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***Basic Study***

***Bifidobacterium infantis* CGMCC313-2’s protective effect on ovalbumin-induced airway asthma and ß-lactoglobulin-induced intestinal food allergy mouse models**

Mengyun L *et al.* Effect of *B. infantis* on allergy diseases

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**Abstract**

***AIM***

To investigated whether oral administration of *Bifidobacterium infantis* CGMCC313-2 (*B. infantis* CGMCC313-2) can inhibit allergen-induced airway inflammation and food allergies in a mouse model.

***METHODS***

Ovalbumin (OVA)-induced allergic asthma and ß-lactoglobulin-induced food allergy mouse models were used in this study. Following oral administration of *B. infantis* CGMCC313-2 during or after allergen sensitization, histopathologic changes in the lung and intestine were detected by hematoxylin and eosin (H&E) staining. For the allergic asthma mouse model, we evaluated the proportion of lung-infiltrating inflammatory cells. OVA-specific IgE and IgG1 levels in the serum and cytokine levels in bronchoalveolar lavage fluids (BALF) were also assessed. For the food allergy mouse model, the levels of total IgE and cytokinesin serum were measured.

***RESULTS***

Oral administration of *B. infantis* CGMCC313-2 during or after allergens sensitization suppressed allergic inflammation in lung and intestine tissues, while the proportion of infiltrating inflammatory cells was significantly decreased in the BALF of allergic asthma mice. Moreover, *B. infantis* CGMCC313-2 decreased the serum levels of total IgE in food allergy mice, while the reduction of IgE and IgG1 was also observed in OVA-induced allergic asthma mice. The expressions of interleukin-4 (IL-4) and IL-13 in both serum and BALF were suppressed after the administration of *B. infantis* CGMCC313-2, while its effect on IL-10 levels in serum was not observed.

***CONCLUSION***

*B. infantis* CGMCC313-2 can inhibit the secretion of allergen-induced IgE, IL-4 and IL-13, and attenuate allergic inflammation.

**Key words:** *Bifidobacterium infantis*; Asthma; Allergy; Ovalbumin; ß-lactoglobulin

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**Core tip:** *Bifidobacterium infantis* CGMCC313-2 could decrease the serum concentration of IgE and IgG1 significantly in asthma and food allergy mice. The number of infiltrating cells in BALF was reduced, and the symptom of eosinophil infiltration on lungs was relieved in asthma mice with *B. infantis* CGMCC313-2. The weight of food allergy mice was regained, and the inflammation of intestine was relieved in food allergy mice with *B. infantis* CGMCC313-2. With the administration of *B. infantis* CGMCC313-2, the concentration of IL-4 and IL-13 were reduced in both asthma and food allergy mice.

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**INTRODUCTION**

The prevalence of asthma, food allergy, eczema, and allergic rhinitis in developed countries has been increased over the last three decades. Specifically in China, childhood allergic diseases are generally lower than that in Western countries; however, the prevalence of asthma, allergic rhinitis, and eczema in children has increased markedly during the past two decades[1-4]. A number of environmental factors including air pollution, cigarette smoking, and allergen exposure have been proposed to explain the changes in the prevalence of allergic diseases; however, no major risk factors have been identified. A common explanation for the increased incidence rates of childhood allergy and asthma observed in industrialized countries during the past few decades is the ‘hygiene hypothesis,’ which state that a lack of early childhood exposure to infectious agents, [symbiotic](http://en.wikipedia.org/wiki/Symbiotic) microorganisms, and parasites increases susceptibility to allergic diseases by suppressing the natural development of the [immune system](http://en.wikipedia.org/wiki/Immune_system)[5,6]. Recent epidemiological and experimental studies have both renewed the “hygiene hypothesis” and extended it to a more specific theorem, the “microflora hypothesis”[6-8].

Probiotics are live microorganisms that confer a health benefit to the host when administered in adequate amounts[9]. In other words, ingested probiotics can modify microbial flora, which benefit the host[10,11]. Previous studies have shown that probiotics can reduce allergic diseases by modifying the immune system of the host. Some probiotic genera including *Lactobacilli* and *Bifidobacteria* are intensively investigated as novel alternative options for the management of allergic diseases including asthma and food allergy[12,13].

Experimental studies have shown that probiotics have strain-specific effects. In this study, the mice which incubated with nebulized ovalbumin were used as asthma model, while the mice which feed with ß-lactoglobulin were used as food allergy model (details in Materials and Methods). And the effect of *Bifidobacterium infantis* CGMCC313-2, which is extensively used as a probiotic drug in China, was investigated in these two mouse models during (prevention) or after allergen sensitization (pre-treatment).

**MATERIALS AND METHODS**

***Mice***

Male BALB/c mice aged 6–8 weeks were obtained from the Laboratory Animal Center of the Fourth Military Medical University. All experimental procedures involving animals were approved by the Ethics Committee for Animal Studies of the Fourth Military Medical University and performed in accordance with their guidelines (approval ID: 20150902).

***Probiotic bacterial preparations***

*Bifidobacterium infantis* CGMCC313-02 powder (Kexing Biotech Company Limited, Shenzhen, China) was stored at -20 °C. Solutions were prepared using normal saline only or normal saline plus *B. infantis* CGMCC313-2. *B. infantis* CGMCC313-2 preparations were adjusted at concentrations of 5 × 1010 colony-forming units (CFU)/mL.

