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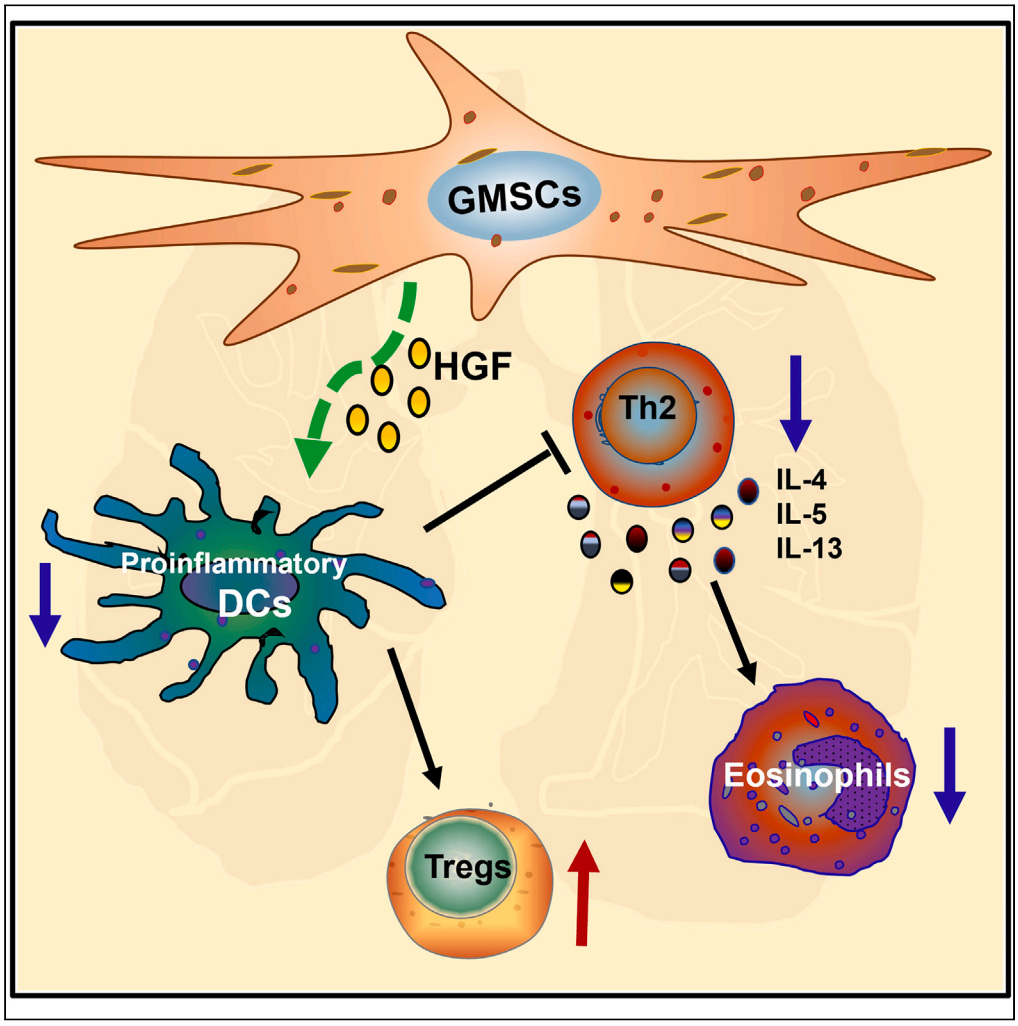


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## Highlights

GMSCs alleviate airway inflammation in an OVA-induced asthma model

GMSCs decrease DCs mediated Th2 differentiation and eosinophil infiltration

The beneficial effects of GMSCs are mediated by the secretion of HGF

Fang et al., iScience 27, 109818
May 17, 2024 
https://doi.org/10.1016/j.isci.2024.109818

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# Gingival-derived mesenchymal stem cells alleviate allergic asthma inflammation via HGF in animal models

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## SUMMARY

**Allergic asthma is a chronic non-communicable disease characterized by lung tissue inflammation. Current treatments can alleviate the clinical symptoms to some extent, but there is still no cure. Recently, the transplantation of mesenchymal stem cells (MSCs) has emerged as a potential approach for treating allergic asthma. Gingival-derived mesenchymal stem cells (GMSCs), a type of MSC recently studied, have shown significant therapeutic effects in various experimental models of autoimmune diseases. However, their application in allergic diseases has yet to be fully elucidated. In this study, using an OVA-induced allergic asthma model, we demonstrated that GMSCs decrease $CD11b^{+}CD11c^{+}$ proinflammatory dendritic cells (DCs), reduce Th2 cells differentiation, and thus effectively diminish eosinophils infiltration. We also identified that the core functional factor, hepatocyte growth factor (HGF) secreted by GMSCs, mediated its effects in relieving airway inflammation. Taken together, our findings indicate GMSCs as a potential therapy for allergic asthma and other related diseases.**

## INTRODUCTION

Allergic asthma is a heterogeneous lung disease characterized by chronic inflammation, influenced by both genetic and environmental factors.[1,2] Immunological response indicators in allergic asthma include an increased production of IgE antibodies, infiltration of eosinophils, and elevated levels of $CD4^{+}$ T helper 2 cells.[3] While current treatment methods can alleviate clinical symptoms such as wheezing, chest tightness, and shortness of breath, there is no cure for allergic asthma. Furthermore, at least 5%–10% of patients experience inadequate symptoms control with existing approaches.[4] Therefore, it is crucial to gain a deeper understanding of the potential pathogenesis of allergic asthma and develop new approaches that offer improved efficacy and fewer side effects.

Th2 cells are major participants in the immune response to allergic asthma, secreting inflammatory cytokines that recruit eosinophils and promote the activation and differentiation of B cells to produce antibodies.[5,6] This pathological response involves the initiation and activation of dendritic cells (DCs), which are highly specialized antigen-presenting cells.[7,8] Previous studies have documented that $CD11b^{+}$ $CD11c^{+}$ DCs are the main proinflammatory DCs in lung tissues, and their numbers are significantly increased in allergic asthma, closely related to Th2 differentiation.[9] Thus, targeting proinflammatory DCs may provide a potential approach to treating allergic asthma.

Mesenchymal stem cells (MSCs) have shown efficacy as treatments for a variety of immune disease experimental models and clinical studies due to their strong immunomodulatory functions and multipotential differentiation capacity.[10] Gingival mesenchymal stem cells (GMSCs), as one of the newly developed types of MSCs, offer specific advantages such as autologous extraction, minimal ethical controversy, no tumorigenesis, and rapid proliferation and propagation independent of serum, potentially making a new breakthrough in the treatment of autoimmune and chronic inflammatory diseases.[11]

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https://doi.org/10.1016/j.isci.2024.109818

Previous studies have demonstrated the regulatory potential of GMSCs on various immune cells *in vitro* and *in vivo*, including Th cells producing different cytokines, B cells, macrophages, and neutrophils.[12–17] GMSCs have also been found to regulate the effects on osteoclasts and synovial cells.[18] In terms of the target molecules involved in GMSC-mediated immune regulation, researchers have identified that PD-L1-positive GMSCs exhibit stronger immunosuppressive effects.[19] Meanwhile, CD39-positive GMSCs are more effective in inhibiting osteoclasts, promoting osteogenesis, and alleviating osteoporosis symptoms.[20] Moreover, several animal models have confirmed the efficacy of GMSCs in various autoimmune and inflammatory diseases, such as rheumatoid arthritis, lupus nephritis, diabetes, graft-versus-host disease, aplastic anemia, type 1 diabetes, and atherosclerosis.[12–17,21] However, it remains unknown whether GMSCs can be utilized for the treatment of allergic diseases. If that is the case, it would be interesting to learn which target cell(s) will be modulated by GMSCs and how GMSCs regulate those target cells. Indeed, since GMSCs secrete various functional factors, including cytokines, growth factors, and chemokines, it is worth exploring which core functional factor secreted by GMSCs plays a vital role in treating diseases.

In this study, we investigated the therapeutic potential of GMSCs in treating allergic asthma using an OVA-induced allergic asthma model. The results demonstrated that the infusion of GMSCs effectively reduced airway inflammation by suppressing the population of $CD11b^+CD11c^+$ inflammatory DCs, which in turn led to a decrease in Th2 cell differentiation and eosinophil infiltration. Additionally, we identified hepatocyte growth factor (HGF), a multifunctional cytokine secreted by GMSCs, as a key mediator of the beneficial effects of GMSCs in allergic asthma. HGF was found to regulate the immune responses initiated by $CD11b^+CD11c^+$ proinflammatory DCs. Overall, our findings suggest that GMSC-based cell therapy holds promise as a potential treatment strategy for patients with allergic asthma and other allergic diseases.

## RESULTS

### GMSCs alleviate OVA-induced allergic airway inflammation

To investigate the therapeutic effect of GMSCs on airway inflammation in allergic asthma, $2\times10^6$ GMSCs or control prepuce-derived fibroblast (PDF) cells were injected through the tail vein into OVA-sensitized mice during the first challenge with a 1% OVA solution (Figure 1A). Infusion of this dose has previously resulted in ideal therapeutic effects on experimental arthritis and other inflammatory disease models.[13,19] The elevated total IgE, an important feature of allergic asthma, was significantly reduced in both the bronchoalveolar lavage fluid (BALF) (Figure 1B, left) and the sera (Figure 1B, right) after treatment with GMSCs. In addition, the pathological changes in lung tissues in mice are important indicators of airway inflammation in an allergic asthma model. As shown in Figure 1C, compared with the OVA model, GMSCs significantly reduced inflammatory cell inflammation in the lung tissue and improved alveolar structure. Meanwhile, mice treated with GMSCs also exhibited distinctly reduced mucus hypersecretion via Schiff's periodate (PAS) staining (Figure 1D).

