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Semaphorin 3E Deficiency Exacerbates Airway Inflammation, Hyperresponsiveness, and Remodeling in a Mouse Model of Allergic Asthma

Hesam Movassagh,* Lianyu Shan,* Ashfaque Mohammed,* Andrew J. Halayko,*[†] and Abdelilah S. Gounni*

Semaphorin 3E (Sema3E) plays a crucial role in axon guidance, vascular patterning, and immune regulation. Nevertheless, the role of Sema3E in asthma is still elusive. In this study, we show that genetic ablation of Sema3E in mice results in increased lung granulocytosis, airway hyperresponsiveness, mucus overproduction, collagen deposition, and Th2/Th17 inflammation. Transfer of *Sema3e*^{-/-} bone marrow progenitor cells to irradiated wild-type (WT) recipients exacerbates airway hyperresponsiveness and inflammation, whereas transfer of WT bone marrow progenitor cells ameliorates asthma pathology in *Sema3e*^{-/-} recipients. *Sema3e*^{-/-} mice display a higher frequency of CD11b⁺ pulmonary dendritic cells than their WT controls at the baseline and after sensitization with house dust mite. Adoptive transfer of CD11b⁺ pulmonary dendritic cells from *Sema3e*^{-/-} mice into WT recipients increases house dust mite-induced Th2/Th17 inflammation in the airway. Together, these findings identify Sema3E as a novel regulatory molecule in allergic asthma that acts upstream of proallergic events and suggest that targeting this molecule could be a novel approach to treat allergic asthma. *The Journal of Immunology*, 2017, 198: 000–000.

Asthma is a heterogeneous chronic disorder of the airways in which the inflammation is associated with airway hyperresponsiveness (AHR) leading to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing. It is among the commonest chronic conditions in Western countries affecting ~14% children and 8% adults. Pathophysiological features of asthma include eosinophil-rich inflammatory cell infiltrates, mucus hypersecretion, and airway wall remodeling (1). In allergic asthma, the most common form of the disease, inhalation and subsequent presentation of allergens by dendritic cells (DCs),

induces Th2 immune deviation and recruitment of inflammatory cells into the lungs, which lead to tissue damage in atopic individuals (2). Allergen-specific pulmonary Th2 cells producing IL-4, IL-5, and IL-13 play critical roles in IgE synthesis, eosinophil recruitment, mast cell growth, and AHR. However, the containment of the Th2 response by activation of Th1, Th17, and T regulatory cells is important in pathogenesis of allergic asthma (3). Considering the essential role of DCs in induction of Th2 and Th17 responses, interference with their proallergic functions is considered as a potential new therapeutic strategy in allergic asthma (4).

Semaphorins, initially discovered as axon guidance molecules, are ubiquitously expressed beyond the nervous system and control immune regulation (5). Among them, semaphorin 3E (Sema3E) and its receptor plexinD1 have emerged as an essential axis involved in cell migration, proliferation, and angiogenesis (6–8), which are key features associated with inflammation and tissue remodeling. We have previously demonstrated that Sema3E inhibits human airway smooth muscle (ASM) cell proliferation and migration via modulation of ERK1/2, Akt, and Rac1 pathways, suggesting a potential role of this pathway in airway remodeling (9). However, the *in vivo* function of Sema3E in allergic asthma has not been investigated.

In this study, we aimed to address the role of Sema3E in the development and maintenance of allergic asthma. We demonstrate that *Sema3e*-deficient mice exhibit enhanced basal accumulation of myeloid cells in their lungs. Upon house dust mite (HDM) sensitization and challenge, *Sema3e*^{-/-} mice display an exacerbated Th2/Th17 inflammatory response, airway remodeling, and AHR compared with wild-type (WT) counterpart. Collectively, our data suggest that Sema3E plays a pivotal role in pathogenesis of allergic asthma, which is mediated by regulation of pulmonary DC subsets.

Materials and Methods

Animals

The 129 P2 *Sema3e*^{-/-} mouse was a gift of F. Mann (Developmental Biology Institute of Marseille Luminy, Université de la Méditerranée,

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H.M. designed, performed, and analyzed the experiments and prepared the manuscript; L.S. and A.M. contributed in conducting experiments; A.J.H. contributed in mouse lung mechanics studies; A.S.G. designed the experiments, analyzed data, and prepared the manuscript; and all authors revised and approved the final version of the manuscript.

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Abbreviations used in this article: AHR, airway hyperresponsiveness; ASM, airway smooth muscle; BALF, bronchoalveolar lavage fluid; BM, bone marrow; DC, dendritic cell; HDM, house dust mite; i.n., intranasal(ly); MLN, mediastinal lymph node; Sema3E, semaphorin 3E; VEGFR2, vascular endothelial growth factor receptor 2; WT, wild-type.

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Marseille, France), which was described previously (10), and 129P2 WT littermates were used as the control group. All of the mice were maintained at the Central Animal Care Services, University of Manitoba facility, under specific pathogen-free conditions and used according to guidelines stipulated by the Canadian Council for Animal Care.

Immunophenotyping of the lung inflammatory cells

Lungs were removed from *Sema3e*^{-/-} and WT naive mice, minced, and enzymatically digested using 1 mg/ml collagenase IV (Worthington Biochemical, Lakewood, NJ) and 0.5 mg/ml DNase from bovine pancreas in RPMI 1640 medium. After RBC lysis and Fc blocking, cells were stained with a mixture containing the following anti-mouse Abs: CD45-Pacific blue (clone 30-F11; eBioscience, San Diego, CA), Siglec F-PE (clone E50-2440; BD Biosciences), CD11b-allophycocyanin (clone M1/70; eBioscience), CD11c-PE (clone N418; eBioscience), CD3-FITC (clone 17A2; eBioscience), B220-eFluor 450 (clone RA3-6B2; eBioscience), CD4-allophycocyanin (clone RM4-5; eBioscience), CD8-PE (clone 53-6.7; eBioscience), $\gamma\delta$ -TCR (clone GL3; BioLegend), and NK1.1-PE Cy7 (clone PK136; eBioscience). Then the samples were acquired using a BD FACSCanto-II flow cytometer and analyzed using FlowJo software.

HDM exposure model

A total of 25 μ g HDM extract (*Dermatophagoides pteronyssinus*, Lot 259585; LPS, 615 EU/vial; protein, 5.35 mg/vial; dry weight, 23.73 mg/vial; Greer Laboratories, Lenoir, NC) in 35 μ l saline was administered intranasally (i.n.) for 5 d/wk during consecutive 2 wk under gaseous anesthesia in *Sema3e*^{-/-} and WT mice (11). The mice were sacrificed 48 h after the last HDM challenge to measure the outcomes. Control *Sema3e*^{-/-} and WT groups were exposed to the sterile saline in all experiments. All the mice were female and 6–8 wk old.

Methacholine challenge test

AHR parameters, including airway resistance, tissue resistance, and tissue elastance, were measured by using FlexiVent small animal ventilator system (SCIREQ, Montreal, QC, Canada). Briefly, HDM or saline-sensitized and challenged mice underwent thoracotomy. Then an increasing gradient of methacholine dose (0, 3, 6, 12, 25, and 50 mg/ml) was administered intratracheally with 5-min interval between the doses, and lung function measurements were performed.

Collection and processing of bronchoalveolar lavage fluid

After measurement of AHR, bronchoalveolar lavage fluid (BALF) was collected from tracheally cannulated *Sema3e*^{-/-} or WT mice with two instillations of 1 ml sterile saline containing 0.1 mM EDTA. After lysis of RBC with ACK buffer, total BALF cells were counted and cytopspins were prepared. Then cytopspins were fixed and stained to perform differential inflammatory cell count. BALF supernatants were stored at -80°C for airway cytokine measurements.

Lung histology

Lower left lobe of the lung was dissected, inflated, and fixed in formalin overnight, followed by embedding in paraffin. Basal and HDM-induced airway inflammation, mucus production, and collagen deposition in lung tissue sections obtained from *Sema3e*^{-/-} versus WT mice were studied by performing H&E, periodic acid-Schiff, and sirius red, respectively. The severity of lung inflammation was determined by histological scoring of H&E-stained slides in a blind manner (12). Morphometric analysis of periodic acid-Schiff-stained slides was used to quantify the mucus overproduction in the airways (13).

Measurement of cytokines and Igs

ELISA of mouse BALF for IL-4, IL-5, IL-9, IL-10, IL-12, IL-13, IL-17A, and IFN- γ was performed, according to the manufacturer's instructions. ELISA plates were read with SpectraMax plate reader and analyzed with SoftMax Pro software (Molecular Devices). All cytokine ELISA kits were from BioLegend (San Diego, CA), except IL-13, which was from eBioscience.

Serum was obtained from saline- and HDM-exposed WT and *Sema3e*^{-/-} mice 48 h after the last allergen challenge. Total and HDM-specific IgE, IgG1, and IgG2a levels were quantified using commercial ELISA kits, according to manufacturer's instructions, as we described previously (14). ELISA kits for measuring total and HDM-specific IgE, IgG1, and IgG2a levels in serum samples were purchased from Southern Biotech (Birmingham, AL).

Intracellular staining of cytokines and transcription factors

Lung-draining mediastinal lymph nodes (MLN) were collected from HDM-exposed mice, followed by preparing single-cell suspension using a cell strainer. The cells were resuspended at a concentration of 4×10^6 cells/ml in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 U/ml streptomycin, and 5×10^{-5} M 2-ME, plated in 24-well tissue culture plates. Then MLN cells were incubated with a freshly prepared mixture containing 50 ng/ml PMA, 500 ng/ml ionomycin, and 10 μ g/ml brefeldin A (Sigma-Aldrich, Oakville, ON, Canada) for 4 h at 37°C and 5% CO₂. Extracellular staining was performed by using anti-mouse CD3 e-Fluor 450 (clone 17A2) and CD4-FITC (both from eBioscience). For staining cytokines, fixed and surface-stained MLN cells were permeabilized with 0.1% saponin in flow cytometry buffer and then stained with specific anti-mouse IFN- γ PE (clone XMGI.2), IL-4 allophycocyanin (clone 11B11), and IL-17A PE (clone eBio17B7), all from eBioscience. Staining for transcription factors was performed with Foxp3 Staining Buffer Set (eBioscience) by using anti-mouse T-bet (clone 4B10) from BioLegend and GATA3 (clone TWAJ) and ROR γ t (clone AFKJS-9) from eBioscience, according to the manufacturer's instructions. Samples were acquired on a FACSCanto II and analyzed using FlowJo software.

