

Both living and dead *Faecalibacterium prausnitzii* alleviate HDM-induced allergic asthma through the modulation of gut microbiota and short-chain fatty acid (SCFAs) production

Running title: Living and dead *Faecalibacterium prausnitzii* alleviate HDM-induced allergic asthma

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Abstract

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1002/jsfa.11207](https://doi.org/10.1002/jsfa.11207)

Background: Asthma is increasingly prevalent worldwide, and novel strategies to prevent or treat this disease are needed. Probiotic intervention has recently been reported to be effective for asthma prevention. Here, we explored the effects of *Faecalibacterium prausnitzii* on the development of allergic airway inflammation in a murine model of house dust mite (HDM)–induced allergic asthma.

Results: Supplementation with living and dead *F. prausnitzii* blocked eosinophils, neutrophil, lymphocytes, and macrophages influx and alleviated the pathological changes. Moreover, both living and dead *F. prausnitzii* administration decreased the levels of IL-4, IL-5, IL-13, and IgG1, elevated the regulatory T cells (Tregs) ratio, improved the microbial dysbiosis, and enhanced SCFAs production. The network correlation analysis revealed that the immune indicators were strongly associated with SCFAs production. Based on the linear discriminant analysis effect size (LEfSe), *Turicibacter* was found as the core genus related to HDM-induced asthma. Living *F. prausnitzii* treatment enriched *Faecalibaculum*, *Dubosiella*, and *Streptococcus*, while dead *F. prausnitzii* treatment increased *Muribaculaceae* and *Parabacteroides*. Interestingly, both living and dead *F. prausnitzii* administration enriched *Lachnospirillum* and normalized the pathways involving carbohydrate and lipid metabolisms, which might be related to SCFAs production.

Conclusion: *F. prausnitzii* exerts an anti-asthmatic effect partly by gut microbiota modulation and SCFAs production, suggesting its promising potential as a probiotic agent for allergic asthma prevention.

Keywords: *F. prausnitzii*; House dust mite; Asthma; Short-chain fatty acids; Gut microbial composition

1. Introduction

Asthma is a long-term inflammatory respiratory disease, characterized by

reversible airflow obstruction and easily triggered bronchospasms.¹ The severity and prevalence of asthma have substantially increased its public health burden worldwide.² According to the estimates from WHO, about 235 million people suffer from asthma.³ As predicted by the disease prevalence over the last decades, the number of asthmatic patients worldwide will increase by 100 million people by 2025.⁴ Previous epidemiological studies revealed that estimates on the asthma prevalence in China ranged from 1.2% to 5.8% in the investigated area.⁵ Furthermore, the investigators estimated that 45 million adults suffer from asthma by extrapolating epidemiological data to the entire Chinese population.

Allergic asthma is known to be caused by the excessive activation of helper T cells to differentiate into T helper type 2 (Th2) cells, which impact the inflammation and tissue damage caused by asthma through the production of multiple cytokines, such as IL-4, IL-5, and IL-13.^{6, 7} Asthma is difficult to diagnose due to the inherent heterogeneity of asthma symptoms and the various influencing factors, including lung function, risk alleles, and environmental exposure.⁸ Although asthma is incurable, symptoms can typically be improved. Corticosteroids are well accepted to be the most effective drugs for improving asthma symptoms.⁹ However, long-term use of inhaled corticosteroids brings a risk of adverse reactions including thrush, the development of cataracts, and a slightly slower rate of growth.¹⁰⁻¹² Therefore, it is urgent to develop effective and safe therapeutic agents for preventing allergic asthma.

Emerging evidence suggests that the composition and function of the human microbiome play a crucial role in the onset of asthma, supporting the concept that the

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bacterial community composition can shape the development of immune function that promotes or prevents allergic sensitization.^{13, 14} Due to the importance of the microbiota in the development of asthma, increasing attention has recently been paid to the application of microbiota-modulating therapies, including the use of probiotics.¹⁵ *F. prausnitzii* is considered a candidate probiotic bacterium which has been proposed as an intestinal health sensor.¹⁶ *F. prausnitzii* is one of the predominant bacterial groups in human feces (accounts for 5~15% of total fecal bacteria) and can shape gut immune responses.¹⁷ A decreased gut abundance of *F. prausnitzii* is strongly associated with inflammation-related diseases, such as inflammatory bowel diseases (IBD).¹⁸⁻²⁰ Although the effect of *F. prausnitzii* on IBD has been well evaluated, its beneficial role in preventing allergic asthma has not been investigated yet.