We next analyzed the different cell subsets in BALF using flow cytometry, with the gating strategy as shown in Figure S1. As depicted in Figures 1E and 1F, in normal mice, over 90% of cells are resident alveolar macrophages (AM), which are the most important immune cells in the alveoli for defending against invasion by foreign pathogens. In the OVA-induced asthma model, the proportions of eosinophils and neutrophils increase remarkably, while the percentage of alveolar macrophage relatively decreases. Treatment with GMSCs significantly decreased the percentages of eosinophils and neutrophils, thus resulting in a relative increase in the proportions of AM compared with the model group. However, due to the differences in the total cell numbers between the model group and GMSC-treated group, only the absolute numbers of eosinophils and neutrophils showed significant differences, not the AM. Additionally, flow cytometry analysis revealed a slight increase in the proportions of lymphocytes, although their total numbers remained unchanged. Moreover, no significant differences in the population of classic $CD11b^+F4/80^+$ macrophages in the lung tissue were observed after GMSC treatment, as indicated by flow cytometry (Figure S2). The balance and changes in these subpopulations reflect the severity of the asthma model and demonstrate that the ameliorative effects of GMSCs on OVA-induced allergic asthma.

### GMSC treatment inhibits cytokine production and $CD11b^+$ $CD11c^+$ DCs

Th2 lymphocytes produce a type 2 immune response mediated by cytokines such as IL-4, IL-5, and IL-13, which are the main causes of IgE antibody production and eosinophilic infiltration in allergic asthma.[6] Flow cytometry was conducted to detect cytokine-secreting $CD4^+$ T cells in lung tissues to further investigate whether GMSCs could inhibit cytokine secretion by inflammatory $CD4^+$ T cells. As shown in Figure 2A, compared with the OVA model, GMSC treatment prominently decreased the proportions of IL-4/IL-5-secreting Th2 cells and IL-17-secreting Th17 cells. Meanwhile, GMSCs also significantly increased the proportions of T regulatory cells (Tregs), which negatively regulate the immune response.[22,23] In addition, markedly decreased levels of IL-4 (Figure 2B, top) and IL-13 (Figure 2B, bottom) cytokines in BALF (Figure 2B, left) and sera (Figure 2B, right) were observed.

DCs, as powerful antigen-presenting cells, initiate the adaptive immune response after identifying and processing antigen, playing a key role in the pathogenesis of allergic asthma.[24] $CD11b^+CD11c^+$ DCs in lung tissues are considered the main proinflammatory DCs that lead in the initiation of the type 2 immune response.[9] Our data showed that infusion of GMSCs could effectively reduce the ratios of $CD11b^+CD11c^+$ proinflammatory DCs in lung tissues (Figure 2C). As expected, the infusion of control PDF cells did not regulate the immune status of the allergic asthma model (Figure S3). These results indicate that GMSC-based cell therapy may reduce the cytokines produced by Th2 cells by regulating $CD11b^+CD11c^+$ proinflammatory DCs, thereby reducing the inflammatory responses in the entire airway.

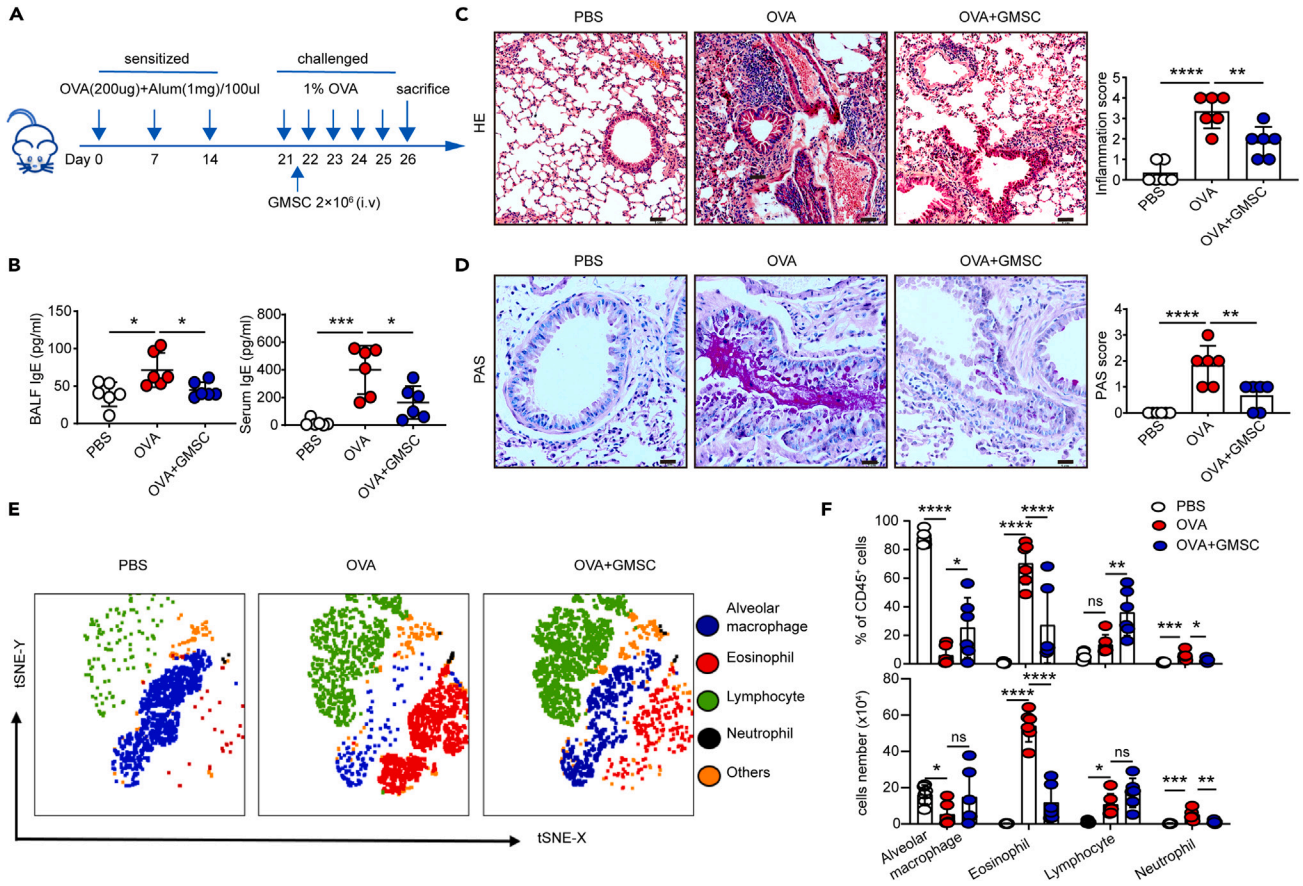


**Figure 1. GMSCs alleviate OVA-induced allergic airway inflammation**

(A) Schematic diagram illustrating the experiment procedure. OVA-sensitized mice were administrated $2\times10^6$ GMSCs during the initial challenge with a 1% OVA solution. The mice were sacrificed on the 26th day after 5 days of continuous aerosol challenge.

(B) Quantification of total IgE antibodies in bronchoalveolar lavage fluid (BALF) and serum using ELISA.

(C and D) Representative H&E and PAS staining images of lung tissues. Infiltrated inflammatory cells and mucus-secreting cells were scored.

(E and F) Flow cytometry characterized the cell populations in BALF, classifying them as alveolar macrophages, eosinophils, lymphocytes, or neutrophils. The distribution and statistics of different cell populations are presented. (Data are presented as mean ± SD; $n = 6$ per group. A one-way ANOVA with Tukey's multiple comparisons test was applied for statistical analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.).

## GMSC-CM regulates DC-mediated immune responses *in vivo*

Given that GMSCs can effectively control the inflammatory responses of allergic asthma induced by OVA, we next sought to determine whether GMSCs exhibit their modulatory roles through a humoral mechanism. The culture medium of GMSCs (GMSC-CM) was concentrated by ultrafiltration and infused intraperitoneally into mice sensitized with OVA during challenge with a 1% OVA solution. In line with the effects of GMSCs, GMSC-CM also significantly reduced the proportion of eosinophils and neutrophils in BALF compared to the OVA-induced model, implying that some functional factors from GMSC-CM could moderate airway inflammation (Figure 3A).

Next, flow cytometry analysis provided further evidence that GMSC-CM could reduce the proportions of $CD11b^+CD11c^+$ proinflammatory DCs in lung tissues, akin to the effects of GMSCs (Figure 3B). Moreover, the phenotypes of DCs in lung tissues and mediastinal lymph nodes, including CD80, CD86, major histocompatibility complex (MHC) II, CD40, and CD69, were all decreased, indicating that the maturation and activation levels of DCs in allergic asthma models were inhibited, which eventually resulted in a reduced occurrence and development of immune responses (Figure 3C).

The inflammatory responses induced by OVA are mainly concentrated in the lung tissues, but the initiation of adaptive immune responses usually occurs in the mediastinal lymph nodes. Therefore, we detected and analyzed various $CD4^+$ T cell subtypes secreting different cytokines in the mediastinal lymph nodes by flow cytometry. As illustrated in Figure 3D, the proportions of Th2 cells secreting IL-4 and IL-5 and Th17 cells secreting IL-17 in mice treated with GMSC-CM were significantly decreased compared to those in the OVA model mice. Conversely, the proportions of Treg cells were significantly increased, thus leading to a restoration of the immune balance from immune disorder. It is likely that the functional molecule(s) secreted from GMSC-CM regulate the maturation and activation of DCs and then further affect the differentiation and development of Th cells.