Bone marrow chimera experiments

Sema3e^{-/-} and WT mice were irradiated with 500 rad for 7.5 min and were injected the following day with 5×10^6 bone marrow (BM) progenitor cells obtained from either *Sema3e*^{-/-} or WT mice in a crisscross manner. The donor cells were established in the recipient mice during 6 wk, and then recipient mice were exposed to i.n. HDM sensitization and challenge for 2 wk, followed by AHR and inflammation measurements.

Analysis of lung DC subsets

Major pulmonary conventional DC subsets from *Sema3e*^{-/-} or WT mice were characterized 3 d after i.n. exposure with saline or a single high dose (100 μ g per mouse) of HDM. Briefly, lungs were enzymatically digested, as mentioned above, followed by RBC lysis and Fc blocking. Then DCs were stained by anti-mouse F4/80-FITC (clone BM8; eBioscience), anti-mouse CD11c-allophycocyanin (clone 418; eBioscience), MHC class II (I-A/I-E) eFluor 450 (clone M5/114.15.2; eBioscience), CD11b PE-Cy7 (clone M1/70; BioLegend), and CD103 PerCP-Cy5.5 (clone 2E7; BioLegend) Abs. Next, the samples were acquired using a BD FACSCanto-II flow cytometer and analyzed using FlowJo software. IL-23 was intracellularly stained in MHCII⁺ CD11c⁺ pulmonary conventional DCs by using anti-mouse IL-23 Ab (clone C15.6; BioLegend) after enzymatic digestion of the lungs.

Adoptive transfer of pulmonary DCs

To evaluate the differential ability of CD11b⁺ pulmonary DCs from *Sema3e*^{-/-} and WT mice to drive Th2/Th17 cell polarization, mice were first challenged with a single high dose of i.n. HDM (100 μ g), and then lungs were harvested and enzymatically digested after 2 d. CD11b⁺ pulmonary DCs were sorted from the population pregated on MHCII^{hi} and CD11c⁺ cells. A total of 15,000 CD11b⁺ pulmonary DCs from either *Sema3e*^{-/-} or WT mice was administered into 129P2 WT littermates through the nasal route. One week later, mice were challenged with 10 μ g i.n. HDM for consecutive 5 d. After 2 d, BALF cellularity, lung tissue inflammation, MLN DC frequency, and cytokine production were evaluated, as described above.

Statistical analyses

GraphPad Prism 5.0 software was used for statistical analysis, and values were presented as the mean $\pm$ SEM. Depending on the number of groups and treatments, data were analyzed by unpaired *t* test, one-way ANOVA, or two-way ANOVA, followed by the Bonferroni's multiple comparisons post hoc test. Differences were considered to be statistically significant at **p* $\leq$ 0.05, ***p* $\leq$ 0.01, and ****p* $\leq$ 0.001.

Results

Sema3E-deficient mice demonstrate exaggerated allergic asthma

To understand how *Sema3E* could contribute to pulmonary inflammatory response, immune cells in the lungs were first compared between *Sema3e*^{-/-} and WT mice at the baseline. At homeostatic conditions, the pulmonary hematopoietic compartment (CD45⁺ cells) (Fig. 1A) revealed significant eosinophil (Fig. 1B)

infiltrate in *Sema3e*^{-/-} mice than those of WT controls, whereas comparable numbers were observed in the spleen (data not shown). Furthermore, alveolar macrophages, T, B, NK, and NKT cells of *Sema3e*^{-/-} mice were found at comparable numbers to WT mice (Fig. 1C–H). Our data suggest that *Sema3E* deficiency leads to elevated granulocyte numbers in the lungs, potentially predisposing mice to allergic airway inflammation.

Repeated inhalational exposure to HDM induces AHR and inflammation (11, 15, 16). We compared the development of allergic asthma in the presence or absence of *Sema3E* in a HDM acute murine model, as depicted in Fig. 2A (11). After exposure to nebulized methacholine, HDM-exposed *Sema3e*^{-/-} mice showed a significant increase in AHR parameters, including airway resistance (Fig. 2B), tissue resistance (Fig. 2C), and tissue elastance (Fig. 2D), compared with WT littermates. *Sema3e*^{-/-} mice had higher total inflammatory cells in BALF than those of WT mice in response to HDM (Fig. 2E) characterized by a significant increase in granulocytes, eosinophils, and neutrophils, upon HDM challenge (Fig. 2F). Increased recruitment of inflammatory cells to the airways was further confirmed by performing FACS analysis on BALF cells (data not shown). Concomitantly, lung histology

studies revealed exacerbated peribronchial inflammation (Fig. 2G, Supplemental Fig. 1A), goblet cell hyperplasia (Fig. 2H, Supplemental Fig. 1B), and collagen deposition (Fig. 2I) in HDM-exposed *Sema3e*^{-/-} compared with WT mice.

Corresponding to their having an exaggerated allergic response, HDM-exposed *Sema3e*^{-/-} mice showed a significant increase in the levels of BALF IL-4, IL-5, IL-13, and IL-17A compared with WT controls. However, IFN- γ and IL-9 levels did not change significantly (Fig. 3A). Intracellular staining of IFN- γ , IL-4, and IL-17A on lung-draining MLN cells further confirmed the Th2/Th17-biased cytokine response (Supplemental Fig. 2). Then we compared the level of essential transcription factors T-bet, GATA3, and ROR γ t involved in regulation of Th1, Th2, and Th17 responses, respectively (Fig. 3B–D). As revealed in Fig. 3B and 3C and in line with IFN- γ secretion data in BALF, T-bet level does not differ between *Sema3e*^{-/-} and WT mice after HDM challenge (Fig. 4). However, the levels of both GATA3 (Fig. 3C) and ROR γ t (Fig. 3D) are significantly higher in MLN CD4⁺ T cells from *Sema3e*^{-/-} mice than those of WT controls upon HDM challenge, which could explain the Th2/Th17-skewed phenotype in the absence of *Sema3E*. The basal expression of

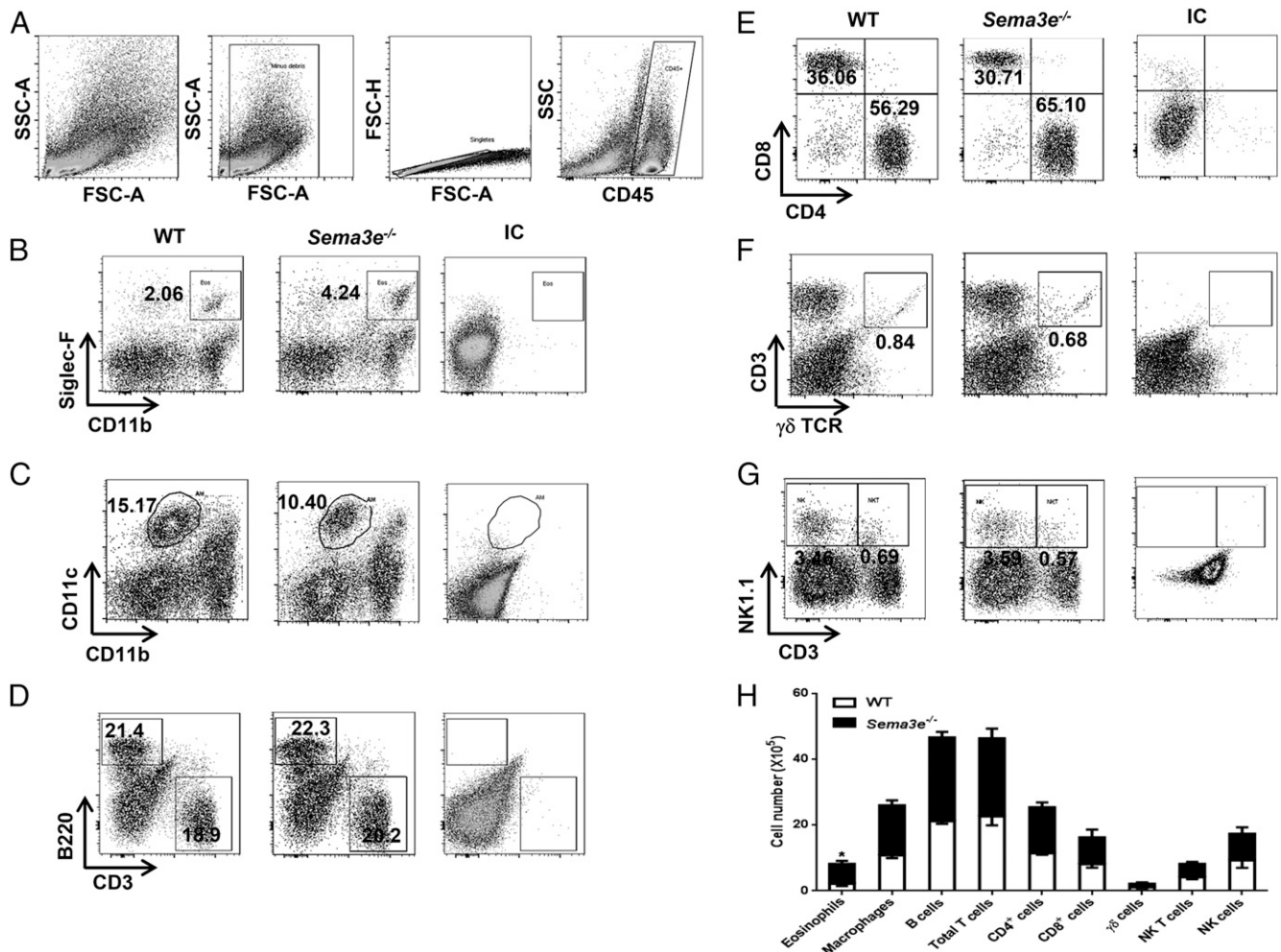


FIGURE 1. Immunophenotyping of pulmonary inflammatory cells in *Sema3e*^{-/-} and WT mice. FACS analysis by using Abs against specific surface markers was used to characterize inflammatory cell populations in the lung single-cell suspensions from *Sema3e*^{-/-} or WT mice that underwent enzymatic digestion. General gating strategy includes exclusion of debris and doublet cells, followed by selection of hematopoietic cells from total lung cells (A). Eosinophils characterized by expression of Siglec-F and CD11b pregated on Gr-1⁻ population (B). Alveolar macrophages expressing CD11b and CD11c pregated on auto-fluorescent F4/80⁺ population (C). T and B cells were characterized by surface expression of CD3 or B220, respectively (D), followed by further identification of CD4⁺ and CD8⁺ cells out of CD3-expressing ones (E). $\gamma\delta$ T cells were characterized by expression of both CD3 and $\gamma\delta$ -TCR (F). CD3⁺ NK1.1⁺ cells represent pulmonary NK cells, whereas NKT cells express both CD3 and NK1.1 (G). The number of each cell type was compared between *Sema3e*^{-/-} and WT mice (H). Staining with isotype control Ab showed no cross-reactivity. $n = 3$. * $p < 0.05$.