The present study aimed to explore the protective effect of *F. prausnitzii* on asthma. To make a murine model mimicking human allergic asthma, mice were intranasally administered a dose of 100 µg of *Dermatophagoides pteronyssinus* dissolved in 25 µL of PBS. The hallmarks of asthma phenotype, immune indicators, Tregs level, and SCFAs production were measured. In addition, we further evaluated the alteration of gut microbiota and predicted the microbial functional pathways to investigate the potential mechanism underlying the protective effect of *F. prausnitzii* treatment on allergic asthma.

2. Materials and methods

2.1. Bacterial culture

F. prausnitzii strain A2-165 (DSMZ 17677, Braunschweig, Germany) pure cultures were grown on yeast extract, tryptone, sodium acetate, and glucose (YCFA)

agar plates in an anaerobic workstation. The cultured bacterial cells and the supernatants were prepared at the anaerobic workstation using a filter upper cup (0.22 μm , Zhejiang Sorfa Life Science Research Co., Ltd., Zhejiang, China). The cell solutions were prepared by suspending the cultured bacterial cells in PBS at an approximated cell density of 4×10^9 CFU/mL. The dead cells were obtained using mild heating treatment (80 °C, 30 min), and were immediately stored at -80 °C.

2.2. Animals and experimental protocol

Female 6-week-old BalB/c mice (14-16 g, SPF grade, Certificate No. SCXK (Zhe) 2018-0001) were housed at a suitably controlled temperature (23 ± 2 °C) and humidity (55 ± 10 %) under 12-h light/dark cycles (Beijing Vital River Laboratory Animal Technology CO., Ltd., Beijing, China). The mice were fed water and a commercial stock diet *ad libitum*. All experimental procedures involving animals were approved by the Animal Care and Use Committee of the Jiangnan University (JN. No20181215b0500125).

Mice were randomly divided into the following groups (n = 6 mice per group, three mice per cage): control group (treated with vehicle), model group (treated with vehicle), supernatant group (treated with supernatant of *F. prausnitzii*), dead bacterium group (DBG, treated with dead *F. prausnitzii*), and living bacterium group (LBG, treated with living *F. prausnitzii*). Mice were housed in individually ventilated cages under specific-pathogen-free conditions. The murine model of asthma was established according to a previous study with minor modifications.²¹ Briefly, after an acclimatization period of five days, the mice were intranasally sensitized with 100 μg of *Dermatophagoides pteronyssinus* extract (Hamburg, Germany) dissolved in 25 μL of PBS on days 0, 7, 14,

and 21; control animals received 25 μ L of PBS only. From day 0 to day 22, vehicle (PBS), the supernatant of *F. prausnitzii*, a dead *F. prausnitzii* suspension ($\sim 1 \times 10^9$ CFU/mL), and a living *F. prausnitzii* suspension ($\sim 1 \times 10^9$ CFU/mL) of 0.25 mL were orally administrated once a day. The experimental flow chart is shown in Figure 1A.

2.3 Differential cell counts in bronchoalveolar lavage fluid (BALF)

To collect the BALF samples, the airways of mice were lavaged three times with 0.5 mL of PBS using a tracheal tube. Total cell counts and differential cell counts in BALF were measured using the trypan blue staining method and Diff-Quick method, respectively (Slabio, Beijing, China). Macrophages, eosinophils, neutrophils, and lymphocytes were identified based on the staining and morphological characteristics.

2.4 Lung histology

After inflated with 0.1% agarose, the left lung was removed, and immediately fixed with 4% (w/v) phosphate-buffered paraformaldehyde for 36 h. Then, the fixed lung tissues were dehydrated using a graded series of ethanol (70 to 100%), hyalinized with xylene, and embedded in paraffin. Sections (5 μ m-thick) were cut and stained using hematoxylin and eosin (H&E) to evaluate the lung tissue pathology.

