prebiotic exposure and sensitization to allergen on the expression of an intestinal biomarker related to IgE transport across the mouse gut barrier. CD23 protein expression was assayed using pooled jejunum samples. Mice were fed either a prebiotic-supplemented diet (black bar) or a control diet during perinatal life (white bar). All samples were collected from 12-week-old mice that had received four intraperitoneal injections of either allergen (bar with pattern) or vehicle (bar without pattern). The data represent the mean ± SD; *, $p < 0.05$; **, $p < 0.01$.

was observed. Intraperitoneal sensitizations to wheat allergens induced an expression of CD23 in mouse jejunum. Prebiotics did not seem to exert an impact on the expression of this intestinal biomarker.

Effects of the Prebiotic-Enriched Diet on the Allergy-Triggering Phase after Allergen Challenge. *Evaluation of Clinical Symptoms (Figure 5A).* After ip challenge with allergen, no difference in symptom severity was observed between sensitized mice, regardless of their diet. Also independent of diet, sensitized mice displayed more severe symptoms (score mean 3/5, characterized by wheezing, labored breathing, and cyanosis around tail and legs) than unsensitized mice (score mean 1/5, characterized by scratching and rubbing around the nose and head) (+Prb, $p < 0.005$; −Prb, $p < 0.01$). Prebiotics did not seem to alleviate clinical symptoms in mice.

Evaluation of Serum Histamine Release (Figure 5B). One hour after ip challenge, histamine levels from sensitized mice were the same for both diets. Serum histamine was significantly more concentrated in sensitized animals (+Prb, $p < 0.005$; −Prb, $p < 0.01$) than in unsensitized animals. Prebiotics did not appear to modulate serum histamine release.

Morphological Alterations of the Jejunum (Figure 5C). After allergenic challenge, jejunum from sensitized mice showed the same rate of morphological alterations regardless of diet. Jejunum from sensitized mice of both diets displayed stronger morphological alterations than jejunum from unsensitized mice ($p < 0.01$). Therefore, ip sensitizations to wheat allergens caused severe intestinal alterations. Prebiotics did not seem to alleviate this jejunal inflammation.

Effects of Prebiotic Exposure on Microbiota Metabolism and Host/Microbiota Interface. *SCFA Production in Mice Ceca (Figure 6A).* Without sensitization, cecal content from +Prb mice displayed higher levels of propionate than that of −Prb mice ($p < 0.05$). In sensitized animals, +Prb mice also exhibited stronger cecal levels of propionate than −Prb mice ($p < 0.05$). Regardless of diet, sensitized and unsensitized mice showed the same amount of propionate in their cecum. Levels of other SCFA (acetate and butyrate) were the same regardless of diet and were not modified after sensitization. Prebiotic feeding had an impact only on propionate, increasing the levels of this SCFA. Allergenic sensitization did not seem to exert an effect on SCFA production by the cecal microbiota.

MyD88 Expression in Mice Jejunum (Figure 6B). Without sensitization, jejunum from mice of both diets showed the same levels of MyD88. After sensitization, mice given the −Prb diet expressed lower amounts of MyD88 in the jejunum ($p < 0.05$) than +Prb mice. In mice given the +Prb diet, MyD88 levels were the same regardless of sensitization status. In mice given the −Prb diet, sensitized animals exhibited weaker levels of MyD88 compared to unsensitized animals ($p < 0.05$). Thus, ip sensitizations to wheat allergens appeared to down-regulate MyD88 expression in the jejunum, whereas prebiotics seemed to maintain high levels of this protein after sensitization.

DISCUSSION

The purpose of the present study was to determine if perinatal consumption of prebiotics can be considered a good approach

