

ORIGINAL ARTICLE

Oral administration of *Clostridium butyricum* CGMCC0313-1 reduces ovalbumin-induced allergic airway inflammation in mice

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ABSTRACT

Background and objective: Probiotic bacteria can induce immune regulation or immune tolerance in patients with allergic diseases, but the underlying mechanisms are still unclear. There has been a growing interest in the use of beneficial bacteria for allergic diseases recently. This study aimed at exploring whether *Clostridium butyricum* CGMCC0313-1 (*C. butyricum*) can reduce ovalbumin (OVA)-induced allergic airway inflammation in a mouse model.

Methods: Mouse model of allergic airway inflammation induced via OVA was used in this study. *C. butyricum* was administered daily by the oral route during or after the sensitization. Airway function, pulmonary airway inflammation, mast cell degranulation, T helper (Th)-specific and anti-inflammatory cytokines, OVA-specific Ig, matrix metalloproteinase 9 (MMP-9) and histopathological alterations were examined.

Results: *C. butyricum* significantly reduced lung resistance in the asthmatic mice. Pulmonary airway inflammation, mast cell degranulation, airway remodelling and the expression of OVA-specific IgE/G1 were suppressed by oral *C. butyricum*. It also reversed the imbalance of Th1/Th2 and increased the anti-inflammatory cytokine IL-10.

Conclusion: *C. butyricum* reduces OVA-induced allergic airway inflammation in mice and might be an additional or supplementary therapy for allergic asthma.

Key words: asthma, *Clostridium butyricum*, mice, ovalbumin, probiotics.

Abbreviations: AHR, airway hyperresponsiveness; ANOVA, analysis of variance; BALF, bronchoalveolar lavage fluid; *C. butyricum*, *Clostridium butyricum* CGMCC0313-1; CFU, Colony-Forming Units; ELISA, enzyme-linked immunosorbent

SUMMARY AT A GLANCE

Clostridium butyricum CGMCC0313-1 (*C. butyricum*) was administered orally to mouse models of allergic airways disease. We found that *C. butyricum* can reduce ovalbumin (OVA)-induced allergic airway inflammation in mice.

assay; HE, haematoxylin/eosin; IACUC, Institutional Animal Care and Use Committee; IFN- γ , interferon-gamma; Ig, immunoglobulin; IL, interleukin; *L. paracasei*, *Lactobacillus paracasei* NCC 2461; *L. rhamnosus*, *Lactobacillus rhamnosus* Lcr35; MCh, acetyl- β -methylcholine chloride; MCPT-1, mast cell protease 1; mMCP-1, mouse mast cell protease 1; MMP, matrix metalloproteinase; NC, normal control; NS, normal saline; OVA, ovalbumin; PBS, phosphate-buffered saline; PC, positive control; Pre, preventive; Rrs, total lung resistance; Th, T helper; TLR, Toll-like receptor; Tre, treated.

INTRODUCTION

Allergic diseases have high prevalence and are increasing rapidly throughout the world, especially in children and in the developing countries. Asthma affects 300 million individuals worldwide, most probably as a result of changing environment and reduced exposure to microbial antigens during infancy.^{1,2} Allergic asthma is a chronic inflammatory disease featured by mucus hyperproduction, airway obstruction, airway hyperresponsiveness (AHR) and airway remodelling.^{1,3}

Alteration of microbiota contributes to the development of allergies and asthma.^{4,5} Changes in the microbiota could result in differences to downstream immune responses or immune development, both of which can affect immune mechanisms in distal mucosal sites such as the lung and result in asthma. However, deficiencies in the immune system can also affect the microbiota composition, which can affect distal mucosal sites and have a role in the development of atopic conditions.⁶ Therefore, some probiotic strains including *Lactobacilli* and *Bifidobacteria* have been intensively investigated as novel alternative options for the management of allergic diseases.^{7,8} Probiotics are

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defined as live microorganisms which confer a health benefit on the host when administered in adequate amounts.⁹ It could reduce allergic disease by modifying the immune system of the host.⁶ *Clostridium butyricum* CGMCC0313-1 (*C. butyricum*) has been widely used for improving gastrointestinal function as probiotics. However, the benefit of *C. butyricum* on allergy is rarely reported. This study aimed to explore whether *C. butyricum* can reduce allergic airway inflammation in mice. Findings from this study will contribute to a better understanding of the protective effects of the beneficial bacteria in allergic diseases.

METHODS

Experimental animal model

Male BALB/c mice of 6–8 weeks were purchased from the Laboratory Animal Center of the Fourth Military Medical University and acclimated to their new environment for 1 week. Animals were housed under conventional conditions, fed standard mouse pellets and water ad libitum. Experimental procedures were approved by the Ethics Committee for Animal Studies of the Fourth Military Medical University and performed in accordance with their guidelines of the Institutional Animal Care and Use Committee (IACUC).

Probiotics

The *C. butyricum* powder (Kexing Biotech Company limited, Weifang, China) was stored at -20°C . Drinks were prepared using normal saline (NS) only or NS plus *C. butyricum*. The concentration of *C. butyricum* was 5×10^8 CFU/mL.