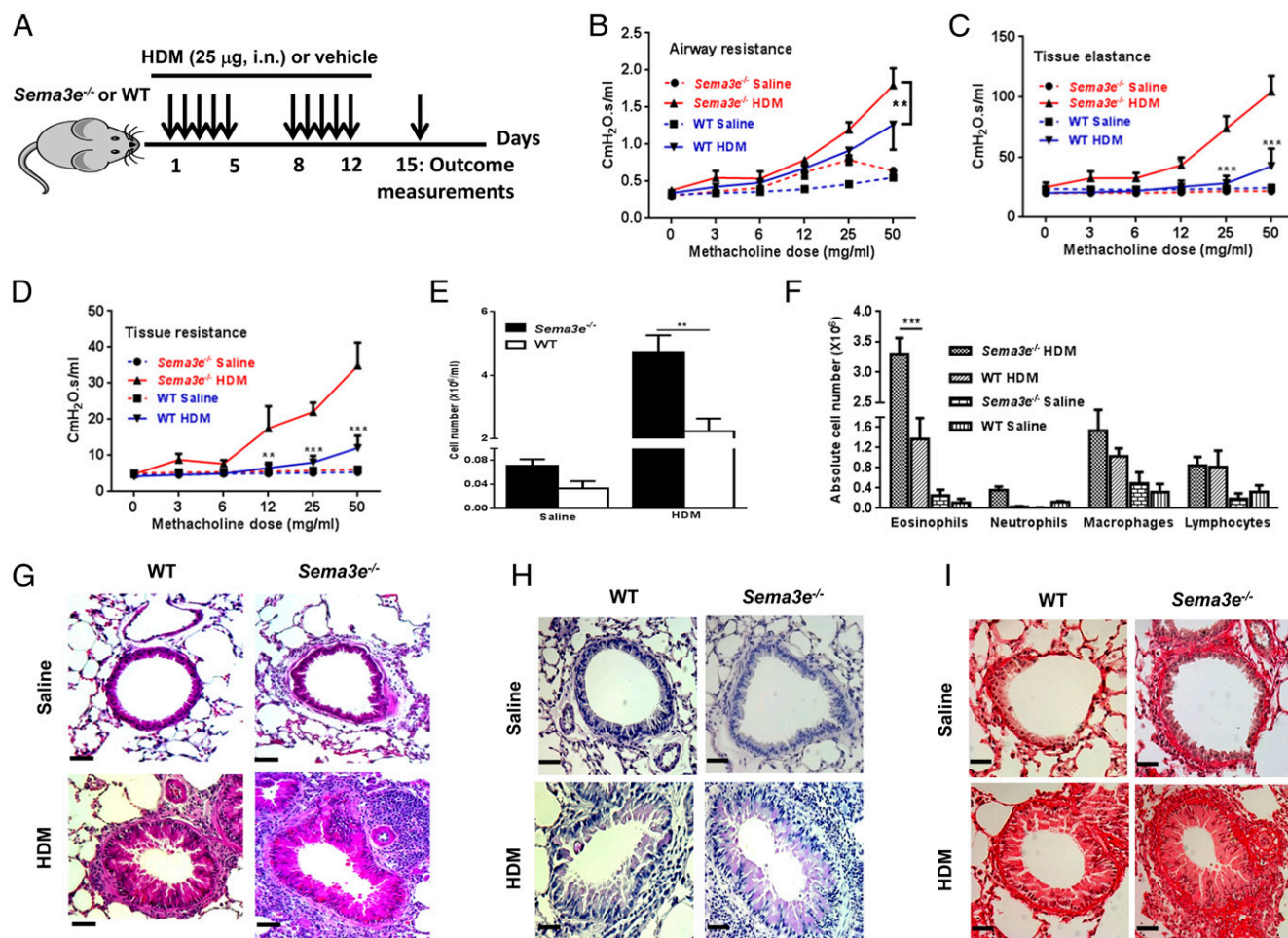


FIGURE 2. Sema3E-deficient mice have elevated airway hyperresponsiveness, inflammation, and remodeling. Allergic airway disease model was established by i.n. exposure to HDM for 2 wk, and control mice received saline (A). Either HDM- or saline-exposed *Sema3E*^{-/-} or WT mice underwent tracheotomy accompanied by methacholine challenge to measure airway resistance (B), tissue resistance (C), and tissue elastance (D). Total (E) and differential (F) count of inflammatory cells was performed on BALF. Lung inflammation (G), mucus overproduction (H), and collagen deposition (I) were further studied by performing H&E, periodic acid-Schiff, and sirius red staining, respectively. Scale bars, 100 µm. All data are representative of four to five mice per group. ***p* < 0.01, ****p* < 0.001.

these transcription factors was not significantly different in the lungs of *Sema3E*^{-/-} versus WT mice (data not shown).

Concomitantly, total and HDM-specific IgE (Fig. 4A, 4B) as well as IgG1 (Fig. 4C, 4D) levels were significantly increased in HDM-exposed *Sema3E*^{-/-} mice in the serum. However, the IgG2a level remained unchanged between *Sema3E*^{-/-} and WT mice upon HDM sensitization and challenge (Fig. 4E, 4F). These data collectively indicate an enhanced Th2/Th17-biased inflammation and tissue remodeling in *Sema3E*^{-/-} mice.

Transfer of Sema3E-deficient BM progenitors exacerbates airway inflammation and hyperresponsiveness

Previous reports show that hematopoietic progenitor cells play critical roles in the pathophysiology of allergic asthma (17, 18). Therefore, we wished to determine whether the production of Sema3E by these cells contributes to disease exacerbation. We created chimeras by injecting BM progenitor cells from either *Sema3E*^{-/-} or WT mice into sublethally irradiated *Sema3E*^{-/-} or WT recipients, followed by HDM exposure for 2 wk. In contrast to irradiated *Sema3E*^{-/-} mice receiving WT BM progenitor cells, transfer of *Sema3E*^{-/-} BM progenitors to WT irradiated recipients exacerbated HDM-induced AHR (Fig. 5A–C), enhanced total and differential BALF cell count (Fig. 5D, 5E), and lung inflammation (Fig. 5F, 5G). BALF cytokine measurements indicated a type 2

response characterized by higher levels of IL-4 and IL-5 in the airways of WT mice receiving BM cells from *Sema3E*^{-/-} mice, which was reversed in WT BM-injected *Sema3E*^{-/-} mice (data not shown). These results show that exacerbation of HDM-induced allergic response in *Sema3E*^{-/-} mice is driven at least partly by BM-derived immune cells.

Sema3E regulates recruitment and function of DC subsets in the lungs

We aimed to address which inflammatory cell compartment is involved in Sema3E-mediated regulation of allergic airway disease. Our immunophenotypic analysis of the lung inflammatory cells revealed that *Sema3E*^{-/-} mice have higher total and CD11b⁺ but lower CD103⁺ DCs than those of WT littermates at homeostatic conditions (Fig. 6A, 6B). DCs are professional APCs that are recruited into the airways upon allergen encounter and migrate into the draining lymph node to present Ag to naive T cells. Given that CD11b⁺ DC subset promotes pulmonary Th2 (19, 20) and Th17 (21) responses, whereas CD103⁺ DC subset regulates Th1/Th17 responses (20) and also induces tolerance to inhaled allergens (22), basal differences in DC subsets might be responsible for the hyperreactivity of *Sema3E*^{-/-} mice following HDM exposure. To test this hypothesis, we sensitized either *Sema3E*^{-/-} or WT mice with a single high dose (100 µg) of HDM for 72 h. We