***Mouse model of OVA-induced allergic asthma***

The mice were divided into four experimental groups, and each group consisted of 10 mice. Four groups of mice were treated as follows: (Group 1) the normal control group received normal saline plus 1.5 mg alum intraperitoneally. The mice were placed in an atomizing chamber (20 cm × 20 cm × 35 cm), and 8 mL saline was nebulized for inhalation. The mice were incubated 30 min each time for 7 continuous days; (Group 2) the positive group (as shown in Figure 1A) received 100 µg ovalbumin (OVA) (Sigma, Buchs, Switzerland) plus 1.5 mg alum intraperitoneally from Day 0 to Day 7, and subsequently challenged with 1% OVA inhaled by nebulizer from Day 21 to Day 28; and (Group 3) the prevention and (Group 4) pre-treatment groups received 100 µg OVA plus alum intraperitoneally and 1% OVA inhaled, and were fed 0.2 mL/d (5 × 1010 CFU/mL) of *B. infantis* CGMCC313-2 from Day 0 to Day 14 (prevention group, as shown in Figure 1B), or from Day 15 to Day 28 (pre-treatment group, as shown in Figure 1C). Serum and BALF samples were collected from mice at sacrifice on Day 29.

***Mouse model of ß-lactoglobulin-induced food allergy***

The mice were divided into four experimental groups, and each group consisted of 10 mice. Four groups of mice were treated as follows: (Group 1) the normal control group were fed normal saline (2 mL each time for 7 continuous days); (Group 2) the positive group (as shown in Figure 2A) received the mixture of 20 mg ß-lactoglobulin(BLG) (Sigma, Buchs, Switzerland) and 10 µg CTX (Cholera toxin, List Biological Laboratories, Campbell, Calif) at days 0, 7, 14 by intragastric gavage (2 mL of the mixture was used each time). Subsequently the mice were challenged with 100 mg BLG (3 mL) on day 21 by intragastric gavage; and (Group 3) the prevention and (Group 4) pre-treatment groups received 20 mg BLG plus 10 µg CTX and challenged by intragastric gavage with 100mg BLG, and were fed 0.2 mL/d (5 × 1010 CFU/ml) of *B. infantis* CGMCC313-2 from Day 0 to Day 21 (prevention group, as shown in Figure 2B), or from Day 22 to Day 28 (pre-treatment group, as shown in Figure 2C). Mice body weight was measured on Day 29, and then the serum samples were collected after the mice sacrificed.

***Measurement of immunoglobulins in serum***

Serum samples from mouse model of OVA-induced allergic asthma were assayed for the levels of OVA-specific IgE and IgG1 using ELISA kits (Chondrex Inc. United States) following the manufacturer’s protocol. The level of total IgE was assayed in the serum of BLG-induced food allergy mice using ELISA kits (Chondrex, Inc. United States).

***Measurement of cytokines***

IL-4, IL-10, IL-13, and IFN-γ levels in serum (from mouse model of BLG-induced food allergy) or in BALF (from mouse model of OVA-induced allergic asthma) were assayed using the ELISA Kits (R&D Systems, Boston, MA, United States) according to the manufacturer’s protocol.

***Cell counts of BALF***

For mouse model of OVA-induced allergic asthma, BALF was isolated in 1ml of phosphate buffered saline (PBS). The BALF cellularity was determined using a hemocytometer. A 10ul aliquot of centrifuged cells (4000 rpm, 5 min) was transferred onto slides, and all leukocytes were fixed for staining using Giemsa. The observer counted 200-300 cells per slide, and the standard morphological criteria was adopted to identify the individual leukocyte populations. The number of leukocytes was counted 2 times, and the average value was maintained.

***Histological analysis***

To assess the pathological changes, samples from either lungs (OVA-induced allergic asthma) or intestine (BLG-induced food allergy) were collected. The samples were fixed in neutrally buffered 10% formaldehyde and embedded in paraffin. -4 µm sections were stained with H&E to detect inflammatory cell infiltration in intestinal tissue (BLG-induced food allergy), or assess the extent of inflammation in lungs (OVA-induced asthma) at 200 × magnification.

***Statistical analysis***

All data points represent the mean ± SEM in each mouse group. Analysis was performed using the software SPSS 19.0 for Windows. Variance analysis of single factor and multi factor (ANOVA) was conducted to determine the statistical significance. *P* value lower than 0.05 was considered statistically significant.

**RESULTS**

***B. infantis decreased the levels of IgE and IgG1 in OVA-induced asthma and BLG-induced food allergy mouse models***

We detected whether oral *B. infantis* CGMCC313-2 would affect serum levels of allergen-induced serum specific IgE and IgG1, and ELISA was used for data analysis in OVA-induced allergic asthma mouse model. The serum levels of OVA-specific IgE and IgG1 were significantly elevated after OVA sensitization/challenge (Group 2) compared with the normal control group (Group 1). In groups which received *B. infantis* CGMCC313-2 for prevention (Group 3) and pre-treatment (Group 4) during the OVA sensitization/challenge, the serum levels of IgE and IgG1 were significantly decreased (*P* < 0.05; Figure 3A and B). Moreover, the levels of serum IgE in the prevention group was also significantly decreased compared with pre-treatment group (*P* < 0.05; Figure3A).