Study design

The BALB/c mice were randomly assigned into four experimental groups with 10 in each group. The four groups of mice were treated as follow: the positive control (PC) group received 10 μg ovalbumin (OVA, grade V, Sigma, St Louis, MO, USA) plus 1.5 mg Alum intraperitoneally on days 7 and 14, and were then subjected to airway allergen challenges by exposure to 1% OVA aerosols for 30 min from day 21 to day 28 with an INQUA NEB plus (Omron Company limited, Dalian, China). The preventive (Pre) group and treated (Tre) group received 10 μg OVA plus 1.5 mg Alum intraperitoneally and 1% OVA inhaled, and were given 200 μL of *C. butyricum* feeding from day 1 to day 14 or from day 15 to day 28, respectively. The normal control (NC) group received 200 μL NS plus 1.5 mg Alum intraperitoneally and NS inhaled (Fig. S1, Supplementary Information).

Airway hyperresponsiveness

AHR was assessed by measuring the total lung resistance (Rrs, cm H₂O.s/mL) using the FlexiVent system (SCIREQ, Montreal, Canada) on day 29, as described previously.^{10,11} Anaesthetized (pentobarbital sodium, 90 mg/kg) and tracheostomized (stainless steel cannula, 18 G) mice were nebulized to increasing doses

1.5–100 mg/mL of acetyl- β -methylcholine chloride (MCh, Sigma). Recorded values were averaged for each dose and used to obtain dose–response curves for each group.

Bronchoalveolar lavage

Bronchoalveolar lavage was performed on day 29 after mice sacrificed. Briefly, the airways of the mice were lavaged three times with 0.8 mL of PBS via a tracheal cannula. The total number of inflammatory cell in the bronchoalveolar lavage fluid (BALF) was determined under light microscopy, and then the BALF were centrifuged at 2500 g for 5 min at 4°C . The cells at the bottom were stained with Wright Giemsa stain. One hundred cells per slide were counted to classify individual leucocyte populations using the standard morphological criteria.

Measurement of mMCP-1

To assess mast cell activation, BALF and blood samples were collected on day 29. The blood was coagulated for 2 h at room temperature and subsequently centrifuged at 4000 g for 10 min at 4°C . Serum samples were stored at -80°C for analysis. Protein expression of mouse mast cell protease 1 (mMCP-1) was determined by ELISA using the mouse MCPT-1 (mMCP-1) ELISA Ready-SET-Go! Kit (eBioscience, San Diego, CA, USA) according to the manufacturer's protocol.

Measurement of cytokines and Igs

Serum and BALF samples were collected to assay the presence of cytokines and Igs. The levels of BALF IL-4, IL-5, IL-10, IL-13, IL-17, INF- γ and serum IL-5, IL-10, IL-17, OVA-specific IgE and IgG1 were measured by ELISA Kits (RD system, Boston, MA, USA; Usn Life, Wuhan, China) following the manufacturer's protocol.

Measurement of MMP-9

Both BALF and serum matrix metalloproteinase 9 (MMP-9) levels were measured by ELISA Kits (Usn Life) in accordance with the manufacturer's instructions, and the ELISA plate was read at 450 nm using an ELISA Reader.

Histopathology

The lungs were fixed with 10% formalin for at least 24 h, after which the lungs were embedded in paraffin. Five-micrometre sections were stained with HE for inflammation, Masson's trichrome for collagen fibres, rabbit polyclonal anti- α -smooth muscle actin antibody (Abcam, Cambridge, MA, USA) for smooth muscle cells and rabbit polyclonal anti-Ki67 antibody (Abcam) for proliferating cells according to standard methods. Slides were reviewed in blinded fashion by two observers according to Ashcroft score in the following way: 0, no positive staining; 1, > 25% of cells stained positive; 2, 25–50% cells stained positive; 3, 50–75% cells stained positive.

Statistical analysis

Data were expressed as means $\pm$ SEMs. All data were analysed with SPSS 17.0 software (SPSSInc, Chicago, Illinois, USA). One-way analysis of variance (ANOVA) and Mann-Whitney U non-parametric tests were conducted to determine the statistical significance, where appropriate. A P -value of <0.05 was considered statistically significant.

RESULTS

C. butyricum inhibits AHR

We measured the total lung resistance to confirm whether *C. butyricum* could influence AHR (Fig. 1). Mice in the PC group showed a significant increase in the total lung resistance (basal, 1.5, 3, 12, 25, 50 and 100 mg/mL MCh) compared with that in the NC group. Prevention usage of *C. butyricum* resulted in a significant decrease in the total lung resistance (25 and 50 mg/mL MCh) as compared with the PC group, treatment usage of *C. butyricum* also significantly reduced the total lung resistance (12, 25, 50 and 100 mg/mL MCh).