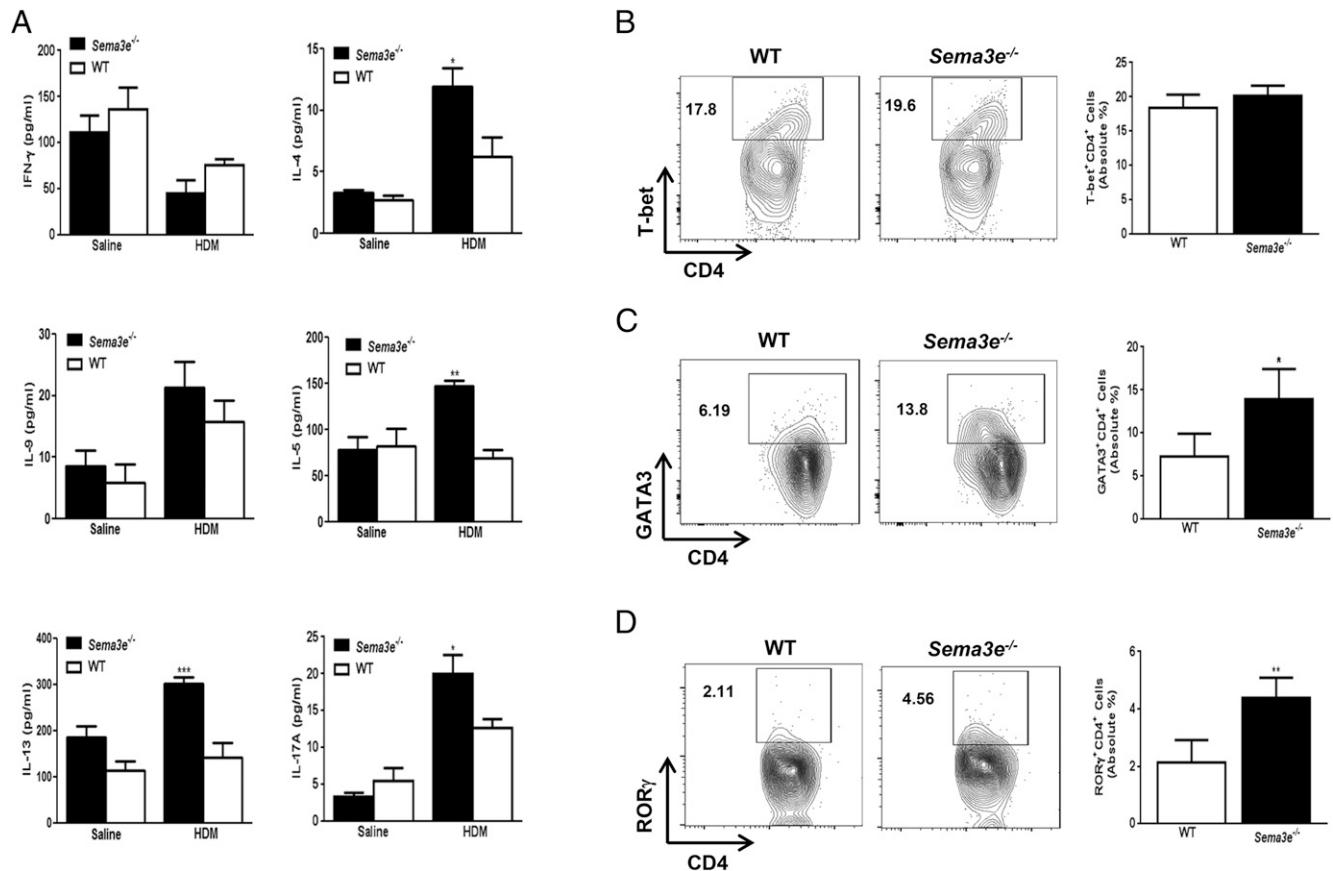


FIGURE 3. HDM promotes an enhanced Th2/Th17 cytokine response in *Sema3e*^{-/-} mice. Airway levels of IL-4, IL-5, IL-9, IL-13, IL-17A and IFN-γ were measured by ELISA on BALF supernatants obtained from *Sema3e*^{-/-} or WT mice after intranasal exposure to saline or HDM (A). T-bet (B), GATA3 (C) and RORγ (D) production was compared between MLN CD4⁺ T cells obtained from HDM-exposed *Sema3e*^{-/-} and WT mice by flow cytometry. *n* = 4–5 per group. **p* < 0.05, ***p* < 0.01, and ****p* < 0.001.

observed that HDM sensitization provokes significantly higher recruitment of Th2-skewing CD11b⁺ pulmonary DCs in *Sema3e*^{-/-} mice compared with WT littermates. In contrast, the lungs of *Sema3e*^{-/-} mice contained significantly lower numbers of tolerogenic CD103⁺ DCs than the control group (Fig. 6C, 6D). The expression of IL-23, as a key cytokine in DC development, was significantly higher in total MHCII^{hi}CD11c⁺ pulmonary DCs from *Sema3e*^{-/-} than WT mice after HDM challenge, but not at the baseline (Supplemental Fig. 3).

Next, we compared the capacity of *Sema3e*^{-/-} versus WT CD11b⁺ pulmonary DCs to induce Th2/Th17 immunity in vivo. We sorted WT or *Sema3e*^{-/-} pulmonary CD11b⁺ DCs from HDM-sensitized mice, adoptively transferred them to WT recipients via i.n. route, and challenged them with HDM after 7 d (Fig. 7A). WT mice receiving CD11b⁺ pulmonary DCs from *Sema3e*^{-/-} mice displayed a significant increase in BALF cells, particularly eosinophils and to lesser extent neutrophils (Fig. 7B, 7C). Similarly, peribronchial inflammatory cell infiltrate was more pronounced in mice receiving CD11b⁺ pulmonary DCs from *Sema3e*^{-/-} mice compared with those of WT littermates (Fig. 7D, 7E). These events were accompanied by an enhanced frequency of IL-4 and IL-17A, but not IFN-γ, producing CD4⁺ T cells in MLN upon adoptive transfer of Sema3E-deficient CD11b⁺ pulmonary DCs (Fig. 7F–I). Adoptive transfer of *Sema3e*^{-/-} CD11b⁺ pulmonary DCs into *Sema3e*^{-/-} mice induced the highest level of airway inflammation upon HDM challenge (Fig. 7B–I). Collectively, these findings indicate that Sema3E deficiency increases pulmonary CD11b⁺ DC numbers and function, leading to en-

hancement of Th2/Th17 response upon HDM sensitization and challenge.

Discussion

In the current study, we demonstrate the significance of Sema3E expression in the airways using a well-described HDM model of allergic asthma wherein *Sema3e*^{-/-} mice displayed exaggerated airway inflammation, AHR, and goblet cell hyperplasia compared with WT littermates. We further found that the essential role of Sema3E in allergic asthma model was mediated by regulation of pulmonary DC subsets.

One of the key findings of our study is that *Sema3e*^{-/-} mice displayed an enhanced pulmonary granulocytosis at the steady state that may account for the exacerbation of the allergic response upon HDM challenge. As such, it will be enticing to suggest that Sema3E pathway may be a predisposing factor for human asthma, and some asthmatics may have dysregulated Sema3E expression. Along the same line, the impact of Sema3E on expression of predisposing mediators involved in development of eosinophils and neutrophils should be investigated. We have recently demonstrated that Sema3E inhibits CXCL-8/IL-8-induced migration of human neutrophils, which is mediated by reduced polymerization of filamentous actin and Rac1 GTPase activity in vitro (23).

Increased levels of IL-5 (24, 25) and IL-17A (26) might be part of the mechanism underlying higher eosinophil and neutrophil accumulation in the airways of *Sema3e*^{-/-} mice, respectively. IL-5 level correlates with eosinophilic asthma and controls eosinophil maturation, differentiation, recruitment, and survival. Targeting

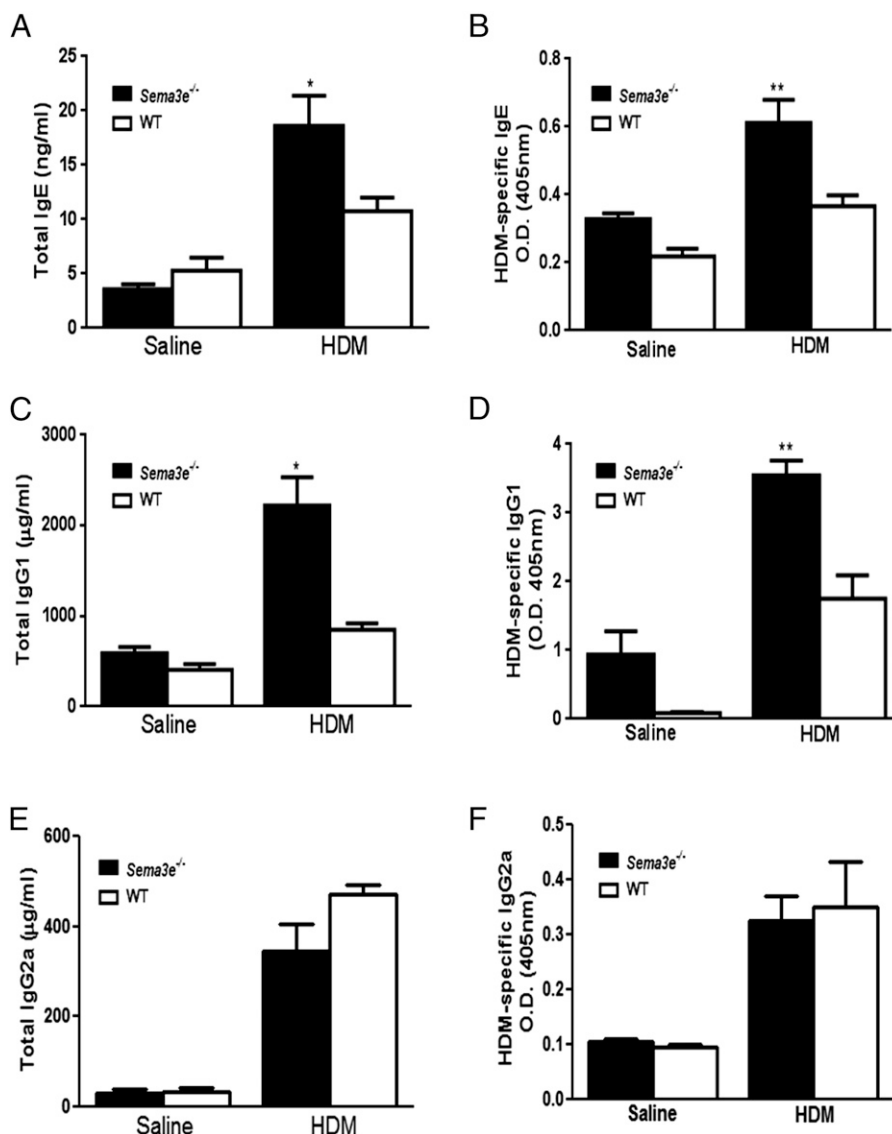


FIGURE 4. Sema3E is involved in serum Ab response to HDM. Serum samples were collected from *Sema3e*^{-/-} and WT mice after i.n. sensitization and challenge with either saline or HDM. Then the levels of total and HDM-specific IgE (**A** and **B**), IgG1 (**C** and **D**), and IgG2a (**E** and **F**) were measured by ELISA. All data are representative of four to five mice per group. **p* < 0.05, ***p* < 0.01.

IL-5/IL-5R signaling has been developed with some benefit to subsets of asthmatic patients (27–29). Similarly, IL-17A level is elevated in severe asthmatic patients, which is associated with neutrophilic asthma (30, 31). Targeting IL-17A has been shown to result in an impaired neutrophilic allergic airway inflammation as well as lower airway remodeling and AHR in mice (32, 33). We also found that, upon allergen challenge, *Sema3e*^{-/-} mice have an increased goblet cell hyperplasia and AHR, which could be in part due to increased IL-13 secretion. IL-13, as a pleiotropic cytokine, plays a major role in the IgE synthesis, goblet cell hyperplasia, smooth muscle remodeling, subepithelial fibrosis, and AHR in allergic asthma (34). Our data suggest that Sema3E could be considered as a novel treatment for allergic asthma via regulation of Th2, for example, IL-4, IL-5, and IL-13, as well as Th17, IL-17A cytokines.