Due to the unavailability of reagents for BLG-specific IgE and IgG1 detection, the serum levels of total IgE were evaluated instead in BLG-induced food allergy mouse model. The serum levels of total IgE were significantly increased after the BLG sensitization/challenge (Group 2) compared with the normal control group (Group 1). In the groups challenged with *B. infantis* CGMCC313-2 for prevention (Group 3) and pre-treatment (Group 4), the levels of total IgE were significantly decreased. Moreover, the total IgE serum levels in pre-treatment group were also significantly decreased compared with prevention group (*P* < 0.05; Figure 3C).

***B. infantis administration increases body weight of BLG-induced food allergy mice***

Compared with normal control, mice in the BLG-sensitized/challenge group showed weight loss. However, the prevention and pre-treatment groups with *B. infantis* CGMCC313-2 showed weight gains (Figure 4), while the pre-treatment group gained more weight than the prevention group.

***B. infantis alters the proportions of lung-infiltrating cells in OVA-induced allergic asthma mice***

In order to evaluate the degree of inflammatory cell infiltration in the lungs of OVA-induced allergic asthma mice, leukocytes counts were conducted in BALF tissue. The cell number was significantly increased in the OVA- sensitized/challenged mice compared to the control group. However, the proportion of infiltrating cells in the lung was significantly decreased in the groups treated with *B. infantis* CGMCC313-2 (Figure5). Differential cell counts which used Giemsa staining failed to identify cell types in our study.

***Impact of B. infantis on allergic inflammation in OVA-induced asthma and BLG-induced food allergy mouse models***

The effect of *B. infantis* CGMCC313-2 on the OVA or BLG-sensitized/challenged mice was evaluated from the perspective of overall lung or intestine inflammation, using histological hematoxylin and eosin staining (Figures 6 and 7, respectively). Compared with normal control mice (Figures 6A and 7A), allergen sensitized/challenged mice (Figures 6B and 7B) had severe inflammation; while the prevention (Figures 6C and 7C) and pre-treatment (Figures 6D and 7D) mice with *B. infantis* CGMCC313-2 treatment exhibited significantly diminished inflammation signs.

***Effect of B. infantis on cytokines in serum and BALF***

To further elucidate possible mechanisms that were responsible for the effects of *B. infantis* CGMCC313-2 on systemic sensitization and allergic inflammation, ELISA was used to determine the expression of IL-4, IL-10, IL-13, and IFN-γ in serum and BALF was collected from BLG-induced food allergy mice and OVA-induced allergic asthma mice respectively. IL-4 and IL-13 in serum (Figure8A and B) or BALF (Figure8D and E) increased significantly in the positive control (PC; Group 2) group compared with normal control (NC; Group 1) group. In the groups that received *B. infantis* CGMCC313-2, the levels of serum IL-4 and IL-13 in the prevention group were significantly decreased. Additionally, the reduction of IL-4 and IL-13 in prevention and treatment groups was also observed in OVA-induced allergic asthma mice. IL-10 in serum (Figure 8C) decreased significantly in the positive control (PC; Group 2) group, prevention (Pre; Group 3) group, and treatment (Tre; Group 4) group compared to normal control (NC; Group 1) group. IL-10 was not detected in BALF from OVA-induced allergic asthma mice, and IFN-γ was not detected in either serum or BALF from any mice.

**DISCUSSION**

There is increasing evidence that intestinal microbiota and ingested probiotics may induce important metabolic and physiological reactions in the host, and drive the maturation of immune system in early life. Among the diverse probiotics, *Lactobacilli* and *Bifidobacteria*, which are part of gut flora in infants, are the most promising candidates that naturally affect immune system development[14,15]. For the same reason, *Lactobacilli* and *Bifidobacteria* are the most frequently used probiotics for clinical intervention studies[16–20]. However, the most important characteristic of probiotics is their strain-specificity effects[21]. In this study, we investigated the role of *Bifidobacterium infantis* CGMCC313-2 in allergic disease prevention and treatment in two mouse models, for the reason that *B. infantis* CGMCC313-2 has been extensively applied for the treatment and prevention of diarrhea including antibiotic-associated diarrhea (AAD) in China. In OVA-sensitized/challenged mice, severe lung inflammation and infiltrating cells in the lungs were observed, and the application of *B. infantis* CGMCC313-2 could significantly diminish inflammation. Similarly, in ß-lactoglobulin-induced food allergy mice, the engagement of *B. infantis* CGMCC313-2 could decrease intestine inflammation, and ameliorate weight loss in BLG-sensitized/challenged mice. These results demonstrate that oral administration of *B. infantis* CGMCC313-2 during or after allergen sensitization may relieve allergic inflammation in airway and intestine.

In the allergen sensitized/challenged mice, IL-4, IL-13, total IgE, and allergen-induced serum specific IgE and IgG1 levels were highly expressed. Based on immunological basis of allergy, the overexpression of IL-4 and IL-13, which is modulated by type 2 T helper cells, could promote IgE production and eosinophil infiltration in target organs. In the present study, following the oral administration of *B. infantis* CGMCC313-2, the levels of IL-13 and total IgE were significantly decreased, which was accompanied by relief of inflammation symptoms. We deduced that the metabolites of *B. infantis* CGMCC313-2, including butyrate and short-chain fatty acids (SCFA), could suppress the inflammatory responses triggered by Th2 cytokines[22-28]. But the level of IL-4 was higher in treatment group, which opposed to the results of IL-13 and IgE. Because of the complexity of immune system and response, the role of IL-4 as allergic disorder marker needed to be detected in our future study. Additionally, the oral administration of probiotics helped in prevention and treatment of airway and intestine allergy.