C. butyricum reduces the infiltration of inflammatory cells

Inflammatory cell infiltration of the lung is a hallmark of allergic airways disease. To analyse the infiltration of inflammatory cells into the lung, BALF was examined for leucocyte accumulation. The BALF of the PC group showed a significant increase in the number of total inflammatory cell ($P < 0.05$), which was due to a relative increase in the number of eosinophils and neutrophils as compared with the NC group. Both the prevention and treatment usage of *C. butyricum* significantly decreased the total inflammatory cell number ($P < 0.01$) and the relative number of eosinophils and neutrophils. (Fig. 2)

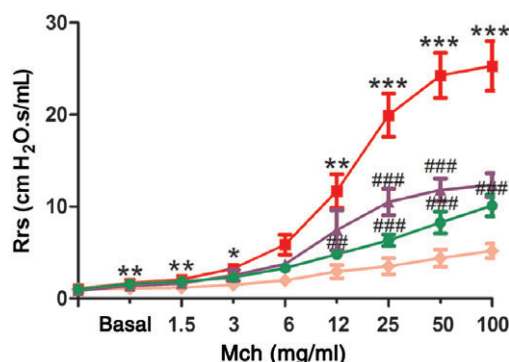


Figure 1 *Clostridium butyricum* CGMCC0313-1 (*C. butyricum*) inhibits airway hyperresponsiveness (AHR). Airway response to increasing doses of acetyl- β -methylcholine chloride (MCh) measured 24 h after the last challenge was expressed by the total lung resistance (Rrs, cm H₂O.s/mL). Basal airway response is also shown. NC, $n = 5$ for the normal control group ($\diamond$); PC, $n = 5$ for the positive control group ($\square$); Pre, $n = 5$ for the preventive group ($\triangle$); Tre, $n = 5$ for the treated group ($\circ$). Results are shown as means $\pm$ standard error of the means. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus the NC group, and # $P < 0.05$, ### $P < 0.01$, #### $P < 0.001$ versus the PC group.

C. butyricum inhibits mucosal mast cell degranulation in asthmatic mice

To examine the effect of *C. butyricum* on mast cells activity, ELISA was employed to measure mMCP-1 protein levels in both BALF (Fig. 3A) and serum (Fig. 3B). The expression of mMCP-1 was significantly increased in the mice of PC group as compared with the control group in both BALF ($P < 0.001$) and serum ($P < 0.01$). *C. butyricum* was effective in suppressing mucosal mast cell degranulation in serum of the Pre ($P < 0.001$) and Tre groups ($P < 0.01$); however, the suppressive effect was not significant in BALF ($P > 0.05$).

Effect of C. butyricum on cytokines and Igs

Cytokines (Fig. S2, Supplementary Information) and OVA-specific IgE/G1 (Fig. 4) were determined using ELISA Kits. Compared with the NC group, the levels of inflammatory cytokines in the BALF (Fig. S2A–D: IL-4, IL-5, IL-13 and IL-17, Supplementary Information) and serum (Fig. S2G–H: IL-5, IL-17, Supplementary Information) were significantly increased in the PC group and decreased in the Pre and Tre groups. However, the BALF IL-13 of the Pre group was not significantly different from that of the PC group ($P > 0.05$). Both INF- γ (Fig. S2E, Supplementary Information) and IL-10 (Fig. S2F,I, Supplementary Information) in the PC group were decreased compared with the counterparts of the NC group. They increased in the groups that received *C. butyricum* orally except for BALF IL-10 in the Tre group.

Compared with the NC group, IgE (Fig. 4A) and IgG1 (Fig. 4B) were significantly elevated in the PC group ($P < 0.001$). However, they decreased dramatically in the Pre and Tre groups, especially the level of IgG1 in the Tre group.

C. butyricum suppresses the expression of MMP-9 in asthmatic mice

MMP-9 was highly correlated with airway remodelling of asthma. Therefore, we detected the levels of MMP-9 in BALF (Fig. 5A) and serum (Fig. 5B) to analyse the effect of *C. butyricum* on the expression of MMP-9. The mice of the PC group had stronger expression of MMP-9 in BALF ($P < 0.01$) and serum ($P < 0.05$) than that of the NC group. MMP-9 decreased in the groups that received *C. butyricum* in BALF ($P < 0.01$) and serum ($P < 0.05$) compared with the PC group (Fig. 5).

C. butyricum reduces histopathological changes in asthmatic mice

To examine the effect of *C. butyricum* on histopathological changes of the lungs, histopathological and immunohistochemical analyses of lung tissues were performed according to Ashcroft score (Figs 6,S3 (Supplementary Information)). Inflammation scope ($P < 0.001$), the number of collagenous fibres ($P < 0.001$), airway smooth muscle ($P < 0.001$) and proliferating cells ($P < 0.01$) in the lung sections of PC mice were significantly increased as compared with those of the NC group. Oral administration of *C. butyricum*, however, reduced inflammation score, the

