Bifidobacterium infantis Relieves Allergic Asthma in Mice by Regulating Th1/Th2

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Background: *Bifidobacteria* are among the probiotics used in treating intestinal diseases and are rarely used for allergic asthma treatment. The present study investigated the mechanism of *B. infantis* in treating allergic asthma in mice.

Material/Methods: A total of 40 male Balb/c mice were randomized into control, ovalbumin (OVA), montelukast (Mon), and *B. infantis* (B10) groups, and allergic asthma was induced in the OVA, Mon, and B10 groups. Airway reactivity was measured on day 29 by methacholine at various doses. The numbers of total cells and inflammatory cells in bronchoalveolar lavage fluid (BALF) were counted by blood cell counter and Diff-Quik staining. Hematoxylin-eosin (HE) staining was performed to observe inflammatory cell infiltration in lung tissues. Total IgE and OVA-specific IgE in serum were measured by ELISA. Mucin 5AC expression was detected by Western blot to evaluate airway obstruction. The levels of Th1 (IFN-γ, IL-2) and Th2 (IL-4, IL-5, IL-13) cytokines in BALF and tissues were detected by ELISA and qRT-PCR, respectively.

Results: The mice in the OVA group had airway hyperreactivity, while the symptoms in the B10 group and Mon group were effectively relieved. B10 reduced the number of inflammatory cells in BALF as well as inflammatory cell infiltration in tissues. Moreover, the levels of total serum IgE, OVA-specific IgE, and Mucin 5AC were increased in the OVA group, but were reduced in the Mon group and B10 group. *B. infantis* increased the levels of Th1 cytokines and decreased those of Th2 cytokines.

Conclusions: *B. infantis* can reduce the infiltration of inflammatory cells induced by OVA-specific antibodies in mice. *B. infantis* has therapeutic effects on allergic asthma by promoting Th1 and inhibiting Th2 immune responses.

MeSH Keywords: **Allergy and Immunology • Asthma • Bifidobacteriales Infections • Ovalbumin • Th1-Th2 Balance**

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Background

Allergic asthma is a chronic respiratory disease characterized by chest tightness, shortness of breath, and coughing after exposure to allergens [1]. If not properly controlled, the disease can lead to anaphylactic shock or even death. The incidence of allergic asthma has been increasing in recent years, and is highest among children. At least 300 million people suffer from allergic asthma worldwide [2]. The main method of clinical treatment is still inhaled glucocorticoids, but this method has certain adverse effects and limitations [2]. Therefore, finding a safe and reliable treatment has become a clinical challenge.

Probiotics can change the composition of certain microbiota in the host, and is a type of beneficial microbial flora [3]. Studies found that supplementing with various probiotic preparations can balance intestinal microbiota, regulate the immune system, and reduce allergies [4]. Many studies have explored new strains of probiotics, probiotic genomics characteristics, *in vivo* mechanism of action, formulation processes, and safety of using probiotics [5]. Researchers discovered that probiotics can treat digestive tract diseases and also reduce the inflammatory mediators and enhance immunity, thereby playing an important role in the treatment of respiratory diseases [6]. Probiotics can be divided into original bacteria, symbiotic bacteria, and fungi according to their sources and modes of action of the strains, and *bifidobacteria* are an important source of bacteria [7].

Bifidobacteria exist in the intestines within a short period of time after birth [8]. According to LPSN, the genus Bifidobacterium comprises 70 established species and 10 subspecies [9,10]. A recent study found that *bifidobacteria* can regulate intestinal microbiota, prevent constipation, and alleviate gastrointestinal disorders [11]. As *bifidobacteria* do not produce endogenous, exotoxin, or harmful substances and gases, they are seen as effective probiotics for the prevention and treatment of human intestinal diseases. In addition, *bifidobacteria* also have certain therapeutic effects on respiratory diseases [12]. Test results on stools from asthmatic patients found that the content of *bifidobacteria* is small compared with normal patients [13]. Moreover, *Bifidobacterium breve* MRx0004 could reduce eosinophils and neutrophil infiltration in tissues of asthmatic mice [14], and *Bifidobacterium longum* could reduce lung inflammation in mice, which is related to T lymphocytes [15]. *B. infantis* is mainly used to prevent digestive tract diseases such as infantile diarrhea. Research showed that *B. infantis* can alleviate allergic inflammation by inhibiting allergen-induced secretion of IgE, IL-4, and IL-13 [16], and reduce IgE in serum, as well as reducing expressions of IL-5 and IL-10 in bronchoalveolar lavage fluid (BALF) [17]. However, more data are required to specifically determine whether *B. infantis* has therapeutic effects on airway allergic inflammation.

Balb/c mice, which are immunodeficient mice highly sensitive to carcinogens, are commonly used in immunological research [18]. Sensitization with ovalbumin (OVA) can cause symptoms such as shortness of breath, restlessness, and even convulsions and incontinence in experimental animals [19]. OVA stimulation causes a large amount of inflammatory cell infiltration in lung tissues and significantly increases serum IgE levels, which mimics the clinical symptoms of allergic asthma. Moreover, OVA has is highly stable and reproductive, and is therefore widely used to induce allergic asthma in animal models [20]. As a potent specific leukotriene receptor antagonist, montelukast can reduce eosinophils, macrophages, and lymphocytes in the airway, and is also commonly used for inducing allergic asthma [21]. In the present study, an OVA-induced mouse model of allergic asthma was established and mice were then treated with *B. infantis* to observe the effects of *B. infantis* on allergic asthma in the mice. We also explored the possible mechanism underlying the effect of *B. infantis* treatment in allergic asthma.

