



Immunopharmacology and inflammation

Semaphorin 7A plays a critical role in IgE-mediated airway inflammation in mice

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ABSTRACT

Elevated allergen-specific IgE levels are a hallmark of allergic asthma, a disease involving chronic airway inflammation characterized by airway hyperresponsiveness (AHR); neutrophilic airway inflammation is found in patients with severe asthma. Furthermore, we have reported that interleukin (IL)-33 and IL-17A contribute to IgE-mediated AHR through neutrophilic inflammation in mice. Meanwhile, semaphorins regulating neuronal and immune function have been focused on in several diseases. Here, we investigated whether semaphorin 7A (SEMA7A) is related to IgE-mediated neutrophilic inflammation in mice. BALB/c mice sensitized with antigen-specific IgE monoclonal antibody were repeatedly challenged by the antigen. When anti-SEMA7A antibody was administered during the fourth to seventh challenges, the infiltration by macrophages, lymphocytes, neutrophils, and eosinophils in the lungs was reduced at the seventh challenge ($P < 0.05$, 0.05 , 0.01 , and 0.05 , respectively). However, the increased production of IL-4, IL-5, IL-13, IL-33, IL-17A, IL-6, and CXCL1 in the lungs was not suppressed. In histological analysis, the epithelial cells, blood vessels, and inflammatory cells in the lungs of IgE-sensitized mice showed SEMA7A expression; plexin C1 for the receptor was expressed in the inflammatory cells. Meanwhile, we examined the effect of anti-SEMA7A antibody on AHR and neutrophilic inflammation enhanced by the collaborative action of IL-33 and IL-17A in normal mice, resulting in the suppression of these responses ($P < 0.05$ and 0.01 , respectively). Collectively, we demonstrated that SEMA7A plays a critical role in IgE-mediated neutrophilic airway inflammation. Therefore, SEMA7A may be a potential therapeutic target for severe allergic asthma showing neutrophilic airway inflammation.

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1. Introduction

Allergic asthma involves chronic airway inflammation related to an increased level of allergen-specific IgE (Cohn et al., 2004; Robinson et al., 1992; Mizutani et al., 2015). Allergen-induced airway hyperresponsiveness (AHR) is the unifying pathophysiological feature of asthma. However, the inflammatory status is complex: the airway granulocytic infiltration is governed by eosinophils in most asthmatic patients (Robinson et al., 1992; Wills-Krap, 1999; Bousquet et al., 2000), whereas neutrophilic airway inflammation is predominantly recognized in patients with severe asthma (Jatakanon et al., 1999; Louis et al., 2000). It has been reported that IL-33 elicits Th2 cytokine production such as IL-5 and IL-13 (Schmitz et al., 2005; Kearley et al., 2009; Mizutani et al.,

2013), which contributes to the infiltration by eosinophils in the lungs (Hogan et al., 1998; Wills-Kraps et al., 1998; De Sanctis et al., 1997). Meanwhile, patients with severe asthma have AHR and neutrophils accompanied by IL-17A production (Al-Ramli et al., 2009; Barczyk et al., 2003); IL-17A has been shown to cause neutrophilic inflammation and AHR in experimental mouse models of asthma (Mizutani et al., 2012a, 2014).

Semaphorins are a large family of secreted and membrane-bound proteins, which contribute to both the nervous and the immune systems (Kolodkin et al., 1992; Hall et al., 1996; Lange et al., 1998; Suzuki et al., 2007; Spriggs, 1999). Among them, semaphorin 7A (SEMA7A) was originally shown to promote axon outgrowth and contribute to the formation of lateral olfactory tracts (Pasterkamp et al., 2003). However, SEMA7A is expressed broadly by lymphoid cells, eosinophils, myeloid cells, bone cells, the nervous system, epidermal keratinocytes, fibroblasts, and endothelial blood vessels (Holmes et al., 2002; Sato and Takahashi, 1998; Mine et al., 2000; Esnault et al., 2014). Additionally, it is a potent stimulator of monocytes and neutrophils and induces

Abbreviations: AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; OVA, ovalbumin; SEMA7A, semaphorin 7A

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cytokine production (Holmes et al., 2002); T-cell responses are negatively regulated by it (Czopik et al., 2006), suggesting the possibility that SEMA7A contributes to the development of allergic diseases. However, the roles of SEMA7A in allergic diseases including asthma remain unclear.

