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RESEARCH ARTICLE

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MFG-E8/integrin β3 signaling contributes to airway inflammation response and airway remodeling in an ovalbumin-induced murine model of asthma

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Abstract

Asthma is the most common chronic childhood disease worldwide, characterized by airway remodeling and chronic inflammation, orchestrated primarily by Th2 cytokines. The aim of the current study was to explore the influences of milk fat globule epidermal growth factor 8 (MFG-E8)/integrin β3 signaling involved in airway inflammation and remodeling in asthma. BALB/c mice were sensitized by intraperitoneal injection of ovalbumin (OVA), followed by OVA nebulization. The levels of MFG-E8 expression were declined markedly in the OVA-induced allergy murine model. In addition, administration of MFG-E8 strongly reduced the accumulation of T-helper type 2 (Th2)-associated cytokines (such as interleukin-4, -5, and -13) as well as chemokine CCL11 (eotaxin) in bronchoalveolar lavage fluid and tissues in the OVA-sensitized mice. Moreover, MFG-E8 remarkably repressed the total immunoglobulin E and OVA-specific immunoglobulin E in serum in OVA-challenged mice. Meanwhile, treatment with recombinant murine MFG-E8 noticeably prevented inflammatory cell infiltration into the airways, as showed by a marked decrease in the numbers of total immune cells, eosinophils, neutrophils, macrophages, and lymphocytes in the bronchoalveolar lavage fluid in response to OVA challenge. Importantly, MFG-E8 apparently alleviated OVA-driven airway remodeling, which were evidenced by declined secretion of important mediators of airway remodeling, including transforming growth factor-β1, matrix metalloproteinase 9, ADAM8, and vascular endothelial growth factor, and reduced airway collagen deposition and inhibited goblet cell hyperplasia in OVA-induced asthma in mice. Mechanistically, integrin 3 contributes to the protective effect of MFG-E8 in inhibiting airway inflammation and remodeling in OVA-driven features of allergic asthma. Overall, MFG-E8, as a candidate molecule to evaluate airway inflammation and remodeling, could be a potential target for the management and prevention of asthma exacerbations, suggesting that MFG-E8/integrin β3 signaling may serve as a promising therapeutic agent for childhood asthma.

KEYWORDS

airway inflammation, airway remodeling, childhood asthma, milk fat globule epidermal growth factor 8 (MFG-E8)

Abbreviations: AHR, airway hyperresponsiveness; BAL fluid, bronchoalveolar lavage fluid; CCL11, eotaxin; MFG-E8, milk fat globule epidermal growth factor 8; MMP-9, matrix metalloproteinase 9; OVA, ovalbumin; rmMFG-E8, recombinant murine MFG-E8; TGF-β1, transforming growth factor; VEGF, vascular endothelial growth factor.

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1 | INTRODUCTION

Asthma is the most common chronic inflammation of airways disorder in children, characterized by Th2-typed inflammation, eosinophilic infiltration, airway remodeling, and airway hyperresponsiveness (AHR).[1,2] These changes are induced mostly by Th2-typed inflammation, resulting in eosinophil infiltration into lung tissue, and contribute to airway remodeling and the deterioration of lung function. The pathogenesis of asthma is still unclear due to its complex disease process. Therefore, it is important to explore the new strategies for the prevention and treatment of asthma.

Milk fat globule epidermal growth factor 8 (MFG-E8) is a multifunctional glycoprotein originally identified as part of the milk fat globule membrane. MFG-E8 appears to be instrumental in cell-cell interactions and has been involved in diverse physiological and pathophysiological functions. An increasing body of evidence indicates that the expression of MFG-E8 is ubiquitously found in spleen, lungs, liver, kidneys, intestine. and mammary glands,[3] whereas a marked decrease in its content is noted in various inflammatory diseases, causing exaggerated inflammation and abnormal tissue homeostasis.[3-5] A previous study reported that MFG-E8 levels in the plasma, lungs, and spleen notably decreased after hemorrhage and resuscitation.[6] In addition, MFG-E8 levels in plasma were apparently lower in patients with chronic obstructive pulmonary disease.[7] Recently, it has been indicated that MFG-E8 attenuates inflammation responses and acute lung injury.[8] Another recent study also showed that MFG-E8$^{-/-}$ mice exhibited extensive lung damage due to exaggerated infiltration of neutrophils and production of tumor necrosis factor-α and macrophage inflammatory protein-2 (MIP-2).[9] A recent report demonstrated that MFG-E8 suppresses AHR in asthma by regulating smooth muscle contraction.[10] Nevertheless, the precise role of MFG-E8 and its receptor integrin β3 in chronic lung inflammation has not been established. This study was conducted to evaluate the ability of MFG-E8 to prevent airway inflammation responses and to improve airway remodeling in ovalbumin (OVA)-induced asthma model in mice.

2 | MATERIALS AND METHODS

2.1 | Animals

Six-week-old BALB/c female mice were obtained from the Affiliated Children's Hospital of Zhengzhou University. All mice were housed in ventilated shelves under pathogen-free conditions with air conditioning and a 12-hour light/dark cycle. All animal experimental procedures followed protocols that were approved by the Ethical Committee for Animal Research of the Affiliated Children's Hospital of Zhengzhou University.