2.5 Determination of BALF inflammatory cytokines and serum IgG1 levels

The BALF IL-4, IL-5, IL-13, and serum IgG1 levels were determined using reagent kits (Elisa kits of IL-4, IL-5, and IL-13, SenBeiJia Biological Technology Co., Ltd., Nanjing, China; Elisa kit of IgG1, Chondrex Inc., Redmond, WA).

2.6 Flow cytometry analysis

Single-cell suspensions were immediately prepared from spleen after the sacrifice of mice. Tregs staining was performed using a reagent kit (eBioscience Mouse

Regulatory T cell staining Kit #1, Invitrogen Corporation, Carlsbad, CA, USA).

2.7 Determination of colonic SCFAs levels

The analysis of SCFAs was carried out referring to a previous study.²² SCFAs concentrations were determined using the external standard method.

2.8 16S rRNA amplicon sequencing of gut microbiota

The total fecal DNA was acquired using a FastDNA Spin Kit for Feces (MP Biomedicals, Santa Ana, USA). The V3-V4 region of 16S rRNA was amplified and sequenced using the Illumina Miseq platform (Illumina, Santiago, CA, USA). The multiplex sequenced reads were deposited in the NCBI Sequence Read Archive under BioProject (PRJNA705641).

2.9 Statistical analysis

Statistical analysis was performed using SPSS software (version 20.0). The results were expressed as means $\pm$ SEM. The statistical difference was evaluated using one-way ANOVA and Duncan's new multiple range tests (* $P < 0.05$, ** $P < 0.01$ vs. model group). Microbiota-related analyses were carried out using R (version 3.5.0), QIIME2, and online platform (<http://huttenhower.sph.harvard.edu/galaxy>).

3. Results

3.1 Living and dead *F. prausnitzii* treatment prevented asthma phenotype in an HDM-induced murine model

As shown in Figure 1B, HDM intervention evidently increased the numbers of eosinophils, neutrophils, lymphocytes, and macrophages when compared to the control group ($P < 0.01$). However, both living and dead *F. prausnitzii*-treated mice exhibited

markedly lower numbers of eosinophils, neutrophils, lymphocytes, and macrophages in BALF compared to the asthmatic mice without treatment ($P < 0.05$). Although there was no statistical difference in the numbers of eosinophils, neutrophils, lymphocytes and macrophages between the supernatant group and the model group, a decreased tendency could obviously be observed.

Furthermore, we observed histological changes in the lungs of mice. As seen in Figure 1C, the model group mice showed apparent hemorrhage and inflammatory cell infiltration, compared with the mice that received no treatment. However, these histological changes were effectively improved by dead and living *F. prausnitzii* treatment. Although the supernatant of *F. prausnitzii* alleviated the hemorrhage in the lungs, inflammatory cell infiltration could still be observed.

3.2 Living and dead *F. prausnitzii* treatment regulated the immune responses in asthmatic mice

To explore the effect of *F. prausnitzii* treatment on immune responses in asthmatic mice, we determined the levels of IL-4, IL-5, IL-13, IgG1, and Tregs. As shown in Figure 2A-C, the model group showed a significantly elevated production of IL-4, IL-5 and IL-13 in comparison with the control group ($P < 0.01$). However, treatment with living or dead *F. prausnitzii* could remarkably decrease the levels of IL-5 and IL-13 compared with those of the model group ($P < 0.01$). Further, only living *F. prausnitzii* could notably decrease the production of IL-4 ($P < 0.05$). Moreover, there was a significant decrease in the level of HDM-specific IgG1 in the LBG and DBG groups, compared with that in the model group (Figure 2D, $P < 0.01$). The supernatant of *F.*

prausnitzii reduced the levels of IL-4, IL-5, IL-13, and IgG1 compared with the model group, but no statistical differences were observed. The spleen Tregs were further determined to evaluate the inflammation status. As shown in Figure 2J, the LBG group showed a significant higher level of Tregs than the model group ($P < 0.05$). However, the DBG and supernatant groups exhibited a comparable level of Tregs to that of the model group.