On the other hand, there was a decrease in serum levels of IL-10 in mice that were sensitized/ challenged with BLG. There is strong evidence indicating that the production of IL-10, which was affected by antigens exposure, was associated with T cell tolerance and Treg secretion, which in turn plays important roles in controlling allergic diseases. However, the administration of *B. infantis* CGMCC313-2 did not promote the secretion of IL-10. This phenomenon was inconsistent with previous preclinical studies in which probiotic strains could promote Treg responses[29,30]. We deduced that the different probiotic strains adopted in different studies may have strain-specific effects, or the immunomodulatory effect of *B. infantis* CGMCC313-2 exhibited a suppression of Th2 responses. In our study, the levels of IFN-γ in both serum and BALF were too low to be detected in all mice, and this may be due to the poor sensitivity of determination technique. Admittedly, this is a limitation of our study.

In the present study, which utilized asthma and food allergy mouse models, we found that *B. infantis* CGMCC313-2 could inhibit the secretion of allergen-induced IgE and Th2 cytokines, and further attenuate the allergic inflammation. Our study also suggested that the modulatory activity of *B. infantis* CGMCC313-2 was not only confined to intestinal allergic diseases, but also to allergic airway disease. Therefore, *B. infantis* CGMCC313-2 may be regarded as a candidate probiotic strain in the prevention and treatment of allergic diseases. However, further clinical and experimental studies are required to delineate the potential preventive and treatment effects of *B. infantis* CGMCC313-2.

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**COMMENTS**

***Background***

Probiotics have exhibited beneficial effects on allergies diseases based on “microflora hypothesis”, and experimental studies have also shown that probiotics have strain-specific effects. In this study, they detected the specific effects of *B. infantis* CGMCC313-2, which widely used as a probiotic drug in China, on asthma and food allergy with mouse models.

***Research frontiers***

Previous studies indicated that ingested *Lactobacilli* and *Bifidobacteria* could modify microbial flora, and it would reduce the symptoms of allergic diseases by modifying the immune system of the host. Meanwhile some probiotic drugs are intensively investigated as novel alternative options for the management of allergic diseases including asthma and food allergy.

***Innovations and breakthroughs***

In the study, *B. infantis* CGMCC313-2 was feed to asthma and food allergy mouse models. After probiotic treatment, they found that *B. infantis* CGMCC313-2 could decrease the serum concentration of IgE and IgG1 significantly, and the concentration of IL-4 and IL-13 were reduced in asthma and food allergy mice too. Meanwhile the symptoms of cell infiltration or inflammation were relieved.

***Applications***

*B. infantis* CGMCC313-2 could inhibit the secretion of allergen-induced IgE and Th2 cytokines, and further attenuate the allergic inflammation. *B. infantis* CGMCC313-2 provided important reference for the prevention and treatment of intestinal and airway allergic diseases.

***Terminology***

IgE and IgG, which closely related with anaphylaxis, were higher in the patients with allergies diseases. IL-4 and IL-13, which secreted by Th2 cells, involve humoral immune response and indicate the degree of allergy diseases.

***Peer-review***

The authors performed clear experiments on asthma mouse model and food allergy mouse model to detect the effects of *B. infantis* on allergy diseases. They found that *B. infantis* could inhibit the secretion of allergens induced IgG, IgE, IL-4 and IL-13, and allergic inflammation were also attenuated. The study shed the light on the prevention and treatment of intestinal and airway allergic diseases. However, further clinical studies are still required.

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**P-Reviewer:** Classen CF, Islek A, Watanabe T **S-Editor:** Qi Y **L-Editor: E-Editor:**