2.2 | OVA sensitization and challenge

The experimental protocol for allergen sensitization and challenge was modified from previously described procedures. Briefly, BALB/c mice were initially sensitized by intraperitoneal injection of 10 μg OVA (Sigma-Aldrich, St. Louis, MO) emulsified in 100 μg of aluminum hydroxide (Sigma-Aldrich) on day 0 and day 14. For the allergen challenge, mice were challenged with 2% OVA for 20 minutes on days 28, 29 and 30. Control mice were subjected to the same protocol but received phosphate buffered saline (PBS) instead of OVA in the challenge phase. Each experiment in our study was repeated 3 times with 5 mice per group. The recombinant murine MFG-E8 (rmMFG-E8; R&D Systems, Minneapolis, MN) was administered intraperitoneally at 20 μg/kg body weight dose before the challenge. For integrin β3 knockdown treatment, integrin β3 small interfering RNA (si-integrin β3; Santa Cruz Biotechnology, Santa Cruz, CA), which was resolved with sterile PBS, was administered intraperitoneally accompanied with rmMFG-E8 treatment.

2.3 | Collection of serum and bronchoalveolar lavage fluid

Mice were anesthetized 24 hours after the last OVA challenge, and blood, bronchoalveolar lavage (BAL) fluid, and lungs were collected. Blood samples were collected and centrifuged for 2 minutes (Eppendorf, Hamburg, Germany), and the serum was separated and stored at −80°C until subsequent measurements. BAL fluid was collected by lung lavage using 1 mL of PBS administered via the tracheal catheter. After 3 lavages, the BAL fluid was centrifuged, and the supernatant was frozen at −80°C for subsequent cytokine analysis. Total cell counts were performed using an automated cell counter (Invitrogen, Waltham, MA). The cell pellets were resuspended in PBS, and differential cell were counted under a light microscope.

2.4 | Real-time quantitative polymerase chain reaction assay

Total RNA was extracted from the tissues using the TRIzol Reagent (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA), following the manufacturer's protocol. The purity and concentration of the RNA were detected using a NanoDrop 2000 (Thermo Fisher Inc.). Complementary DNA was synthesized using

cDNA Synthesis Kit (Applied Biosystems, Carlsbad, CA). Real-time quantitative polymerase chain reaction was performed using an ABI PRISM 7700 sequence detection system with Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA) based on the manufacturer's instructions. Glyceraldehyde 3-phosphate dehydrogenase was used as a loading control.

2.5 | Western blot analysis

The lung tissue and cells were homogenized in a protein extraction buffer (Biyuntian, Shanghai, China), and the total protein concentration was determined using a bicinchoninic acid assay (BCA assay) kit (Thermo Scientific, Waltham, MA), according to the manufacturer's directions. Then, the loading buffer was added in the extracted proteins, followed by 10 minutes of boiling at 95°C. Equal amounts of protein were separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA), which was blocked with 5% nonfat milk in TBS containing 0.1% Tween 20, and then incubated overnight at 4°C with the appropriate primary antibody: anti–MFG-E8, anti–integrin-β3, anti–vascular endothelial growth factor (VEGF), anti–matrix metalloproteinase 9 (MMP-9), anti-ADAM8, and anti–glyceraldehyde 3-phosphate dehydrogenase antibody (Santa Cruz Biotechnology, Inc). After incubation, membranes were washed and incubated with a horseradish peroxidase–conjugated secondary antibody (Santa Cruz Biotechnology) at room temperature for 1 hour. Finally, the bands were visualized using the ECL Plus chemiluminescence reagent kit (Amersham Bioscience, Arlington Heights, IL) and quantified by optical density methods using the Image J software (National Institutes of Health, Bethesda, MD). The relative protein expression levels were normalized to glyceraldehyde 3-phosphate dehydrogenase.

2.6 | Measurement of levels of cytokines in BAL fluid and total immunoglobulin E and OVA-specific immunoglobulin E in the serum

The concentrations of interleukin (IL)-4, IL-5, IL-13, transforming growth factor-β1 (TGF-β1), VEGF, and MMP-9 in BAL fluid were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using a Mouse CytoSet Kit (Invitrogen, CA), according to the manufacturer's instructions. Serum total immunoglobulin E (IgE) was quantitated with an IgE ELISA kit (BD Biosciences, CA). Serum OVA–specific IgE was quantitated with Mouse OVA-specific IgE ELISA assay kit (DS Pharma Biomedical Co., Ltd, Osaka, Japan). All assays were conducted in triplicate.

2.7 | Histological analysis

Lungs were fixed with 10% formalin, embedded in paraffin, sectioned into 5-μm slices, and stained with Masson's trichrome for the detection of collagen deposition and periodic acid-Schiff (PAS) for the analysis of mucus secretion in the goblet cells of the lung epithelium.

2.8 | Statistical analysis

The statistical analysis was performed using SPSS 21.0 software (SPSS Inc, Chicago, IL), and graphs were generated using GraphPad prism 5.0 software (San Diego, CA). All data were presented as the mean ± standard deviation of 3 independent experiments. Comparisons among the study groups were performed by 1-way analysis of variance, followed by Tukey's post hoc test. $P < .05$ were considered statistically significant.