3.3 Living and dead *F. prausnitzii*-treated mice shared a different gut microbial composition

To determine whether the gut microbiota composition is related to the protective effect of *F. prausnitzii* on allergic asthma, we utilized 16S rRNA-amplicon sequencing for bacterial identification. The model group showed a dramatic alteration of the gut microbial structure compared with that of normal control mice, with a decreased abundance of *Bacteroidetes* phylum and an increased abundance of *Firmicutes* phylum (Figure 3A). Alpha diversity was evaluated based on the observed species and Shannon indexes to measure the diversity of species within the mice gut. As shown in Figure 3B, there was a significant difference in observed species and Shannon index between the model group and the probiotic-treated groups (DBG and LBG groups). Principal component analysis (PCA) reflected a beta diversity (Figure 3C). The top twenty-five abundant genera are shown in Figure 3D.

LEfSe analysis was further applied to find the intestinal flora biomarkers among all groups (Figure 3E). The cladogram indicated the different taxa with an LDA score >2 and $P < 0.05$. The relative abundance of statistically different taxa is shown in Figure

3F. The model group mice showed a higher abundance of *Turicibacter* and a lower abundance of *Lachnoclostridium* than those of the control group, whereas the DBG and LBG groups had significantly reversed results ($P < 0.05$). Moreover, dead *F. prausnitzii* remarkably increased the abundance of *Parabacteroides* and *Muribaculaceae*, while living *F. prausnitzii* significantly enriched the abundance of *Faecalibaculum*, *Streptococcus*, and *Dubosiella*. However, there was no statistical difference in these taxa between the model group and the supernatant group, except for the genus *Turicibacter*.

3.4 Living and dead *F. prausnitzii* treatment altered gut microbial functional pathways and SCFAs production in asthmatic mice

To reveal the effect of *F. prausnitzii* treatment on the alteration of gut microbial functional pathways, phylogenetic investigation of communities by reconstruction of unobserved states (PICRUST) analysis was performed. A PCA of the functional pathways distinctly clustered the model group from the DBG and LBG groups (Figure 4A-B). As shown in Figure 4C-D, twenty-one pathways were significantly differentially abundant between the DBG and model groups, and thirteen significantly different pathways were observed between the LBG and model groups ($P \leq 0.05$, effect size ≥ 1). Many of the functional pathways with a higher abundance in LBG and DBG groups belong to the carbohydrate metabolism (i.e., fructose and mannose metabolism, pyruvate metabolism, and pentose phosphate pathway), lipid metabolism (i.e., biosynthesis of unsaturated fatty acids, fatty acid biosynthesis, and linoleic acid metabolism), and metabolism of cofactors and vitamins (i.e., folate biosynthesis, biotin

metabolism, and lipoic acid metabolism).

We further examined the difference in SCFAs production between the model group and probiotic-treated groups. The levels of acetate, propionate, and butyrate were significantly decreased in asthmatic mice compared to those of the control group (Figure 4E-G, $P < 0.01$). However, living or dead *F. prausnitzii*-treated mice showed higher contents of propionate and butyrate than the asthmatic mice without treatment. Further, there was no statistic difference in the level of acetate between the model and the treatment groups (Supernatant, DBG, and LBG).

3.5 The network analysis among different variations

Interaction network analysis was performed to explore the association among BALF differential cell counts, immune indicators, SCFAs production, and gut microbiota. As shown in Figure 5, the core microbiome and SCFAs production were closely correlated with the BALF differential cell counts and immune indicators ($P < 0.05$, $r > 0.9$, or $r < -0.9$). Propionate was significantly negatively related to the numbers of lymphocytes, eosinophils, and macrophages, as well as the contents of IL-4, IL-5, and IL-13 ($P < 0.05$, $r < -0.9$). Acetate was only negatively related to IL-4 level. However, butyrate exhibited no statistically different correlation with BALF differential cell counts and immune indicators.