Material and Methods

Animals and medicines

Forty male SPF healthy Balb/c mice (age 6 weeks, weight 18±1 g) were purchased from the Beijing Vital Lihua Experimental Animal Center. The mice were housed in the SPF facility of Zhejiang Provincial People's Hospital Animal Laboratory at 22±1°C in 55±5% humidity with a 12-h day/night cycle. Animal experiments were approved by the Zhejiang Provincial People's Hospital Animal Ethics Committee (No. AEC20180912067). *B. infantis* CGMCC313-2 purchased from Shandong Kexing Biological Company was confirmed by the Institute of Microbiology of the Chinese Academy of Sciences and stored at −20°C. *B. infantis* CGMCC313-2 was adjusted to a concentration of $5×10^{10}$ CFU/mL by physiological saline before use. Ovalbumin (OVA, HZB1412, Sigma, USA) and montelukast (T1677-25 mg, Target Mol, USA) were stored at 4°C.

Establishing an animal model of OVA-induced allergic asthma

All Balb/c mice were adaptively fed for 7 days and randomly divided into 4 groups – a control group, an OVA-induced allergic asthma group (OVA group), a montelukast (Mon) group, and a *B. infantis* (B10) group, with 10 mice in each group. As shown in Figure 1, mice in the control group inhaled 8 mL saline daily for 14 days, while those in the OVA, Mon, and B10 groups were intraperitoneally injected daily with 100 μg OVA [22]. From day 21 to day 28, the mice in the OVA group, Mon group, and B10 group were aerosolized with 1% OVA by a nebulizer (NE-C900, Omron, China). The mice were placed in an atomization chamber and aerosolized 30 minutes (min) a day for
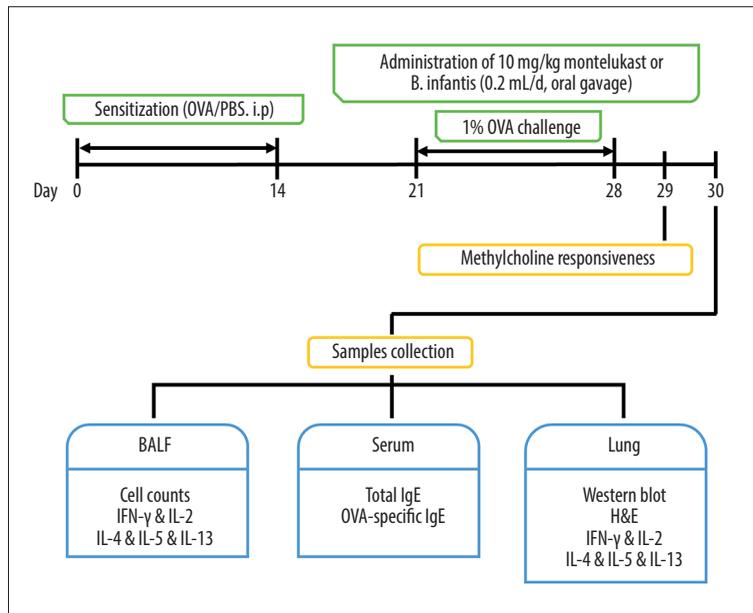
Figure 1. Technical roadmap for the experimental study of *B. infantis* in the treatment of ovalbumin-induced mice with allergic asthma.

7 days. From day 21 to day 28, the Mon group was orally administered montelukast once a day (10 mg/kg/d), while the B10 group was orally administered *B. infantis* CGMCC313-2 (0.2 mL/d) [23]. The mice in the other 2 groups were orally administered 0.2 ml saline at the same time and frequency.

Airway reactivity test

Airway responsiveness refers to response of the airway to various physical and chemical stimuli or allergens [24]. Under normal circumstances, the airway does not induce smooth-muscle contraction or only has weak contraction response to the above-mentioned micro-stimulation, but asthma patients can be over-reactive to airway inflammation and show sensitive and excessive bronchus smooth-muscle contraction response, which results in narrowing of the airway and increase of airway resistance. Airway hyperreactivity (AHR) is one of the important characteristics of asthma [24]. On day 29 of the experiment, we performed airway reactivity tests on all the experimental mice. A non-invasive pulmonary function meter (gyd-003, EMKA, France) was used to measure the level of exhalation pause (Penh) of the mice after nebulizing the animals with different concentrations of methacholine (0, 6.25, 12.5, and 25 mg/mL). Thus, the changes in Penh relative to the baseline level reflect the degree of AHR.

Sample collection

Samples were collected from the experimental animals on day 31. We prepared 0.5% sodium pentobarbital solution (P3761-25G, Sigma, USA) with physiological saline and administered it at 50 mg/kg to anesthetize the mice via intraperitoneal injection. After the limb muscles relaxed, the abdominal aorta and blood were collected. After centrifugation, the supernatant (serum) was collected and stored at −20°C. Bronchoalveolar lavage fluid (BALF) was collected immediately after blood collection. The trachea was punctured using a venous indwelling needle. Wes slowly injected 1 ml of pre-cooled PBS into the lungs of the mice, and the liquid was then pumped back and collected into a centrifuge tube. These steps were repeated 10 times to fully collect BALF [25]. Finally, sterilized ophthalmic scissors (18-0110, LAIYUE, China) were used to open the chest of the mice, and the lung tissues were removed. After washing in physiological saline, the right lungs were fixed in 4% paraformaldehyde (KGIHC016, KeyGen, China) and the left lung was stored in a cryotube at −80°C.