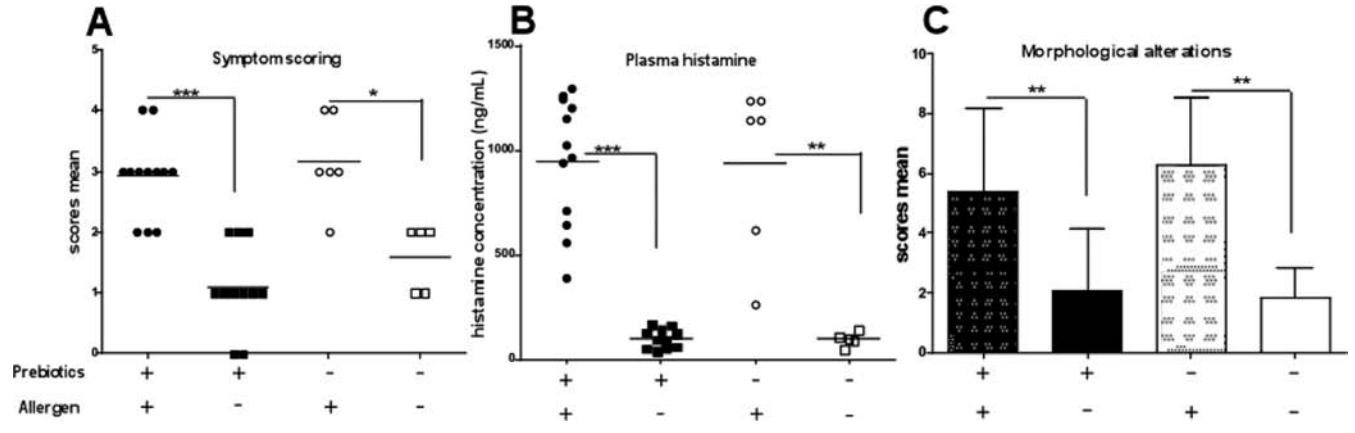

Figure 5. Effects of prebiotic exposure and sensitization to allergen on different biomarkers related to the triggering phase of allergic reaction. Mice were fed either a prebiotic-supplemented diet (black bar or shapes) or a control diet during perinatal life (white bar or shapes). Symptom scores (A), blood histamine levels (B), and jejunum morphological alteration scores (C) were evaluated in mice challenged either with (bar with pattern or circles) or without wheat allergen (bar without pattern or squares). The data represent the mean ± SD; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$.

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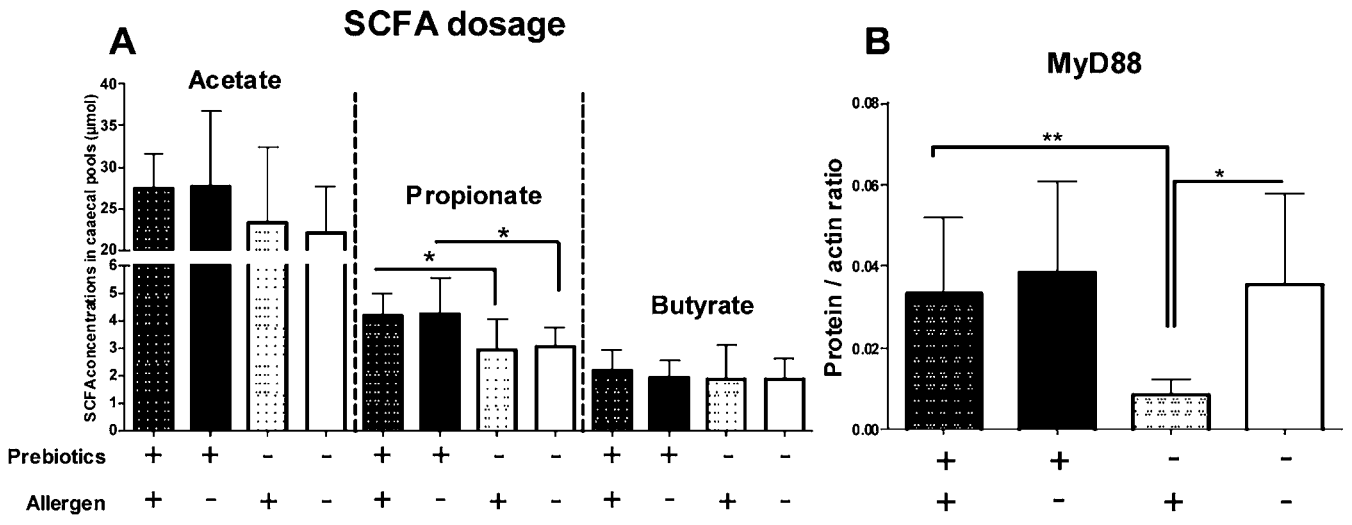

Figure 6. Effects of prebiotic exposure and sensitization to allergen on various biomarkers related to the host/microbiota interface. SCFA cecal levels (acetate, propionate, and butyrate (A) and the jejunal expression of a Toll-like receptor pathway biomarker (MyD88; B) were determined using pooled samples from mice challenged either with (bar with pattern or circles) or without wheat allergen (bar without pattern or squares). Mice were fed either a prebiotic-supplemented diet (black bar) or a control diet during perinatal life (white bar). The data represent the mean ± SD; ∗, $p < 0.05$; ∗∗, $p < 0.01$.