We previously reported that AHR was involved in BALB/c mice passively sensitized by intraperitoneal (i.p.) injections of ovalbumin (OVA)-specific IgE monoclonal antibody (mAb) through neutrophilic inflammation by repeated intratracheal OVA challenges (Mizutani et al., 2012a). In this study, we showed that treatment with anti-SEMA7A antibody during the fourth to seventh challenges inhibited IgE-mediated neutrophilic inflammation at the seventh challenge. Furthermore, the increased expression of SEMA7A and plexin C1 (PLXNC1) for the receptor occurred in the lungs of IgE-sensitized mice. Meanwhile, we reported that IL-33 and IL-17A contribute to IgE-mediated AHR through neutrophilic inflammation; IL-17A promotes the exacerbation of IL-33-induced AHR by enhancing neutrophilic inflammation (Mizutani et al., 2012a, 2013, 2014). Therefore, we examined whether the exacerbation of IL-33-induced neutrophilic inflammation and AHR by IL-17A is suppressed by treatment with anti-SEMA7A antibody; the results indeed showed the inhibition of these responses. Thus, we newly showed that SEMA7A is critical for IgE-mediated neutrophilic airway inflammation, suggesting that it may contribute to neutrophilic inflammation in patients with severe asthma.

2. Materials and methods

2.1. Animals

Male 7-week-old BALB/c mice were obtained from Japan SLC (Hamamatsu, Japan). These mice were maintained in a temperature-controlled environment with free access to standard rodent chow and water. All of the experimental procedures were approved by the Experimental Animal Research Committee at Kobe Pharmaceutical University.

2.2. Passive sensitization with OVA-specific IgE mAb and treatment with anti-SEMA7A antibody

OVA-specific IgE mAb (OE-1) was derived from a B-cell hybridoma producing murine IgE, as described previously (Mizutani et al., 2012b). The hybridoma was grown in CELLline CL1000 with BD-Cell-MAB medium (BD Biosciences, San Diego, CA, USA) supplemented with 20% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin–streptomycin. The level of OE-1 in culture supernatants of hybridoma was assessed by ELISA (Mizutani et al., 2012b).

Passive sensitization with OE-1 was performed with the protocol described previously (Mizutani et al., 2013). As shown in Fig. 1A, BALB/c mice were passively sensitized with i.p. injections of a supernatant containing OE-1 (100 µg/mouse) of hybridoma on days 0, 1, and 2. Both the sensitized and the nonsensitized mice were challenged on days 1, 2, 3, 8, 9, 10, and 15 under anesthesia