Inflammatory cell count

The collected BALF was centrifuged at 120 g for 10 min at 4°C, and the supernatant obtained was stored at −80°C for later use. A portion of the remaining cell pellets was resuspended in PBS, while the rest was made into cell smears. Total cell number was counted from 6 observation fields using a hemocytometer (YA0811, Solarbio, China). Different types of inflammatory cells were observed by Diff-Quik staining (D030-1-1, Nanjing Jiancheng, China) [26]. The cell smears were passively dried, and then fixed by RI reagent for 10 s at room temperature. After removing the excess liquid, the slices were immediately transferred to the R2 reagent for 8 s and then removed immediately. The liquid on the slices was then removed again and then slices were rinsed in the R3 reagent for 8 s. Finally, the dye solution was carefully removed from the cell smears under running water. Cell smears were then dehydrated twice with 100% ethanol solution and sealed. Finally, types of inflammatory cells were identified and counted under a microscope (TS100, Nikon, Japan).

Table 1. The summary results inflammation cytokines level by ELISA.

BALF (pg/mL)	Control	OVA	Mon	B10
IFN-γ	76.31±18.30	44.68±7.23[#]	67.45±12.07[*]	62.16±10.07[*]
IL-2	131.21±9.68	98.73±4.07[##]	121.03±6.52[**]	116.83±5.12[**]
IL-4	40.26±9.77	158.97±26.48[##]	62.15±14.79[**]	71.34±18.22[**]
IL-5	22.37±6.24	89.65±19.81[##]	28.93±7.11[**]	33.56±8.42[**]
IL-13	31.76±8.38	197.78±33.75[##]	99.67±15.45[**]	105.34±15.64[**]

Th1 (IFN-γ, IL-2) and Th2 (IL-4, IL-5, IL-13) cytokines in mouse BALF, detected by ELISA (n=3 for each group). * $P<0.05$; ** $P<0.01$. [#] *vs.* Control; [##] *vs.* OVA.

Hematoxylin-eosin (HE) staining

Paraformaldehyde-fixed lung tissues were dehydrated, paraffin-embedded, and sectioned. The sections were dewaxed and maintained in hematoxylin solution (RH018-100, RuiTaibio, China) for 10 min. The sections were rinsed in tap water to remove blue color and then restored to blue using 1% aqueous ammonia solution (DH0063, Leagene, China) and washed under running water. Next, the lung tissue sections were stained by eosin solution (1570, Dingguo, China) for 5 min, and conventionally gradient-dehydrated. Finally, the sections were sealed with neutral gum (N116470-100g, Aladdin, China). The pathological images of the sections were observed and collected under a microscope (TS100, Nikon, Japan) [27].

Detection of serum immunoglobulin E (IgE) levels

Total IgE and OVA-specific IgE in the serum of each group of mice were measured using a Mouse IgE ELISA Kit (CSB-E07983m, Cusabio, China) and a Mouse OVA sIgE ELISA Kit (CSB-E08914m, Cusabio, China). The collected blood was allowed to stand at room temperature for 2 h and then centrifuged for 15 min (1000 g, 4°C). The serum was diluted according to the kit instructions, and the reaction system was added. Optical density value (OD) at 450 nm was assessed using a microplate reader (3-6550-01, ASONE, Japan). Finally, the concentrations of total IgE and OVA-specific IgE (ng/ml) in the serum of each group were calculated [28].

Western blot analysis

Proteins were extracted from the lung tissues. Then, 100 mg lung tissues, 40 µl of 25× protease inhibitor (M167-50MG/YZ, Amresco, USA), 100 µl 10× phosphatase inhibitor (C0003-1ml-100, Topscience, China), and 860 µl RIPA lysate (BDIT0037-50, Biodragon, China) were added to a centrifuge tube and thoroughly ground using a tissue disrupter at 30 times/s for 10 min (TissueLyser II, QIAGEN, Germany). Next, the mixture was allowed to stand on ice for 10 min. The supernatant in the original

centrifuge tube was pipetted into a new tube and centrifuged at 16 000 g and 4°C for 10 min. The protein concentration was determined using a BCA kit (SLF-23227, Thermo, USA). We isolated 100 µg of proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, P0014A, Beyotime, China) and transferred it to an NC membrane (SA1609, SAB, USA), which was washed 3 times (at room temperature) with 1×TBST for 10 min. Then, the bands were blocked in 5% blocking solution (KGP108, KeyGene, China) for 2 h. After washing, anti-Mucin 5AC antibody (ab24071, 130 kDa, 1: 1000, Abcam, UK) and β-actin (ab8226, 42kDa, 1: 10 000, Abcam, UK) were incubated with the membrane overnight at 4°C, and β-actin served as the internal reference of the experiment. The NC membrane was washed by TBST 3 times, then Rabbit Anti-Mouse IgG H&L (ab6728, 1: 10 000, Abcam, UK) was added and incubated together for 1 h at room temperature. After washing, we added luminescent liquid (WBKLS0100, Coolaber, China) to the NC membrane. Finally, The membrane was placed in a gel imaging system (SDW-1008, Qiagen, Germany) for detection, and the images were analyzed using Image J software (National Institutes of Health, USA) [29].

Detection of cytokine levels in BALF

IFN-γ (CSB-E04578m), IL-2 (CSB-E04627m), IL-4 (CSB-E04634m), IL-5 (CSB-E04637m), and IL-13 (CSB-E04602m) in BALF were detected by enzyme-linked immunosorbent assay (ELISA, Cusabio, Wuhan, China). The BALF supernatant was first dissolved on ice and then diluted. The OD values at a wavelength of 450 nm were detected using a microplate reader (3-6550-01, ASONE, Japan). A standard curve was drawn from the OD values, and the concentration (pg/ml) of each cytokine in BALF of different groups was calculated [28]. The levels of inflammatory cytokines are shown in Table 1.

Extraction of total RNAs and quantitative real-time polymerase chain reaction (qRT-PCR)

Lung tissues (50 mg) were cut into pieces and placed in a centrifuge tube. We added 1 ml Trizol (15596-026, Invitrogen, USA)

Table 2. The summary results of gene expression by qRT-PCR.