to prevent allergies. We chose to use our current mouse model of ip sensitization with deamidated gliadins because it displayed two major advantages: first, it was similar to allergic patients in terms of IgE reactivity patterns, and, second, mouse responses showed high homogeneity in each group.[32,33] Moreover, additional results obtained during the current study demonstrate that this model is reproducible: levels of immunoglobulins and histamine and symptom scores were the same as what has already been observed.[33] For the first time, we observed in this study that intestinal physiology is modified by ip injection of wheat allergens, that is, the increased expression of CD23, which is responsible for IgE transport across the gut mucosa,[2] and evident alterations of gut morphology. Therefore, this mouse model of sensitization to food allergen exhibits a typical Th2 response culminating in clinical symptoms and intestinal alterations.

In a healthy context, we previously showed that prebiotic feeding during perinatal and postweaning periods increased the expression of some biomarkers related to Th1 and Treg responses in mice that are potentially involved in tolerance mechanisms.[31] In the current study, in which allergy was induced in mice, we tested the effects of prebiotic exposure during perinatal and postweaning periods on the expression of biomarkers related to tolerance (IgG2a, IgA, IFN-γ, TGF-β, and IL-10). Our results showed that IgA, IgG2a, IL-10, TGF-β, and IFN-γ were highly expressed after allergen sensitization in prebiotic-fed mice. Interestingly, these molecules are related to tolerance and would play a crucial role in the prevention of allergy. Therefore, under nonallergic conditions,[31] and now under allergic conditions, we have demonstrated that prebiotic consumption during the entire perinatal and postweaning periods allows the induction of the expression of various tolerance-related biomarkers. The action mechanism responsible for this GOS/inulin effect on mouse immune responses is still not clearly understood. Until now, it was generally thought that prebiotic action was mainly indirectly mediated by microbiota in different ways: SCFA production by fermentation of oligosaccharides and/or pattern recognition receptor signaling. Thus, to evaluate prebiotic action on the microbiota, we quantified SCFA in the cecum of mice and the jejunal expression of a biomarker related to TLR pathways, MyD88. Our results showed that SCFA production was modified by prebiotic feeding. Under these conditions, the propionate concentration in the cecum was higher, regardless of the sensitization status of the mice. Propionate jointly with acetate and butyrate can have immunomodulatory effects; it has notably been described to stimulate the in vitro secretion of IL-10 by lymph node lymphocytes.[37] Thus, on the basis of our study, we can suggest that the increase in IL-10 secretion in prebiotic-sensitized mice may be linked to propionate. We also noted that, after sensitization, +Prb mice maintained a normal level of MyD88 expression, which should, however, have been reduced by the sensitization, as observed for −Prb-sensitized mice. Some studies have highlighted the potential of allergy development to down-regulate PRR receptors and signalization. In the case of a mouse model of respiratory allergy to ovalbumin, it has been evidenced that TLR2 receptor was down-regulated in both lung cells and lung dendritic cells.[38] This effect was not necessarily correlated to the allergen; it may be induced by Th2 response itself. Indeed, other authors have demonstrated that Th2 cytokines such as IL-4 and IL-13 can down-regulate other receptors such as TLR3 and TLR4 in intestinal epithelial cells.[39] This mechanism might explain the down-regulation of MyD88 observed in sensitized mice fed the control diet. This prebiotic effect was also detected for IgG2a, the secretion of which was maintained after sensitization in +Prb mice. This result is interesting because MyD88 is an adaptor molecule for TLR pathways that initiates downstream signaling events and leads to the secretion of various inflammatory cytokines including IFN-γ.[20] We can thus hypothesize that, in our study, the increase in IFN-γ secretion in prebiotic-sensitized mice may have been related to the induction of MyD88-dependent pathways and may have led to higher IgG2a secretion after sensitization. Recent data also suggest a direct interaction between prebiotic oligosaccharides and immune cells via carbohydrate-binding proteins such as C-type lectins or galectins,[40] especially galectin-9.[41,42] Thus, we suggest that the pro-tolerogenic effect of GOS/inulin feeding

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might also be mediated by galectin-9. In conclusion, prebiotics may have induced tolerance-related biomarkers either directly by binding some galectins or indirectly through microbiota metabolism and its ability to bind some receptors of the gut barrier.