3 | RESULTS

3.1 | Characterization of OVA-mediated MFG-E8 induction in mouse lung

To confirm the role of MFG-E8 in the development of asthma, we established an OVA-sensitized/-challenged murine asthmatic model and investigated the functional role of MFG-E8 in the pathogenesis of asthma. We initially evaluated the expression of MFG-E8 in models of OVA-triggered asthma, as assessed by reverse transcription polymerase chain reaction and Western blot analysis. The results of reverse transcription polymerase chain reaction, as shown in Figure 1A, indicate that the messenger RNA expression of MFG-E8 was markedly declined in the mice challenged with OVA. The similar results were obtained by using Western blot analysis of MFG-E8 protein (Figure 1B). These results were similar to previously published data,[10] showing that MFG-E8 expression is decreased in airways of patients with asthma.

3.2 | Effects of MFG-E8 on the production of cytokines and chemokine in OVA-immunized mice

Airway inflammation is central to the pathogenesis of asthma. Allergic asthmatic inflammation is known to be caused by the secretion of a series of Th1 cytokines

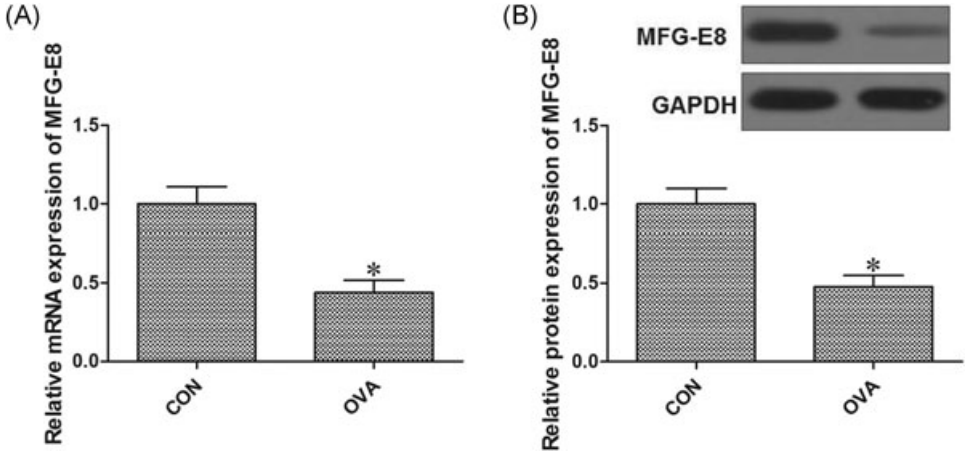

FIGURE 1 The expression of MFG-E8 is decreased after OVA sensitization and challenge. (A) The messenger RNA expression of MFG-E8 in lung tissue was assessed using the RT-PCR assay. (B) MFG-E8 protein expression was measured by Western blot analysis and quantified by densitometry. *$P < .05$ versus CON. CON, PBS-treated; OVA, OVA-challenged. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MFG-E8, milk fat globule epidermal growth factor 8; OVA, ovalbumin; PBS, phosphate buffered saline; RT-PCR, reverse transcription polymerase chain reaction

(interferon-γ) and Th2 cytokines.[11] To investigate the blocking effects of MFG-E8 on the OVA-triggered Th2-predominant immune response, the levels of IL-4, IL-5, and IL-13 were examined using the ELISA assay. As expected, the secretion of IL-4, IL-5, and IL-13 was markedly elevated after OVA sensitization and challenge, whereas rmMFG-E8 caused a remarkable attenuation in the production of these cytokines in BAL fluid from OVA-sensitized mice (Figure 2A-C). Meanwhile, we collected lung tissues to evaluate gene expressions of these Th2 cytokines. In line with the results of the ELISA assay, rmMFG-E8 led to a strong reduction in the messenger RNA expression of Th2-associated cytokines in lung tissues (Figure 2E-G). Given that CCL11 plays an important role in eosinophil chemotaxis in asthma, the release of CCL11 was also measured with an ELISA kit, and we observed that OVA sensitization exhibited a notable increase in the level of CCL11; however, treatment with rmMFG-E8 distinctly downregulated the level of CCL11 production induced by OVA challenge. These findings collectively indicated that MFG-E8 impeded the development of the Th2 pathogenic immune response in OVA-induced murine asthmatic model.

3.3 | Administration of rmMFG-E8 alleviates airway inflammation in OVA-sensitized mice

Aiming to investigate the airway inflammation, the number of total and specific cells in the BAL fluid was assessed. An important component of the allergic asthma model is the production of IgE. We set out to investigate the effects of MFG-E8 on adaptive immune responses to OVA sensitization; the levels of total and OVA-specific IgE as well as OVA-specific IgG1 (a Th2-associated antibody) in the serum were analyzed. As illustrated in Figure 3A-C, marked elevation in serum total IgE, OVA-specific IgE, and IgG1 was observed in OVA-challenged mice, whereas the overexpression of MFG-E8 resulted in a dramatic reduction in the secretion of total IgE and OVA-specific IgE and IgG1 levels in the serum. Eosinophils increase is generally considered as the hallmark of asthma onset, and neutrophils is considered the indicator of inflammation. As summarized in Figure 3D-H, the number of total cells, eosinophils, neutrophils, macrophages, and lymphocytes were robustly accumulated in the BAL fluid from OVA-immunized mice, whereas rmMFG-E8 remarkably diminished in the influx of inflammatory cells, including eosinophils, neutrophils, macrophages, and lymphocytes, into BAL fluid. These findings strongly implied that MFG-E8 showed a greater suppressive effect on inflammatory cell recruitment in a model of OVA-sensitized asthma, indicating that a specific inhibition of the Th2 response by MFG-E8 in asthmatic mice.