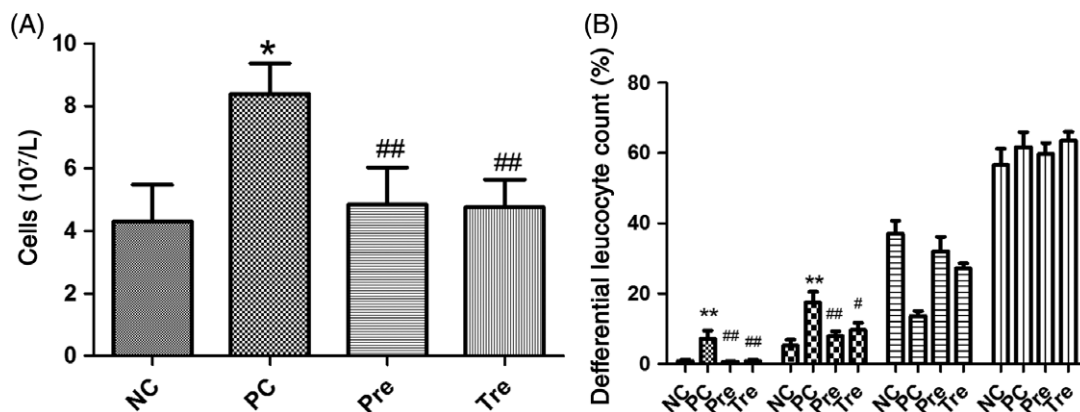


Figure 2 *Clostridium butyricum* CGMCC0313-1 reduces the infiltration of inflammatory cells. The total inflammatory cells number (A) were counted (10⁷/L) in the bronchoalveolar lavage fluid (BALF) by the haemocytometer. Differential leucocyte (B) counts are shown as percentages of the total cell count for each group. NC, *n* = 10 for the normal control group; PC, *n* = 10 for the positive control group; Pre, *n* = 10 for the preventive group; Tre, *n* = 10 for the treated group. Results are shown as means $\pm$ standard error of the means. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus the NC group, and #*P* < 0.05, ##*P* < 0.01, ###*P* < 0.001 versus the PC group. [checkered] Eosinophil; [cross-hatched] neutrophil; [horizontal lines] lymphocyte; [vertical lines] macrophage.

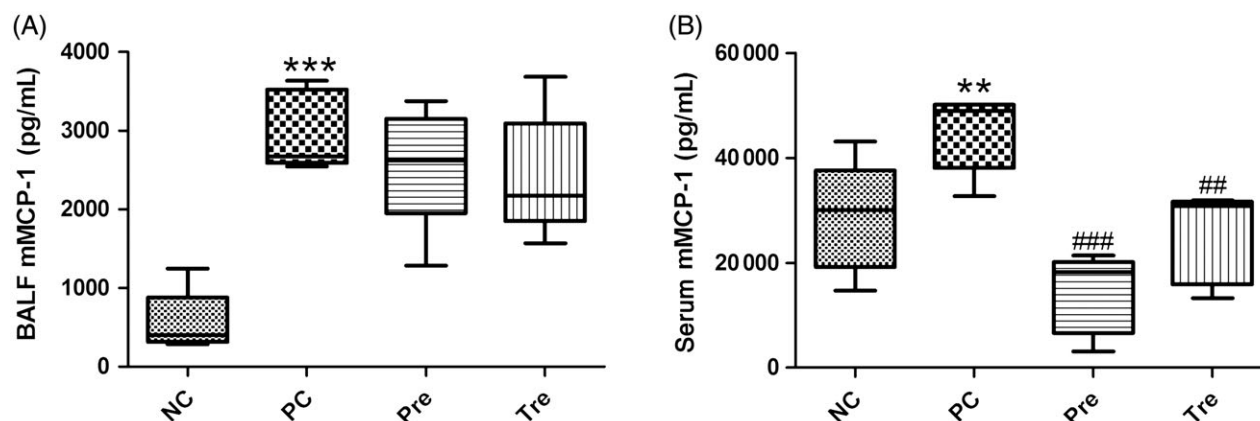


Figure 3 *Clostridium butyricum* CGMCC0313-1 (*C. butyricum*) inhibits mucosal mast cell degranulation in asthmatic mice. Mouse bronchoalveolar lavage fluid (BALF) (A) and serum (B) were isolated on day 29 and mouse mast cell protease-1 (mMCP-1) levels were measured by ELISA. NC, *n* = 10 for the normal control group; PC, *n* = 10 for the positive control group; Pre, *n* = 10 for the preventive group; Tre, *n* = 10 for the treated group. Results are shown as means $\pm$ standard error of the means. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus the NC group, and #*P* < 0.05, ##*P* < 0.01, ###*P* < 0.001 versus the PC group.

number of collagenous fibres, airway smooth muscle and proliferating cells in the Pre and Tre groups.

DISCUSSION

In this study, the total number of inflammatory cells in BALF was significantly increased in the PC group as compared with the NC group. Inflammation scope, the number of collagenous fibres, airway smooth muscle and proliferating cells were increased in the lung section of PC mice. Additionally, the lung resistance and OVA-specific IgE/G1 were significantly increased in the asthmatic mice. Therefore, we successfully mimicked OVA-induced allergic airway inflammation in our mouse model.

Various strains of probiotics have been used in animal models of asthma. In a mouse model of birch pollen allergy, perinatal administration of *Lactobacillus*

paracasei NCC 2461 (*L. paracasei*) to pregnant/lactating mothers protects their offspring against the development of airway inflammation by activating regulatory pathways, likely through TLR2/4 signalling.¹² Oral administration of *Lactobacillus rhamnosus* Lcr35 (*L. rhamnosus*) to mice attenuates airway inflammation of allergic asthma and induces immune regulation by a CD4⁺CD25⁺Foxp3⁺Treg cell-mediated mechanism.^{13–15} A recent study suggests that for an OVA-sensitized/challenged mouse, oral *L. rhamnosus* GG (LGG) can not only reduce lung resistance and pulmonary inflammation, but also suppress the expression of T helper 2 (Th2) cytokines, MMP-9 and OVA-specific IgE.¹⁶ Similarly, oral administration of *C. butyricum* to asthmatic mice is effective in reducing allergic airway inflammation and remodelling in our study.