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** China

**Peer-review report classification**

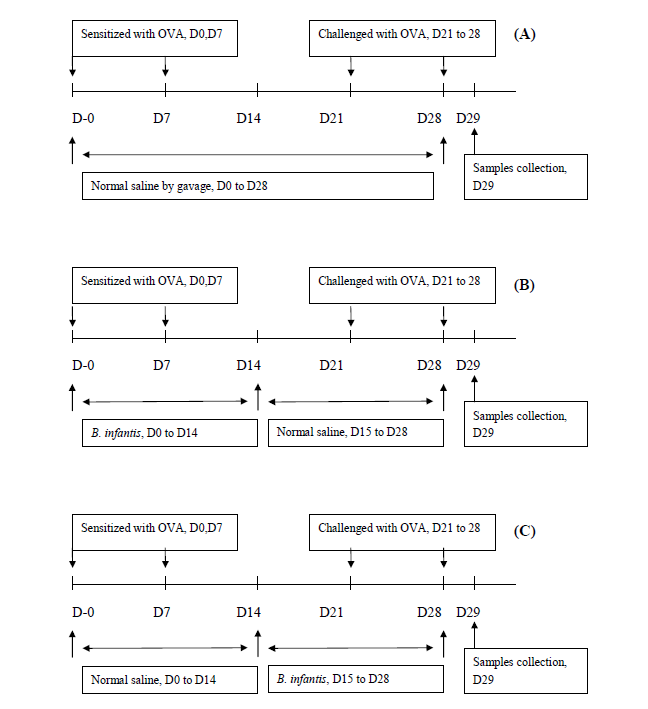
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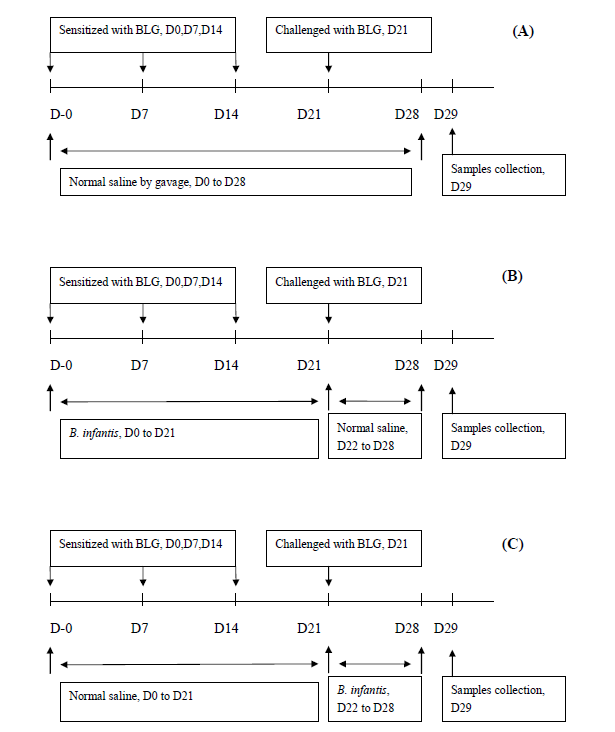
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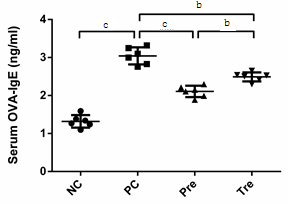
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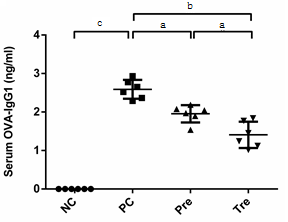
**Figure 1 Protocols used for the establishment of mouse models.** A: Allergic asthma; B: Prevention; C: Pre-treatment of OVA-induced airway allergy with *B. infantis* CGMCC313-2.

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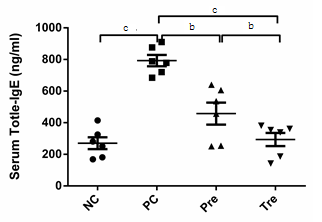
**Figure 2 Protocols used for the establishment of mouse models.** A: Food allergy; B: Prevention; C: Pre-treatment of ß-lactoglobulin-induced food allergies with *B. infantis* CGMCC313-2.

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**A**

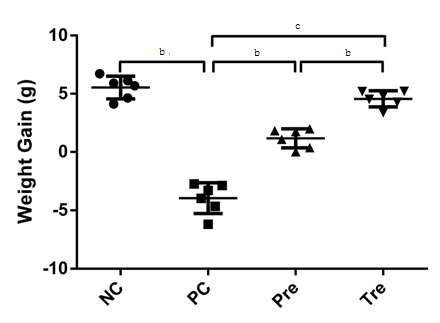
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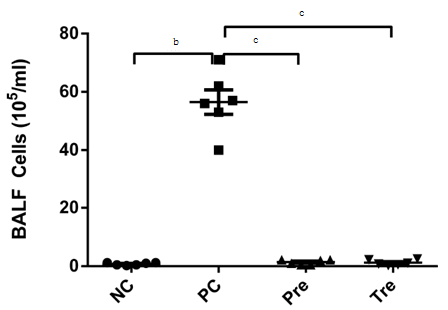
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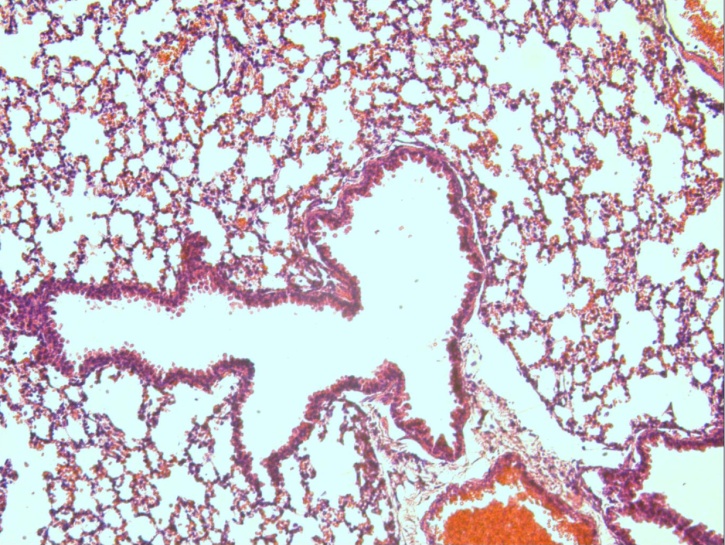
**Figure 3 Effect of *B. infantis* CGMCC313-2 on the reversal of IgE and IgG1 in OVA-induced asthma and BLG-induced food allergy mouse models.** A and B: There were significant increase of OVA-specific IgE and IgG1 expression in positive control (PC; Group 2) group compared with normal control (NC; Group 1) group in allergic asthma mouse model. While prevention (pre; Group 3) and pre-treatment (tre; Group 4) groups with *B. infantis*CGMCC313-2 administration had decreased expression. **C:** There was significant increase of total IgE expression in positive control (PC; Group 2) group compared with normal control (NC; Group 1) group in BLG-induced food allergy mouse model. While prevention (pre; Group 3) and pre-treatment (tre; Group 4) groups with *B. infantis* CGMCC313-2 administration had decreased expression. The statistical differences are represented as follows: a*P* < 0.05; b*P* < 0.01, and c*P* < 0.001.

