

## Basic Study

**Protective effect of *Bifidobacterium infantis* CGMCC313-2 on ovalbumin-induced airway asthma and  $\beta$ -lactoglobulin-induced intestinal food allergy mouse models**

Meng-Yun Liu, Zhen-Yu Yang, Wen-Kui Dai, Jian-Qiong Huang, Yin-Hu Li, Juan Zhang, Chuang-Zhao Qiu, Chun Wei, Qian Zhou, Xin Sun, Xin Feng, Dong-Fang Li, He-Ping Wang, Yue-Jie Zheng

Meng-Yun Liu, Jian-Qiong Huang, He-Ping Wang, Yue-Jie Zheng, Department of Respiratory, Shenzhen Children's Hospital, Shenzhen 518026, China

Zhen-Yu Yang, Wen-Kui Dai, Yin-Hu Li, Chuang-Zhao Qiu, Qian Zhou, Xin Feng, Dong-Fang Li, WeHealthGene Co., Shenzhen 518129, China

Juan Zhang, Chun Wei, Xin Sun, Xijing Hospital, The Fourth Military Medical University, Xi'an 7100032, Shann'xi Province, China

**Author contributions:** Zheng YJ designed the study; Liu MY, Yang ZY and Li YH interpreted the data and wrote the manuscript; Qiu CZ, Zhou Q and Feng X conducted the bioinformatics analysis; Li DF, Huang JQ, Zhang J, Wang HP, Wei C and Sun X contributed to the study design and animal experiments; Liu MY, Yang ZY and Dai WK contributed to this work equally.

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**Correspondence to:** Dr. Yue-Jie Zheng, Director, Department of Respiratory, Shenzhen Children's Hospital, No. 7019 Yitian Road, Shenzhen 518026, China. [shine1990@sina.com](mailto:shine1990@sina.com)  
**Telephone:** +86-755-83936101  
**Fax:** +86-755-83009800

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**Abstract****AIM**

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

**METHODS**

Ovalbumin (OVA)-induced allergic asthma and  $\beta$ -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

evaluated by hematoxylin and eosin (HE) staining. In the allergic asthma mouse model, we evaluated the proportion of lung-infiltrating inflammatory cells. OVA-specific IgE and IgG1 levels in serum and cytokine levels in bronchoalveolar lavage fluid (BALF) were also assessed. In the food allergy mouse model, the levels of total IgE and cytokines in serum were measured.

## RESULTS

Oral administration of *B. infantis* CGMCC313-2 during or after allergen sensitization suppressed allergic inflammation in lung and intestinal 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, and reductions in IgE and IgG1 were also observed in OVA-induced allergic asthma mice. The expression of interleukin-4 (IL-4) and IL-13 in both serum and BALF was suppressed following the administration of *B. infantis* CGMCC313-2, while an effect on serum IL-10 levels was not observed.

## CONCLUSION

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

**Key words:** *Bifidobacterium infantis*; Asthma; Allergy; Ovalbumin;  $\beta$ -lactoglobulin

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**Core tip:** *Bifidobacterium infantis* CGMCC313-2 significantly decreased the serum concentration of IgE and IgG1 in asthma and food allergy mouse models. The number of infiltrating cells in bronchoalveolar lavage fluid was reduced, and eosinophil infiltration in lungs was relieved by *B. infantis* CGMCC313-2 in allergic asthma mice. Body weight was regained in food allergy mice, and intestinal inflammation was attenuated by *B. infantis* CGMCC313-2. Following administration of *B. infantis* CGMCC313-2, the concentrations of interleukin-4 (IL-4) and IL-13 decreased in both allergic asthma and food allergy mice.

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## INTRODUCTION

The prevalence of asthma, food allergies, eczema, and

allergic rhinitis in developed countries has increased over the last three decades. In China, childhood allergic diseases are generally lower than those in Western countries; however, the prevalence of asthma, allergic rhinitis, and eczema in children has increased markedly during the past two decades<sup>[1-4]</sup>. 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 states that a lack of early childhood exposure to infectious agents, symbiotic microorganisms, and parasites increase susceptibility to allergic diseases by suppressing the natural development of the immune system<sup>[5,6]</sup>. Recent epidemiological and experimental studies have both renewed the "hygiene hypothesis" and extended it to a more specific theorem, the "microflora hypothesis"<sup>[6-8]</sup>.