Network analysis revealed that the differential genus not only affected SCFAs production, but also regulated BALF differential cell counts and immune indicators. The relative abundance of *Parabacteroides* and *Muribaculaceae* showed a positive correlation with the production of butyrate, and the *Lachnoclostridium* was positively

related to the propionate level ($P < 0.05$, $r > 0.9$). Moreover, *Lachnoclostridium* and *Muribaculaceae*, the core microbiome in the DBG group, were negatively related to the number of neutrophils and eosinophils ($P < 0.05$, $r < -0.9$). Interestingly, *Dubosiella*, the differential genus in the LBG group, showed a positive association with the Tregs level ($P < 0.05$, $r > 0.9$). Additionally, the relative abundance of *Streptococcus* was negatively related to the relative abundance of *Turicibacter* ($P < 0.05$, $r < -0.9$), which was the core genus in the model group. These results indicated that, during the intervention, the amelioration of asthma was closely associated with SCFAs production and the regulation of distinct genus in the gut.

4. Discussion and conclusion

Emerging evidence suggests that the microbiome plays a pivotal role in the progress of airway disease. A distinct insight coming from germ-free (GF) animals revealed the mechanistic role of the microbiome in the onset of allergic airway inflammation.²³ This insight firmly supports the concept that the balance between immune tolerance and inflammation within tissues is partly influenced by the crosstalk between immune cells and the microbiome.²⁴⁻²⁶ The disruption of communication between the host and the microbiome can be detrimental to the efficient maturation of the immune system, resulting in a risk of development of inflammatory disorders, such as asthma and respiratory infections.^{27, 28} Therefore, strategies to ensure the proper development of the immune system and to preserve a health-promoting microbiota are considered in the prevention of asthma and allergy-related diseases.

The living *F. prausnitzii* can still maintain metabolism and normal physical

activities in the host intestinal tract, showing the potential to impact the gut microbial micro-ecology, whereas the dead *F. prausnitzii* is inanimate and can only be considered “bioactive compounds”. Moreover, Quévrain et al. have found an anti-inflammatory protein from *F. prausnitzii*, suggesting that the dead *F. prausnitzii* might exert bioactive function.²⁹ Therefore, to determine whether *F. prausnitzii* itself, its components or its secreted metabolites exert anti-asthmatic effect, we tested freshly cultured *F. prausnitzii*, heat-killed *F. prausnitzii*, and the supernatant of *F. prausnitzii* in a well-established HDM-induced asthma mouse model. Our findings demonstrated that living and dead *F. prausnitzii* treatment significantly reduced BALF differential cell counts (Figure 1B, $P < 0.05$). Moreover, H&E staining indicated that the mice treated with dead or living *F. prausnitzii* showed much lower inflammatory cell infiltration and hemorrhage than the asthmatic mice without treatment (Figure 1C), suggesting that dead *F. prausnitzii* was as effective as living *F. prausnitzii* on attenuating the phenotypes of HDM-induced asthma. Although the supernatant of *F. prausnitzii* didn't show evident protective effect on BALF differential cell influx, alleviated pathological changes can be observed.

Allergic asthma is clinically defined as reversible airway obstruction and immunologically characterized by an excessive activation of Th2 cells and an overexpression of the inflammatory cytokines IL-4, IL-5, and IL-13.³⁰ Therefore, we further estimated the contents of these Th2-driven cytokines, as well as the level of Tregs. Our findings revealed that treatment with dead or living *F. prausnitzii* induced a significant reduction of Th2-driven cytokines. Surprisingly, HDM-specific IgG1 was also decreased by the administration of dead or living *F. prausnitzii* (Figure 2D, $P <$

0.01). Moreover, LBG and DBG groups showed a higher Tregs ratio than the model group (Figure 2J). Tregs are closely associated with the limitation of the inflammatory response in peripheral tissues.³¹ Interestingly, *F. prausnitzii* has been reported to be able to induce Tregs production.³² These results demonstrated that administration of dead or living *F. prausnitzii* reversed the Th2-driven cytokines and IgG1 levels and elevated the Tregs ratio in the spleen, resulting in the alleviation of HDM-induced allergic asthma.