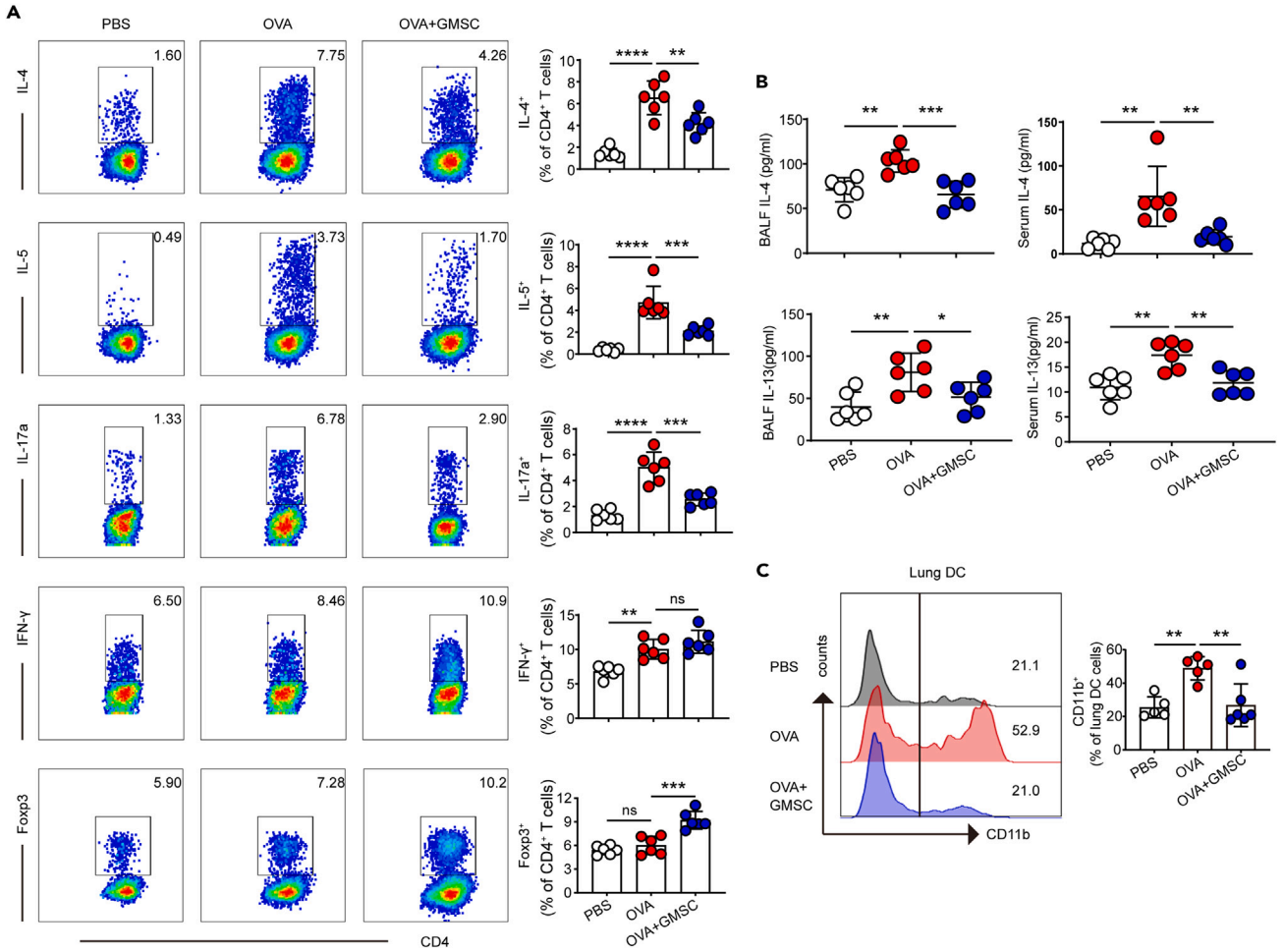


**Figure 2. GMSC treatment inhibits cytokine production and CD11b$^+$ CD11c$^+$ DCs**

OVA-sensitized mice were treated with GMSCs during the initial challenge with a 1% OVA solution. The mice were sacrificed on the 26th day after 5 days of continuous aerosol challenge.

(A) Flow cytometry analysis of various cytokine-secreting CD4$^+$ T cells.

(B) ELISA assessment of cytokine levels, including IL-4 and IL-13 in BALF and serum.

(C) Flow cytometry analysis of the percentages of CD11b$^+$CD11c$^+$ proinflammatory DCs in lung tissue. (Data are presented as mean ± SD; $n = 5$–6 per group. A one-way ANOVA with Tukey's multiple comparisons test was applied for statistical analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns indicates no significance.).

## GMSC-CM regulates DC-mediated immune responses *in vitro*

To verify the effect of GMSC-CM on DCs, bone marrow cells from normal mice were induced to differentiate into bone marrow-derived dendritic cells (BMDCs) *in vitro* by rm Granulocyte-Macrophage Colony-Stimulating Factor (rm GM-CSF) and rm IL-4.[25] These DCs were then stimulated with OVA at 1 mg/ml to induce maturation and antigen capture. During this process, GMSC-CM was simultaneously added to determine its effect on the activation state of BMDCs. The results, shown in Figures 4A and 4B, indicated that the expression levels of surface molecules CD80, CD86, MHC II, CD40, and CD69 were significantly reduced in BMDCs treated with GMSC-CM compared with control BMDCs. Additionally, there cells failed to effectively stimulate T cell proliferation (Figure 4C).

To further investigate the effect of GMSC-CM on the functions of BMDCs in antigen presentation and disease induction, naive CD4$^+$ T cells were selected from the spleens of normal mice and co-cultured with BMDCs treated with or without GMSC-CM. Under the induction of specific cytokines, CD4$^+$ T cells were differentiated into Th2 and Treg cells. However, BMDCs treated with GMSC-CM demonstrated a reduction in Th2 cell polarization and an increase in Treg cell polarization compared to control BMDCs (Figures 4D and 4E). Additionally, $2\times10^6$ OVA-treated BMDCs, with or without GMSC-CM, were injected into mice via the tail vein. The mice were then sacrificed on the 11th day after a continuous 4-day challenge with a 1% OVA solution aerosol. As depicted in Figures 4F and 4G, BMDCs treated with GMSC-CM induced lower levels of inflammatory cell infiltration and mucus hypersecretion, as confirmed by H&E and Schiff's periodate (PAS) staining, when compared to the control group. These results suggest that GMSC-CM can affect the differentiation and development of CD4$^+$ T cells by regulating the maturation and activation of DCs.

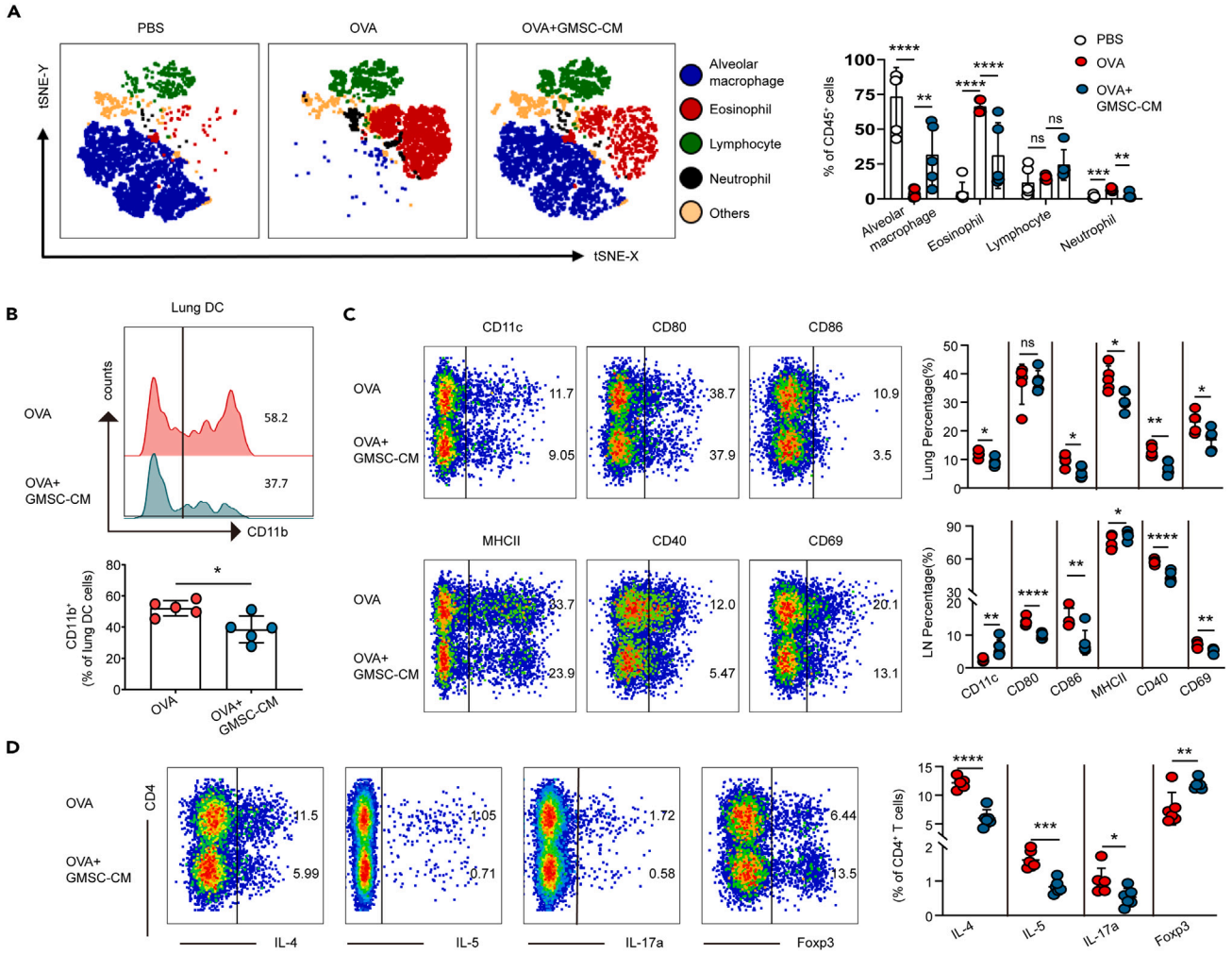


**Figure 3. GMSC-CM regulates DCs-mediated immune responses *in vivo***

OVA-sensitized mice were continuously intraperitoneally injected with GMSC-CM during the challenged with a 1% OVA solution. The mice were sacrificed on the 26th day after 5 days of continuous aerosol challenge.