Allergy-induced airway inflammation and subsequent airway remodelling involve various cells including eosinophils, neutrophils, macrophage, T

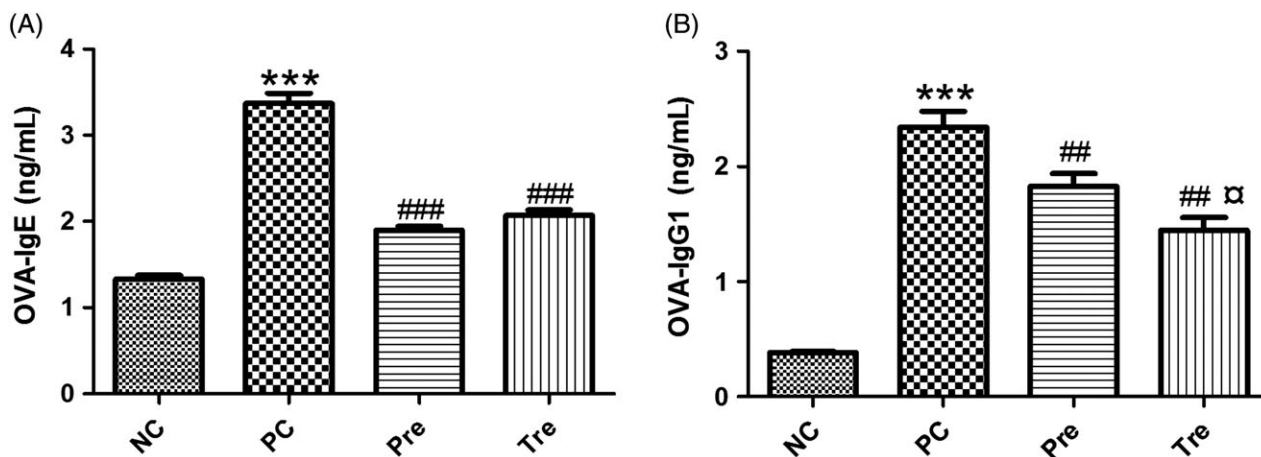


Figure 4 The effect of *Clostridium butyricum* CGMCC0313-1 (*C. butyricum*) on Igs. The expression of ovalbumin (OVA)-specific IgE/G1(A,B) was determined using the ELISA Kits. NC, $n = 10$ for the normal control group; PC, $n = 10$ for the positive control group; Pre, $n = 10$ for the preventive group; Tre, $n = 10$ for the treated group. Results are shown as means $\pm$ standard error of the means. *** $P < 0.001$ versus the NC group, ## $P < 0.01$, ### $P < 0.001$ versus the PC group and $^{\circ}P < 0.05$ versus the Pre group.

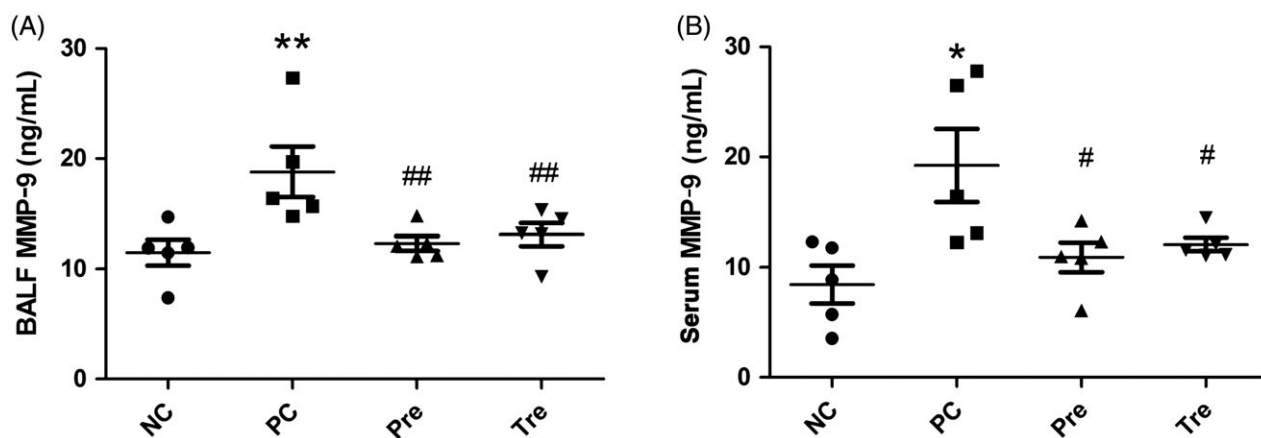


Figure 5 *Clostridium butyricum* CGMCC0313-1 (*C. butyricum*) suppresses the expression of matrix metalloproteinase 9 (MMP-9) in the mouse model of allergic airways inflammation. Bronchoalveolar lavage fluid (BALF) (A) and serum (B) MMP-9 were measured by ELISA Kits. NC, $n = 10$ for the normal control group; PC, $n = 10$ for the positive control group; Pre, $n = 10$ for the preventive group; Tre, $n = 10$ for the treated group. Results are shown as means $\pm$ standard error of the means. * $P < 0.05$, ** $P < 0.01$ versus the NC group, # $P < 0.05$, ## $P < 0.01$ versus the PC group.