3.4 | MFG-E8 have an inhibitory effect on airway remodeling triggered by OVA challenge

TGF-β1 expression and activation are commonly increased in asthma. VEGF is an important mediator of airway remodeling. MMP-9 is a significant pathogenic factor in asthmatic airway remodeling. To further investigate the functions of MFG-E8 in regard to airway remodeling, we examined the expression of

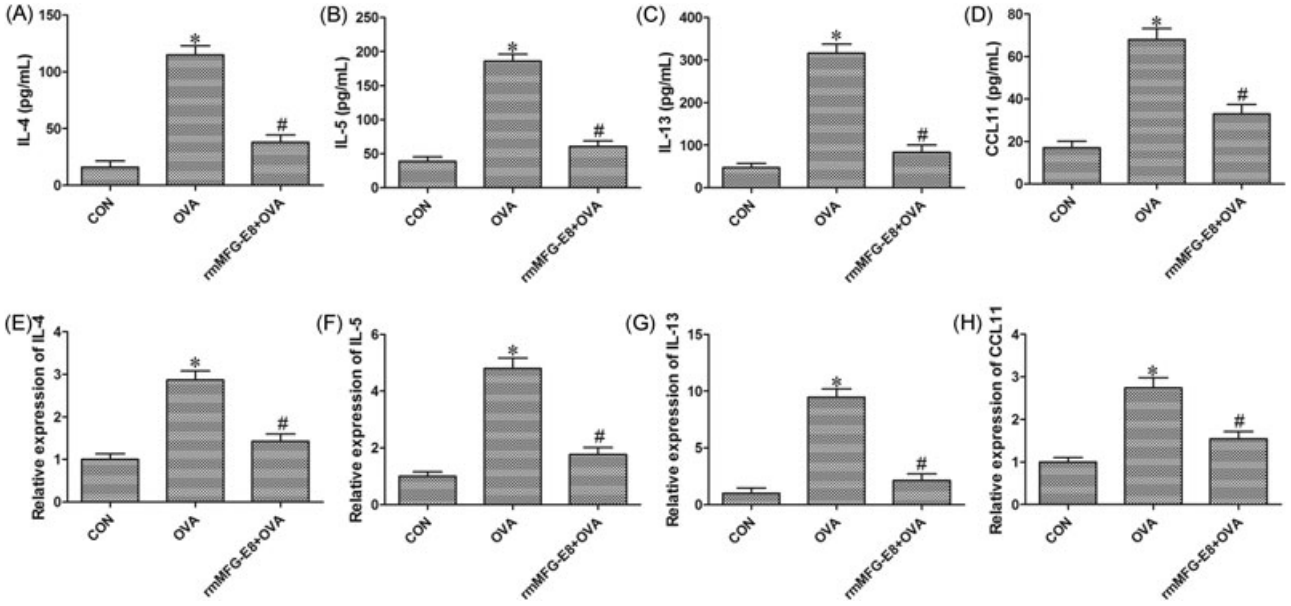

FIGURE 2 MFG-E8 caused a reduction in the level of Th2 cytokines and chemokine in OVA-immunized mice. The concentrations of (A) IL-4, (B) IL-5, (C) IL-13, and (D) CCL11 were determined using the ELISA assay in BAL fluid. The gene expression of (E) IL-4, (F) IL-5, (G) IL-13, and (H) CCL11 in lung tissues was detected by RT-PCR analysis. Data represent the mean ± standard deviation of at least 3 independent experiments. $^{*}P < .05$ versus CON; $^{\#}P < .05$ versus OVA. CON: PBS-treated; OVA: OVA-challenged; MFG-E8 + OVA: OVA-challenged mice treated with rmMFG-E8. BAL, bronchoalveolar lavage; IL, interleukin; MFG-E8, milk fat globule epidermal growth factor 8; OVA, ovalbumin; PBS, phosphate buffered saline; rmMFG-E8, recombinant murine MFG-E8; RT-PCR, reverse transcription polymerase chain reaction