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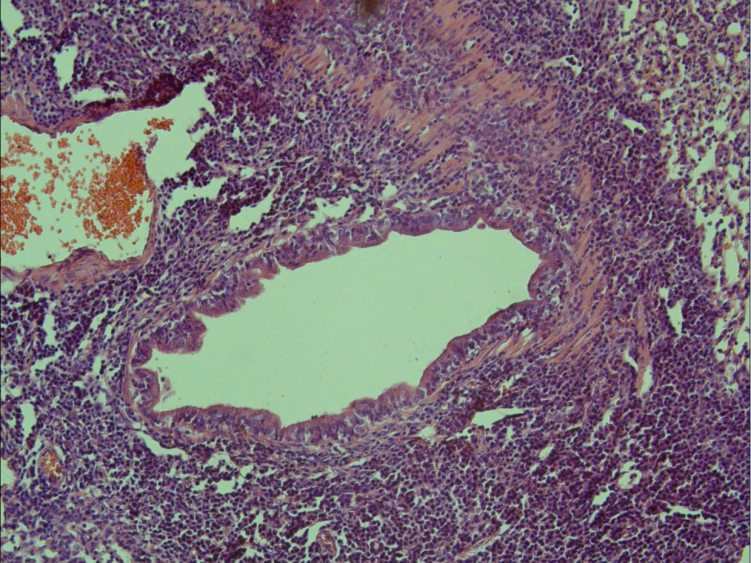
**Figure 4 Effect of *B. infantis* CGMCC313-2 on body weight in BLG-induced food allergy mice.** Average body weight decreased significantly in the positive control (PC; Group 2) group compared with normal control (NC; Group 1) group. Prevention (pre; Group 3) and pre-treatment (tre; Group 4) groups with *B. infantis* CGMCC313-2 administration had increased. The statistical differences are represented as follows: a*P* < 0.05; b*P* < 0.01, and c*P* < 0.001.

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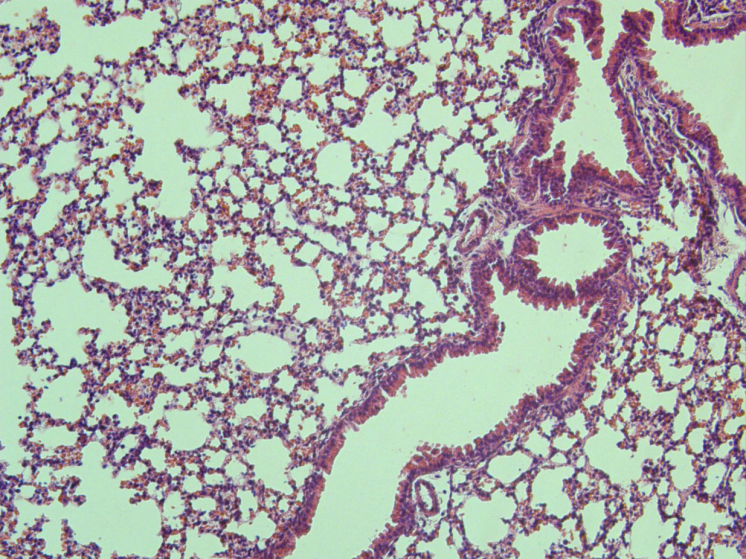
**Figure 5** **Effects of *B. infantis* CGMCC313-2 on infiltrating cells in the lungs of ovalbumin-induced allergic asthma mice.** Total cell number in BALF increased significantly in the positive control (PC; Group 2) group compared with normal control (NC; Group 1) group. Prevention (pre; Group 3), and pre-treatment (tre; Group 4) groups with *B. infantis* CGMCC313-2 administration had decreased. The statistical differences are represented as: a*P* < 0.05; b*P* < 0.01, and c*P* < 0.001.