lymphocytes and mast cells.^{17,18} Infiltration of mast cells into the airway smooth muscle cell layer of allergic asthmatic patients could result in increased airway resistance and disease severity, which is a key feature of asthma.^{19–21} A host of pleiotropic autacoid mediators, proteases and cytokines will be released in response to mast cell degranulation.^{22,23} It was reported that mMCP-1 was a key protease of mast cell degranulation and could enhance airway narrowing in mice through effects on the epithelium.^{24,25} In our study, the expression of mMCP-1 was significantly increased in the asthmatic mice. Our results are consistent with those previous studies.^{26–28} We found that *C. butyricum* was effective in inhibiting mucosal mast cell degranulation, as shown by the significant decrease in the protein levels of mMCP-1 in serum. There is no clear explanation why *C. butyricum* does suppress the expression of mMCP-1 only in serum but not in the BALF, further research is warranted.

Asthma was considered a Th2 cell-mediated disease. Pro-inflammatory and Th2 cytokines including IL-4, IL-5, IL-13 and IL-17 drive the accumulation of eosinophils in the lung of asthmatic patients, which are closely related to the severity of asthma.²⁹ Interferon-gamma (IFN- γ) released by Th1 can inhibit the development of Th2 and alleviate the symptoms of asthma.³⁰ Th17 cells release IL-17^{31,32} and recruit neutrophils which attract eosinophils indirectly, further exacerbating asthma attacks.^{29,33} Subjects with asthma have reduced levels of the anti-inflammatory cytokine IL-10 in the sputum.³⁴ CD4⁺CD25⁺Treg can induce immune tolerance in an IL-10-dependent manner. Previous study showed that mice with less expression of IL-10 have decreased CD4⁺CD25⁺Treg and present serious allergic inflammation.^{35,36} Increased Th2 but decreased Th1 and IL-10 were observed in our study. Therefore, the beneficial effect of *C. butyricum* are possibly achieved through the regulation of Th1/Th2 and



Figure 6 Ashcroft score of histopathological changes. Slides were reviewed in blinded fashion by two observers independently and slides were scored on the basis of the percentage of positive stained cells according to Ashcroft score in the following way: 0, no positive staining; 1, <25% of cells stained positive; 2, 25–50% cells stained positive; 3, 50–75% cells stained positive. Results are presented as average score of 5–10 animals/group. ■, HE; ▨, Masson; ▩, α-smooth muscle; ▪, Ki67. ** $P < 0.01$, *** $P < 0.001$ versus the normal control group, ## $P < 0.01$, ### $P < 0.001$ versus the positive control group, and ° $P < 0.05$ versus the preventive group.

CD4⁺CD25⁺Treg in an IL-10-dependent manner; however, we lack the data in CD4⁺CD25⁺Treg and we will explore it in the future study.

MMPs are members of a family of enzymes that cleave extracellular matrix proteins.¹⁶ Among all MMPs, MMP-9 is closely related to asthmatic pathologies.^{37,38} The metalloprotease increases the inflammatory cell recruitment and the release of inflammatory cytokines, which exacerbates airway inflammation.³⁹ In addition, MMP-9 induces the degradation of the extracellular matrix, resulting in airway remodelling in asthma.⁴⁰ Asthma patients showed significantly elevated MMP-9 expression compared with the healthy controls.^{41,42} Moreover, MMP-9 levels are correlated with severity of asthma symptoms.^{43,44} Particularly, mice deficient in MMP-9 manifest decreased airway inflammation and AHR.⁴⁵ In our study, *C. butyricum* was effective in suppressing the expression of MMP-9.

We expected the preventive effect works better than the treatment. However, in all our experiments, prevention and treatment seem to work equally well except for the AHR experiments, in which the treatment seems to be more efficacious. The possible explanation for this is the use of the acute model in our study, in which model the phenotypic features of the disease may be not fully manifested.

To our knowledge, this is the first report in which the preventive and therapeutic effects of *C. butyricum* on allergic airway inflammation were investigated. We found that *C. butyricum* was effective in suppressing pulmonary airway inflammation, mast cell degranulation, OVA-specific IgE/G1 and airway remodelling in our study. Additionally, *C. butyricum* can reduce lung resistance, reverse the imbalance of Th1/Th2 and increase the expression of the anti-inflammatory cytokine IL-10. In conclusion, we confirmed the beneficial effect of *C. butyricum* on mouse model of allergic

airway inflammation, and we believe that *C. butyricum* might be an additional or supplementary therapy not only for allergic airway inflammation but also for other allergic diseases.

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Supplementary Information

Additional supplementary information can be accessed via the [html](#) version of this article at the publisher's website.

Figure S1 Time schedule of the allergic asthma mouse model.

Figure S2 The effect of *Clostridium butyricum* CGMCC0313-1 on cytokines.

Figure S3 *Clostridium butyricum* CGMCC0313-1 reduces histopathological changes in asthmatic mice.