Lung	Control	OVA	Mon	B10
IFN-γ	1.00±0.28	0.42±0.09#	2.08±0.65**	1.75±0.52**
IL-2	1.00±0.18	0.61±0.11#	1.19±0.22**	1.42±0.27**
IL-4	1.00±0.31	6.76±1.23##	3.49±0.62**	4.03±0.57**
IL-5	1.00±0.32	5.04±1.23##	1.96±0.43**	3.03±0.64*
IL-13	1.00±0.34	8.89±2.20##	3.02±0.81**	3.24±0.89**

Th1 (IFN-γ, IL-2) and Th2 (IL-4, IL-5, IL-13) cytokines in mouse lung tissue, detected by qRT-PCR (n=3 for each group). * $P<0.05$; ** $P<0.01$. # *vs.* Control; ## *vs.* OVA.

to disrupt the tissue. The total RNAs from the aspirated supernatant were then extracted by chloroform and isopropanol method. RNA concentration was measured using NanoDrop2000 (YQ1633128263, Thermo, USA). CDNAs were obtained by reverse transcription of RNAs using a reverse transcription kit (k1622, Thermo Fisher, USA). The QRT-PCR reaction system (A100, LongGene, China) consisted of 6 µl DEPC water (R1600, Solarbio, China), 2 µl diluted cDNAs, 10 µl FastStart Universal SYBR Green Master (04913914001, Roche, Switzerland), and 1 µl of each of diluted upstream and downstream primers in a 96-well plate. The amplification conditions of PCR were: pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, and annealing at 60°C for 1 min, for a total of 40 cycles. The expression level of each gene mRNA was obtained by $2^{-\Delta\Delta CT}$ calculation [30]. The gene primer sequences used in this experiment were:
IFN-γ-F, 5'-ATGAACGCTACACACTGCATC-3', and
IFN-γ-R, 5'-ATGAACGCTACACACTGCATC-3';
IL-2-F, 5'-TGAGCAGGATGGAGAATTACAGG-3', and
IL-2-R, 5'-GTCCAAGTTCATCTTCTAGGCAC-3';
IL-4-F, 5'-ATCATCGGCATTTTGAACGAGG-3', and
IL-4-R, 5'-TGCAGCTCCATGAGAACACTA-3';
IL-5-F, 5'-CTCTGTTGACAAGCAATGAGACG-3', and
IL-5-R, 5'-TCTTCAGTATGTCTAGCCCCTG-3';
IL-13-F, 5'-CAGCCTCCCCGATACCAAAAT-3', and
IL-13-R, 5'-GCGAAACAGTTGCTTTGTGTAG-3';
GAPDH-F, 5'-TGGATTTGGACGCATTGGTC-3', and
GAPDH-R, 5'-TTTGCACTGGTACGTGTTGAT-3'.
GAPDH served as an internal reference. The gene expressions are shown in Table 2.

Statistical analysis

The data were analyzed using SPSS 22.0 software (SPSS, Inc., USA) and are shown as mean ± standard deviation of the mean. Differences between 2 groups were compared by two-tailed *t* test, while differences between multiple groups were compared by one-way analysis of variance (ANOVA). $P<0.05$ was considered to be a statistically significant difference.
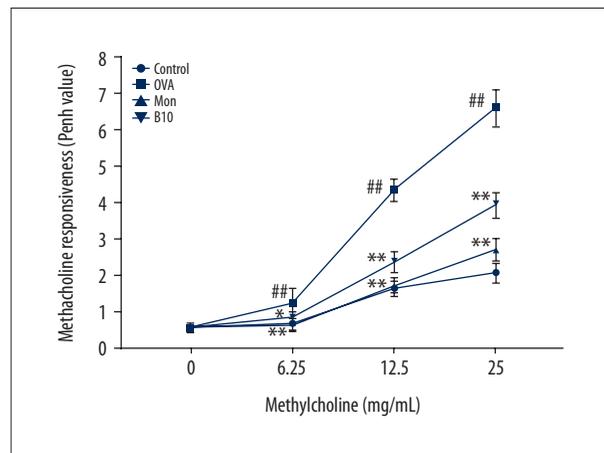

Figure 2. *B. infantis* reduced airway hyperreactivity (AHR) in the mice with allergic asthma (n=10). All experiments were repeated in triplicate to average. ## $p<0.001$ *vs.* control group (control); * $p<0.05$, ** $p<0.001$ *vs.* Ovalbumin-induced allergic asthma group (OVA).

Results

Technical roadmap for the experimental study of *B. infantis* in the treatment of ovalbumin-induced mice with allergic asthma