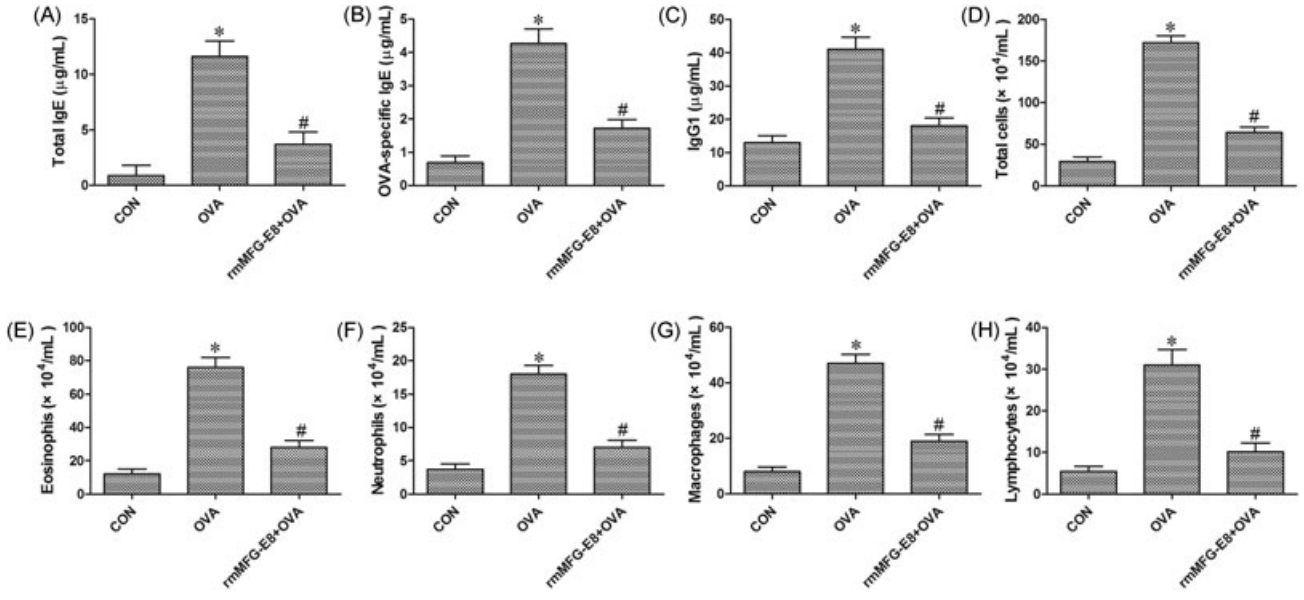

FIGURE 3 Effects of MFG-E8 on the recruitment of cell infiltrate and inflammatory cells in BAL fluid from OVA-induced allergic asthmatic mice. The serum levels of total IgE (A), OVA-specific IgE (B), and OVA-specific IgG1 (C) were determined using ELISA kits. The total cells number (D) as well as the number of eosinophils (E), neutrophil (F), macrophages (G) and lymphocytes (H) in the BAL fluid were counted. Results are pooled data from 3 independent experiments. $^{*}P < .05$ versus CON; $^{\#}P < .05$ versus OVA. CON: PBS-treated; OVA: OVA-challenged; MFG-E8 + OVA: OVA-challenged mice treated with rmMFG-E8. BAL, bronchoalveolar lavage; ELISA, enzyme-linked immunosorbent assay; IgE, immunoglobulin E; MFG-E8, milk fat globule epidermal growth factor 8; OVA, ovalbumin; PBS, phosphate buffered saline; rmMFG-E8, recombinant murine MFG-E8

genes associated with airway remodeling. As shown in Figure 4A,B, the levels of TGF-β1 remarkably elevated in both BAL fluid and serum from OVA-sensitized asthmatic mice, whereas administration of rmMFG-E8 profoundly diminished the level of TGF-β1. At the same time, OVA sensitization led to a prominent increase in the production of VEGF and MMP-9 in serum. In contrast, treatment with rmMFG-E8 caused a large downregulation in the secretion of VEGF and MMP-9 (Figure 4C,D) in the serum of OVA-sensitized asthmatic mice. Consistent with the results of ELISA analysis, the expression of VEGF and MMP-9 with a smaller decrease in ADAM8 was assessed by Western blot analysis (Figure 4E-H). The effects of MFG-E8 on the OVA-challenged mice with collagen deposition in the lung were evaluated through histological staining with Masson's trichrome stain, and we observed that the administration of MFG-E8 exerted a substantial effect on reducing extracellular matrix (ECM) deposition in bronchiolar subepithelial regions (Figure 4I). To evaluate the effects of MFG-E8 extract on goblet cell hyperplasia, lung tissues were stained using PAS. The results showed that MFG-E8 obviously reduced the number of mucus-secreting goblet cells (Figure 4J). Taken together, these data clearly demonstrate that MFG-E8 may be effective in alleviating immune-mediated airway remodeling in OVA-induced asthmatic mice.

3.5 | Knockdown integrin β3 abolished the effects of rmMFG-E8 on airway inflammation and remodeling

To further explore mechanisms of MFG-E8, we first investigated the effects of MFG-E8 on its receptor integrin β3 in mice with OVA-challenged asthma. As can be seen, the results of Western blot detection showed that the knockdown of integrin β3 resulted in a dramatic decrease in the expression of integrin β3 and MFG-E8 after OVA challenge (Figure 5A), whereas quantitative