SUPPLEMENTARY INFORMATION

Oral administration of *Clostridium butyricum* CGMCC313-1 reduces ovalbumin-induced allergic airway inflammation in mice

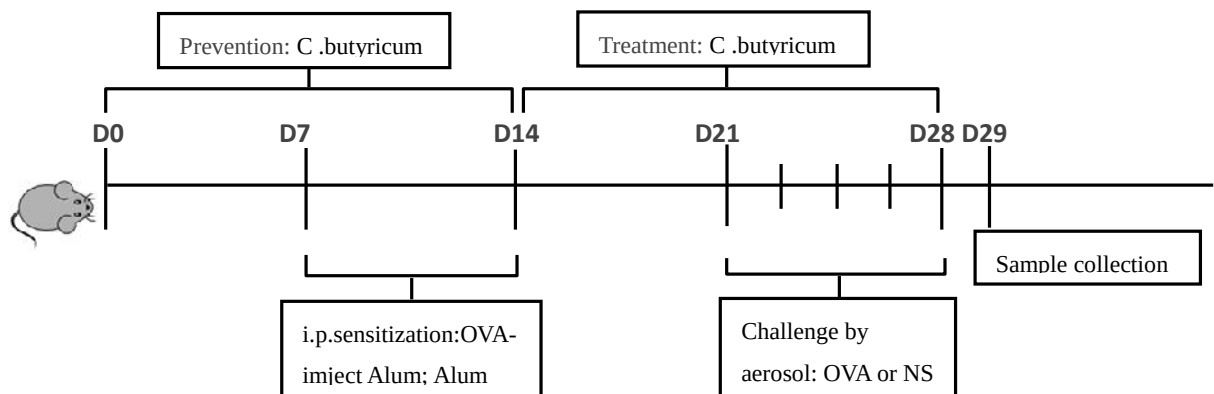
Juan ZHANG^{1*}, Zhao-Ling SHI^{1*}, Ming-Hua ZENG¹, Chun WEI¹, Hai-Xia WU¹, Meng-Yun LIU², Jian-Qiong HUANG², Yue-Jie ZHENG², Xin SUN¹

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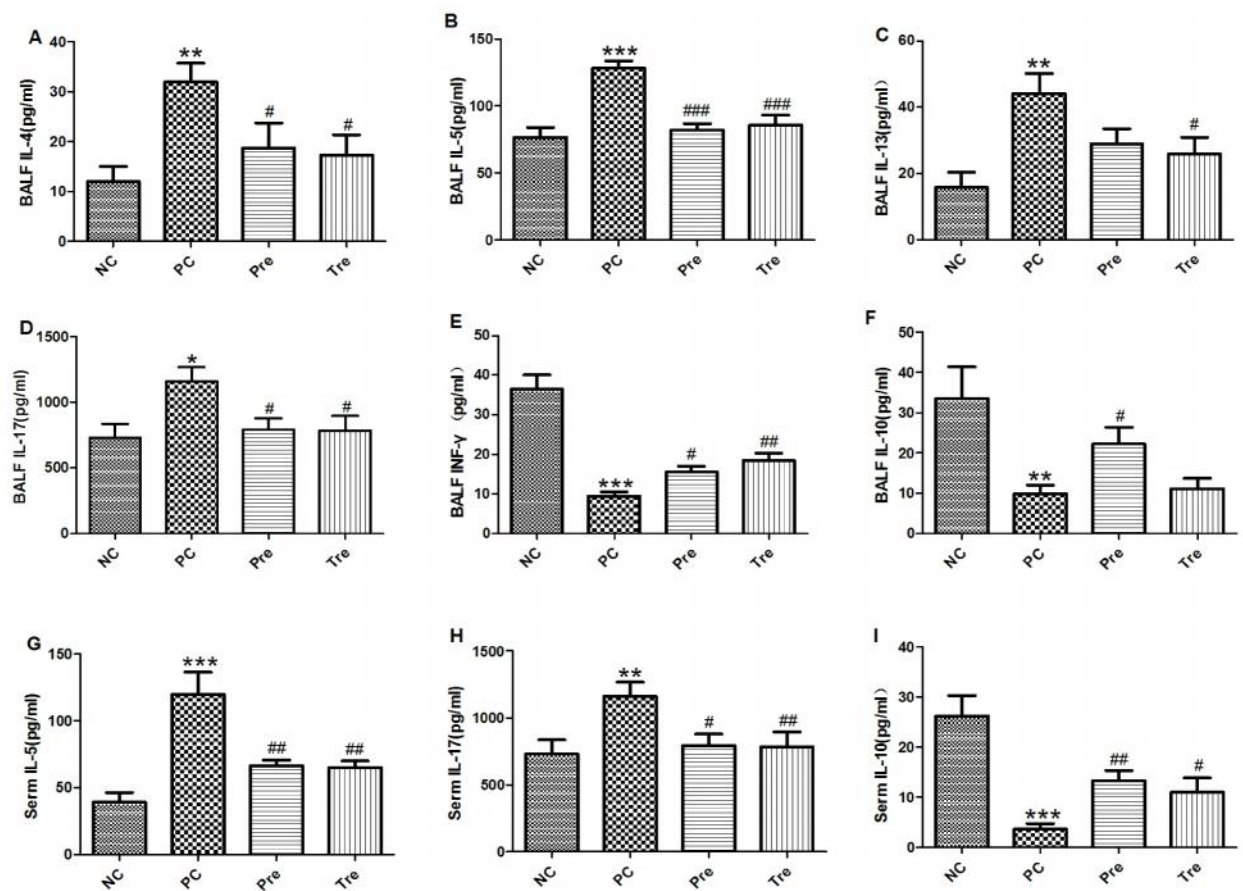
*JZ and Z-L S contributed equally to this study.