Higher levels of both total and HDM-specific IgE and IgG1, as the Abs associated with type 2 immunity, in *Sema3e*^{-/-} mice suggest a negative regulatory role for Sema3E in Ig class-switching as a cardinal feature of B cell development and humoral response. These findings could be explained by elevated levels of IL-4 in *Sema3e*^{-/-} mice upon HDM exposure, which is considered as a key player for Ig class-switching to IgE (35, 36). Xue et al. (37) have recently reported that sema4c-deficient mice

display an augmented Th2 response, including an increased level of IgE in the serum after allergen challenge. Transfer of sema4c-deficient B cells was shown to induce severe allergic Th2 inflammation in WT mice characterized by high level of IgE in the serum (37). It suggests that semaphorin family members could be essential to regulate B cell response, for example, IgE synthesis. The relative importance of Sema3E versus Sema4C in regulating IgE response is not known and warrants further investigation.

Sema3e^{-/-} mice revealed higher DC recruitment into the airways both at the baseline and after HDM sensitization. In particular, a higher frequency of pulmonary CD11b⁺ DCs observed in *Sema3e*^{-/-} mice is consistent with previous studies in which CD11b⁺ DCs induced Th2 response upon HDM exposure (19), whereas CD103⁺ DCs induced pulmonary tolerance to inhaled allergens (22) and Th1 deviation (20). Along with more Th2/Th17-biased response in the airways of *Sema3e*^{-/-} mice, we found that the higher frequency of pulmonary CD11b⁺ DCs from *Sema3e*^{-/-} mice was also accompanied by privileged secretion of Th2/Th17 cytokines in our adoptive transfer model in vivo. This finding implies that Sema3E play a role in regulating Th2/Th17 response in allergic airway inflammation.

AHR, the most characteristic clinical facet of asthma, is triggered mainly by chronic airway inflammation (38), which is ini-

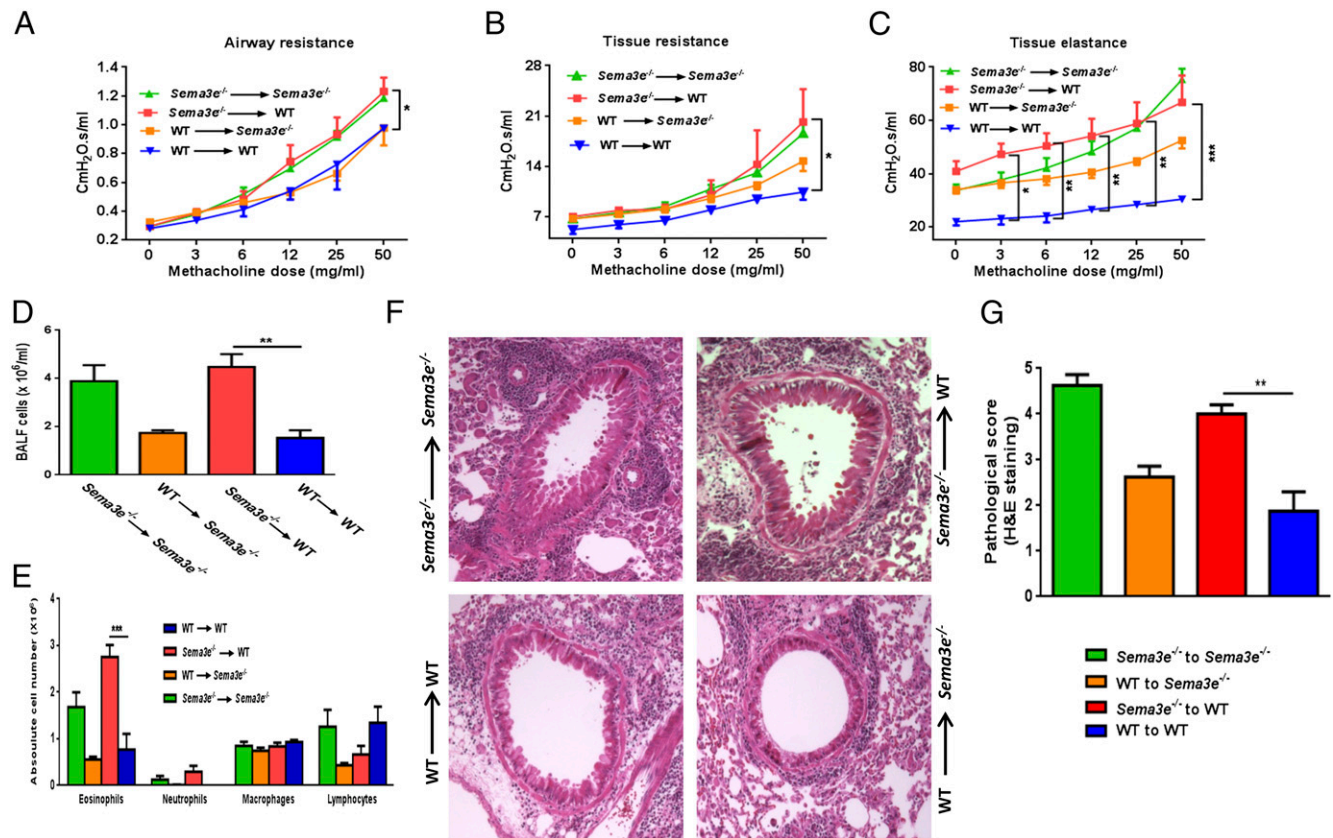
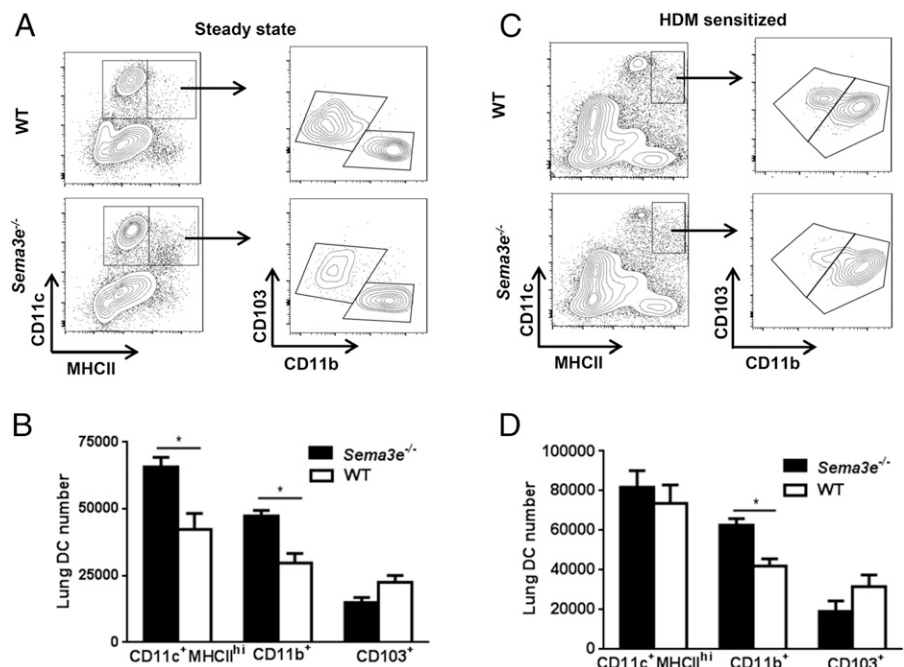


FIGURE 5. Lack of Sema3E in immune cells contributes to allergic AHR and airway inflammation. $Sema3e^{-/-}$ or WT BM cells were transferred into irradiated recipients via i.v. route and then sensitized and challenged with HDM for 2 wk. Lung function parameters, including airway resistance (A), tissue resistance (B), and tissue elastance (C), were measured. Total (D) and differential (E) inflammatory cells were counted in BALF. Airway inflammation was investigated by H&E staining of lung sections (F and G). Data represent four to five mice per group. Original magnification $\times 100$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

tiated by activated pulmonary DCs via induction of Th2 response (39). Allergen challenge provokes substantial recruitment of DC into the airways (40), and specific elimination of conventional DCs can prevent allergic airway inflammation (3, 4). Therefore,

regulated recruitment of DC subsets or modulation of their functions by Sema3E may be linked to the increased AHR observed in our $Sema3e^{-/-}$ model. Importantly, Sema3E receptor, plexinD1, has been previously demonstrated to be highly expressed on BM-

FIGURE 6. Sema3E implicates in basal and HDM-induced pulmonary DC recruitment. $Sema3e^{-/-}$ or WT mice were sensitized with a single high dose of HDM or saline for 2 d, and then pulmonary DC subsets were stained by specific Abs and acquired, followed by flow cytometry. Baseline (A and B) and HDM-induced (C and D) recruitment of total ($\text{MHCII}^{\text{hi}} \text{CD11c}^+$) pulmonary DCs as well as CD11b^+ and CD103^+ DC subsets was compared between $Sema3e^{-/-}$ and WT mice. Pulmonary DC data represent three independent experiments generated from three pooled whole-lung samples in each group per experiment. $n = 9$ per group. * $p < 0.05$.