(A) Flow cytometry analysis of the distribution and statistics of different immune cells in BALF.

(B) Flow cytometry analysis of the percentages of $CD11b^{+}CD11c^{+}$ proinflammatory DCs in lung tissue.

(C) Flow cytometry analysis of DCs ratios and the activation makers, including CD80, CD86, MHC II, CD40, and CD69, in lung tissue and mediastinal lymph nodes.

(D) Different cytokine-secreting $CD4^{+}$ T cells were determined by flow cytometry. (Data are presented as mean ± SD; $n = 5–6$ per group. One-way ANOVA with Tukey's multiple comparisons test or unpaired Student's t test were used for statistical analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns means no significance.).

## HGF contributes to the immunomodulation of GMSCs on DCs

We have documented that GMSC-CM can effectively inhibit the inflammatory responses of allergic asthma by modulating DC-mediated immune responses. Herein, we further explored the specific functional molecules and mechanisms underlying these effects. A series of MSC-related immunomodulatory molecules were screened and verified by qPCR. As shown in Figure 5A, GSMCs showed a higher mRNA expression of various immune regulatory molecules, among which the expression of HGF was significantly higher compared to that of PDF cells. Concurrently, the protein expression of HGF was also upregulated in GMSCs and GMSC-CM (Figures 5B and 5C). Moreover, the HGF-specific receptor c-Met is expressed on DCs, and HGF have been shown to inhibit the incidence of allergic asthma in models.[26,27] We hypothesized that GSMCs or GMSC-CM regulates DCs to inhibit the development of allergic asthma by secreting HGF.

The role of HGF in the regulation of DCs by GMSC-CM was evaluated in an *in vitro* experiment where BMDCs were stimulated with OVA at 1 mg/ml. The addition GMSC-CM effectively inhibited the expression levels of activation molecules CD80, CD86, CD40, and CD69 in BMDCs. However, the inhibitory effect of GMSC-CM was significantly reduced after blocking HGF signaling (Figures 5D and 5E). Moreover, GMSC-CM

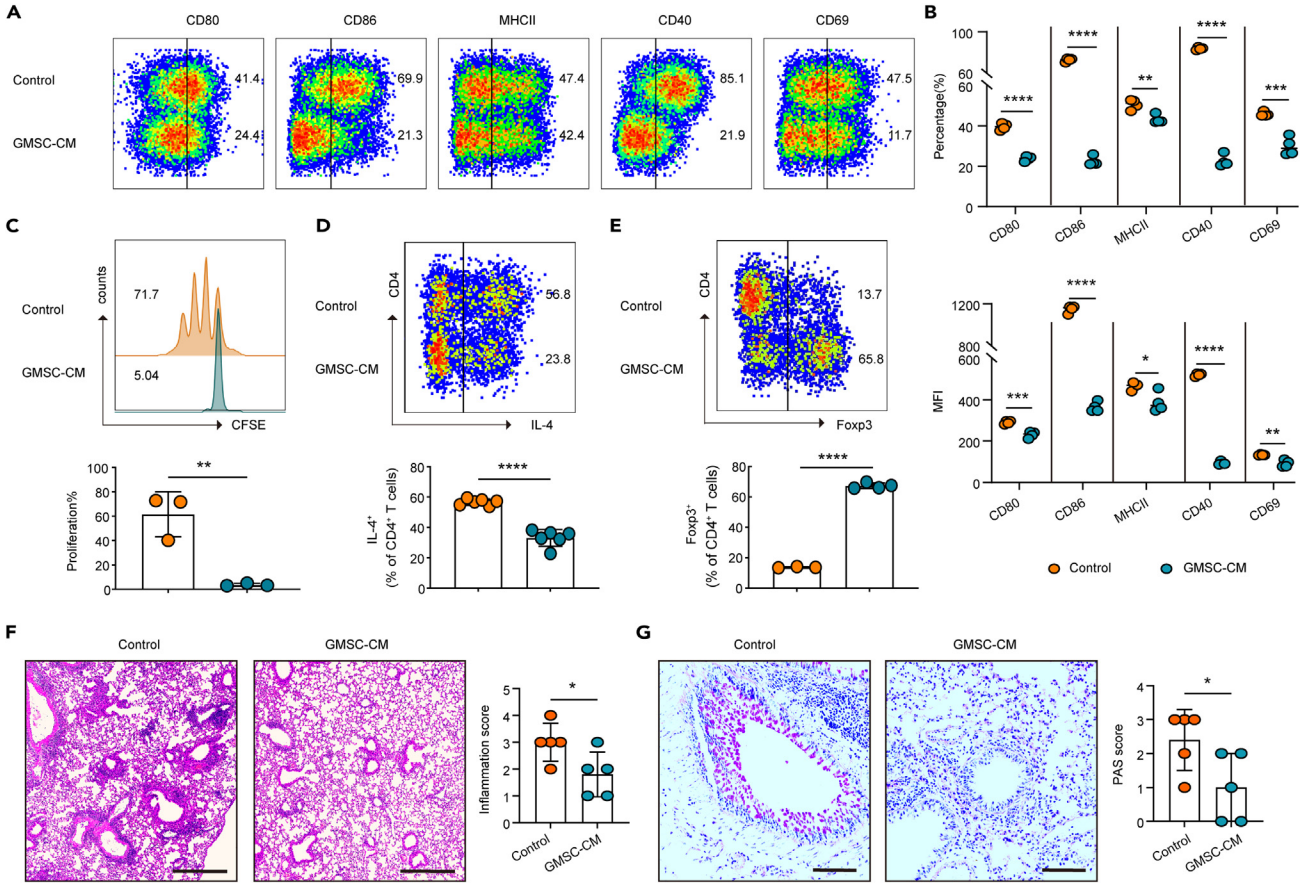


**Figure 4. GMSC-CM regulates DC-mediated immune responses *in vitro***

Bone marrow-derived dendritic cells (BMDCs) from C57BL/6 mice were treated with OVA at 1 mg/ml for 24 h to induce their maturation and antigen capture, with or without GMSC-CM.

(A) Flow cytometry analysis of surface molecular markers, including CD80, CD86, MHC II, CD40, and CD69.

(B) The percentage and mean fluorescence intensity (MFI) of those makers.

(C) CFSE-labeled T cells were co-cultured with the different types of DCs. T cell proliferation, which represents the antigen-presenting capacity of DCs, was analyzed using CFSE.

(D and E) Naive T cells from the spleen were cultured with the different DCs in the present of rm IL-4 for Th2 cells (D) or rh TGF-β/IL-2 for Treg cells (E). Three days later, the T cells were collected and tested by flow cytometry.

(F and G) $2\times10^6$ OVA-treated BMDCs, with or without GMSC-CM, were injected into mice via the tail vein. The mice were then sacrificed on the 11th day after a continuous 4-day challenge with a 1% OVA solution aerosol. Representative H&E (F) and PAS (G) staining images of lung tissue. The scores of infiltrated inflammatory cells and mucus-secreting cells were measured in each group. (Data are presented as mean ± SD; $n$ = 3–6 per group. Unpaired Student's t test was used for statistical analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns means no significance.).

could enhance the phosphorylation of STAT3, which is the critical transcription factor mediating the tolerance of DCs. The enhancing effect of GMSC-CM was reduced after the blockade of HGF signaling (Figure S4). Thus, HGF could be an important functional molecule in the modulation of DCs by GMSC-CM.

## GMSC-CM alleviates allergic airway inflammation via HGF

The OVA-induced allergic asthma model was used to further explore whether GMSC-CM controls disease development via HGF *in vivo*. As illustrated in Figures 5F and 5G, GMSC-CM effectively reduced the pathogenic changes and inhibited the infiltration of eosinophils in lung tissues, similarly to the infusion of GMSCs. Interestingly, compared to mice treated with GMSC-CM, mice in which HGF signaling was largely blocked showed that the therapeutic effects of GMSC-CM were significantly diminished. Those mice exhibited more severe pathological changes and increased eosinophil infiltration, closely resembling those of the control asthma model. We therefore believe that GMSC-CM primarily alleviates airway inflammation mostly through its key component, HGF.

As HGF in GMSC-CM suppresses the maturation and activation of DCs *in vitro*, we then sought to determine whether HGF in GMSC-CM also mediates $CD11b^+CD11c^+$ proinflammatory DCs and Th2 type cells in an asthma model *in vivo*. Flow cytometry analysis revealed that the