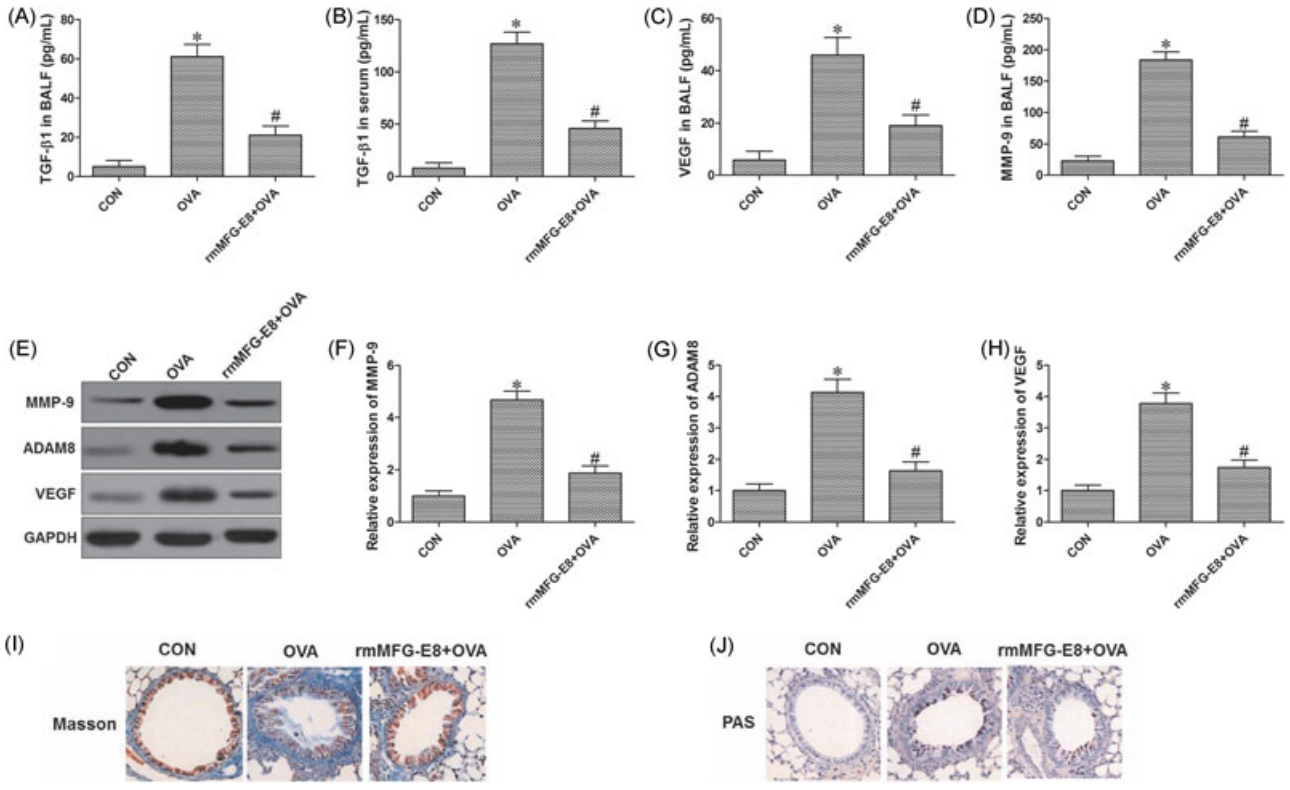

FIGURE 4 Effects of MFG-E8 on airway remodeling in OVA-induced model of asthma. ELISA was used to measure TGF-β1 in BAL fluid (A) and serum (B). The level of VEGF (C) and MMP-9 (D) in the BAL fluid, as detected by ELISA analysis. (E-H) The protein expression of VEGF and MMP-9 was assessed by Western blot analysis in the lung tissue. (I) Representative photomicrographs of lung sections with Masson's trichrome were obtained. (J) Lung sections stained with periodic acid-Schiff (PAS) to identify mucin-containing (purple) cells. *$P < .05$ versus CON; #$P < .05$ versus OVA. CON: PBS-treated; Data shown are means ± standard deviation from 3 determinations. OVA: OVA-challenged; MFG-E8 + OVA: OVA-challenged mice treated with rmMFG-E8. BAL, bronchoalveolar lavage; ELISA, enzyme-linked immunosorbent assay; MFG-E8, milk fat globule epidermal growth factor 8; MMP-9, matrix metalloproteinase 9; OVA, ovalbumin; PBS, phosphate buffered saline; rmMFG-E8, recombinant murine MFG-E8; VEGF, vascular endothelial growth factor