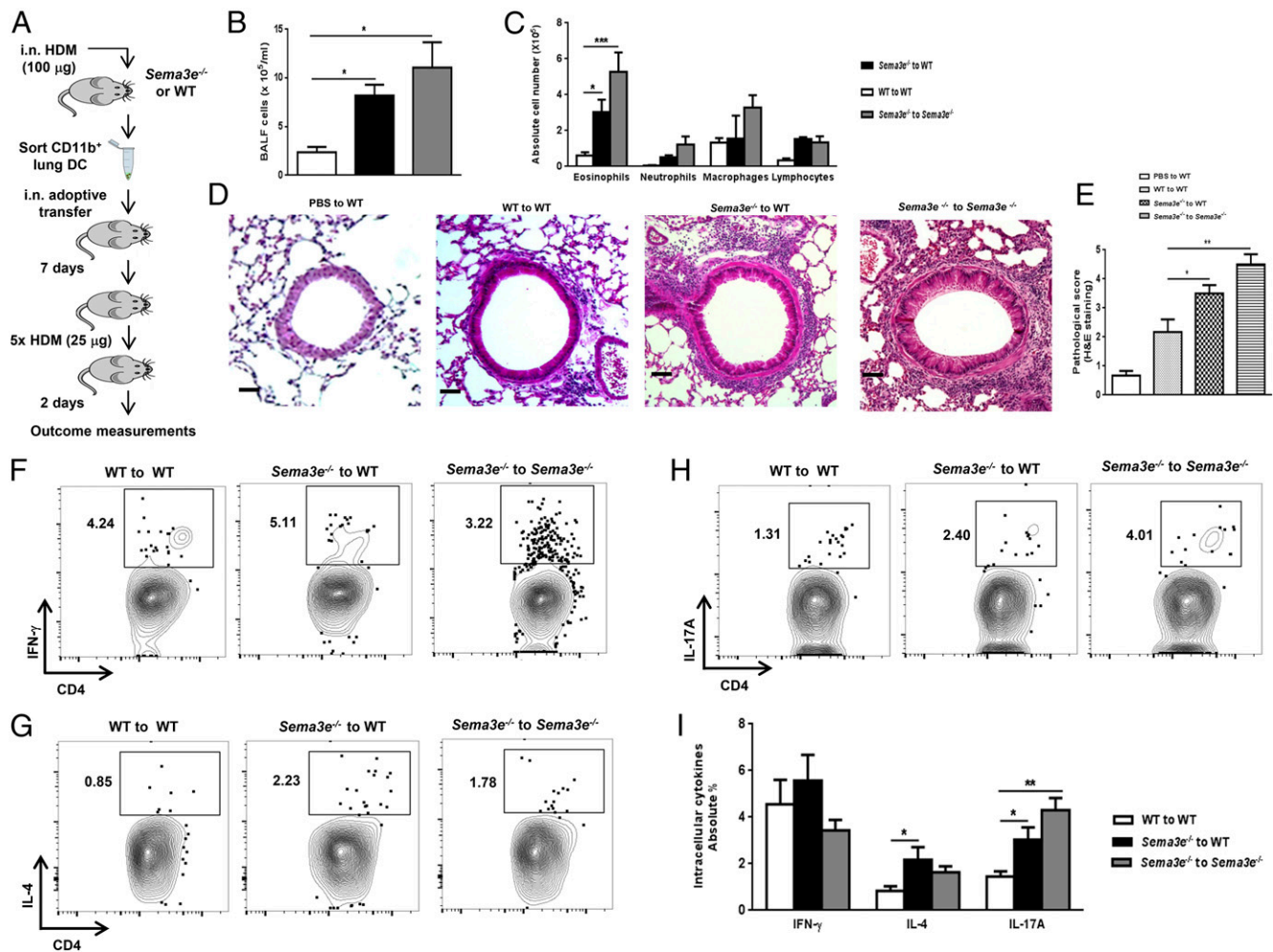


FIGURE 7. Adoptive transfer of CD11b⁺ pulmonary DCs from *Sema3e*-deficient mice exacerbates allergic airway inflammation. Cartoon depicting allergic airway induction by adoptively transferred CD11b⁺ DCs from HDM-sensitized *Sema3e*^{-/-} or WT mice into naive WT or *Sema3e*^{-/-} recipients (**A**). Total (**B**) and differential (**C**) counts of inflammatory cells recovered in the BALF after HDM exposure. Histological examination of lung sections of recipient mice stained with H&E. Scale bars, 100 µm. (**D** and **E**) Intracellular IFN-γ (**F**), IL-4 (**G**), and IL-17A (**H**) production in MLN CD4⁺ T cells after adoptive transfer and HDM challenge was investigated and statistically compared (**I**). Data are representative of four independent experiments. *n* = 5–9 per group. **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

derived DCs (41) and pulmonary DCs (data not shown), indicating that these cells can respond to Sema3E. However, it is important to highlight that AHR can be driven directly by structural cells (42). As we previously reported an inhibitory effect of Sema3E on the proliferation and migration of human ASM cells (9), it is plausible that the absence of Sema3E may drive ASM cell proliferation and/or migration, leading to exaggerated AHR in vivo, hence another layer of complexity to Sema3E role in allergic asthma. We also found that adoptive transfer of BM progenitor cells from WT mice to *Sema3e*^{-/-} recipients did not recapitulate the tissue elastance observed in the WT condition. These data suggest that Sema3E derived from resident compartment play a role in tissue elastance and thus a component of AHR.

Higher airway inflammation upon adoptive transfer of CD11b⁺ pulmonary DCs from HDM-sensitized *Sema3e*^{-/-} compared with those of WT mice in WT recipients suggests an essential role of Sema3E in allergic asthma, which could be mediated at least in part by regulation of this specific DC subset. One of the key issues to be considered in future studies is the controversial roles of CD11b⁺ versus CD103⁺ pulmonary DCs wherein CD103⁺ subset has been shown to prime Th2 response to HDM or cockroach allergens, whereas CD11b⁺ pulmonary DCs induce Th1 differentiation (43).

Increased recruitment of CD11b⁺ DCs into the airways of *Sema3e*^{-/-} mice suggests that Sema3E acts as a guidance cue for DCs, which is analogous to the original function of semaphorins in axon guidance (44). This finding is in line with the previous studies on the repulsive effect of Sema3E on human ASM cell (9) and atherosclerotic plaque macrophage (45) migration. However, Sema3E seems to act as a macrophage chemoattractant to adipose tissue, leading to inflammation in obesity model (46). In that study, the mechanism of Sema3E chemoattraction is mediated through coligation of plexinD1 with coreceptors neuropilin 1 and vascular endothelial growth factor receptor 2 (VEGFR2), which revert the repulsive activity to chemoattraction (46). However, VEGFR2 surface expression was not detected in spleen, blood, BM, and pulmonary DCs (47). In fact, the Immunological Genome Project has revealed a high expression of VEGFR2 on plasmacytoid DC, but not other hematopoietic cell types, including conventional DCs (48). In addition, we were not able to detect surface expression of VEGFR2 on mouse lung MHCII⁺ CD11c⁺ DCs after HDM exposure by flow cytometry. Nevertheless, VEGFR2 expression has been induced upon VEGF stimulation on pulmonary myeloid DCs characterized as MHCII^{lo} CD11c⁺ cells (49). In our experiments, neither neuropilin 1 nor VEGFR2 was expressed on the surface of pulmonary DCs from

HDM-exposed mice (data not shown), suggesting that the coligation is less likely to occur in our system.

It has been previously reported that PlexinD1 could be ligated by other semaphorins such as Sema4A, suggestive of a potential competition between these ligands to bind PlexinD1 as the receptor. Because Sema4A deficiency leads to an exacerbated allergic response in the mouse airways (50, 51), there might be a synergistic role of Sema3E and Sema4A in the context of asthma, which could be mediated by PlexinD1. Considering the significant impact of Sema3E gene deletion on inflammatory cells, as evident in our BM chimera data, and the crucial role of Sema4A in allergic asthma, as another PlexinD1 ligand, it would be very insightful to investigate the role of PlexinD1 deficiency in lung resident and also inflammatory cell function.

In summary, we report that Sema3E plays an essential immunoregulatory role in experimental allergic asthma. The absence of Sema3E led to higher airway inflammation in allergic asthmatic mice, which was associated with exacerbated features of AHR and remodeling. Sema3E deficiency resulted in uncontrollable recruitment and function of DCs into the airways upon allergen encounter. This previously unknown role for Sema3E in allergic asthma may lead to develop therapeutic strategies in other diseases involving AHR, airway inflammation, and remodeling.

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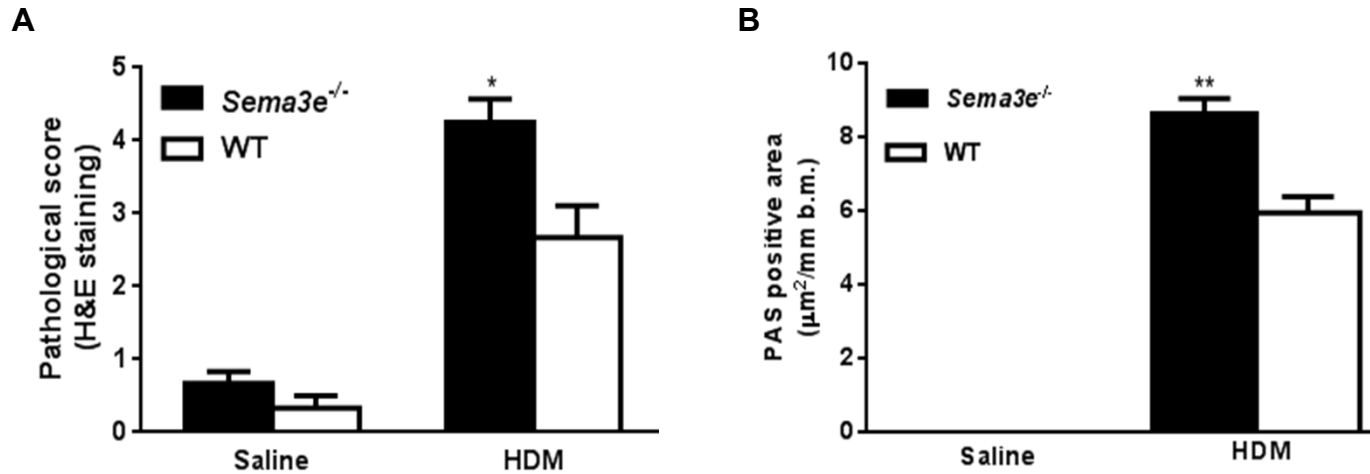
Disclosures

The authors have no financial conflicts of interest.