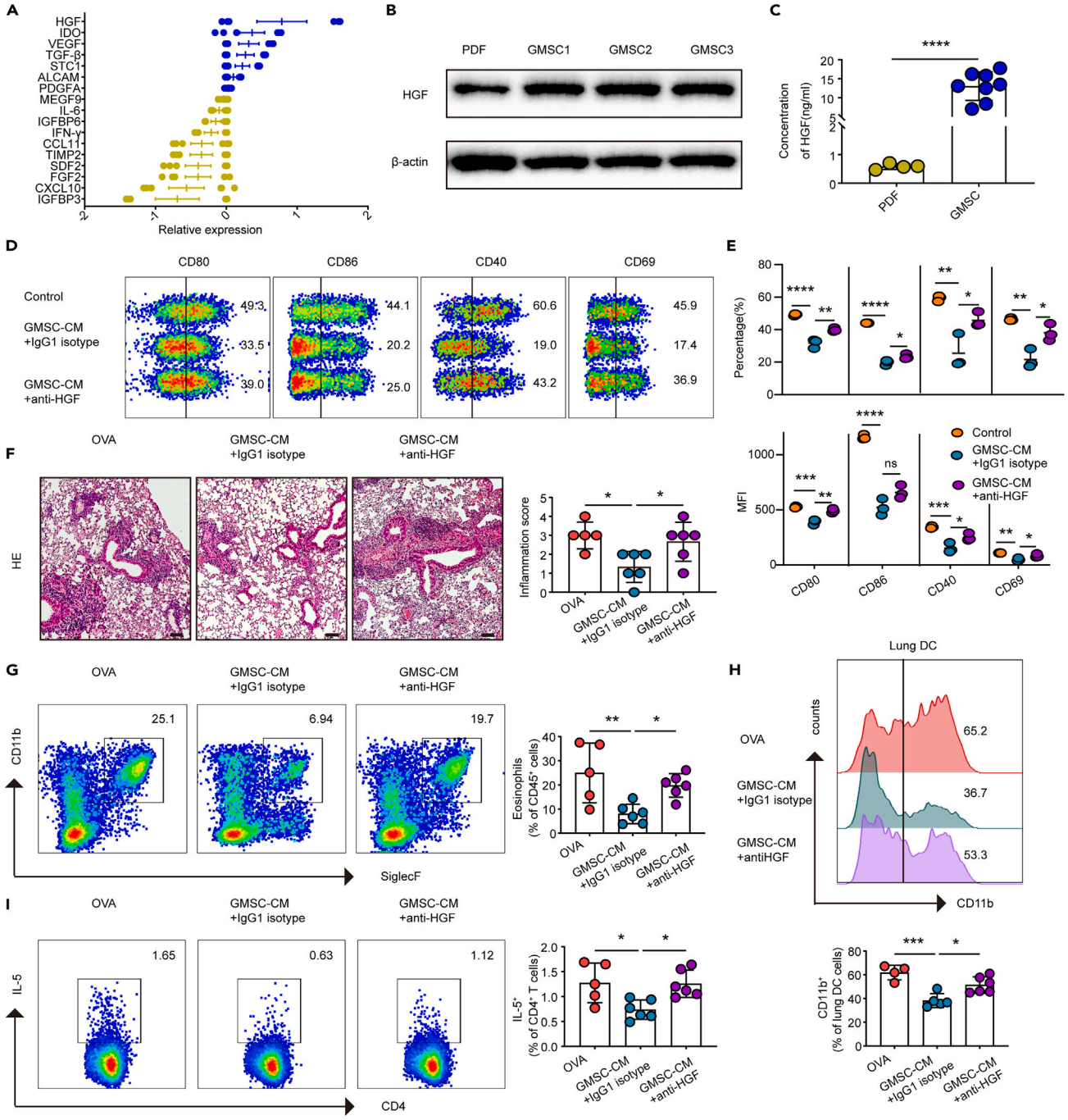


**Figure 5. HGF mediates the alleviation of allergic airway inflammation by GMSC-CM**

(A) Relative expression levels of GMSCs molecules determined by quantitative RT-PCR array.

(B) Western blotting analysis of HGF levels in prepuce-derived fibroblast cells (PDF) and GMSCs derived from different individuals.

(C) ELISA measurement of HGF levels in the culture medium of PDF and GMSCs.

(D) BMDCs were treated with GMSC-CM in the presence or absence of an HGF neutralizing antibody or control IgG1 isotype, and were stimulated with OVA at 1 mg/ml for 24 h. Flow cytometry analysis of DC surface makers, including CD80, CD86, CD40, and CD69.

(E) The percentage and mean fluorescence intensity (MFI) of those makers.

(F–I) OVA-sensitized mice were continuously injected intraperitoneally with GMSC-CM in the presence or absence of an HGF neutralizing antibody or control IgG1 isotype during the challenge, and the mice were sacrificed on the 26th day after 5 days of continuous aerosol challenge.

(F) Lung tissue pathological changes assessed by H&E staining.

**Figure 5. *Continued***

(G) Eosinophil infiltration into lung tissue was analyzed by flow cytometry.

(H) Analysis of $CD11b^+CD11c^+$ proinflammatory DCs in the lung tissue by flow cytometry.

(I) IL5-secreting $CD4^+$ T cells were determined by flow cytometry. (Data are presented as mean ± SD; $n = 3–6$ per group. A one-way ANOVA with Tukey's multiple comparisons test was used for statistical analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.).

levels of $CD11b^+CD11c^+$ proinflammatory DCs in the lung tissues (Figure 5H) and IL5-secreting $CD4^+$ T cells in the lymph nodes (Figure 5I) were significantly increased in the group where HGF signaling was blocked compared with the GMSC-CM treatment group. This indicates that GMSC-CM could inhibit $CD11b^+CD11c^+$ inflammatory DCs mainly by secreting HGF, eventually affecting the occurrence of Th2 type immune responses and the progression of allergic asthma.

### Intervening in the expression of HGF affects the function of GMSCs *in vivo*

To further confirm whether HGF was the core molecule responsible for inhibiting airway inflammation in GMSCs, we used HGF-knockdown or HGF-overexpressing GMSCs to verify their function *in vivo*. First, HGF gene overexpression and knockdown lentiviral vectors were constructed *in vitro* (Figure S5). Subsequently, we successfully constructed HGF-knockdown and HGF-overexpressing GMSCs. The expression level of HGF in HGF-overexpressing GMSCs ($GMSC^{HGF}$) was much higher than that in overexpression vector control GMSCs ($GMSC^{oe\text{-}con}$) and normal untreated GMSCs ($GMSC^{blank}$) (Figures 6A–6C). Similarly, the expression level of HGF in HGF-knockdown GMSCs ($GMSC^{shHGF}$) was significantly lower than that in the knockdown vector control GMSCs ($GMSC^{sh\text{-}con}$) (Figures 6D–6F).

We immediately administered GMSCs with different HGF expression levels to mice with allergic asthma. The results showed that, consistent with the previous findings, HGF-knockdown GMSCs significantly reduced the effectiveness of the treatment on allergic asthma. This included in effectiveness included impacts on improving lung tissue pathology (Figure 6G), suppressing eosinophil infiltration (Figure 6H), reducing the number of $CD11b^+CD11c^+$ proinflammatory DCs (Figures 6I and 6K), and decreasing the percentage of IL-5-secreting $CD4^+$ T cells (Figures 6J and 6L). In contrast, HGF-overexpressing GMSCs more effectively inhibited the airway inflammatory responses of allergic asthma, significantly reduced the ratio of $CD11b^+CD11c^+$ proinflammatory DCs and decreased the percentage of IL-5-secreting $CD4^+$ T cells (Figures 6G–6L), further demonstrating that the therapeutic effect of GMSCs on experimental allergic asthma is mostly dependent on HGF signaling. Thus, manipulating HGF expression on MSCs might strengthen the application of GMSC-based cell therapy, at least in the context of asthma.

## DISCUSSION

The current treatment methods for allergic asthma include controlling the onset, treatment, achieving remission, and managing long-term or potential recurrence. However, the long-term side effects of drug use and patient compliance are also important factors in the failure to effectively control the occurrence of allergic asthma. To control acute allergic asthma, bronchial spasmolytic agents, such as β2 agonists, theophylline, anticholinergic drugs, corticosteroids, and leukotriene modulators are commonly used. It is important to note that there are currently no cues for allergic asthma.[28] Furthermore, the long-term and repeated episodes of allergic asthma can lead to complications such as chronic bronchitis, pulmonary interstitial fibrosis, obstructive emphysema, and respiratory arrest. It is, therefore, crucial to explore the underlying pathological mechanisms of allergic asthma and discover new therapeutic approaches.

Currently, the transplantation of MSCs has been documented as an attractive approach to treating allergic asthma.[29] One specific advantage of MSCs in lung disease treatment is that most exogenous MSCs remain in the lung tissue after transplantation into mice. The migration of MSCs in the lung relies on the stromal cell-derived factor 1α (SDF-1α)/CXCR4 axis.[30] Numerous studies have demonstrated the positive effects of MSCs in allergic asthma. MSCs can inhibit pulmonary inflammation and improve airway remodeling by regulating different target cells, including enhancing Th1 cell differentiation, increasing the proportions of Treg cells and inhibiting proinflammatory DCs.[31–33] However, current research mainly focuses on MSCs derived from bone marrow, adipose tissue, and umbilical cord blood, which have some concerns, such as low yield, potential tumorigenicity, and ethical issues, which may affect their clinical application. GMSCs, as a newly developed type of MSC, offer several advantages, including easy acquisition of materials, rapid cell growth, no serum dependence for cell cultures, no tumorigenicity, no retention, as well as stable phenotypes and telomerase activity even after long-term culture. These features suggest that GMSC-based cell therapy could be a better option for clinical application.[34,35]

Our past work has focused on the study of new molecules and mechanisms related to the immunomodulatory effect of GMSCs and has confirmed the therapeutic effects in various autoimmune diseases and inflammatory diseases.[12–16,18–21,34] We have also established a standardized protocol for the extraction, culture, and identification of clinical-grade GMSCs.[36] However, the impact of GMSCs on allergic disease has yet to be determined. In this study, we conducted a comprehensive evaluation of the therapeutic effects of GMSCs on an OVA-induced allergic asthma model. This model represents a type 2 immune response characterized by significant eosinophil infiltration and elevated IgE antibody production.[37] Our findings demonstrated that GMSCs were capable of effectively improving lung tissue pathology and alleviating airway inflammation.