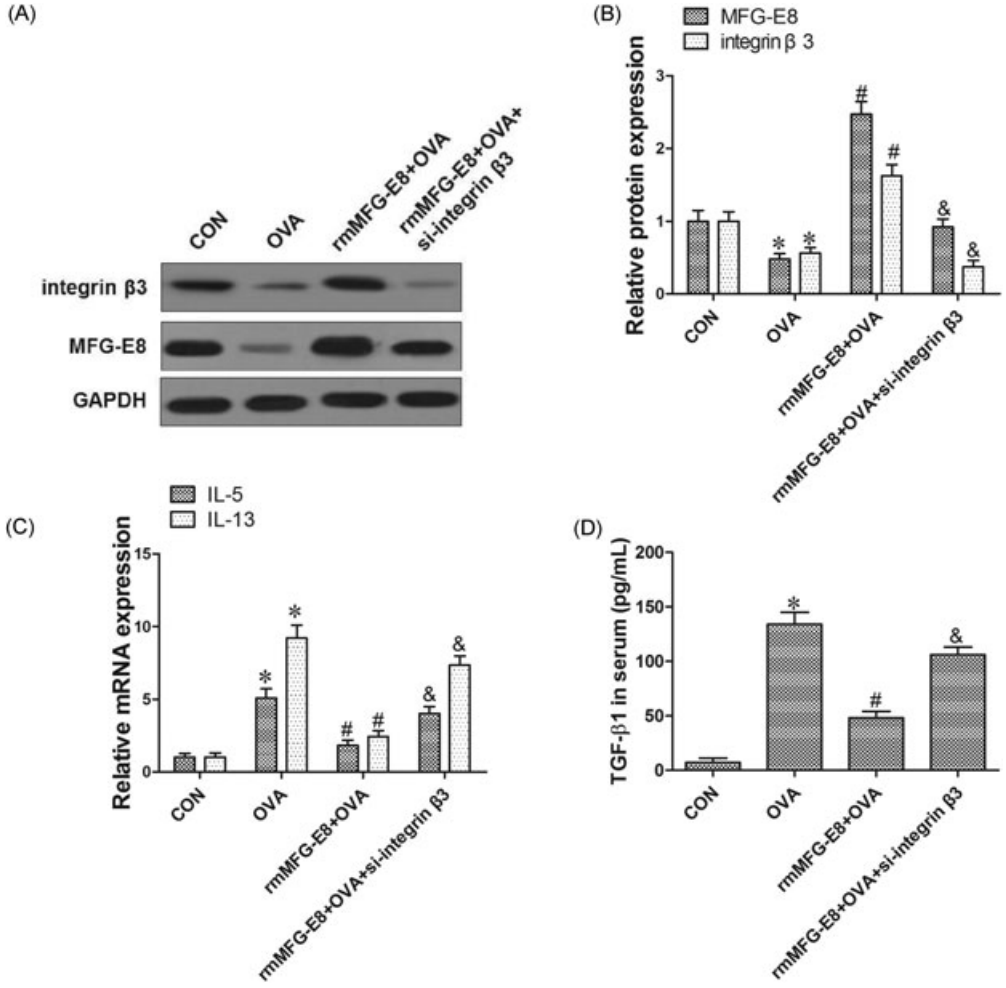

FIGURE 5 Integrin β3 contributes to the anti-inflammatory response and anti-airway remodeling effects of MFG-E8. (A) Integrin β3 and MFG-E8 were assessed by Western blot analysis assay. Quantification of the bands is shown in panel (B). The ELISA assay was used to measure the level of (C) IL-5, (D) IL-13, and (E) TGF-β1. Data shown are mean ± standard deviation from 3 independent experiments. $^{*}P < .05$ versus CON; $^{\#}P < .05$ versus OVA. $^{\&}P < .05$ versus rmMFG-E8 + OVA. ELISA, enzyme-linked immunosorbent assay; IL, interleukin; MFG-E8, milk fat globule epidermal growth factor 8; OVA, ovalbumin; rmMFG-E8, recombinant murine MFG-E8

data are shown in Figure 5B. In addition, silencing of integrin β3 obviously impaired inhibitory effects on the accumulation of inflammatory mediators IL-5 and IL-13 (Figure 5C,D). Meanwhile, suppression of integrin β3 completely abolished the protective effect of MFG-E8 on airway remodeling through downregulating the TGF-β1 expression (Figure 5E).The findings showed that MFG-E8 effectively ameliorated the pathogenesis of OVA-induced asthma via the repression of the Th2-mediated inflammation response and airway remodeling might be dependent on its receptor integrin β3.

4 | DISCUSSION

Asthma, an increasingly common chronic disease among children, is a chronic airway inflammatory disease which is associated with airway inflammation, airway remodeling, reversible airway obstruction, and airway AHR, and that involves coordinate expression of multiple inflammatory genes in the lungs. MFG-E8, a secretory protein, plays important roles in several biological processes, including apoptotic cell clearance, angiogenesis, and anti-inflammation. However, the potential effects of MFG-E8 on the pathogenesis of asthma remains elusive. In the current study, we used a mouse model of asthma characterized by airway inflammation and remodeling as a representation of patients with chronic asthma to evaluate the role of MFG-E8 in the pathogenesis of asthma. We first evaluated the anti-inflammatory response and anti-airway remodeling effects of MFG-E8 in OVA-challenged asthma models. The expression level of MFG-E8 was elevated in a murine model of asthma, and we observed that there is a strong downregulation of

MFG-E8 messenger RNA and protein levels in the lungs in asthma. This finding was in accordance with the results from previous study.[10]

It is well known that the main mechanism of asthma is a Th1/Th2 imbalance. Th2-type inflammation cytokines, such as IL-4, IL-5, and IL-13, are believed to be critically important for the pathogenesis of asthmatic responses.[12] It has previously been shown that children with asthma have increased cytokine levels in BAL fluid.[13] IL-5, a key cytokine in chronic inflammation, was increased in the OVA mice. IL-13 is a Th2 cytokine associated with AHR, IgE production, and airway remodeling.[14] Enhanced levels of IL-4, IL-5, and IL-13 have also been observed in asthmatic human subjects compared with healthy controls.[15] Also, the level of CCL11 (also known as eotaxin-1) is related to the degree of eosinophilic airway inflammation. It has been indicated that the overexpression of MFG-E8 significantly suppressed the inflammation of TNF-α, IL-6, and IL-1β.[8] A previous study also reported that MFG-E8 attenuated inflammation in lung.[9] In addition to investigating the possible anti-inflammatory effects of MFG-E8, it was important to assess whether MFG-E8 modulation of Th2 cytokines could have meaningful effects on allergen-induced airway reactivity. Here, we evaluated the levels of the Th2 inflammatory cytokines, such as IL-4, IL-5, and IL-13, in the BAL fluid and tissues, and the results showed that OVA sensitization resulted in substantial elevation of Th2 cytokines in both BAL fluid and lung tissues, whereas treatment with rmMFG-E8 markedly prevented the release of proinflammatory cytokines after the OVA challenge. We also revealed that administration of MFG-E8 apparently attenuated the release of CCL11, which is an important chemokine in eosinophilic airway inflammation and remodeling. On the basis of the above findings, we conclude that MFG-E8-mediated inflammation responses downregulated Th2-associated cytokines and chemokine production in a mouse model of asthma.