Figure S1 Time schedule of the allergic asthma mouse model.



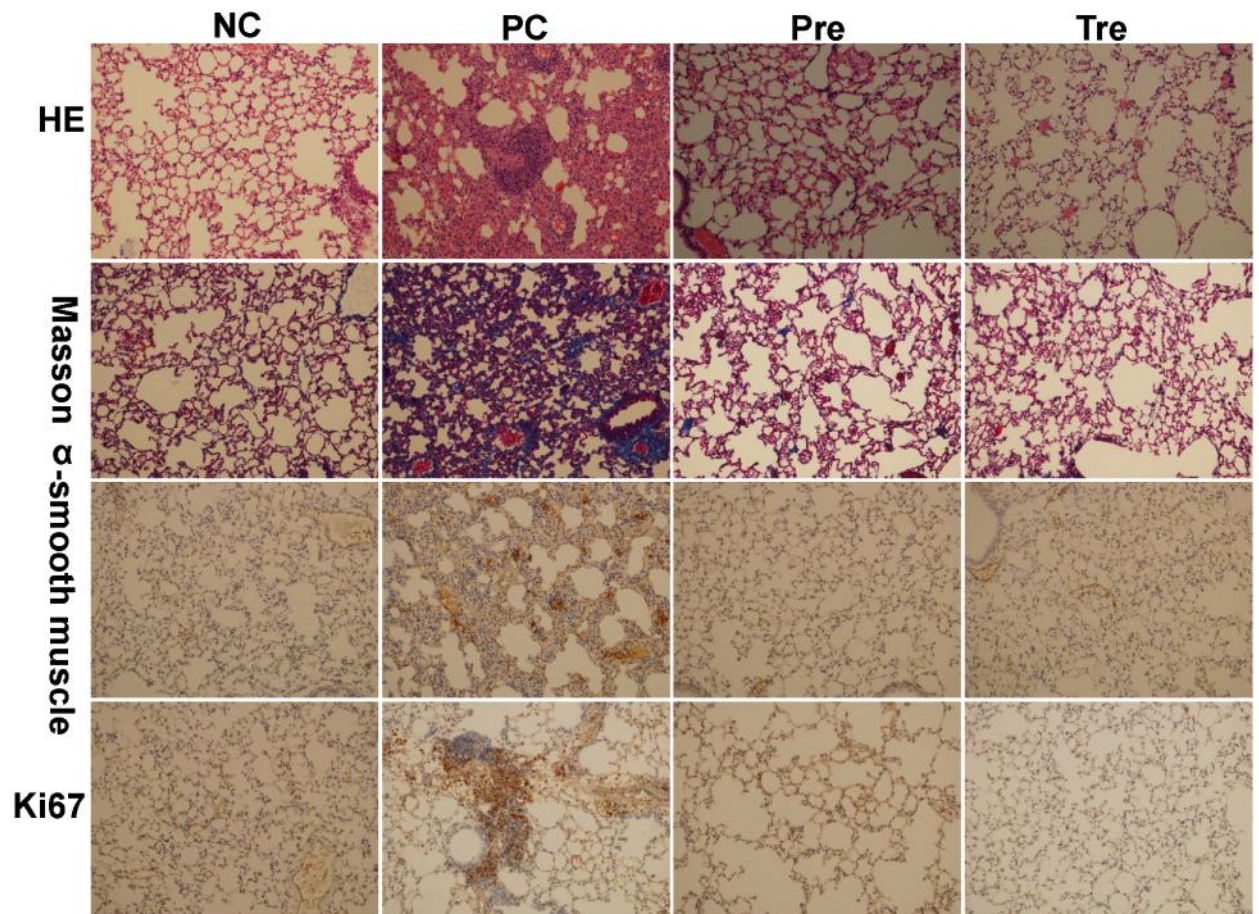
Male BALB/c mice were sensitized intraperitoneally to OVA-imject Alum or only Alum on day 7 and 14, and challenged daily by aerosolised OVA or NS from day 21 to day 28. The animals received *C. butyricum* feeding from day 1 to day14 or day15 to day 28 by oral gavage. OVA, ovalbumin; *C. butyricum*, *Clostridium butyricum* CGMCC313-1; NS, Normal saline

Figure S2 The effect of *C. butyricum* on cytokines



The expression of cytokines (A-I) was determined using the ELISA Kits. NC, n=10 for the normal control group; PC, n=10 for the positive control group; Pre, n=10 for the preventive group; and Tre, n=10 for the treated group. Results are shown as means $\pm$ SEMs. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus the normal control group, and # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ versus the positive control group. ELISA, enzyme-linked immunosorbent assay; *C. butyricum*, *Clostridium butyricum* CGMCC0313-1

Figure S3 *C. butyricum* reduces histopathological changes in asthmatic mice.



Representative photomicrographs for 5-10 mice/group of histological staining for inflammation, collagen fibers, smooth muscle cells and proliferating cells in lung tissue. Original magnification, 200X.