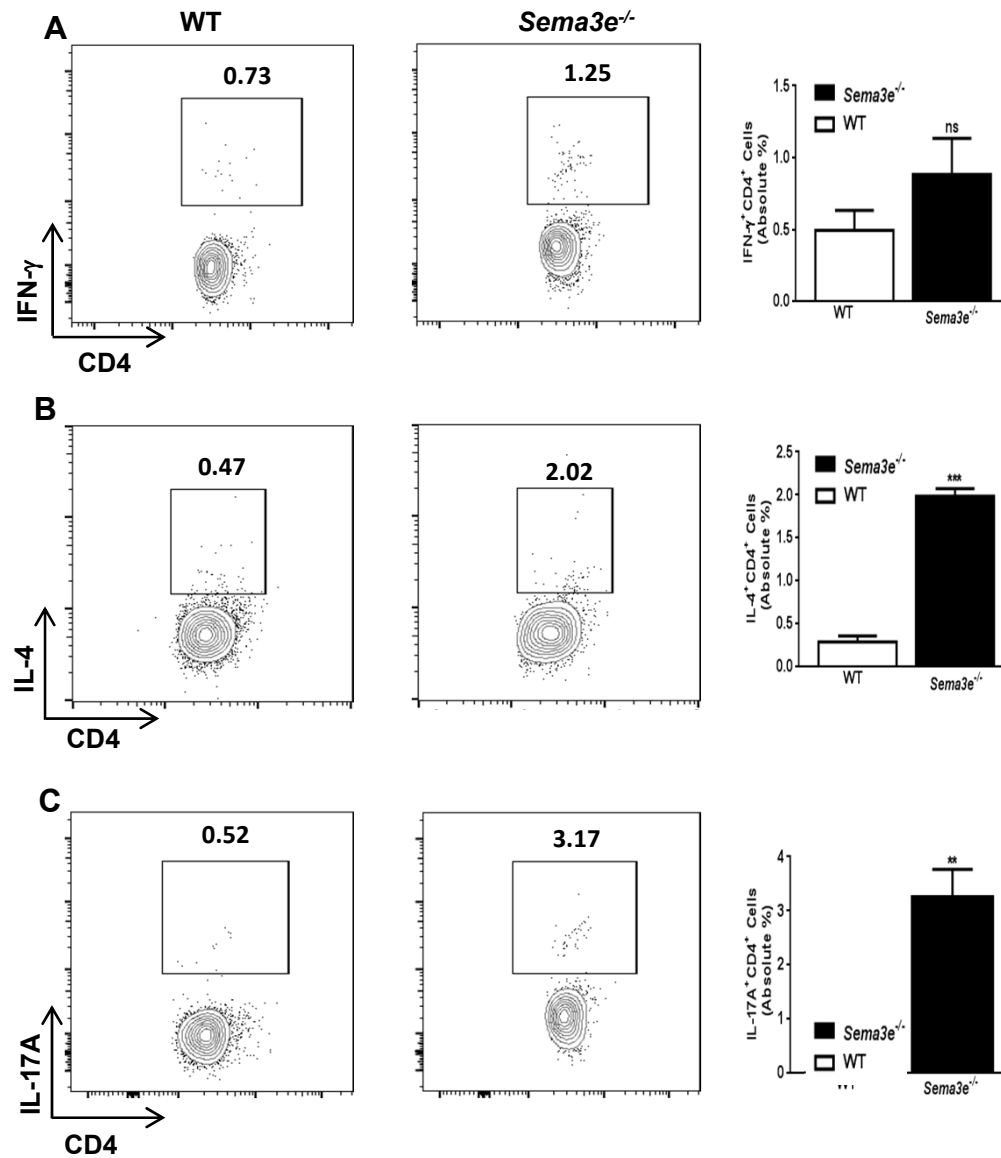
References

- Holgate, S. T., H. S. Arshad, G. C. Roberts, P. H. Howarth, P. Thurner, and D. E. Davies. 2009. A new look at the pathogenesis of asthma. *Clin. Sci.* 118: 439–450.
- Kim, H. Y., R. H. DeKruyff, and D. T. Umetsu. 2010. The many paths to asthma: phenotype shaped by innate and adaptive immunity. *Nat. Immunol.* 11: 577–584.
- Lambrecht, B. N., and H. Hammad. 2015. The immunology of asthma. *Nat. Immunol.* 16: 45–56.
- Kopf, M., C. Schneider, and S. P. Nobs. 2015. The development and function of lung-resident macrophages and dendritic cells. *Nat. Immunol.* 16: 36–44.
- Kumanogoh, A., and H. Kikutani. 2013. Immunological functions of the neuropilins and plexins as receptors for semaphorins. *Nat. Rev. Immunol.* 13: 802–814.
- Choi, Y. I., J. S. Duke-Cohan, W. B. Ahmed, M. A. Handley, F. Mann, J. A. Epstein, L. K. Clayton, and E. L. Reinherz. 2008. PlexinD1 glycoprotein controls migration of positively selected thymocytes into the medulla. *Immunity* 29: 888–898.
- Moriya, J., T. Minamino, K. Tateno, S. Okada, A. Uemura, I. Shimizu, M. Yokoyama, A. Nojima, M. Okada, H. Koga, and I. Komuro. 2010. Inhibition of semaphorin as a novel strategy for therapeutic angiogenesis. *Circ. Res.* 106: 391–398.
- Sabag, A. D., J. Bode, D. Fink, B. Kigel, W. Kugler, and G. Neufeld. 2012. Semaphorin-3D and semaphorin-3E inhibit the development of tumors from glioblastoma cells implanted in the cortex of the brain. *PLoS One* 7: e42912.
- Movassagh, H., L. Shan, A. J. Halayko, M. Roth, M. Tamm, J. Chakir, and A. S. Gounni. 2014. Neuronal chemorepellent semaphorin 3E inhibits human airway smooth muscle cell proliferation and migration. *J. Allergy Clin. Immunol.* 133: 560–567.
- Gu, C., Y. Yoshida, J. Livet, D. V. Reimert, F. Mann, J. Merte, C. E. Henderson, T. M. Jessell, A. L. Kolodkin, and D. D. Ginty. 2005. Semaphorin 3E and plexin-D1 control vascular pattern independently of neuropilins. *Science* 307: 265–268.
- Hirota, J. A., A. Budelsky, D. Smith, B. Lipsky, R. Ellis, Y. Y. Xiang, W. Y. Lu, and M. D. Inman. 2010. The role of interleukin-4Ralpha in the induction of glutamic acid decarboxylase in airway epithelium following acute house dust mite exposure. *Clin. Exp. Allergy* 40: 820–830.
- Mabalirajan, U., R. Rehman, T. Ahmad, S. Kumar, S. Singh, G. D. Leishangthem, J. Aich, M. Kumar, K. Khanna, V. P. Singh, et al. 2013. Linoleic acid metabolite drives severe asthma by causing airway epithelial injury. *Sci. Rep.* 3: 1349.
- Liu, J. N., D. H. Suh, E. M. Yang, S. I. Lee, H. S. Park, and Y. S. Shin. 2014. Attenuation of airway inflammation by simvastatin and the implications for asthma treatment: is the jury still out? *Exp. Mol. Med.* 46: e113.
- Gounni, A. S., K. Spanel-Borowski, M. Palacios, C. Heusser, S. Moncada, and E. Lobos. 2001. Pulmonary inflammation induced by a recombinant *Brugia malayi* gamma-glutamyl transpeptidase homologue: involvement of humoral autoimmune responses. *Mol. Med.* 7: 344–354.
- Fattouh, R., A. Al-Garawi, M. Fattouh, K. Arias, T. D. Walker, S. Goncharova, A. J. Coyle, A. A. Humbles, and M. Jordana. 2011. Eosinophils are dispensable for allergic remodeling and immunity in a model of house dust mite-induced airway disease. *Am. J. Respir. Crit. Care Med.* 183: 179–188.
- Johnson, J. R., R. E. Wiley, R. Fattouh, F. K. Swirski, B. U. Gajewska, A. J. Coyle, J. C. Gutierrez-Ramos, R. Ellis, M. D. Inman, and M. Jordana. 2004. Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am. J. Respir. Crit. Care Med.* 169: 378–385.
- Doherty, T. A., N. Khorram, K. Sugimoto, D. Sheppard, P. Rosenthal, J. Y. Cho, A. Pham, M. Miller, M. Croft, and D. H. Broide. 2012. *Alternaria* induces STAT6-dependent acute airway eosinophilia and epithelial FIZZ1 expression that promotes airway fibrosis and epithelial thickness. *J. Immunol.* 188: 2622–2629.
- Starkey, M. R., R. Y. Kim, E. L. Beckett, H. C. Schilter, D. Shim, A. T. Essilfie, D. H. Nguyen, K. W. Beagley, J. Mattes, C. R. Mackay, et al. 2012. *Chlamydia muridarum* lung infection in infants alters hematopoietic cells to promote allergic airway disease in mice. *PLoS One* 7: e42588.
- Plantinga, M., M. Guillems, M. Vanheerswynghe, K. Deswarte, F. Franco-Madeira, W. Toussaint, L. Vanhoutte, K. Neyt, N. Killeen, B. Malissen, et al. 2013. Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* 38: 322–335.
- Furuhashi, K., T. Suda, H. Hasegawa, Y. Suzuki, D. Hashimoto, N. Enomoto, T. Fujisawa, Y. Nakamura, N. Inui, K. Shibata, et al. 2012. Mouse lung CD103+ and CD11bhigh dendritic cells preferentially induce distinct CD4+ T-cell responses. *Am. J. Respir. Cell Mol. Biol.* 46: 165–172.
- Schlitzer, A., N. McGovern, P. Teo, T. Zelante, K. Atarashi, D. Low, A. W. Ho, P. See, A. Shin, P. S. Wasan, et al. 2013. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* 38: 970–983.
- Khare, A., N. Krishnamoorthy, T. B. Oriss, M. Fei, P. Ray, and A. Ray. 2013. Cutting edge: inhaled antigen upregulates retinaldehyde dehydrogenase in lung CD103+ but not plasmacytoid dendritic cells to induce Foxp3 de novo in CD4+ T cells and promote airway tolerance. *J. Immunol.* 191: 25–29.
- Movassagh, H., A. Saati, S. Nandagopal, A. Mohammed, N. Tatar, L. Shan, J. S. Duke-Cohan, K. R. Fowke, F. Lin, and A. S. Gounni. 2016. Chemorepellent semaphorin 3E negatively regulates neutrophil migration in vitro and in vivo. *J. Immunol.* In press.
- Johansson, M. W., and D. F. Mosher. 2013. Integrin activation states and eosinophil recruitment in asthma. *Front. Pharmacol.* 4: 33.
- Sagani, S., and C. M. Lloyd. 2014. Eosinophils in the pathogenesis of paediatric severe asthma. *Curr. Opin. Allergy Clin. Immunol.* 14: 143–148.
- Chesné, J., F. Braza, G. Mahay, S. Brouard, M. Aronica, and A. Magnan. 2014. IL-17 in severe asthma. Where do we stand? *Am. J. Respir. Crit. Care Med.* 190: 1094–1101.
- Ortega, H. G., M. C. Liu, I. D. Pavord, G. G. Brusselle, J. M. FitzGerald, A. Chetta, M. Humbert, L. E. Katz, O. N. Keene, S. W. Yancey, and P. Chanez. 2014. Mepolizumab treatment in patients with severe eosinophilic asthma. [Published erratum appears in 2015 *N. Engl. J. Med.*] *N. Engl. J. Med.* 371: 1198–1207.
- FitzGerald, J. M., E. R. Bleecker, P. Nair, S. Korn, K. Ohta, M. Lommatzsch, G. T. Ferguson, W. W. Busse, P. Barker, S. Sproule, et al.; CALIMA Study Investigators. 2016. Benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 388: 2128–2141.
- Flood-Page, P., A. Menzies-Gow, S. Phipps, S. Ying, A. Wangoo, M. S. Ludwig, N. Barnes, D. Robinson, and A. B. Kay. 2003. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J. Clin. Invest.* 112: 1029–1036.
- Al-Ramli, W., D. Préfontaine, F. Chouiali, J. G. Martin, R. Olivenstein, C. Lemière, and Q. Hamid. 2009. T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. *J. Allergy Clin. Immunol.* 123: 1185–1187.
- Turato, G., S. Baraldo, R. Zuin, and M. Saetta. 2007. The laws of attraction: chemokines, neutrophils and eosinophils in severe exacerbations of asthma. *Thorax* 62: 465–466.
- Song, C., L. Luo, Z. Lei, B. Li, Z. Liang, G. Liu, D. Li, G. Zhang, B. Huang, and Z. H. Feng. 2008. IL-17-producing alveolar macrophages mediate allergic lung inflammation related to asthma. *J. Immunol.* 181: 6117–6124.
- Nakae, S., Y. Komiyama, A. Nambu, K. Sudo, M. Iwase, I. Homma, K. Sekikawa, M. Asano, and Y. Iwakura. 2002. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 17: 375–387.
- Erle, D. J., and D. Sheppard. 2014. The cell biology of asthma. *J. Cell Biol.* 205: 621–631.
- Erazo, A., N. Kutchukhidze, M. Leung, A. P. Christ, J. F. Urban, Jr., M. A. Curotto de Lafaille, and J. J. Lafaille. 2007. Unique maturation program of the IgE response in vivo. *Immunity* 26: 191–203.