Probiotics are live microorganisms that confer a health benefit to the host when administered in adequate numbers<sup>[9]</sup>. In other words, ingested probiotics can modify microbial flora, which benefit the host<sup>[10,11]</sup>. 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<sup>[12,13]</sup>.

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

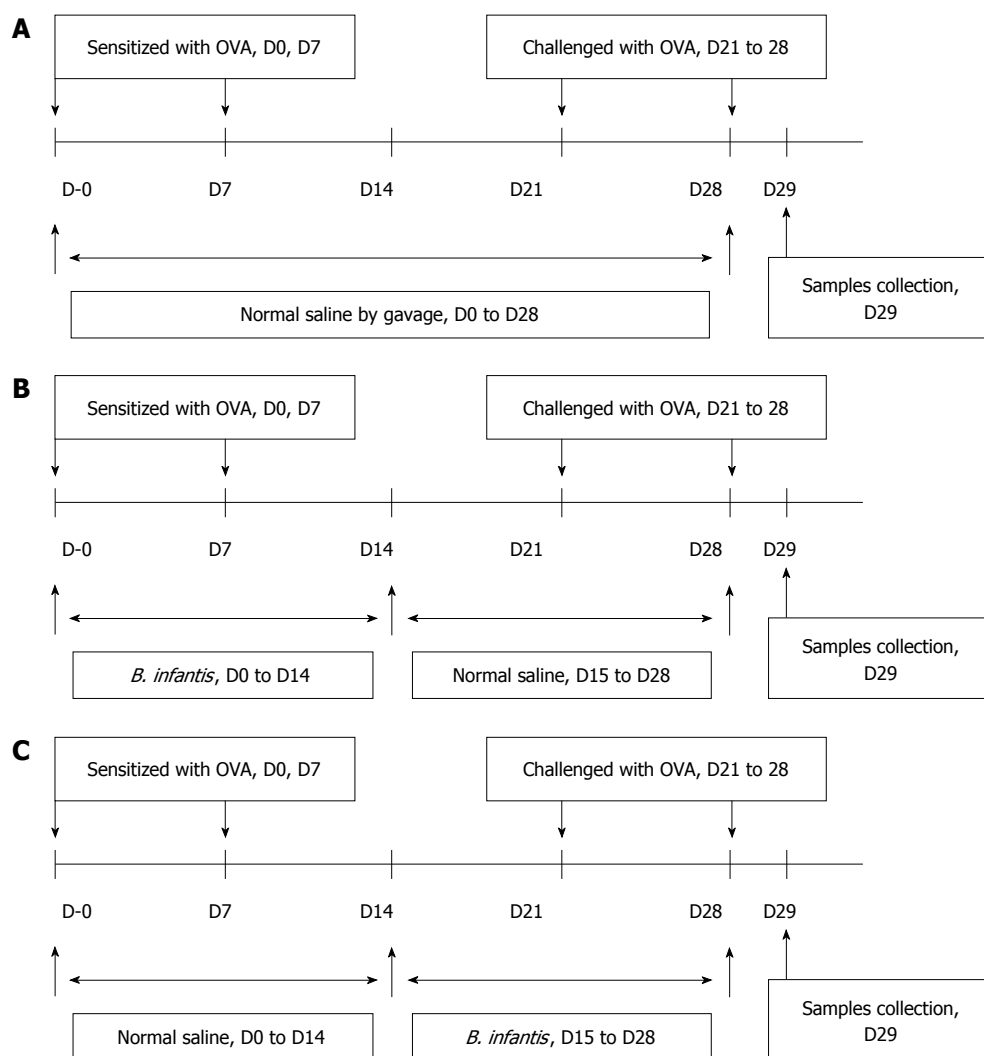
## MATERIALS AND METHODS

### Mice

Male BALB/c mice aged 6-8 wk 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*



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

CGMCC313-2. *B. infantis* CGMCC313-2 preparations were adjusted at concentrations of  $5 \times 10^{10}$  colony-forming units (CFU)/mL.