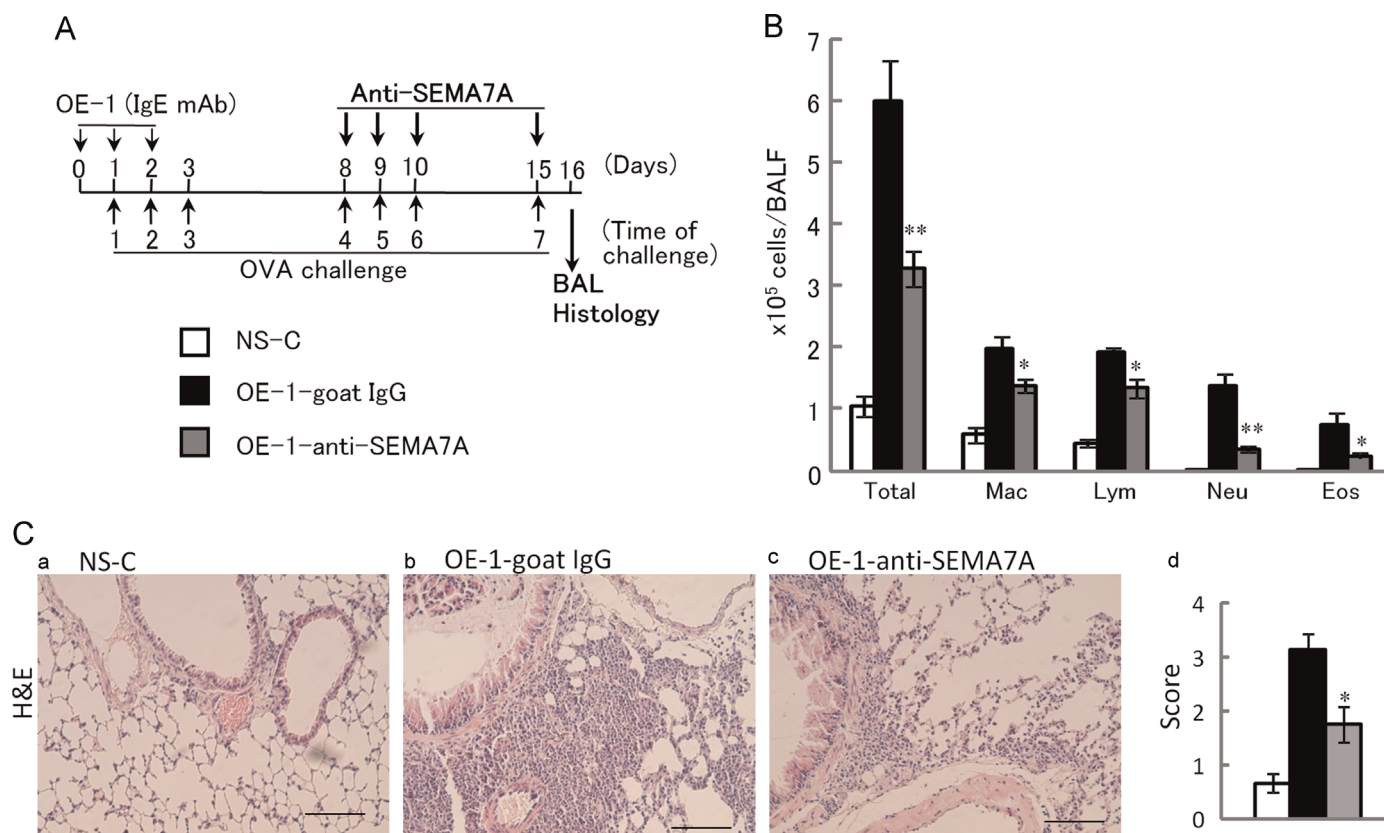


Fig. 1. Effect of treatment with anti-SEMA7A antibody on IgE-mediated airway inflammation. (A) Experimental protocol for sensitization with OE-1 and challenge with antigen, and treatment with anti-SEMA7A antibody. Anti-SEMA7A antibody was intratracheally administered on days 8, 9, 10, and 15 (OE-1-anti-SEMA7A). Negative and positive controls were nonsensitized-challenged (NS-C) and OE-1-sensitized-challenged, control goat IgG-treated mice (OE-1-goat IgG), respectively. (B and C) Effects of treatment with anti-SEMA7A antibody on inflammatory cells in BALF (B) and airway inflammation (H&E) in the lungs (C) 24 h after the seventh challenge. Scale bars, 100 µm. Histological appearance was scored for inflammation (H&E) (Cd). Each value is the mean ± S.E.M. of four or five animals. **P* < 0.05 and ***P* < 0.01 compared with the OE-1-goat IgG group (Student's *t*-test (two-tailed)). Total, all cells; Mac, macrophages; Lym, lymphocytes; Neu, neutrophils; Eos, eosinophils.