Previous preclinical studies have shown that MSCs possess homing ability, are chemotactic, are recruited to inflammatory injury sites in the body, and can replace damaged cells through self-renewal and directional differentiation. However, more recent studies have provided evidence that most transplanted MSCs undergo apoptosis, and the differentiation level at the injured site is low and unstable. However, we have previously monitored the cell fate of GMSCs after cell infusion and demonstrated the GMSC survive well at least 25 days in the lung tissue after

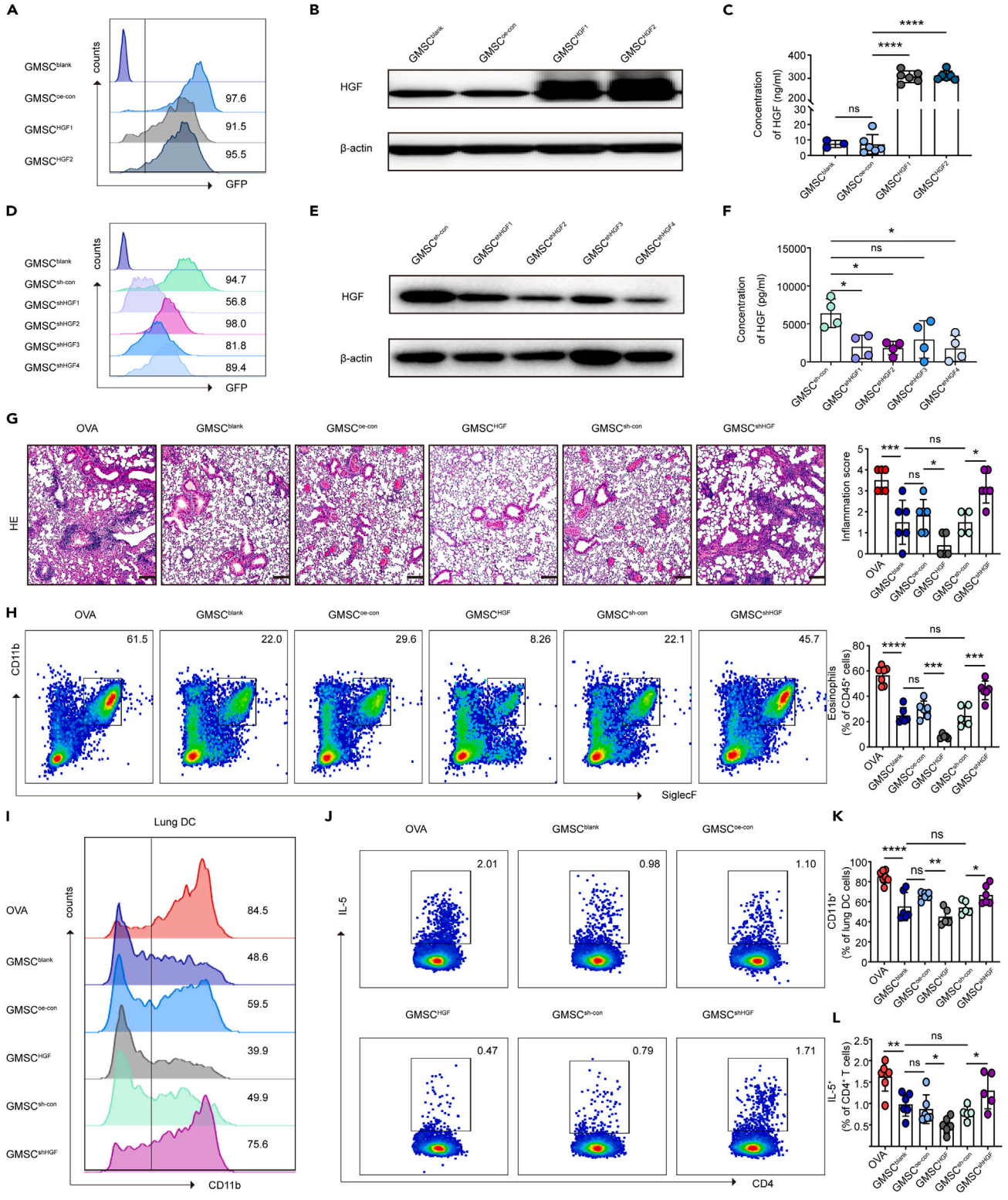


**Figure 6. Intervening the expression of HGF affects the function of GMSCs *in vivo***

(A–F) Evaluation of HGF gene overexpression or knockdown efficiency and effectiveness in GMSCs. (A and D) Flow cytometry analysis of the percentage of GFP-positive cells. (B and E) Western blotting analysis of HGF protein levels in GMSCs after transduction. (C and F) ELISA measurement of HGF secretion by GMSCs after 48 h of culture.

**Figure 6. *Continued***

(G–I) OVA-sensitized mice were treated with GMSCs exhibiting varying levels of HGF during the initial challenge with a 1% OVA solution, and the mice were sacrificed on the 26th day after 5 days of continuous aerosol challenge.

(G) Lung tissue pathological changes assessed by H&E staining.

(H) Eosinophil infiltration in lung tissue analyzed by flow cytometry.

(I and K) Analysis of $CD11b^+CD11c^+$ proinflammatory DCs in lung tissue by flow cytometry.

(J and L) IL5-secreting $CD4^+$ T cells were determined by flow cytometry. (Data are presented as mean ± SD; $n$ = 3–6 per group. A one-way ANOVA with Tukey's multiple comparisons test was used for statistical analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns means no significance.).

cell injection.[13] Considering the asthma model we conducted has a short period, we believe the GMSCs have a sufficient life span in combating asthma *in vivo*.

Nonetheless, the therapeutic effect mediated by MSCs may also partially depend on the substances they secrete.[38,39] Indeed, culture supernatants from MSCs have displayed promising therapeutic effects on a variety of diseases, including allergic asthma. Moreover, most diseases exist in a state of local inflammation or hypoxia, environment that can stimulate MSCs to secrete more cytokines or protein ingredients to regulate various target cells and enhance their immune regulation function. At the same time, a growing number of studies have focused on specific components of MSCs that have therapeutic effects. For example, insulin-like growth factor-2 (IGF2) mediates the immunoregulatory function of MSCs in improving multiple sclerosis by reprogramming macrophages and upregulating Treg cells.[40] Additionally, the exosomes of MSCs, an important medium for information communication, also offer new insights and new methods for cell-free transplantation therapy.[2,41] In our recent study, we specialized in extracting concentrated exosomes from GMSCs and found that GMSC-derived exosomes inhibit T cell responses *in vitro* and protect against the collagen-induced arthritis (CIA) model, which is an autoimmune disease (JCI Insight in press). Herein, we have shown that GMSC-CM could also effectively reduce the infiltration of eosinophils and neutrophils in alveolar lavage fluid, indicating that some secreted components from GMSCs could inhibit the inflammatory and allergic response of lung tissues.

Macrophages, as one of the innate immunocyte, encompass both pro-inflammatory and anti-inflammatory effects in chronic inflammation, such as asthma. Macrophages may contribute to the pathogenesis of asthma by releasing inflammatory cytokines and nitric oxide (NO), which can exacerbate lung injury and airway remodeling. Conversely, macrophages also secret several anti-inflammatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor β (TGF-β). These factors can mitigate the inflammatory response, reduce tissue damage, and help maintain immune system balance. This variability may depend on the disease stage and different macrophage subgroups.[42,43] In lung tissue, there are two important macrophage populations: the alveolar macrophage and the pulmonary interstitial macrophage. In the BALF of normal mice, over 90% of cells are resident AM, which are the most important immune cells in the alveoli for resisting invasion by foreign pathogens. In our previous study, we demonstrated that GMSCs could promote the expression of anti-inflammation macrophages.[14] In this study, the percentage, but not the absolute number, of alveolar macrophage among $CD45^+$ cells had increased in the BALF after GMSC treatment. Furthermore, there were no significant differences in the population of $CD11b^+F4/80^+$ macrophage after GMSC treatment.

DCs located in lung tissues, especially $CD11b^+CD11c^+$ proinflammatory DCs, serve as the primary antigen-presenting cells and migrate to mediastinal lymph nodes to initiate adaptive immune responses upon identifying and processing foreign antigens. The differentiation of naive T cells is strongly influenced by the phenotypic status and cytokine production by DCs.[44] Moreover, phenotypic and functional alterations in DCs play a critical role in the onset and progression of allergic asthma.[24,44] As previously reported, we also observed that $CD11b^+CD11c^+$ proinflammatory DCs were significantly increased in the lung tissues of allergic asthma mice, closely related to cytokine secretion levels from Th2 cells.[45,46] Nonetheless, infusion of GMSC-CM reduced the proportions of $CD11b^+CD11c^+$ inflammatory DCs. Furthermore, the maturity and activation levels of DCs in lung tissues and mediastinal lymph nodes were found to decrease, leading to reduced differentiation of Th2 and Th17 cells and increased differentiation of Treg cells. Th17 and Treg cells have distinct roles in the initiation and progression of asthma.[47] The regulatory effect of GMSCs on DCs was similar *in vitro* with bone marrow-derived DCs. These results indicate that GMSCs can regulate DCs through the secretion of functional molecules, thereby reducing the production of Th2 cells and their related cytokines. Moreover, these components also reduce the proportions of eosinophils. GMSCs alleviate airway inflammatory and allergic responses through their secretory components.

HGF was identified as a highly expressed molecule in GMSCs compared to control cells, and it is reasonable to propose that it may play a critical role in modulating immune responses during experimental allergic asthma. HGF was originally discovered as a mitogen for liver cell growth and is primarily involved in the repair process of liver tissues after injury.[48] Many studies have established that HGF is a multifunctional cytokine that can induce angiogenesis, promote cell proliferation and migration, and inhibit cell apoptosis.[49] Additionally, HGF acts as an important secreted cytokine of MSCs to mediate their immune regulation. MSCs that are primed or overexpress HGF significantly enhance cell survival, migration, and regeneration compared to MSCs alone.[50] For example, MSCs restore lung tissue permeability and improve lung tissue injury by secreting HGF.[51] Moreover, airway epithelial cells secrete HGF to promote lung tissue injury repair. During the challenge phase in allergic asthma mice, exogenous HGF can effectively reduce airway hyperresponsiveness and airway inflammation, while neutralizing endogenous HGF can aggravate disease progression.[27,52] These studies suggest that HGF may serve as a crucial functional molecule in the amelioration of allergic asthma. In our present study, we provide evidence demonstrating that GMSCs play significant roles in mitigating airway inflammation, partially through the secretion of HGF, which can directly regulate inflammatory DCs via the HGF-specific receptor c-Met expressed on DCs. Additionally, GMSCs with HGF overexpression had greater efficiency in treating asthma in mice. It is suggested that GMSCs with HGF overexpression may be a better cell population for cell therapy in allergic asthma and other allergic diseases, demonstrating a "1 + 1>2" effect. It has to be stated that HGF is not only effector molecule GMSCs secreted for controlling asthma. In fact, the

blockade of HGF failed to completely abolish the therapeutic effect of GMSCs on asthma, implying the other components GSMCs secreted also help to combat asthma. However, HGF, secreted by GMSCs, is a key player in treating asthma.