The technical roadmap is shown in Figure 1. On days 1–14, the mice in the OVA, Mon, and B10 groups were used to establish the animal model. From days 21–28, the mice in the Mon and B10 groups were orally administered montelukast once a day (10 mg/kg/d), while those in the other 2 groups were given normal saline. On days 21–28, mice in the OVA, Mon, and B10 groups were subjected to OVA atomization stimulation. Airway reactivity of all mice was measured at the end of the atomization. BALF, serum, and lung tissues were collected for subsequent experimental studies. No mice died during the modeling process.
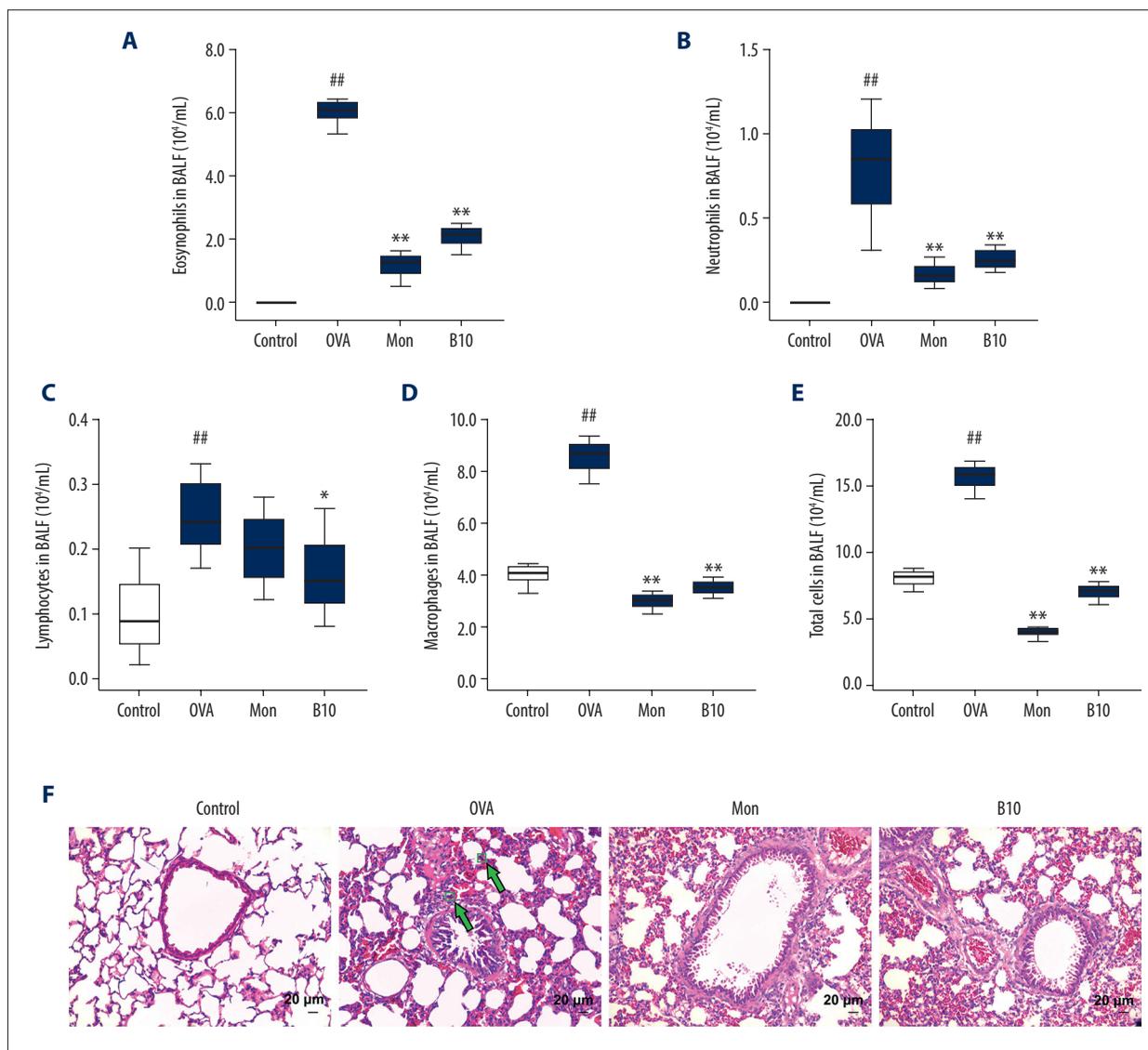
Figure 3. *B. infantis* reduced inflammatory cell infiltration in OVA-induced mice. (**A–E**) Total cells in BALF were counted by a hemocytometer. The number of eosinophils, neutrophils, lymphocytes and macrophages in BALF were counted by Diff-Quik staining. (**F**) Hematoxylin-eosin staining showed inflammatory cell infiltration of lung tissues. All experiments were repeated in triplicate to average (n=10). ## $p < 0.001$ *vs.* control; * $p < 0.05$, ** $p < 0.001$ *vs.* OVA.

B. infantis reduced AHR in the mice with allergic asthma

As shown in Figure 2, the Penh values of each group increased with the increase of methacholine concentrations. The airway responsiveness of the OVA-induced allergic asthma group was increased the most significantly when the methacholine concentration was 6.25 mg/ml. Moreover, the Penh values were lower in the B10 group compared with the OVA group, but this decrease was significantly reversed at the methacholine concentration of 12.5 mg/mL ($p < 0.001$). The positive drug Mon group had a greater reduction than in the B10 group, and was closer to the Penh values in the normal group. These results indicated successful establishment of an OVA-induced model of allergic asthma

in mice and also demonstrated that *B. infantis* can reduce AHR in model mice, which is similar to the effects of montelukast.