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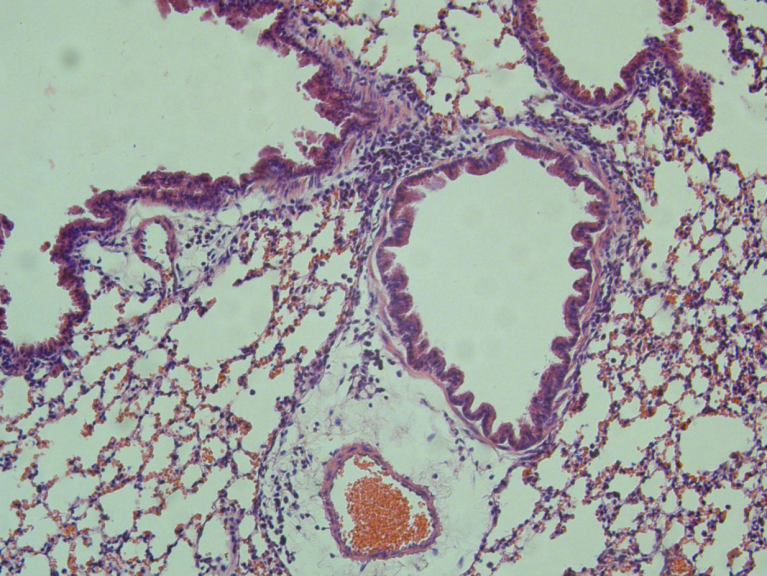
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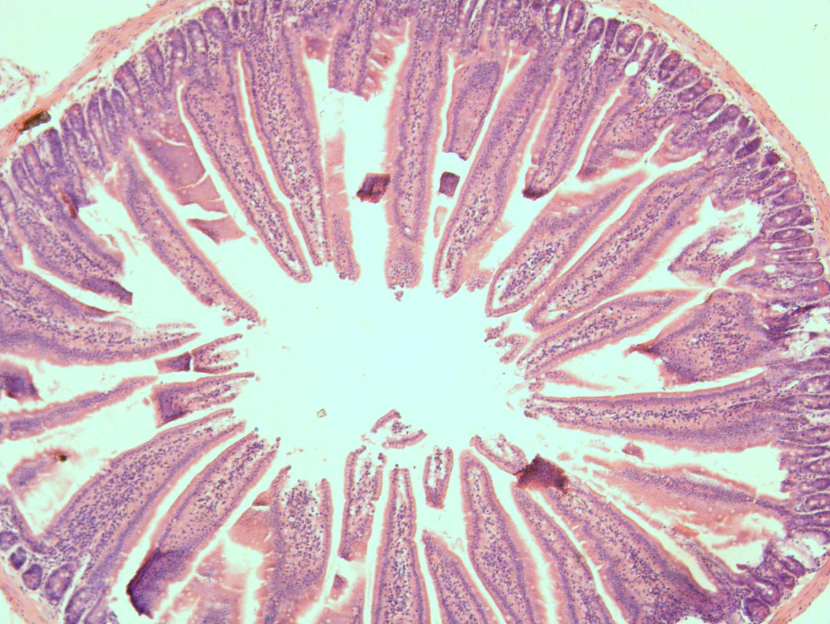
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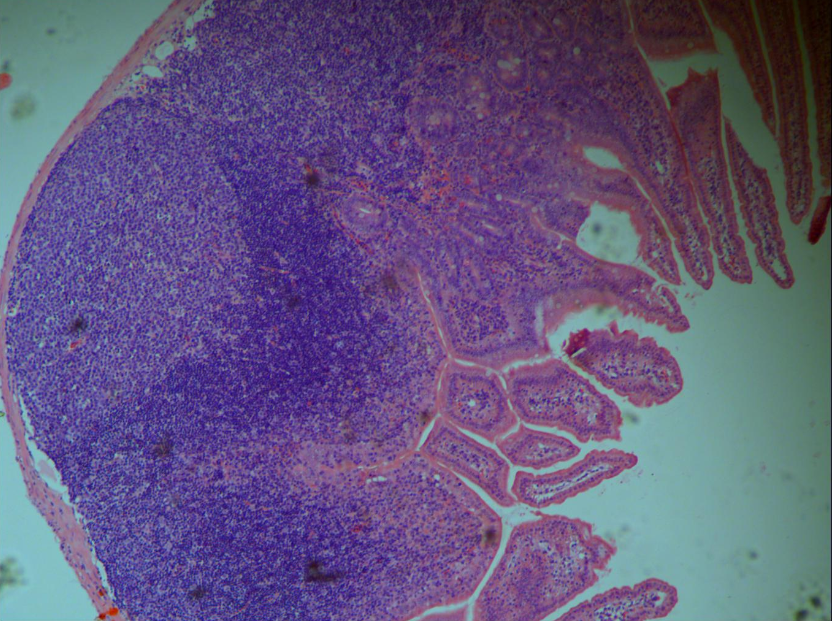
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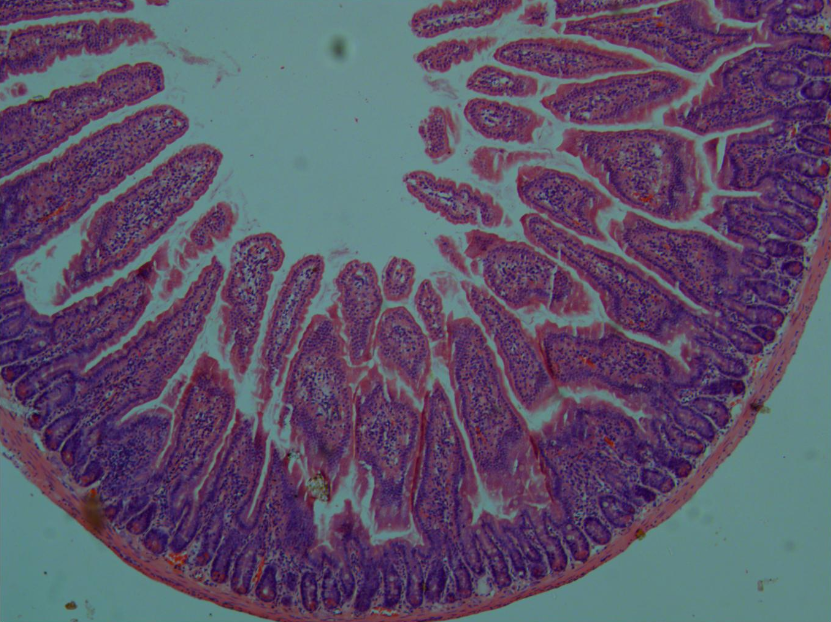
**Figure 6 Effects of *B. infantis* CGMCC313-2 on OVA-induced airway inflammation.** Besides (C) prevention group and (D) pre-treatment group which were treated with *B. infantis* CGMCC313-2, lung tissues were also obtained from (A) the normal control group and (B) the ovalbumin -sensitized/challenged group on Day 29. The tissues were stained and imaged under × 200 magnification. The positive control group shows severe airway inflammation, while the groups with *B. infantis* CGMCC313-2 administration shown relieved airway inflammation.