Because IgE is a hallmark of the Th2-mediated immune response in the pathogenesis of allergic inflammation. In this context, MFG-E8 strongly repressed the levels of serum IgE as well as OVA-specific IgE production in OVA-challenged mice. IgG1, a Th2-associated antibody, has been used as a Th2 marker and is correlated with IgE levels. Interestingly, we observed that OVA-specific IgG1 was also declined exclusively after treatment with rmMFG-E8, providing additional evidence that MFG-E8 attenuated Th2-driven immune response. One of the features of asthma is the accumulation of inflammatory cell, eosinophils, neutrophils, lymphocytes, and macrophages in the lungs.[16] Asthma is characterized by chronic inflammation of the airways and airway remodeling as a result of overproduction of inflammatory cells. The hallmark of chronic asthma is the infiltration of inflammatory cells, not only eosinophils but also neutrophils, into the lung. IL-4 is known to directly promote the features of asthma, including the recruitment of eosinophils, and enhanced production of IgE and upregulation of the IgE-mediated response to inflammatory cells.[17] IL-5 plays a central role in the differentiation, maturation, and survival of eosinophils, which leads to infiltration into the airways.[18] The data presented in this study indicated that MFG-E8 markedly reduced the degree of inflammatory cell infiltration in OVA-induced asthmatic mice. To this point, we demonstrated that MFG-E8 was able to modulate allergic immune response in a murine model of OVA-induced asthma, implying that the suppressive effects of MFG-E8 on OVA-induced airway inflammation are due to the modulation of the Th2-mediated immune response. These effects results from the inhibition of the Th2 response, indicating that MFG-E8 may have pharmacological effects that would be useful in the treatment of asthma.

TGF-β1 is a multifunctional cytokine produced by many types of cells during the airway remodeling process and plays a critical role in regulating the most of the cellular biological processes that lead to airway remodeling.[19] In addition, VEGF is an important mediator of vascular inflammation and airway remodeling.[20] MMP-9 is a significant pathogenic factor in asthmatic airway remodeling.[21] We further investigated the MFG-E8 in a mouse model of OVA-induced remodeling with regard to airway function. ADAM8 is induced by allergens and Th2 cytokines in the lung in experimental asthma and associated with asthma pathogenesis.[22] Our presented results showed that treatment with rmMFG-E8 notably ameliorated OVA-challenged airway remodeling as reflected in inhibiting the expression level of genes associated with airway remodeling, including TGF-β1, MMP-9, ADAM8, and VEGF. Therefore, along with these findings, we concluded that MFG-E8 possesses a potent efficacy on controlling allergic asthma response through repressing airway inflammation and alleviating airway remodeling, proposing a novel mechanism of MFG-E8 on OVA-induced airway remodeling in asthma.

On the basis of the results observed in the current study, we demonstrated that administration of MFG-E8 markedly suppressed the key features of asthmatic by reducing the pulmonary inflammation, OVA-specific IgE production, eosinophil infiltration, and Th2 pathogenic immune response, as well by inhibiting important airway remodeling mediators, including TGF-β1, MMP-9, ADAM8, and VEGF production, indicating that MFG-E8 exerted protective effects against OVA-challenged airway inflammation and remodeling

during the development of asthma. Integrins are a family of heterodimeric cell adhesion molecules, mediating cell-cell interactions or adhesion between cells and ECM.[23] Multiple lines of evidence have indicated that integrin β3 is a putative receptor of MFG-E8 in various cell types.[24-26] A previous study also demonstrated that anti-inflammatory role of MFG-E8 may depend on αvβ3 integrin-mediated intracellular signaling.[27] MFG-E8/integrin β3 may be involved in neuroinflammation and apoptotic changes after SAH.[24] It also has been reported that integrin β3 regulated the expression of Th2 cytokines in the rejecting transplant.[28] In addition, β3 integrin plays a critical role in vascular development/maturation and regulating the vascular sensitivity to VEGF in the heart.[29] We further explored the specific mechanisms involved in MFG-E8 anti-allergic effects, and all the therapeutic effects of MFG-E8 were markedly reversed by silencing of integrin β3. Our data implicated that the protective effects of MFG-E8 on Th2-driven pathologies, such as airway inflammation response and airway remodeling, depend on integrin β3 signaling.

In summary, our results demonstrate that MFG-E8 is a promising therapeutic candidate that is able to improve eosinophilic inflammation and alleviate the development of airway remodeling involving the integrin β3–dependent pathway. Taken together, our findings provide a valuable insight into the potential use of MFG-E8/integrin β3 signaling as novel therapeutics for asthma.

AUTHOR CONTRIBUTIONS

YZ and LL: Conceived and designed the experiments. YZ, HH, and LL: Performed the experiments and analyzed the data. HH and LL: Contributed reagents/materials/analysis tools. YZ, HH, and LL: Wrote the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interests.

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