Table 1

Effect of treatment with anti-SEMA7A antibody on inflammatory cells in BALF 24 h after the seventh challenge in IgE-sensitized mice.

	Total cells ($\times 10^5$ cells/BALF)
NS-C	1.22 $\pm$ 0.16
OE-1-goat IgG	7.25 $\pm$ 0.53
OE-1-anti-SEMA7A (0.6 μ g/mouse)	5.66 $\pm$ 0.47
OE-1-anti-SEMA7A (6 μ g/mouse)	2.95 $\pm$ 0.45 ^a

Anti-SEMA7A antibody was intratracheally administered on days 8, 9, 10, and 15 (OE-1-anti-SEMA7A). Negative and positive controls were nonsensitized-challenged (NS-C) and OE-1-sensitized-challenged, control goat IgG-treated mice (OE-1-goat IgG), respectively. Each value is the mean $\pm$ S.E.M. of three to four animals.

^a $P < 0.01$ compared with the OE-1-goat IgG group (Dunnett's test).

with isoflurane (Wako Pure Chemicals, Osaka, Japan) with 1% OVA (grade V; Sigma-Aldrich, St. Louis, MO, USA) in a volume of 20 μ l by intratracheal administration. Furthermore, to evaluate the role of SEMA7A in IgE-mediated airway inflammation, we administered anti-SEMA7A antibody (6 μ g/mouse) (AF1835, goat IgG; R&D Systems, Minneapolis, MN, USA) intratracheally 30 min before each of the antigen challenges on days 8, 9, 10, and 15 (Fig. 1A). Control mice were given the same amount of goat IgG on days 8, 9, 10, and 15. Additionally, the accumulation of inflammatory cells in the lungs 24 h after the last instillation was evaluated on day 16 (Fig. 1A). Meanwhile, as shown Table 1, the accumulation of inflammatory cells in bronchoalveolar lavage fluid (BALF) 24 h after the seventh challenge was dose-dependently inhibited by treatment with anti-SEMA7A antibody (0.6 and 6 μ g/animal).

2.3. Coadministration of IL-33 and IL-17A and treatment with anti-SEMA7A antibody

As shown in Fig. 5, we assessed the effect of anti-SEMA7A antibody on neutrophilic inflammation and AHR induced by the coadministration of IL-33 and IL-17A (Biolegend, San Diego, CA, USA). As reported previously (Mizutani et al., 2014), IL-33 in solution (200 ng/mouse) was administered intratracheally to normal mice on days 0, 1, and 2 under anesthesia with isoflurane in a volume of 20 μ l; furthermore, on day 7, IL-33+IL-17A (200+40 ng/mouse) was administered intratracheally, and the accumulation of inflammatory cells in the lungs and AHR 24 h after the last instillation was evaluated on day 8 (Fig. 5A). In the negative control group, phosphate-buffered saline (PBS) was administered intratracheally to normal mice on days 0, 1, 2, and 7. Furthermore, to evaluate the role of SEMA7A in neutrophilic inflammation and AHR induced by the coadministration of IL-33 and IL-17A, we administered anti-SEMA7A antibody (6 μ g/mouse) intratracheally 30 min before the last instillation on day 7 (Fig. 5A). Control mice were given the same amount of goat IgG on day 7.

2.4. Analysis of cells recovered by bronchoalveolar lavage

To evaluate airway cellular inflammation, we examined the accumulation of inflammatory cells in BALF, as detailed previously (Mizutani et al., 2012b). The trachea was cannulated and the left bronchi were tied for histological examination. The right airway lumen was washed twice with 0.5 ml of Hanks' balanced salt solution (HBSS) containing 2% FBS. The cell pellet was suspended in a defined volume (200 μ l/sample) of HBSS containing 2% FBS. The total leukocyte count in BALF was determined by staining with Turk's solution. For differential cell counting, BAL cells were stained with Diff-Quik solution (Sysmex International Reagents, Kobe, Japan).