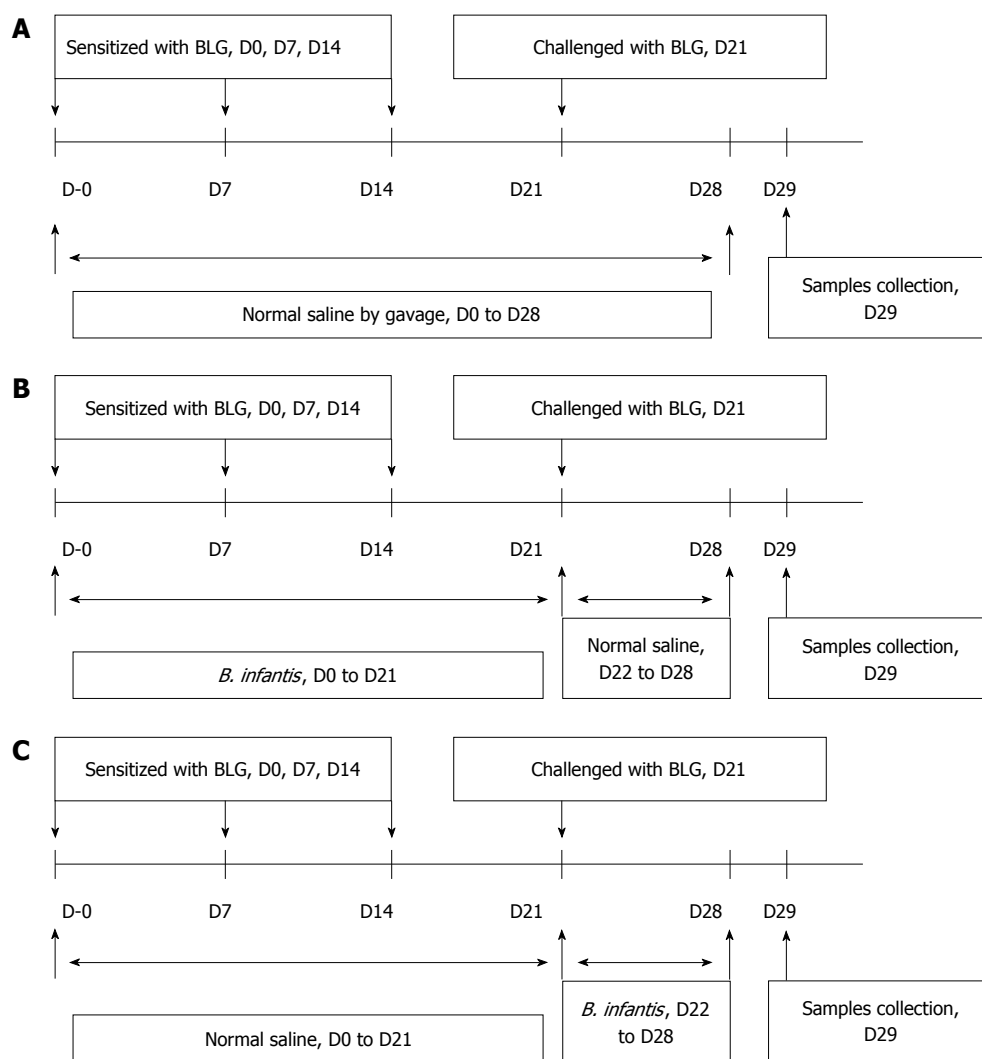
#### Mouse model of OVA-induced allergic asthma

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 administered by nebulization. The mice were incubated for 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 ( $5 \times 10^{10}$  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 β-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 was 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 β-lactoglobulin (BLG) (Sigma, Buchs, Switzerland) and 10 μg CTX (Cholera toxin, List Biological Laboratories, Campbell, CA, United States) on days 0, 7, and 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)



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

pre-treatment groups received 20 mg BLG plus 10  $\mu$ g CTX and challenged with 100 mg BLG by intragastric gavage, and were fed 0.2 mL/d ( $5 \times 10^{10}$  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). Body weight was measured on Day 29, and then serum samples were collected after the mice were sacrificed.