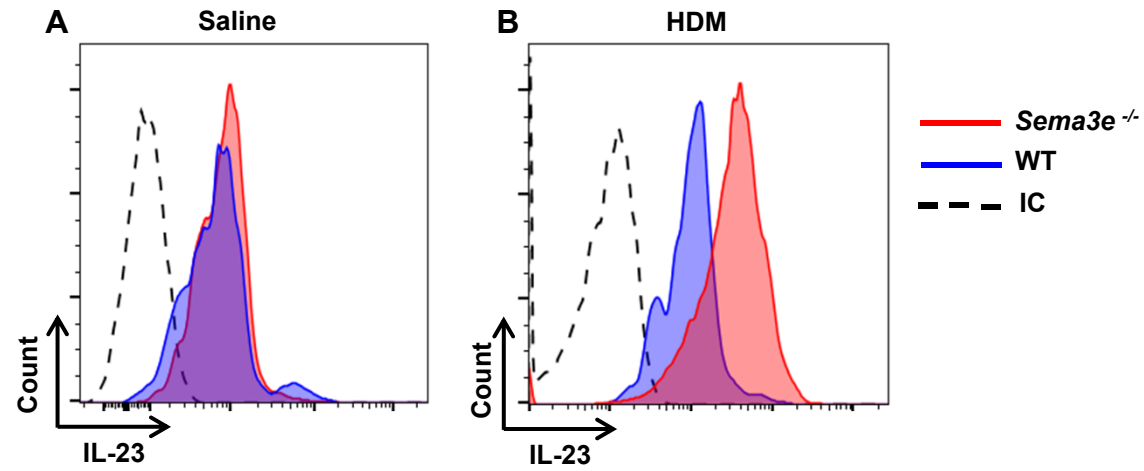
36. Wu, L. C., and A. A. Zarrin. 2014. The production and regulation of IgE by the immune system. *Nat. Rev. Immunol.* 14: 247–259.
37. Xue, D., G. N. Kaufman, M. Dembele, M. Beland, A. H. Massoud, B. C. Mindt, R. Fiter, E. D. Fixman, J. G. Martin, R. H. Friedel, et al. 2017. Semaphorin 4C protects against allergic inflammation: requirement of regulatory CD138+ plasma cells. *J. Immunol.* 198: 71–81.
38. Lommatzsch, M. 2012. Airway hyperresponsiveness: new insights into the pathogenesis. *Semin. Respir. Crit. Care Med.* 33: 579–587.
39. Lambrecht, B. N., and H. Hammad. 2009. Biology of lung dendritic cells at the origin of asthma. *Immunity* 31: 412–424.
40. Bratke, K., M. Lommatzsch, P. Julius, M. Kuepper, H. D. Kleine, W. Luttmann, and J. Christian Virchow. 2007. Dendritic cell subsets in human bronchoalveolar lavage fluid after segmental allergen challenge. *Thorax* 62: 168–175.
41. Holl, E. K., K. E. Roney, I. C. Allen, E. Steinbach, J. C. Arthur, A. Buntzman, S. Plevy, J. Frelinger, and J. P. Ting. 2012. Plexin-B2 and Plexin-D1 in dendritic cells: expression and IL-12/IL-23p40 production. *PLoS One* 7: e43333.
42. Balenga, N. A., W. Jester, M. Jiang, R. A. Panettieri, Jr., and K. M. Druey. 2014. Loss of regulator of G protein signaling 5 promotes airway hyperresponsiveness in the absence of allergic inflammation. *J. Allergy Clin. Immunol.* 134: 451–459.
43. Nakano, H., M. E. Free, G. S. Whitehead, S. Maruoka, R. H. Wilson, K. Nakano, and D. N. Cook. 2012. Pulmonary CD103(+) dendritic cells prime Th2 responses to inhaled allergens. *Mucosal Immunol.* 5: 53–65.
44. Worzfeld, T., and S. Offermanns. 2014. Semaphorins and plexins as therapeutic targets. *Nat. Rev. Drug Discov.* 13: 603–621.
45. Wanschel, A., T. Seibert, B. Hewing, B. Ramkhalawon, T. D. Ray, J. M. van Gils, K. J. Rayner, J. E. Feig, E. R. O'Brien, E. A. Fisher, and K. J. Moore. 2013. Neuroimmune guidance cue semaphorin 3E is expressed in atherosclerotic plaques and regulates macrophage retention. *Arterioscler. Thromb. Vasc. Biol.* 33: 886–893.
46. Shimizu, I., Y. Yoshida, J. Moriya, A. Nojima, A. Uemura, Y. Kobayashi, and T. Minamino. 2013. Semaphorin3E-induced inflammation contributes to insulin resistance in dietary obesity. *Cell Metab.* 18: 491–504.
47. Agudo, J., A. Ruza, N. Tung, H. Salmon, M. Leboeuf, D. Hashimoto, C. Becker, L. A. Garrett-Sinha, A. Baccarini, M. Merad, and B. D. Brown. 2014. The miR-126-VEGFR2 axis controls the innate response to pathogen-associated nucleic acids. *Nat. Immunol.* 15: 54–62.
48. Immunological Genome Consortium. 2012. Deciphering the transcriptional network of the dendritic cell lineage. *Nat. Immunol.* 13: 888–899.
49. Chapoval, S. P., C. G. Lee, C. Tang, A. D. Keegan, L. Cohn, K. Bottomly, and J. A. Elias. 2009. Lung vascular endothelial growth factor expression induces local myeloid dendritic cell activation. *Clin. Immunol.* 132: 371–384.
50. Nkyimbeng-Takwi, E. H., K. Shanks, E. Smith, A. Iyer, M. M. Lipsky, L. J. Detolla, H. Kikutani, A. D. Keegan, and S. P. Chapoval. 2012. Neuroimmune semaphorin 4A downregulates the severity of allergic response. *Mucosal Immunol.* 5: 409–419.
51. Morihana, T., S. Goya, M. Mizui, T. Yasui, D. V. Prasad, A. Kumanogoh, M. Tamura, T. Shikina, Y. Maeda, Y. Iwamoto, et al. 2013. An inhibitory role for Sema4A in antigen-specific allergic asthma. *J. Clin. Immunol.* 33: 200–209.



Supplemental Fig. 1. Sema3E deficiency leads to enhanced inflammation and mucus production in the airways. Lung tissue sections from either saline or HDM-exposed *Sema3E*^{-/-} versus WT mice stained with H&E were pathologically scored from 0-5 to determine the severity of inflammation (A). PAS-stained lung tissue sections underwent morphometric analysis to quantify the impact of Sema3E deficiency on goblet cell hyperplasia (B). Both experiments were performed in a blind manner (N=4-5 per group, * $P < 0.05$ and ** $P < 0.01$).



Supplemental Fig. 2. Intracellular IFN- γ (A), IL-4 (B) and IL-17A (C) production was compared between MLN CD4⁺ T cells obtained from HDM-exposed *Sema3e*^{-/-} and WT mice by flow cytometry. (N=4-5 per group, ** $P < 0.01$ and *** $P < 0.001$).



Supplemental Fig. 3. IL-23 production was investigated in non autofluorescent F4/80⁻ MHCII^{hi} CD11c⁺ pulmonary DC purified from *Sema3e*^{-/-} or WT mice upon saline (A) or HDM (B) challenge (N=4-5 per group).