2.5. Measurement of cytokines in lung tissue supernatants

The frozen right lobe (after BAL) was homogenized in 1 ml of T-PER tissue protein extraction reagent (Thermo Scientific, Rockford, IL, USA) containing a Complete Mini Protease Inhibitor Cocktail tablet (Roche, Mannheim, Germany; 1 tablet/10 ml of T-PER stock reagent), as described previously (Mizutani et al., 2012a). Lung homogenates were centrifuged at 9000 $\times$ g for 10 min at 4 °C. The levels of IL-4, IL-5, IL-13, CXCL1 (R&D Systems), IL-33, IL-17A, and IL-6 (Biolegend) in supernatants of lung homogenates were measured using quantitative colorimetric sandwich ELISA kits.

The levels of sensitivity of these assays were 2 pg/ml (IL-4), 7 pg/ml (IL-5), 1.5 pg/ml (IL-13), 2 pg/ml (CXCL1), 15.3 pg/ml (IL-33), 2.7 pg/ml (IL-17A), and 2 pg/ml (IL-6). Additionally, intra- and inter-assay coefficients of variation were less than 8% and 13% for all assays, respectively.

2.6. Histological analysis

Left lungs were fixed in 10% neutral-buffered formalin, and then dissected, embedded in paraffin, and cut into 4- μ m-thick sections. These sections were stained with hematoxylin & eosin (H&E), periodic acid-Schiff (PAS), and Masson's trichrome (Masson).

Immunohistochemistry was performed with paraffin-embedded sections. Serial 4- μ m-thick sections of lungs were mounted on glass slides, dewaxed, and rehydrated with PBS. Endogenous peroxidase was blocked with 3% H₂O₂ in water for 30 min. After blocking of nonspecific binding with super blocking buffer for 20 min, the sections were incubated for 24 h at 4 °C with a polyclonal antibody against SEMA7A (ab23578, rabbit IgG; Abcam, Cambridge, MA, USA). The slides were developed using horseradish peroxidase (HRP)-conjugated anti-rabbit IgG and the diaminobenzidine (DAB) substrate kit for peroxidase (Vector Laboratories, Burlingame, CA, USA). Furthermore, in immunohistochemistry for PLXNC1, we used a polyclonal antibody against PLXNC1 (AF5375, sheep IgG; R&D Systems) and anti-sheep HRP-DAB Cell & Tissue Staining Kit (CTS019; R&D Systems). Counterstaining was done with Mayer's hematoxyline.

Scoring for each section was performed for inflammation (H&E), goblet cell hyperplasia (PAS), sub-epithelial fibrosis (Masson), and immunohistochemistry (SEMA7A and PLXNC1) on a scale of 0–4 with increments of 0.5 by a blind observer, as described previously (Mizutani et al., 2012b).

2.7. Measurement of AHR to methacholine

To estimate AHR to methacholine (MCh) 24 h after the coadministration of IL-33 and IL-17A, we measured specific airway resistance (sRaw; cm H₂O ml/[ml/s]) before and after the intratracheal instillation of MCh using a two-chambered, double-flow plethysmograph system (Pulmos-I; M.I.P.S., Osaka, Japan) according to the method by Pennock et al. (1979). In brief, MCh (25 μ g/ml) in solution was administered intratracheally to mice under isoflurane-induced anesthesia, and sRaw was measured 2 min after the instillation of MCh solution. Furthermore, we have reported that the instillation of MCh (25 μ g/ml) 24 h after the coadministration of IL-33 and IL-17A strongly induced the increased change in sRaw compared with that in PBS-treated mice when increasingly high doses of MCh (6.25, 12.5, and 25 μ g/ml) in solution were administered consecutively to mice (Mizutani et al., 2014).

2.8. Statistical analysis

Data are shown as the mean $\pm$ S.E.M. Statistical analyses for comparisons between two groups were performed using Student's *t*-test (two-tailed). To compare more than two groups, we used Dunnett's test after conducting one-way ANOVA. A probability value of $P < 0.05$ was considered significant.