#### Measurement of serum immunoglobulins

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

#### Measurement of cytokines

IL-4, IL-10, IL-13, and IFN- $\gamma$  levels in serum (from the

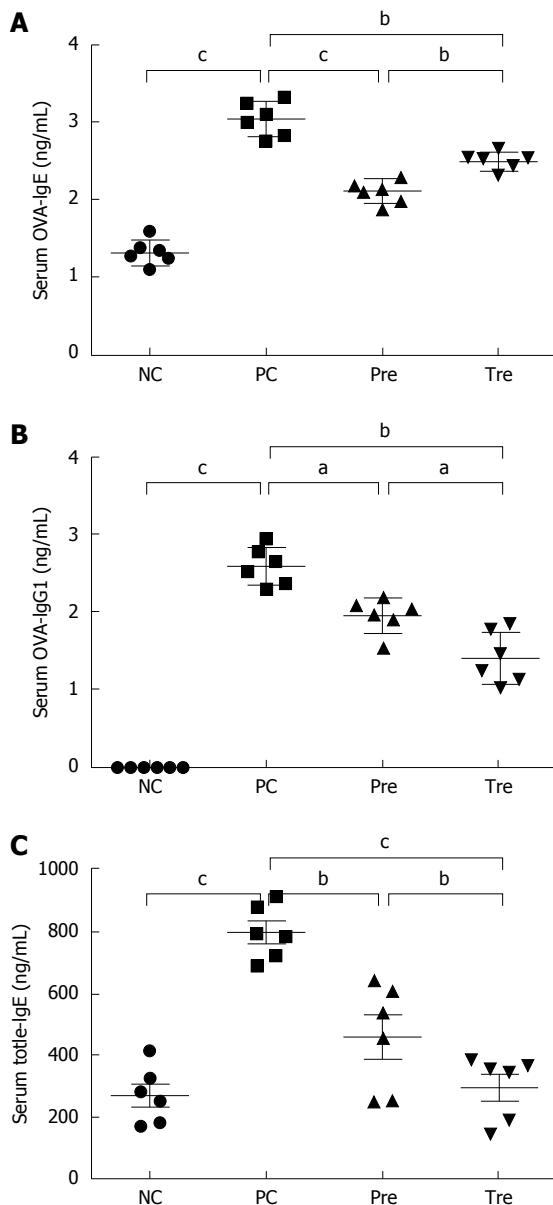
BLG-induced food allergy mouse model) or in BALF (from the OVA-induced allergic asthma mouse model) were assayed using ELISA Kits (R&D Systems, Boston, MA, United States) according to the manufacturer's protocol.

#### Cell counts of BALF

BALF was isolated in 1 mL of phosphate buffered saline (PBS) from the mouse model of OVA-induced allergic asthma. The BALF cellularity was determined using a hemocytometer. A 10  $\mu$ L 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 standard morphological criteria were adopted to identify the individual leukocyte populations. The number of leukocytes was counted twice, and the average value was calculated.

#### Histological analysis

To assess the pathological changes, samples from



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

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. Sections 4  $\mu$ m thick were stained with HE to detect inflammatory cell infiltration in intestinal tissue (BLG-induced food allergy), or to assess the extent of inflammation in the lungs (OVA-induced asthma) at 200  $\times$  magnification.

### Statistical analysis

All data points represent the mean  $\pm$  SEM in each mouse group. Analysis was performed using SPSS 19.0 software for Windows. Variance analysis of single factor and multi factor was conducted to determine the statistical significance. A  $P$  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 determined whether oral *B. infantis* CGMCC313-2 affected serum levels of allergen-induced specific IgE and IgG1, and ELISA was used for data analysis in the OVA-induced allergic asthma mouse model. The serum levels of OVA-specific IgE and IgG1 were significantly elevated in the 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 were also significantly decreased compared with the pre-treatment group ( $P < 0.05$ ; Figure 3A).

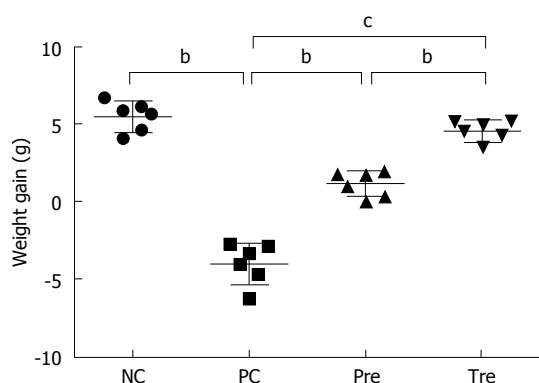
Due to the unavailability of reagents for BLG-specific IgE and IgG1 detection, the serum levels of total IgE were evaluated in the BLG-induced food allergy mouse model. The serum levels of total IgE were significantly increased after the BLG sensitization/challenge (Group 2) compared with the normal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