Several studies have demonstrated that oral administration of probiotics improved the phenotypes of allergic asthma and inflammatory cascade.^{1, 33} Indeed, some strains are effective and can regulate the gut microbiota composition and intestinal microecology. However, the mechanism underlying the roles of intestinal flora in asthma alleviation remains to be elucidated. Here, we performed a comprehensive analysis for gut microbiota using 16S rRNA-amplicon sequencing. HDM intervention notably impacted the overall gut microbiota composition, with the increase of Firmicutes/Bacteroides ratio (Figure 3A). This alteration of Firmicutes/Bacteroides ratio was reversed by the living and dead *F. prausnitzii* treatment but was aggravated by the supernatant of *F. prausnitzii*. Based on the alpha diversity analysis, LBG and DBG groups had a much higher mean species diversity than the model group (Figure 3B, $P < 0.05$). In addition, the beta diversity suggested that there is an evidently structural separation of the microbial composition among these five groups (Figure 3C). These results indicated that living and dead *F. prausnitzii* treatment remarkably altered the gut microbial composition in asthmatic mice.

To further explore the differential taxa among the control, model, supernatant,

DBG, and LBG groups, LEfSe analysis was performed. Here, we found that *Turicibacter* was the core genus in the model group versus other groups (Figure 3E), suggesting that gut microbial dysbiosis was closely related to the increased abundance of *Turicibacter* in the model group. *Turicibacter* has been reported as an opportunistic pathogen.³⁴⁻³⁶ Moreover, Chen et al. found that the *Turicibacter* genera was expanded in rheumatoid arthritis patients, indicating that *Turicibacter* may be treated as an indicator of some inflammatory diseases.³⁷ However, supplementation with living or dead *F. prausnitzii* significantly suppressed the increase of *Turicibacter* in asthmatic mice and restored the intestinal microbiota balance through up-regulating some differential taxa, such as *Lachnoclostridium*, *Muribaculaceae*, *Parabacteroides*, and *Dubosiella* (Figure 3F). Unexpectedly, our results were not in accordance with those of a previous study, which reported that *Lachnoclostridium* was found to be more enriched in food-allergic subjects than that in control subjects.³⁸ Another study revealed that the abundance of *Muribaculaceae* was reduced in high fat diet (HFD)-induced food allergic mice.³⁹ A recent investigation reported a reduced abundance of *Parabacteroides* in the feces from subjects suffering relapsing-remitting multiple sclerosis.⁴⁰ Although a reduced abundance of *Dubosiella* was not observed in the model group, oral administration of living *F. prausnitzii* significantly enriched this genus. A previous patent demonstrated that *Dubosiella* genus can be used for treatment and diagnosis of allergic and autoimmune diseases,⁴¹ suggesting that *Dubosiella* should be considered in future allergic asthma studies.

We further compared the microbial functional pathways between the model group

and probiotic-treated groups (LBG and DBG groups) using PICRUSt to reveal the effect of gut microbiota on metabolic pathways. The significantly different functional pathways were related to carbohydrate metabolism, lipid metabolism, and metabolisms of cofactors and vitamins (Figure 4C-D, $P < 0.05$). A previous study demonstrated that the carbohydrate and lipid metabolisms in asthmatic children were changed when budesonide inhalation treatment was accepted,⁴² suggesting that these two pathways play a crucial role in treating asthma. Vinding et al. also reported that the lipid metabolism is related to asthma, aeroallergen sensitization, and bronchial responsiveness, indicating that asthma is a systemic disorder sharing dyslipidemia with other chronic inflammation disorders.⁴³ Moreover, vitamins, including vitamins C, D and E, could be helpful in improving asthma control or decreasing the amount of medication,^{44, 45} which suggests that metabolisms of cofactors and vitamins should be considered in allergic asthma. Interestingly, supplementation with living or dead probiotics significantly regulated these three pathways. However, the LBG and DBG groups shared different sub-pathways under these three functional pathways.

SCFAs, mainly acetate, propionate, and butyrate, are reported to be a potential immunomodulators to attenuate the symptoms of allergic asthma.^{46, 47} Moreover, the carbohydrate and lipid metabolisms are closely related to the SCFAs production, and the differential taxa (*Lachnospirillum*, *Muribaculaceae*, and *Parabacteroides*) between the model group and the probiotic-treated groups have the ability to produce SCFAs.^{48, 49} Therefore, we placed the emphasis on SCFAs production. Here, we found that there is a significant reduction in the production of acetate, propionate, and butyrate