3. Results

3.1. Effect of anti-SEMA7A antibody on IgE-mediated airway inflammation

We have reported that IgE-mediated airway inflammation was exacerbated during the fourth to seventh challenges (Mizutani et al., 2013). Therefore, we examined the effect of treatment with anti-SEMA7A antibody during the fourth to seventh challenges on the infiltration of inflammatory cells in BALF at the seventh challenge (Fig. 1A). Treatment with anti-SEMA7A antibody (0.6 and 6 μ g/mouse) was found to inhibit total cells in BALF of IgE-sensitized mice in a dose-dependent manner; the treatment (6 μ g/mouse) significantly inhibited this (72% inhibition, $P < 0.01$) (Table 1). Furthermore, treatment with anti-SEMA7A antibody (6 μ g/mouse) significantly inhibited the infiltration by macrophages (44% inhibition, $P < 0.05$), lymphocytes (40% inhibition, $P < 0.05$), neutrophils (76% inhibition, $P < 0.01$), and eosinophils (70% inhibition, $P < 0.05$) in BALF of IgE-sensitized mice (Fig. 1B); in the histological analysis, the treatment suppressed airway inflammation (H&E) (62% inhibition, $P < 0.05$) at the seventh challenge in the lungs of IgE-sensitized mice (Fig. 1C).

Subsequently, we investigated whether treatment with anti-SEMA7A antibody during the fourth to seventh challenges inhibits IgE-mediated airway remodeling such as goblet cell hyperplasia and sub-epithelial fibrosis, which were exacerbated during the fourth to seventh antigen challenges (Mizutani et al., 2013).

Treatment with anti-SEMA7A antibody during the fourth to seventh challenges significantly inhibited sub-epithelial fibrosis (Masson) (35% inhibition, $P < 0.05$) at the seventh challenge in IgE-sensitized mice (Fig. 2A), although goblet cell hyperplasia (PAS) was not affected by the treatment ($P > 0.1$) (Fig. 2B).

3.2. Effect of anti-SEMA7A antibody on IgE-mediated cytokine production

It has been reported that SEMA7A regulates immune responses (Kolodkin et al., 1992; Hall et al., 1996; Lange et al., 1998; Suzuki et al., 2007; Spriggs, 1999). Furthermore, we have reported the increased levels of Th2 cytokines (IL-4, IL-5, and IL-13), IL-33, IL-17A, IL-6, and CXCL1 at the seventh antigen challenge in the lung homogenate supernatants of IgE-sensitized mice (Mizutani et al., 2014, 2015). Therefore, we examined whether treatment with anti-SEMA7A antibody inhibits their production. Interestingly, treatment with anti-SEMA7A during the fourth to seventh challenges failed to suppress the production of these cytokines and chemokines (all data: $P > 0.3$) at the seventh challenge in IgE-sensitized mice (Fig. 3).

3.3. Expression of SEMA7A and PLXNC1 in the lungs of IgE-sensitized mice

Next, we investigated the site of SEMA7A expression in the lungs of IgE-sensitized mice. The expression of SEMA7A in the lungs 24 h after the seventh antigen challenge in IgE-sensitized mice was significantly increased compared with that in non-sensitized-challenged mice ($P < 0.05$) (Fig. 4A). Furthermore, the expression of SEMA7A was observed in epithelial cells and blood vessels in nonsensitized and IgE-sensitized mice; the inflammatory cells in IgE-sensitized mice expressed it (Fig. 4A).