B. infantis reduced inflammatory cell infiltration in OVA-induced mice

By counting the inflammatory cells in BALF, we found that the total numbers of cells, including the numbers of eosinophils, neutrophils, and macrophages, were significantly higher in the OVA group ($p < 0.001$, Figure 3A–3E). Compared with the model group, these inflammatory cells were far lower in the *B. infantis* group and Mon group in BALF after the treatment. To further observe the inflammatory cell infiltration in lung tissues,
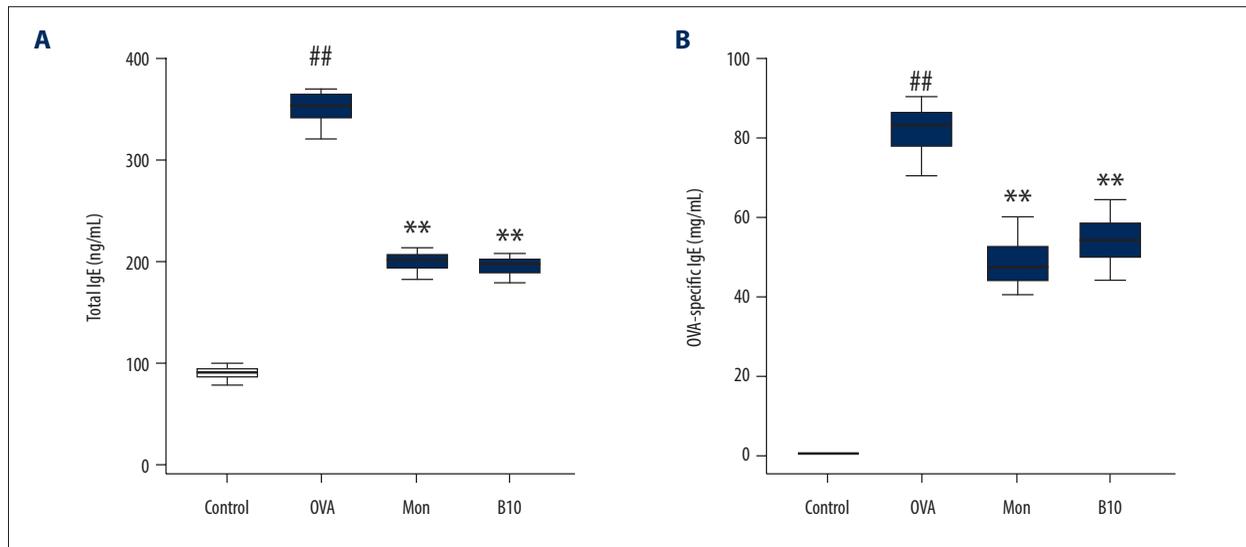
Figure 4. *B. infantis* reduced the expression of IgE in the serum of allergic asthma mice. (**A**) ELISA was used to measure the total IgE content (ng/ml) in the serum of mice. (**B**) ELISA was used to detect the content of OVA-specific IgE (ng/ml) in the serum of mice. All experiments were repeated in triplicate to average (n=10). ## *p*<0.001 *vs.* control; ** *p*<0.001 *vs.* OVA.
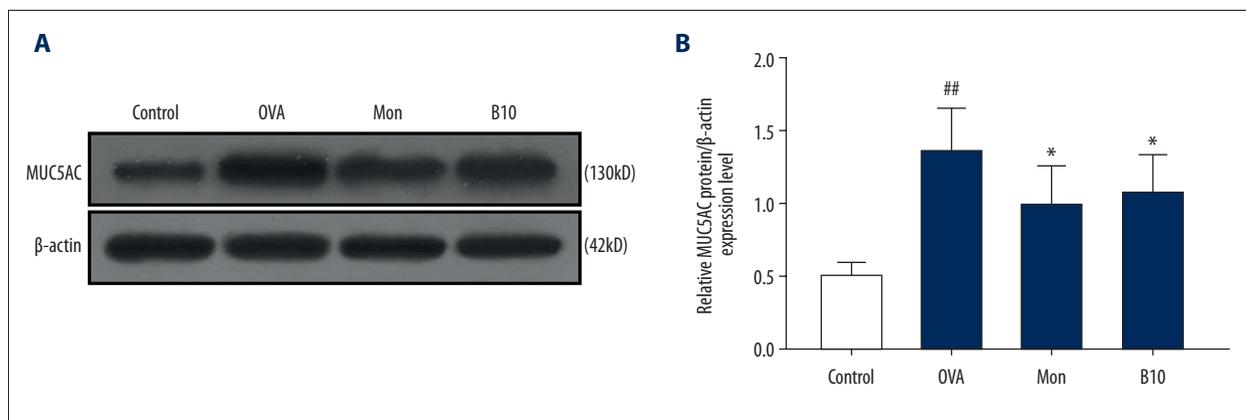

Figure 5. *B. infantis* reduced the expression of MUC5AC in the lung tissue of the mice with allergic asthma. (**A, B**) Protein expression of MUC5AC in lung tissues was detected by Western blot. β-actin served as a reference gene. All experiments were repeated in triplicate to average (n=10). ## *p*<0.001 *vs.* control; * *p*<0.05 *vs.* OVA.

we performed HE staining. The results showed a large amount of inflammatory cell infiltration surrounding the bronchi and alveolar cells in the OVA group (shown by the green arrow in Figure 3F), and the smooth-muscle layer and the airway wall were thickened. In contrast, the inflammatory cell infiltration was significantly lower in the *B. infantis* and Mon treatment groups, and the airway wall and smooth-muscle layer were also thinner than in the OVA group. These results indicated that a great amount of inflammatory cell infiltration occurred in the allergic asthma mice induced by OVA, and the condition was significantly improved in the *B. infantis* and Mon groups.