In summary, our results suggest that HGF expressed by GMSCs is a key player in reducing allergic asthma. It primarily targets $CD11b^{+}CD11c^{+}$ proinflammatory DCs, then restores the balance between Th2 and Treg cells, ultimately combating asthma. Therefore, these findings provide insights that the application of GMSCs or GMSC-CM could be a promising and effective approach for treating allergic diseases, including asthma and other inflammatory allergic conditions.

### Limitations of the study

This study has several limitations. Firstly, experiments investigating the impact of the injected cell count on the therapeutic outcome were not conducted. Additionally, the HGF receptor c-Met was not knocked out on DCs to verify its role in treating asthma with GMSCs.

## STAR★METHODS

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## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.109818.

## ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (NSFC) (82200028 to Q.Fang, 82001760 to W.W., and 81871224 to S.G.Z.); the Guangdong Basic and Applied Basic Research Foundation (2021A1515010251 to Z.X., 2023A1515010002 to W.W., and 202201011085 to W.W.); and the National Key R&D Program of China (2017YFA0105801 to S.G.Z.). We thank Dr. Nancy Olsen, a professor and chief of the Division of Rheumatology and Immunology in the Hershey Medical Center at Penn State University, for her critical comments and language edits for this manuscript.

## AUTHOR CONTRIBUTIONS

Conceptualization, S.G.Z.; methodology, Q.Fang, W.W., and Z.X.; validation, Q.Fang and W.W.; visualization, Q.Fang and S.G.Z.; writing – original draft, Q.Fang and Z.X.; writing – review & editing, S.G.Z., Y.Z., T.L., J.S., and Q.Fu; resources, D.Z. and J.Y; funding acquisition, S.G.Z., Q.Fang, W.W., and Z.X.; supervision, R.L., J.W., W.S., and X. X.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: February 21, 2024
Revised: March 25, 2024
Accepted: April 24, 2024
Published: April 26, 2024

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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Antibodies | | |
| PerCP/Cyanine5.5 anti-mouse CD45 Antibody, Clone, 30-F11 | Biolegend | Cat#103132, RRID: AB_893340 |
| PE anti-mouse CD11c Antibody, Clone, N418 | Biolegend | Cat#117308, RRID: AB_313777 |
| PE/Cyanine7 anti-mouse/human CD11b Antibody, Clone, M1/70 | Biolegend | Cat#101216, RRID: AB_312799 |
| APC anti-mouse CD170 (Siglec-F) Antibody, Clone, S17007L | Biolegend | Cat#155508, RRID: AB_2750237 |
| APC/Cyanine7 anti-mouse Ly-6G Antibody, Clone, 1A8 | Biolegend | Cat#127624, RRID: AB_10640819 |
| PE/Cyanine7 anti-mouse IL-4 Antibody, Clone, 11B11 | Biolegend | Cat#504118, RRID: AB_10898116 |
| APC anti-mouse F4/80 Antibody, Clone, BM8 | Biolegend | Cat# 123116, RRID: AB_893481 |
| PE anti-mouse/human IL-5 Antibody, Clone, TRFK5 | Biolegend | Cat# 504303, RRID: AB_315327 |
| APC/Cyanine7 anti-mouse IL-17A Antibody, Clone, TC11-18H10.1 | Biolegend | Cat#506940, RRID: AB_2565781 |
| APC anti-mouse IFN-γ Antibody, Clone, XMG1.2 | Biolegend | Cat#505810, RRID: AB_315404 |
| Alexa Fluor® 488 anti-mouse FOXP3 Antibody, Clone, MF-14 | Biolegend | Cat#126406, RRID: AB_1089113 |
| FITC anti-mouse CD80 Antibody, Clone, 16-10A1 | Biolegend | Cat#104706, RRID: AB_313127 |
| PE/Cyanine7 anti-mouse CD86 Antibody, Clone, GL-1 | Biolegend | Cat#105014, RRID: AB_439783 |
| PerCP/Cyanine5.5 anti-mouse I-A/I-E Antibody, Clone, M5/114.15.2 | Biolegend | Cat#107626, RRID: AB_2191071 |
| FITC anti-mouse CD40 Antibody, Clone, 3/23 | Biolegend | Cat#124608, RRID: AB_1134096 |
| PE/Cyanine7 anti-mouse CD69 Antibody, Clone, H1.2F3 | Biolegend | Cat#104512, RRID: AB_493564 |
| APC anti-mouse CD4 Antibody, Clone, | Biolegend | Cat#100516, RRID: AB_312719 |
| Biotin anti-mouse CD11c Antibody, Clone, N418 | Biolegend | Cat#117304, RRID: AB_313773 |
| Biotin anti-mouse/human CD11b Antibody, Clone, M1/70 | Biolegend | Cat#101204, RRID: AB_312787 |
| Biotin anti-mouse CD49b Antibody, Clone, HMα2 | Biolegend | Cat#103522, RRID: AB_2566366 |
| Biotin anti-mouse CD25 Antibody, Clone, PC61 | Biolegend | Cat#102004, RRID: AB_312853 |
| Biotin anti-mouse CD8a Antibody, Clone, 53-6.7 | Biolegend | Cat#100704, RRID: AB_312743 |
| Biotin anti-mouse/human CD45R/B220 Antibody, Clone, RA3-6B2 | Biolegend | Cat#103204, RRID: AB_312989 |
| PE-Cyanine7 FOXP3 Monoclonal Antibody, Clone, FJK-16s | Thermo Fisher | Cat#25-5773-82, RRID: AB_891552 |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| HGF Recombinant Monoclonal Antibody, Clone, 7H6L1 | Invitrogen | Cat#701283, RRID: AB_2532457 |
| Phospho-STAT3(Tyr705) XP Rabbit mAb, Clone, D3A7 | CST | Cat#9145s, RRID: AB_2491009 |
| STAT3 Rabbit mAb, Clone, D3Z2G | CST | Cat#12640s, RRID: AB_2629499 |
| Mouse Anti-Actin, beta Monoclonal Antibody, Clone mAbcam 8226 | abcam | Cat#Ab8226, RRID: AB_306371 |
| HRP Conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) | BOSTER | Cat#BA1054, RRID: AB_2734136 |
| **Bacterial and virus strains** | | |
| *E coli* DH5α Competent Cells | Takara | Cat#9057 |
| **Biological samples** | | |
| Serum samples from C57BL/6 mice | This paper | N/A |
| Bronchoalveolar lavage fluid samples from C57BL/6 mice | This paper | N/A |
| Human gingival tissues from healthy individuals | This paper | N/A |
| **Chemicals, peptides, and recombinant proteins** | | |
| Ovalbumin (OVA) | Sigma | Cat#A5503-10G |
| ImjectTM Alum | Thermo Fisher | Cat#77161 |
| Collagenase IV | Sigma | Cat#C5138 |
| PMA | Sigma | Cat#P8139-5MG |
| Ionomycin | InvivoGen | Cat#Inh-Ion |
| Brefeldin A Solution (1000×BFA) | Biolegend | Cat#420601 |
| Foxp3/Transcription Factor Staining Buffer | eBioscience | Cat#00-5523-00 |
| Fixation Buffer | Biolegend | Cat#420801 |
| Intracellular Staining Permeabilization Wash Buffer | Biolegend | Cat#421002 |
| Trypsin | Sigma | Cat#T4049-500ml |
| Murine GM-CSF | PeproTech | Cat#315-03-100 |
| Murine IL-4 | PeproTech | Cat#214-14-20 |
| Anti-biotin Microbeads | Miltenyi | Cat#130-090-485 |
| CD62L Microbeads | Miltenyi | Cat#130-090-701 |
| Mitomycin C | Sigma | Cat#M4287-2mg |
| Ultra-LEAFTM Purified anti-mouse CD3 | Biolegend | Cat#100238 |
| Ultra-LEAFTM Purified anti-mouse CD28 | Biolegend | Cat#102116 |
| Ultra-LEAFTM Purified anti-mouse IFN-γ | Biolegend | Cat#513208 |
| Human TGF-β1 | PeproTech | Cat#AF-100-21-C-10 |
| Human IL-2 | PeproTech | Cat#AF-200-02-50 |
| SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) | TaKaRa | Cat#RR820A |
| Western and IP cell lysates buffer | Beyotime | Cat#P0013 |
| 5× protein loading buffer | Sangon Biotech | Cat#C506032-0005 |
| XbaI | NEB | Cat#R0145V |
| BamHI-HF | NEB | Cat#R3136s |
| AgeI-HF | NEB | Cat#R3552s |
| EcoRI-HF | NEB | Cat#R3101s |
| 10× CutSmart | NEB | Cat#B7204s |
| T4 DNA ligase | NEB | Cat#M0202V |
| OPTI-MEM medium | Thermo Fisher | Cat#31985-070 |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Polyethylenimine Linear (PEI) | Polysciences | Cat#23966-1 |
| **Critical commercial assays** | | |
| IL-4 ELISA kit | Dakowei Biological Co. LTD | Cat#1210402 |
| IgE ELISA kit | Dakowei Biological Co. LTD | Cat#1218202 |
| IL-13 ELISA kit | Abclonal | Cat#RK00107 |
| CFSE Cell Division Tracker Kit | Biolegend | Cat#423801 |
| Tissue RNA Purification Kit | EScience | Cat#RN001 |
| Rapid reverse transcription kit | EScience | Cat#RT001 |
| PAGE gel rapid preparation kit 10% | Epizyme | Cat#PG112 |
| BCA protein quantitative kit | TIANGEN | Cat#PA11502 |
| Plasmid extraction kit | TIANGEN | Cat#DP103-03 |
| **Experimental models: Organisms/strains** | | |
| C57BL/6 mice | GemPharmatech Co, Ltd | N/A |
| GFP-IL-4 BALB/c mice | GemPharmatech Co, Ltd | N/A |
| GFP-Foxp3 C57BL/6 mice | GemPharmatech Co, Ltd | N/A |
| **Oligonucleotides** | | |
| Primer for qPCR | This paper | Table S1 |
| Primer for HGF | This paper | Table S2 |
| Primer for shHGF | This paper | Table S2 |
| **Recombinant DNA** | | |
| pLVX-IRES-ZsGreen1-HGF | This paper | N/A |
| pLKO.1 puro-shHGF | This paper | N/A |
| **Software and algorithms** | | |
| GraphPad Prism v9.0 software | GraphPad | https://www.graphpad.com/ |
| FlowJo V10 | BD Biosciences | https://www.flowjo.com |
| ImageJ | ImageJ | https://imagej.net/ij/ |
| SlideViewer | 3DHISTECH Ltd | https://www.3dhistech.com/ |

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Dr. Song Guo Zheng (Song.Zheng@shsmu.edu.cn).