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between the control and model groups (Figure 4E-G, $P < 0.01$). However, *F. prausnitzii* (LBG or DBG) administration significantly reversed this alteration of propionate and butyrate, but no statistical difference was found regarding acetate. Our results are in accordance with those of previous studies, which reported that probiotic treatment enhanced the production of SCFAs in asthmatic mice.¹ SCFAs have multiple physiological roles in body functions, such as shaping gut microbiota ecology, modulating allergic airway inflammation, and even inducing differentiation of colonic Treg cells.⁵⁰⁻⁵² Thus, we attempted to explore the correlation of SCFAs with immune indicators and BALF differential cell counts. Propionate had a significantly negative correlation with lymphocytes, eosinophils, neutrophils, IL-4, IL-5, and IL-13, and acetate was only negatively correlated with IL-4 (Figure 5, $P < 0.05$, $r < -0.9$). However, no statistical difference between butyrate and these hallmarks was observed. These results demonstrated that propionate may exert a dominant effect within SCFAs on improving HDM-induced allergic asthma.

In addition to SCFAs production, gut microbiota could also directly impact the immune indicators and BALF differential cell counts in asthmatic mice based on the network analysis (Figure 5). The relative abundance of *Lachnoclostridium* and *Muribaculaceae* showed a significantly negative correlation with IL-5 and IL-13 levels ($P < 0.05$, $r < -0.9$). The strategy of suppressing the Th2 type immune responses can contribute to alleviate asthma.³⁰ Moreover, *Lachnoclostridium* and *Muribaculaceae* were negatively related to the numbers of neutrophils and eosinophils ($P < 0.05$, $r < -0.9$). Interestingly, *Dubosiella* showed a positive correlation with the Tregs level ($P <$

0.05, $r > 0.9$), and *Lachnoclostridium* was negatively correlated with HDM-specific IgG1 level ($P < 0.05$, $r < -0.9$). The interaction within the gut microbiota exerts a non-negligible effect on asthma development. *Streptococcus* was negatively correlated with *Turicibacter*, which was the core genus in the model group ($P < 0.05$, $r < -0.9$). In addition, *Muribaculaceae* exhibited a positive correlation with *Lachnoclostridium* ($P < 0.05$, $r > 0.9$). Obviously, supplementation with *F. prausnitzii* stimulated the competition within the intestinal flora and this competition may help balance the microbiota dysbiosis induced by HDM. These results demonstrated that the protective effect of *F. prausnitzii* on HDM-induced asthma could be attributed to the modulation of SCFAs production and gut microbiota.

Based on the comprehensive analysis of asthma phenotype hallmarks, immune indicators, gut microbiota composition, gut microbial functional pathways, and SCFAs metabolism, we found that the living *F. prausnitzii*, dead *F. prausnitzii*, and supernatant differed in their capacity to prevent HDM-induced allergic asthma. Although the supernatant could improve the phenotypes of asthma, no statistical differences were observed. Indeed, *F. prausnitzii* supernatant showed anti-inflammatory properties in a DiNitroBenzene Sulfate (DNBS)-induced colitis model.⁵³ However, the effect of *F. prausnitzii* supernatant cannot be comparable to that of the living and dead *F. prausnitzii*. Moreover, the use of dead *F. prausnitzii* was as effective as that of living *F. prausnitzii*, raising the possibility that the anti-inflammatory protein form *F. prausnitzii* could exert a protective effect on HDM-induced allergic asthma. Further investigation is required to confirm this hypothesis.

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This study still has some limitations that need to be mentioned. For example, the *in vitro* experiment to describe the effects of *F. prausnitzii* on relevant immune cells was not performed due to the uncultured characteristic of *F. prausnitzii* in the cell culture chamber. Also, the detailed mechanism underlying the microbiota-derived SCFAs as the major mediators of the anti-asthmatic effect of *F. prausnitzii* still needs further work to elucidate. In addition, attempts to build a connection between the airway microbiome and the gut microbiome is also our following direction, trying to explore the possible action mechanism through the gut-lung axis.