B. infantis reduced the expression of IgE in serum and MUC5AC expression in the lung tissues of model mice

The expressions of total IgE and OVA-specific IgE were detected by ELISA in serum, showing that the serum levels of OVA-specific IgE and total IgE in the OVA group were significantly higher than those in the control group (*p*<0.001, Figure 4), while the levels in the serum of the *B. infantis* and Mon-treated mice were far lower (*p*<0.001, Figure 4). Western blot analysis demonstrated that the expression of MUC5AC protein was downregulated in the lung tissues from the OVA mice in the *B. infantis* group and the Mon group (*p*<0.05, Figure 5). Thus, *B. infantis* reduced the expression of IgE in the serum of model mice and reduced MUC5AC expression in lung tissues of mice, and the beneficial effect was similar to that of montelukast.
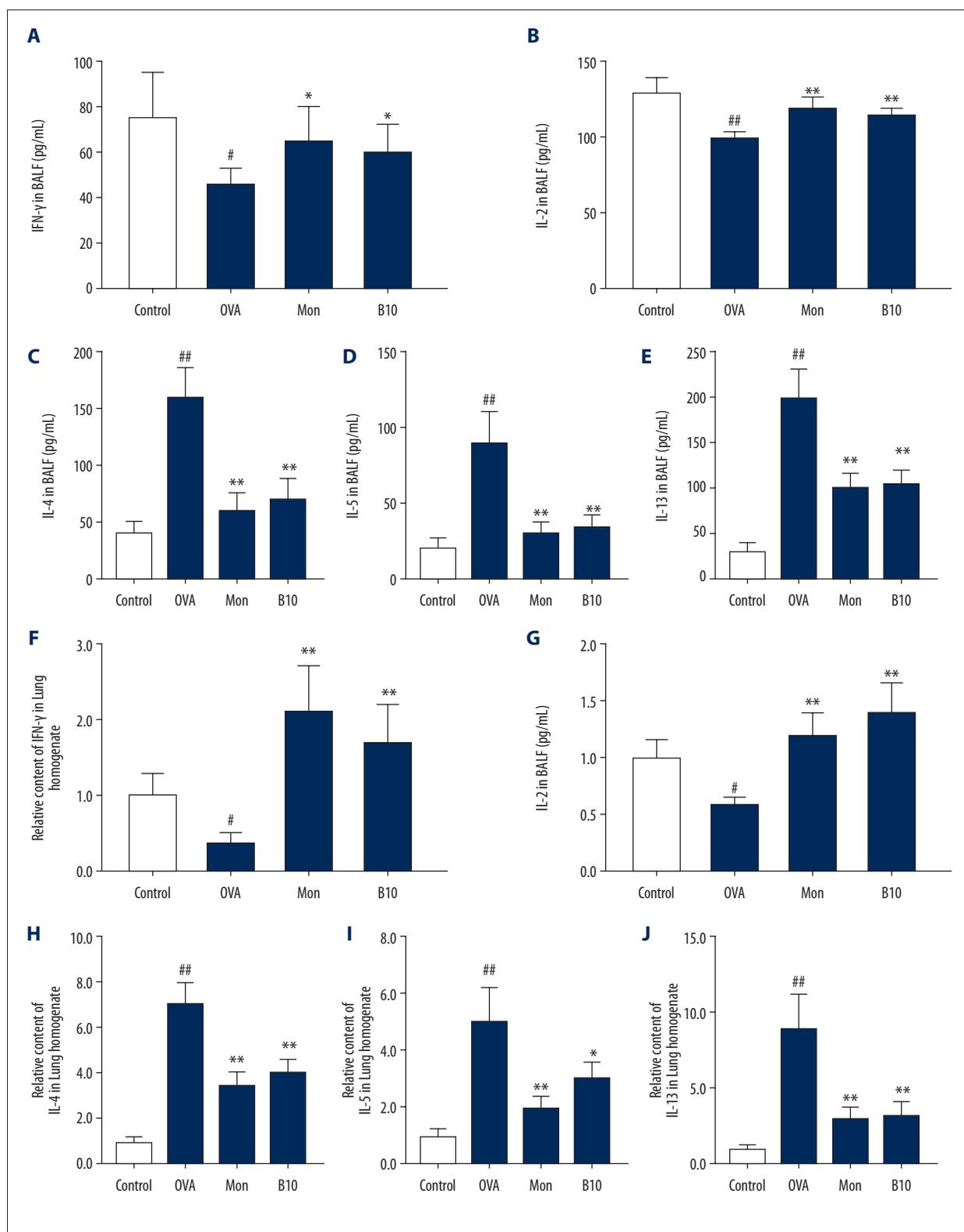
Figure 6. *B. infantis* regulated the balance of Th1/Th2-related cytokines in BALF and lung tissues. (**A–E**) ELISA was used to detect the contents of IFN-γ, IL-2, IL-4, IL-5, and IL-13 in BALF. (**F–J**) QRT-PCR was used to detect the expression of IFN-γ, IL-2, IL-4, IL-5, and IL-13 in lung tissues. β-actin served as a reference gene. All experiments were repeated in triplicate to average (n=10). # $p < 0.05$, ## $p < 0.001$ *vs.* control; * $p < 0.05$, ** $p < 0.001$ *vs.* OVA.

B. infantis regulated the balance of Th1/Th2-related cytokines in BALF and lung tissues

Finally, we explored the possible mechanisms by which *B. infantis* affected allergic asthma. The cytokines of Th1 (IFN-γ and IL-2) and Th2 (IL-4, IL-5, and IL-13) in BALF and lung tissues were detected by ELISA and qRT-PCR, respectively. Compared with the control group, the levels of IFN-γ and IL-2 in the BALF of the OVA group were significantly lower, and the levels of IL-4, IL-5, and IL-13 were far higher. After treatment with *B. infantis*, the levels of IFN-γ and IL-2 in BALF were increased, and the levels of IL-4, IL-5, and IL-13 were decreased (Figure 6A–6E). The results of the qRT-PCR experiment were similar to those shown by ELISA (Figure 6F–6J). We also found that the mRNA expression levels of IFN-γ and IL-2 were reduced in the OVA group ($p<0.05$), but were significantly improved in the *B. infantis* group and Mon group ($p<0.001$). In the OVA group, the levels of IL-4, IL-5 and IL-13 were noticeably higher ($p<0.001$), but were lower after *B. infantis* and Mon treatment. These data indicate that *B. infantis* ameliorated allergic asthma by increasing expression levels of IFN-γ and IL-2 and inhibiting IL-4, IL-5, and IL-13 expression.