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

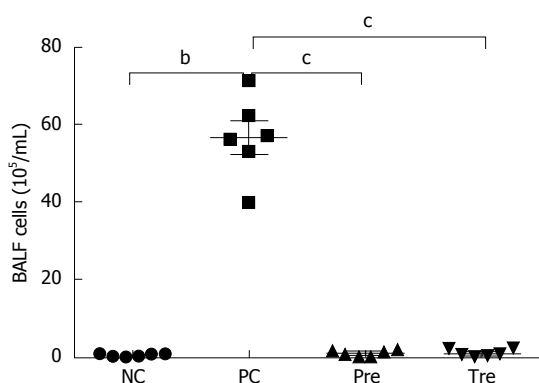
Compared with the normal control group, mice in the BLG-sensitization/challenge group showed weight loss. However, the prevention and pre-treatment groups showed weight gain following *B. infantis* CGMCC313-2 (Figure 4), and the pre-treatment group gained more weight than the prevention group.

### *B. infantis* alters the proportion 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, leukocyte counts were conducted in BALF tissue. Inflammatory cell number was significantly increased in the OVA-sensitized/challenged mice compared to the



**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 the normal control (NC; Group 1) group. The prevention (pre; Group 3) and pre-treatment (tre; Group 4) groups following *B. infantis* CGMCC313-2 administration showed an increase in body weight. The statistical differences are represented as follows: <sup>a</sup>*P* < 0.05; <sup>b</sup>*P* < 0.01, and <sup>c</sup>*P* < 0.001.



**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 the normal control (NC; Group 1) group. The prevention (pre; Group 3), and pre-treatment (tre; Group 4) groups following *B. infantis* CGMCC313-2 administration showed a decrease. The statistical differences are represented as: <sup>a</sup>*P* < 0.05; <sup>b</sup>*P* < 0.01, and <sup>c</sup>*P* < 0.001.

control group. However, the proportion of infiltrating cells in the lung was significantly decreased in the groups treated with *B. infantis* CGMCC313-2 (Figure 5). Differential cell counts using 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 in the OVA or BLG-sensitized/challenged mice was evaluated from the perspective of overall lung or intestinal inflammation using histological HE staining (Figures 6 and 7, respectively). Compared with normal control mice (Figure 6A and Figure 7A), allergen sensitized/challenged mice (Figure 6B and Figure 7B) had severe inflammation; while the prevention (Figure 6C and Figure 7C) and pre-treatment (Figure 6D and Figure

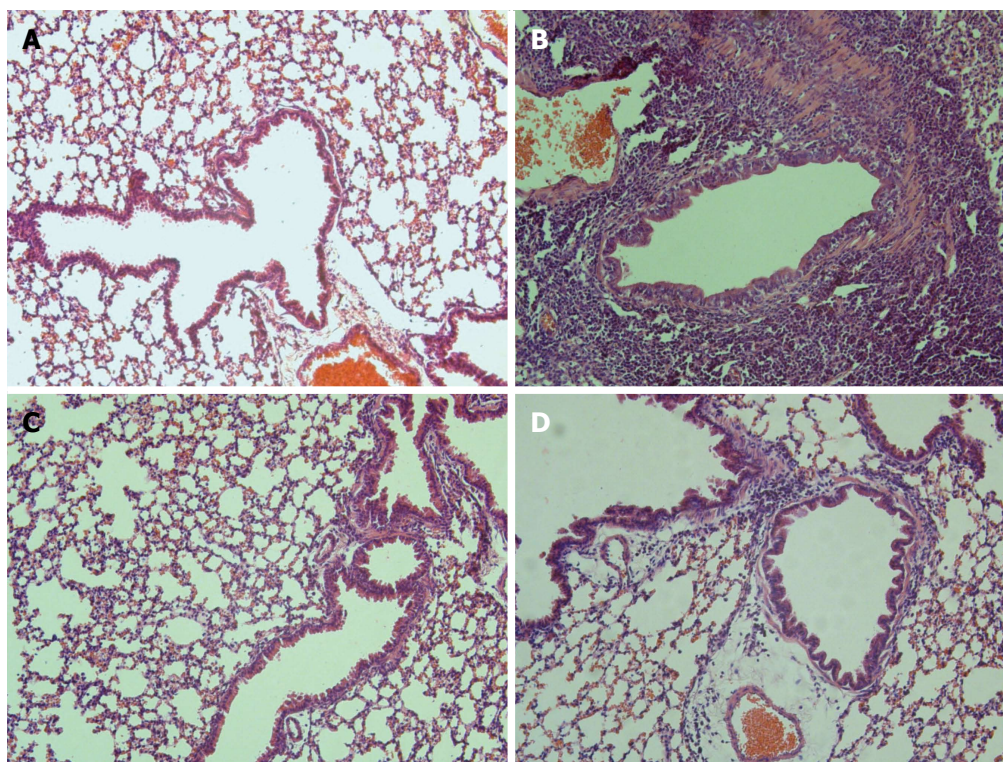
7D) mice showed significantly diminished signs of inflammation following *B. infantis* CGMCC313-2 treatment.