### Materials availability

All reagents generated in this study are accessible from the lead contact with a completed Materials Transfer Agreement.

### Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

## EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

### Animal models

Wild-type C57BL/6 mice, GFP-IL-4 BALB/c mice, and GFP-Foxp3 C57BL/6 mice (female, 6-8 weeks old) were bred and housed at the Guangdong Laboratory Animals Monitoring Institute. No comparison was made regarding the influence of mouse sex on the results and animal

models. All procedures involving animals were performed in accordance with guidelines and regulations set forth by the Institutional Animal Care and Use Committee at Guangdong Provincial People's Hospital (Approval no. KY-Z-2022-024-02).

## METHOD DETAILS

### GMSCs and GMSC-CM

Human gingival tissues were collected from healthy individuals (female or male, 18-45 years old) without periodontal diseases during routine dental surgeries. The study was approved by the medical ethics committees of the Institutional Review Boards (IRB) at Guangdong Provincial People's Hospital. Informed consent was obtained from all participants and/or their representatives. Human GMSCs were isolated from these tissue samples following previously established protocols.[36] A total of $2\times10^6$ GMSCs were cultured in serum-free media for 48 hours, and conditioned growth media (CM) was concentrated 20-fold through 30 kD centrifugal filter devices (Merck Millipore), which was termed GMSC-CM.

### Asthma model and GMSC treatment

On days 0, 7, and 14, female C57BL/6 mice (6-8 weeks old) were intraperitoneally injected with 200 μg of OVA (A5503-10G, Sigma–Aldrich) in 1 mg Imject™ alum adjuvant (77161, Thermo Fisher Scientific) in a total volume of 100 μl. The blank control group was injected with PBS. Fron days 21 to 25, the mice were exposed to aerosolized a 1% OVA for 30 min each day. On day 26, the mice were anesthetized and sacrificed to collect samples for relevant tests. On day 21, GMSCs or control human dermal fibroblasts ($2\times10^6$) were administered to the mice *via* tail vein injection. To investigate the underlying mechanism(s), GMSC-CM or GMSC-CM incubated with functional blocking anti-HGF or IgG1 isotype antibody for 1 hour were intraperitoneally injected on days 21 to 24.

### Bronchoalveolar lavage fluid (BALF)

To collect BALF, the mice's tracheas were cannulated using a syringe, and their lungs were rinsed three times with 1 ml of PBS. After centrifugation, the supernatants were collected for the detection of IgE, IL-4 (1210402), IgE (1218202), and IL-13 (RK00107) using ELISA. The cells present in the BALF were then analyzed by flow cytometry.

### Cell suspension preparation

The mice were sacrificed, and their lungs were digested using 0.5 mg/ml collagenase IV (C5138, Sigma–Aldrich) for 1 h at 37°C. After digestion, lung tissue was filtered through a 70 μm cell strainer. The mediastinal lymph nodes were minced and similarly filtered through a 70 μm cell strainer. All isolated cells were then suspended in PBS supplemented with 2% FBS.

### Generation of bone marrow-derived dendritic cells (BMDCs)

BMDCs were generated using a previously described method.[53,54] Briefly, bone marrow cells were harvested from wild-type C57BL/6 mice and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (10270-106, Gibco), 50 ng/ml recombinant mouse GM-CSF (315-03-100, Peprotech) and 2.5 ng/ml recombinant mouse IL-4 (214-14-20, Peprotech) for 6 days. Loose, adherent cell clusters were obtained and cultured with or without GMSC-CM. BMDC maturation was induced by stimulating the cells with OVA 1mg/ml for 24h.

### T-cell proliferation *in vitro*

Enriched T cells were isolated from the spleens of wild-type mice, stained with carboxyfluorescein succinimidyl ester (CFSE, 423801, BioLegend) at 2 mM, and then incubated with mitomycin at 50 ng/ml for 3 days. The proliferative levels of CFSE-labeled $CD4^+$ cells were measured using flow cytometry.

### Th cell polarization *in vitro*

Naive $CD4^+CD62L^+$ T cells were isolated from the spleens of GFP-IL-4 BALB/c mice or GFP-Foxp3 C57BL/6 mice using autoMACS and then cultured with BMDCs treated with mitomycin at 50 ng/ml. To generate induced regulatory T cells (iTreg), naive $CD4^+$ T cells and BMDCs were stimulated with 1 μg/ml soluble anti-CD3 (100238, BioLegend), 1 μg/ml soluble anti-CD28 (102116, BioLegend), 2 ng/ml rhTGF-β (AF-100-21-C-10, PeproTech), and 50 U/ml rhIL-2 (AF-200-02-50, PeproTech) for 3 days. To induce Th2 cells that secret IL-4, the cell mixtures were stimulated with 1 μg/ml soluble anti-CD3 (100238, BioLegend), 1 μg/ml soluble anti-CD28 (102116, BioLegend), 10 μg/ml anti-IFNγ (513208, BioLegend), and 10 ng/ml rmIL-4 (214-14-20, PeproTech) for 3 days. Subsequently, the cells were harvested and stained for CD4 and other molecular markers.

### BMDCs functions *in vivo*

$2\times10^6$ OVA-treated BMDCs, with or without GMSC-CM, were administered to mice intravenously via the tail vein. The mice were subsequently sacrificed on day 11 following a continuous 4-day challenge with a 1% OVA solution aerosol.

### Transfection

Lentiviral vectors expressing green fluorescence protein, either encoding the HGF gene or shRNA against HGF, were designed, constructed, and amplified as previously described (Beijing Tsingke Biotech Co., Ltd.).[55] The lentivirus particles were produced by co-transfecting 293T cells with the lentiviral vectors and two packaging plasmids. GMSCs were transfected overnight, subsequently washed, and then normal growth medium was added for an additional 72 hours.

### Histological analysis

The lung tissues were fixed in formalin, embedded in paraffin, sectioned, and stained with H&E or periodic acid-Schiff (PAS). The inflammatory infiltration analysis was scored using 0- to 4-point scales: grade 0, no inflammatory infiltration; 1, rare inflammatory infiltration; 2, mild inflammatory infiltration; 3, moderate inflammatory infiltration; and 4, severe inflammatory infiltration.

### Flow cytometry analysis

The cells were resuspended in PBS and stained with anti-CD11b (101216), anti-CD11c (117308), anti-SiglecF (155508), anti-Ly6G (127624), anti-F4/80(123116), and anti-CD45 (103132) antibodies (Biolegend). For intracellular staining of IL-4 (504118), IL-5 (504303), IL-17 (506940), and IFN-γ (505810), cells underwent 5 h of stimulation with PMA (P8139-5MG, Sigma), ionomycin (Inh-Ion, InvivoGen) and BFA (420601, Biolegend). Subsequently, cells were fixed and permeabilized using a fixation/permeabilization solution and stained with the respective antibodies. The results were obtained on a BD FACS Fortessa flow cytometer and analyzed using FlowJo software.

### ELISA

Human HGF in cell culture supernatants from GMSCs and mouse IgE (1218202), IL-4 (1210402), and IL-13 (RK00107) in sera/bronchoalveolar lavage fluids (BALF) were detected by an ELISA kit (Biolegend) according to the instructions provided by the manufacturer.

### Quantitative real-time RT–PCR

Total RNA was isolated from GMSCs and prepuce-derived fibroblast cells PDF using the RNA-Quick Purification Kit (RN001, EScience) following the manufacturer's instructions. cDNA synthesis was carried out using the Fast All-in-One RT Kit (RT001, EScience). Quantitative RT–PCR was conducted using the TB Green Premix Ex Taq II (RR820A, Takara). Each sample was carried out in triplicate, and the abundance of each molecule was evaluated by comparing its relative expression to the reference gene β-actin using the 2-ΔΔ threshold cycle (ΔΔCt) method.

### Western blot

Proteins (25 μg) from each sample were separated by 10% polyacrylamide-SDS gels and transferred to PVDF membranes (Millipore). After blocking with 5% nonfat dry milk, the membranes were incubated with primary antibodies against HGF (1:1000; 701283, Thermo Scientific), β-actin (1:1000; BA1054, BOSTER), P-STST3 (1:1000; 9145s; CST) and STST3 (1:1000; 12640s; CST) overnight at 4°C. Then, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (BA1054, BOSTER) for 1 h at room temperature, followed by visualization using enhanced chemiluminescence (ECL; Thermo Scientific).

## QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were carried out using GraphPad Prism software. One-way analysis of variance (ANOVA) with Tukey's multiple comparisons test or unpaired Student's t-test was used for statistical analysis as appropriate. The data are presented as the mean ± SD. *P* values less than 0.05 were considered to indicate statistical significance. *$p<0.05$; **$p<0.01$; ***$p<0.005$; ****$p<0.0001$, ns means no significance.