Discussion

As the most common type of asthmatic disease, allergic asthma is a chronic inflammatory airway disease that occurs due to repeated stimulation by allergens [31]. Allergic asthma involves a variety of inflammatory cells, and is characterized by elevated expressions of biomarkers such as IgE and cytokines in serum as well as by pathological manifestations such as airway hyperreactivity (AHR) and airway remodeling [31]. The pathogenesis of allergic asthma mainly occurs via airway inflammation and neuroreceptor regulation, genetic mechanisms, and airway remodeling mechanisms [31]. Eosinophils, neutrophils, lymphocytes, and macrophages are important factors in the airway inflammatory response. Amyloid granules released by eosinophils at the site of inflammation stimulate mucus production, causing tissue edema and airway obstruction and releasing Th1 and Th2 cytokines and chemokines that further promote inflammation [32]. Neutrophils can secrete a variety of inflammatory mediators and cytokines and promote inflammation, thus causing airway remodeling [33]. Macrophages are essential in the immune response, and pulmonary macrophages can cause smooth-muscle spasm, leading to AHR by directly releasing histamine and generating oxygen free radicals to inhibit β adrenergic receptor function [34]. Lymphocytes are mainly responsible for regulating the immune response of the body, and secrete pro-inflammatory cytokines and promote IgE synthesis [35]. The present study showed that OVA-induced model mice developed significant AHR, and that the numbers of these inflammatory cells in the BALF of

OVA-induced model mice were greatly increased. Moreover, the pathological results also found a large amount of inflammatory infiltration in the lung tissues. However, after oral administration of *B. infantis* and montelukast into the mice, airway reactivity and development of inflammatory cell infiltration were alleviated, and numbers of various inflammatory cells in BALF were also effectively reduced.

Immunoglobulin E (IgE) is an antibody produced by differentiation of B lymphocytes and is involved in type I allergic reactions [36]. IgE-mediated mast cell activation can cause eosinophils and Th2 cells to aggregate and produce more mucus, thereby leading to airway smooth-muscle contraction [37]. IgE is a biomarker for the diagnosis of allergic asthma, and it is closely related to the severity of disease [36]. In the present study, the total IgE and OVA-specific IgE in the serum of OVA-induced allergic asthma mice were significantly higher than in the normal mice, and the decrease in IgE after *B. infantis* treatment suggested that *B. infantis* had a therapeutic effect on allergic asthma. Mucin 5AC (MUC5AC), which is mainly expressed in the airway epithelium [38], increases mucus secretion and leads to airway obstruction when its expression increases [38]. Previous studies [39] found that the expression of MUC5A involves the NF-B pathway, and that the inflammatory factor IL-13 can promote the expression of MUC5A, causing excessive secretion of airway mucus. In the present study, *B. infantis* significantly reduced the protein expression of MUC5A.

Cytokines such as IL-4, IL-5, and IL-13 are produced by Th2 cells and are the main contributors to initiation of inflammation in asthma [40]. IL-5 is an important cytokine for eosinophil growth, differentiation, and chemotaxis, and plays an important role in inflammatory response and airway remodeling in allergic asthma [41]. Although it has less effect on mucus hyperplasia and airway hyperresponsiveness, IL-13 regulates airway hyperresponsiveness, promotes mucus hyperplasia, and has an important effect on the occurrence and development of allergic asthma [42]. IL-4, which is involved in the regulation of B cells, together with IL-13, induces the conversion of IgG to IgE and also upregulates the expression of vascular cell adhesion factor-1 (VCAM-1) [43]. After the allergic asthma model was induced in T cell immunodeficiency mice (without IL-4 and IL-13) by OVA, AHR and inflammation were significantly attenuated, and serum IgE and IgG1 levels were also greatly reduced [44]. The role of Th2 cell activation in asthma has gradually received increased research attention, and studies found that the levels of Th1 cytokines in serum and BALF are significantly increased in asthmatic patients [45]. Therefore, in addition to the analysis of cytokines produced by Th2 cells, we also assessed IFN-γ and IL-2 produced by Th1 cells, and the results showed that the expressions of IL-4, IL-5, and IL-13 were upregulated in BALF and lung tissues of the mice in the OVA group. In contrast, both IFN-γ and IL-2 levels were lower

in the OVA group mice than in normal mice. IFN-γ and IL-2 are key cytokines secreted by Th1, and it has been reported that IFN-γ inhibits the production of IL-4 and regulates the differentiation and aggregation of eosinophils [46]. IL-2 is a T cell growth factor that induces T cell proliferation and causes the production of IgE antibodies [47], and inhibition of IgE production is reversed by IFN-γ. These previous results are consistent with our findings. In the present study, the expressions of IL-4, IL-5, and IL-13 were significantly downregulated in the mice with allergic asthma after treatment with *B. infantis*, and the expressions of IFN-γ and IL-2 were upregulated, indicating that *B. infantis* can be used to treat allergic asthma through regulating the balance of Th1/Th2 cytokines.

The present study only explored the mechanism of *B. infantis* in the treatment of allergic asthma by detecting the expression levels of inflammatory factors; thus, further research is needed to confirm our findings. The drug resistance of *B. infantis* should also be evaluated, and the safe concentration of *B. infantis* in humans remains to be established.

Conclusions

In the present study, allergic asthma mice developed severe AHR and airway inflammation, and subsequent changes in specific antibodies and cytokine levels in the bodies of the mice led to an imbalance of Th1/Th2 cytokines. However, after oral administration of *B. infantis*, the inflammation and expressions of specific antibodies in mice were improved and reduced, and the attenuation of the Th1 immune response and the hyperactivity of Th2 immune response were significantly alleviated. Therefore, we conclude that *B. infantis* has immunomodulatory effects and could be used in the treatment of allergic asthma through regulating the imbalance of Th1/Th2 cytokines.

Conflict of interest

None.

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