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

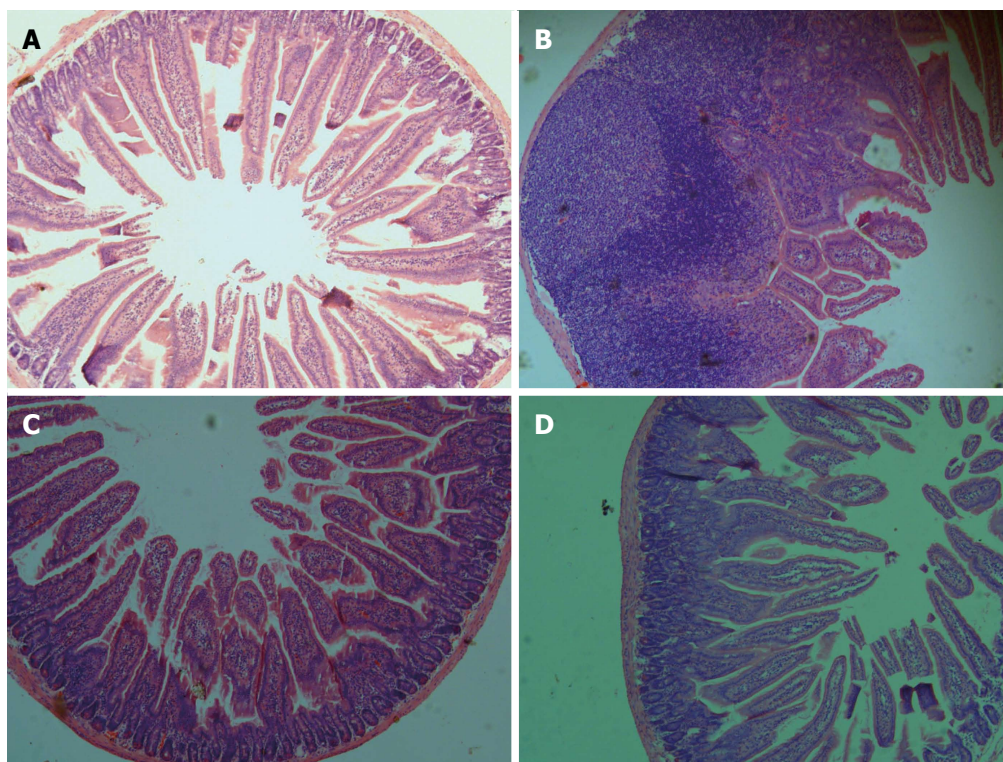
To further elucidate possible mechanisms responsible for the effects of *B. infantis* CGMCC313-2 on systemic sensitization and allergic inflammation, ELISA was used to determine the expression of IL-4, IL-10, IL-13, and IFN- $\gamma$  in serum and BALF was collected from BLG-induced food allergy mice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