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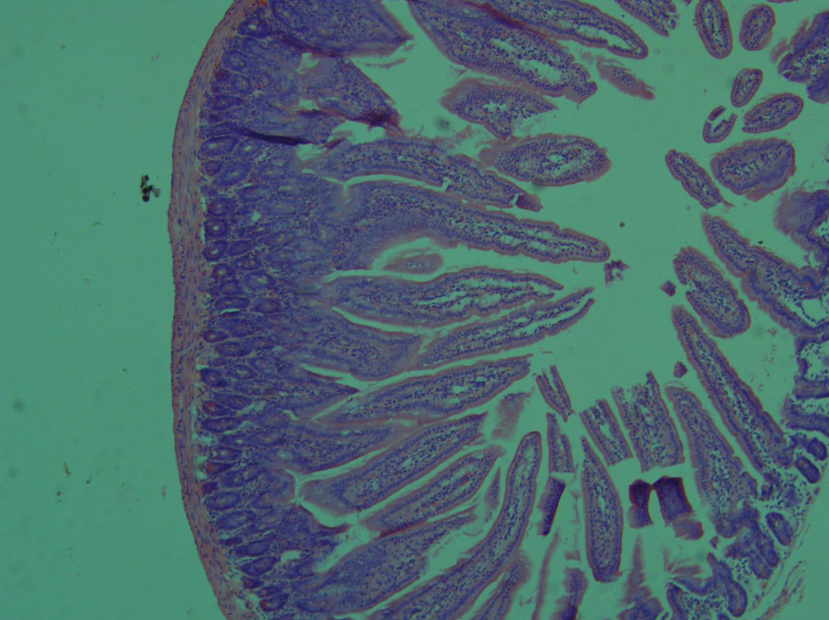
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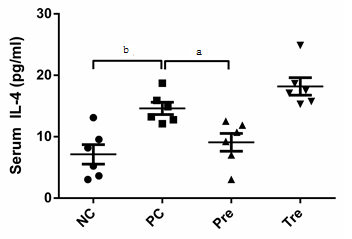
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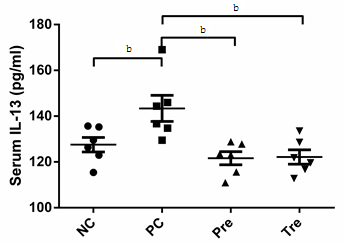
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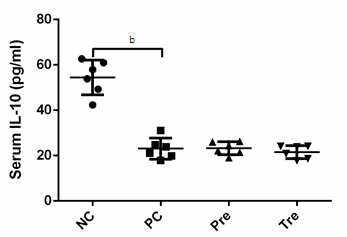
**Figure 7 Effects of *B. infantis* CGMCC313-2 on BLG-induced intestine inflammation.** Intestine tissues were obtained from (A) the normal control group and (B) the BLG-sensitized/challenged group on Day 29, along with (C) prevention group and (D) pre-treatment group which were treated with *B. infantis* CGMCC313-2. The tissues were stained and imaged under200x magnification. The positive control group shows severe intestinal inflammation, while the groups with *B. infantis* CGMCC313-2 administration exhibit relieved intestinal inflammation.

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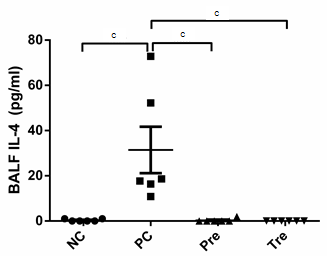
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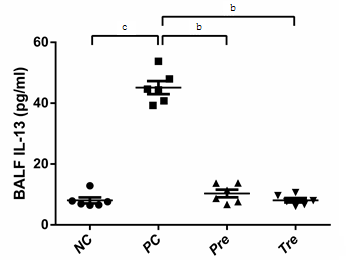
**B**

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**E**

**Figure 8 Effects of *B. infantis* CGMCC313-2 on cytokines in serum and BALF.** IL-4, IL-10, and IL-13 in serum and BALF were detected in BLG-induced food allergy mice and OVA-induced allergic asthma mice, respectively. A: Serum IL-4 in prevention (pre; Group 3) group; B: serum IL-13 in prevention (pre; Group 3) and pre-treatment (tre; Group 4) groups were significantly decreased compared with positive control (PC; Group 2) group; C: There was no significant difference among positive control group (PC; Group 2), prevention group (pre; Group 3), and pre-treatment (tre; Group 4) group on IL-10, and it decreased significantly when compared with normal control (NC; Group 1) group; D: The concentration of BALF IL-4 and (**E**) BALF IL-13 were significantly decreased in the prevention (pre; Group 3) group and pre-treatment (tre; Group 4) group which were treated with *B. infantis*CGMCC313-2. The statistical differences are represented as follows: a*P* < 0.05; b*P* < 0.01, and c*P* < 0.001.