Taken together, our findings demonstrated that supplementation with living or dead *F. prausnitzii* reversed the hallmarks of asthmatic phenotype and immune responses, increased SCFAs production, and improved the enteric dysbacteriosis caused by HDM-induced asthma. The proposed mechanism underlying the anti-asthmatic action of *F. prausnitzii* is illustrated in Figure 6. Therefore, the findings of the present study provide a valuable perspective on the potential usefulness of *F. prausnitzii* as a probiotic agent for the prevention of allergic asthma.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors gratefully acknowledge the financial supports by the National First-Class Discipline Program of Food Science and Technology (JUFSTR20180102), the Fundamental Research Funds for the Central Universities (JUSRP51903B), the Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu

Province, and the Postgraduate Research & Practice Innovation Program of Jiangsu Province, China (No. KYCX18_1779).

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Figure legends

Figure 1. *F. prausnitzii* treatment efficiently prevented the phenotype of house dust mite (HDM)-induced asthma. (A) Experimental design. (B) Differential cell counts in BALF. (C) Representative H&E staining of the lung (400×); blue arrows: inflammatory cells; green arrows: hemorrhage. * $P < 0.05$, ** $P < 0.01$ compared with the model group; $n = 6$.

Figure 2. *F. prausnitzii* treatment improved inflammatory cascades and elevated the Tregs ratio in asthmatic mice. (A-C) IL-4, IL-5, and IL-13 cytokine levels in BALF ($n = 6$). (D) Serum HDM-specific immunoglobulin IgG1 ($n = 6$). (E-I) Representative dot plots and percentage of $CD4^{+}Foxp3^{+}$ cells from the control, model, supernatant, LBG, and DBG groups. (J) The ratio of $CD4^{+}Foxp3^{+}$ cells in spleen lymphocytes ($n = 4$). * $P < 0.05$, ** $P < 0.01$ compared with the model group.

Figure 3. Analysis of gut microbial composition. (A) Alteration at the phylum level. (B) Alpha diversity based on the observed species and Shannon indexes. (C) Principal component analysis (PCA) analysis. (D) The top twenty-five abundant genera (E) Linear discriminant analysis effect size (LEfSe) comparison among all groups ($P < 0.05$, LDA score > 2.0). (F) The relative abundance of Muribaculaceae, *Turicibacter*, *Lachnoclostridium*, *Parabacteroides*, *Faecalibaculum*, *Streptococcus*, and *Dubosiella*. * $P < 0.05$, ** $P < 0.01$ compared with the model group; $n = 6$.

Figure 4. Prediction of microbial functional pathways and the short chain fatty acids (SCFAs) production. (A) Principal component analysis (PCA) of microbial functional

pathways between the model group and the DBG group. (B) Principal component analysis (PCA) of microbial functional pathways between the model group and the LBG group. (C) Differential pathways between the model group and the DBG group (two-sided Welch's t-test at a $P < 0.05$). (D) Differential pathways between the model group and the LBG group (two-sided Welch's t-test at a $P < 0.05$). (E-G) Colonic acetate, propionate, and butyrate concentrations. Data are expressed as the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ compared with the model group; $n = 6$.

Figure 5. The interaction network analysis among the asthma phenotype hallmarks, short chain fatty acids (SCFAs), immune indicators, and differential taxa. The size of the circles represents the contribution, a positive correlation is shown with a red line, and a negative correlation is shown with a blue line ($P < 0.05$, $r > 0.9$, or $r < -0.9$).

Figure 6. Proposed mechanism of *F. prausnitzii* treatment on the alleviation action in asthmatic mice. To make a house dust mite (HDM)-induce asthma model, mice were intranasally administered a dose of 100 μg of *Dermatophagoides pteronyssinus* dissolved in 25 μL of PBS. Supplementation with living or dead *F. prausnitzii* improved the microbial dysbiosis, especially decreasing the abundance of *Turicibacter* and increasing the abundance of *Muribaculaceae*, *Lachnoclostridium*, *Parabacteroides*, and *Dubosiella*. *F. prausnitzii* treatment also increased the production of short-chain fatty acids (SCFAs), which may be attributed to the enrichment of SCFAs-produced bacteria, including the genera *Muribaculaceae*, *Lachnoclostridium*, and *Parabacteroides*. SCFAs activated regulatory T cells (Tregs), which suppressed the Th2 immune responses induced by HDM. The reduced levels of IL-4, IL-5, and IL-13

blocked the activation of eosinophils, thus contributing to the improvement of airway obstruction and airway inflammation.