**DISCUSSION**

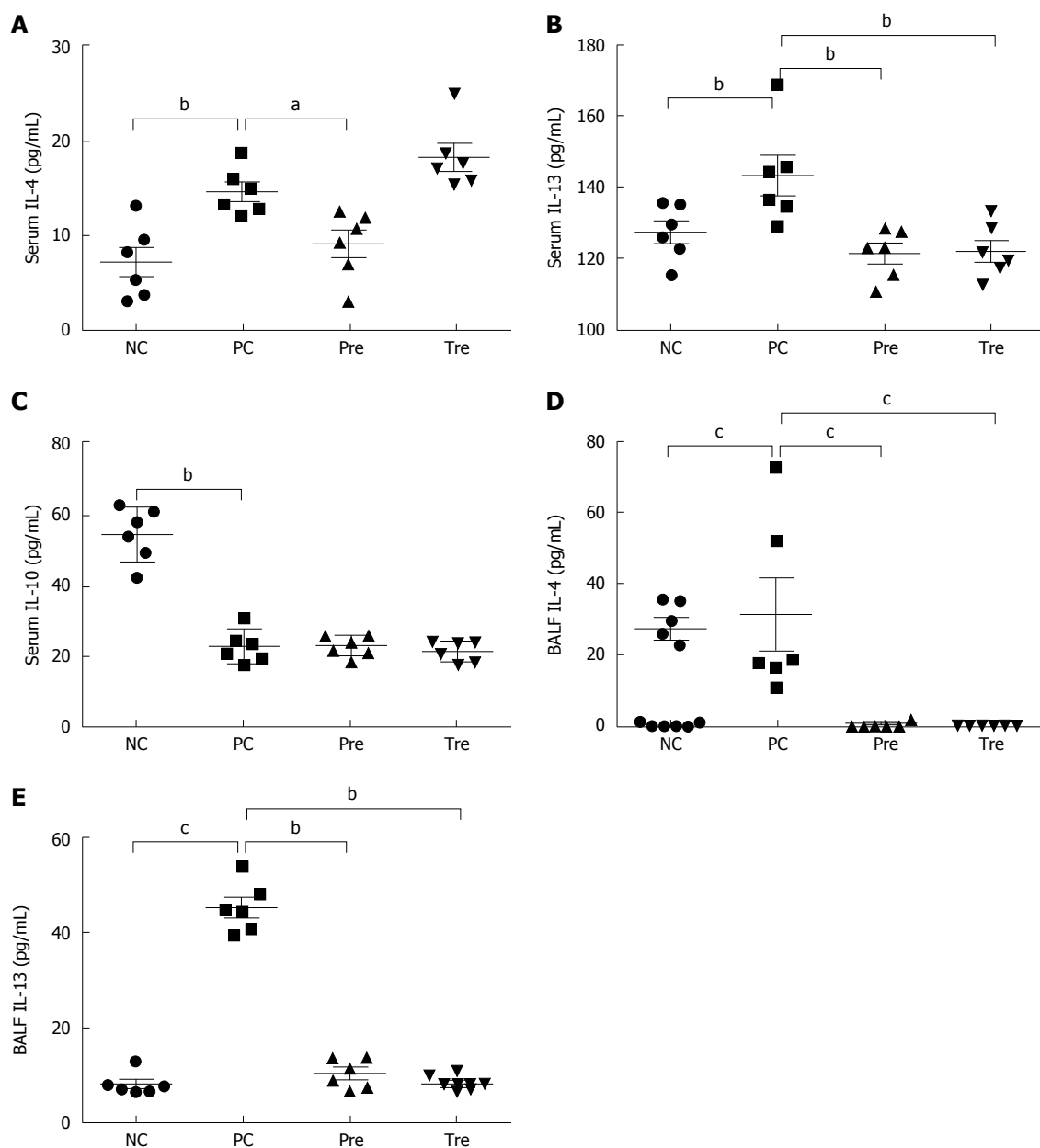
There is increasing evidence to show that intestinal microbiota and ingested probiotics may induce important metabolic and physiological reactions in the host, and drive maturation of the immune system in early life. Of these diverse probiotics, *Lactobacilli* and *Bifidobacteria*, which are part of the gut flora in infants, are the most promising candidates that naturally affect immune system development<sup>[14,15]</sup>. For the same reason, *Lactobacilli* and *Bifidobacteria* are the most frequently used probiotics for clinical intervention studies<sup>[16-20]</sup>. However, the most important characteristic of probiotics is their strain-specificity effect<sup>[21]</sup>. In this study, we investigated the role of *Bifidobacterium infantis* CGMCC313-2 in allergic disease prevention and treatment in two mouse models, as *B. infantis* CGMCC313-2 has been extensively used in the treatment and prevention of diarrhea including antibiotic-associated diarrhea in China. In OVA-sensitized/challenged mice, severe lung inflammation and infiltrating cells in the lungs were observed, and the administration of *B. infantis* CGMCC313-2 significantly diminished inflammation. Similarly, in  $\beta$ -lactoglobulin-induced food allergy mice, *B. infantis* CGMCC313-2 decreased intestinal inflammation, and ameliorated weight loss in BLG-sensitized/challenged mice. These results demonstrate that oral administration of *B. infantis* CGMCC313-2