New data seem to suggest that the perinatal period constitutes a crucial window of opportunity during which both the immune system and the microbiota develop.[43] Therefore, perinatal life appears to be an interesting target for the implementation of preventive strategies for numerous pathologies associated with the immune and microbiota systems. As an example, perinatal strategies with probiotics to prevent allergic diseases have shown very encouraging results in both human and animal models.[44] However, until now, the effects of perinatal prebiotic consumption on allergic disease prevention have never been explored. On the contrary, the effects of postweaning prebiotic consumption on allergy were examined in numerous studies that demonstrated that prebiotics can reduce some immunological and clinical biomarkers of allergic diseases.[28,29] These promising results motivated our choice to feed mice with prebiotics during both the perinatal and postweaning periods to maximize the effects of prebiotics on the immune responses of mice. We thus examined whether prebiotic exposure during the perinatal and postweaning periods can have effects on the expression of immune biomarkers related to allergy (Th2 and Th17) and on the clinical manifestations of allergic reactions after allergen challenge in a mouse model of gluten allergy. In our conditions, prebiotics were not able to modulate the expression of either Th2- or Th17-related biomarkers (high and similar levels of IgE, IgG1, IL-4, IL-17A, and CD23 observed in both +Prb and −Prb groups). In addition, we also noted that feeding a GOS/inulin mix had no impact on the triggering phase of allergy (same symptom severity and same rate of morphological jejunal alteration). This was confirmed in particular by the plasma histamine levels from all sensitized mice, which were equivalent. These results were highly surprising because we observed that prebiotics were able to induce high levels of tolerance-related biomarkers, which were not ultimately capable of modulating allergy mechanisms. In fact, we noted that in our mouse model of gluten allergy, Th2- and Th17-related biomarkers were strongly induced in sensitized mice regardless of diet. We can explain these observations by the harsh experimental conditions for both the sensitization (4 ip injections of allergen with Alum) and challenge phases (also ip route). Intraperitoneal sensitization is in fact known to strongly activate a Th2 response.[45] In addition, in our mouse model of allergy, we left too short of a delay between the end of sensitization and challenge. Perhaps this short time period limited the down-regulation of the Th2 and Th17 responses by both Th1 and Treg biomarkers. Thus, it can be proposed that under less harsh conditions, the Th2 and Th17 responses would be stimulated to a lesser degree, promoting prebiotic effects on the Th1 and Treg pathways that might alleviate symptoms in mice. Indeed, in the context of an oral immunotherapy model, Adel-Patient et al. demonstrated that oral β-lactoglobulin administration can decrease Th2-related biomarkers after only a single ip sensitization to allergen with Alum.[46] In the context of prebiotic effects on allergy prevention, Vos et al. have observed that under less harsh conditions (two ip sensitizations to allergen with alum followed by an intranasal challenge), respiratory symptoms were decreased in the prebiotic group.[29] A potential strategy of interest may be to combine a few sensitizations by ip route followed by oral exposures to allergens. This more physiologic strategy may then allow study of the prebiotic effect on local gut immune responses notably in lamina propria, Peyer patches, and mesenteric lymph nodes. Thus, in our experimental conditions, the sensitization procedure may have been too drastic for prebiotics to unbalance such a large allergic response, even if exposure to these dietary supplements during both the perinatal and postweaning periods allowed the production of high levels of tolerance-related biomarkers.

In conclusion, the strategy of perinatal and postweaning exposure to prebiotics is very encouraging because it allows the induction of several tolerance-related biomarkers after allergen sensitization. Data obtained during the current study did not permit us to prove the efficacy of this strategy for clinical aspects of allergy. Consequently, these results need to be confirmed in a less severe model of sensitization to demonstrate whether immune regulation can lead to an antiallergic effect.

AUTHOR INFORMATION

Corresponding Author

*(M.B.) Phone: +33 2 40 67 50 35. Fax: +33 2 40 67 50 25. E-mail: marie.bodinier@nantes.inra.fr.

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Notes

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ABBREVIATIONS USED

Alum, aluminum hydroxide; F-ELISA, fluorometric enzyme-linked immunosorbent assay; FOS, fructooligosaccharides; GOS, galactooligosaccharides; IFN, interferon; MyD88, myeloid differentiation primary response gene 88; PBS, phosphate-buffered saline; Prb, prebiotic; PRR, pattern recognition receptor; RPMI, Roswell Park Memorial Institute medium; SCFA, short-chain fatty acid; TBS, Tris-buffered saline; TGF, transforming growth factor; TLR, Toll-like receptor; Treg, regulatory T cells

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