**Figure 6** Effects of *B. infantis* CGMCC313-2 on OVA-induced airway inflammation. Lung tissues were obtained from the (C) prevention group and (D) pre-treatment group treated with *B. infantis* CGMCC313-2, and from (A) the normal control group and (B) the ovalbumin sensitized/challenged group on Day 29. The tissues were stained and observed under  $\times 200$  magnification. The positive control group showed severe airway inflammation, while the groups treated with *B. infantis* CGMCC313-2 showed attenuation of airway inflammation.



**Figure 7** Effects of *B. infantis* CGMCC313-2 on BLG-induced intestinal inflammation. Intestinal tissues were obtained from (A) the normal control group and (B) the BLG-sensitized/challenged group on Day 29, and from the (C) prevention group and (D) pre-treatment group which were treated with *B. infantis* CGMCC313-2. The tissues were stained and observed under  $200 \times$  magnification. The positive control group showed severe intestinal inflammation, while the groups treated with *B. infantis* CGMCC313-2 showed attenuated intestinal inflammation.



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

during or after allergen sensitization may relieve allergic inflammation in the 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 the 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 the attenuation of inflammatory symptoms. We deduced that the metabolites of *B. infantis* CGMCC313-2, including butyrate and short-chain fatty acids, can suppress the inflammatory responses triggered by Th2 cytokines<sup>[22-28]</sup>. However, the level of IL-4 was higher in the treatment group, which was opposite to the results of IL-13 and IgE. Due to the complexity of the immune system and response, the role of IL-4 as an allergic disorder marker requires further investigation in our future study. In addition, the oral administration of this



probiotic helped in the prevention and treatment of airway and intestine allergy.

On the other hand, there was a decrease in IL-10 serum levels in mice sensitized/challenged with BLG. There is strong evidence to indicate that the production of IL-10, which is affected by antigens exposure, is 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 promoted Treg responses<sup>[29,30]</sup>. 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 suppressed Th2 responses. In our study, the levels of IFN- $\gamma$  in both serum and BALF were too low to be detected in all mice, and this may have been due to the poor sensitivity of the measurement technique. This is a limitation of our study.

In the present study, which included allergic asthma and food allergy mouse models, we found that *B. infantis* CGMCC313-2 inhibited the secretion of allergen-induced IgE and Th2 cytokines, and further attenuated 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.

## ACKNOWLEDGMENTS

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## COMMENTS

### Background

Probiotics exhibit beneficial effects on allergies based on the "microflora hypothesis", and experimental studies have also shown that probiotics have strain-specific effects. In this study, the specific effects of *B. infantis* CGMCC313-2, which is widely used as a probiotic drug in China, on allergic asthma and food allergy mouse models were determined.

### Research frontiers

Previous studies have indicated that ingested *Lactobacilli* and *Bifidobacteria* can modify microbial flora, and can reduce the symptoms of allergic diseases by modifying the host immune system. Some probiotic drugs have been intensively investigated as novel alternative options for the management of allergic diseases including asthma and food allergy.

## Innovations and breakthroughs

In this study, *B. infantis* CGMCC313-2 was administered to allergic asthma and food allergy mouse models. Following *B. infantis* CGMCC313-2 treatment, the serum concentrations of IgE and IgG1 significantly decreased, and the concentrations of interleukin-4 (IL-4) and IL-13 also reduced in mice with allergic asthma and food allergy. Inflammatory cell infiltration and inflammation were also attenuated.

## Applications

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

## Terminology

IgE and IgG, which are closely related with anaphylaxis, are higher in patients with allergies. IL-4 and IL-13, which are secreted by Th2 cells, are involved in the humoral immune response and indicate the degree of allergic disease.

## 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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