Additionally, we investigated whether the expression of PLXNC1 for the receptor of SEMA7A was increased in the lungs of IgE-sensitized mice. Its expression in the lungs of such mice was

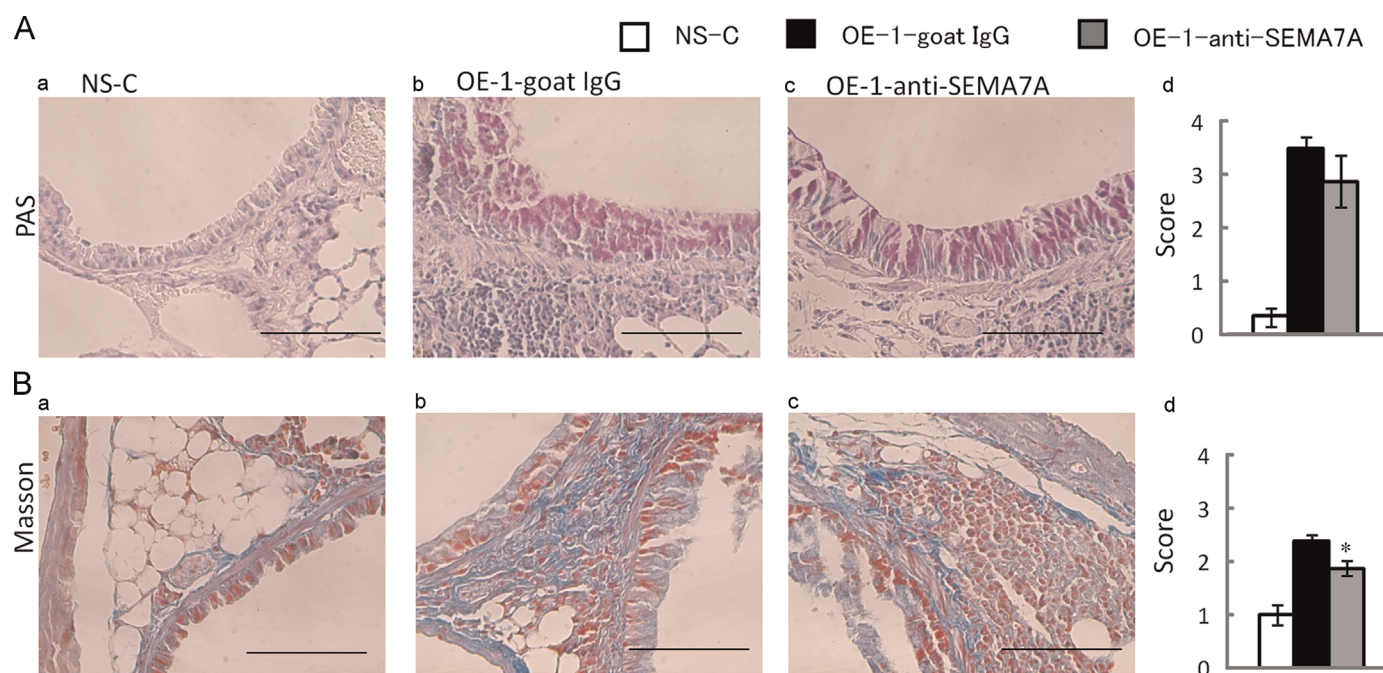


Fig. 2. Effect of treatment with anti-SEMA7A antibody on IgE-mediated airway remodeling in the lungs of IgE-sensitized mice. Anti-SEMA7A antibody was intratracheally administered on days 8, 9, 10, and 15 (c, OE-1-anti-SEMA7A). Negative and positive controls were nonsensitized-challenged (a, NS-C) and OE-1-sensitized-challenged, control goat IgG-treated mice (b, OE-1-goat IgG), respectively. (A and B) Effects of treatment with anti-SEMA7A antibody on goblet cell hyperplasia (PAS) (A) and sub-epithelial fibrosis (Masson) (B) in the lungs 24 h after the seventh challenge. Scale bars, 100 μ m. Histological appearance was scored for goblet cell hyperplasia (Ad) and sub-epithelial fibrosis (Bd). Each value is the mean $\pm$ S.E.M. of four or five animals. * $P < 0.05$ compared with the OE-1-goat IgG group (Student's *t*-test (two-tailed)).

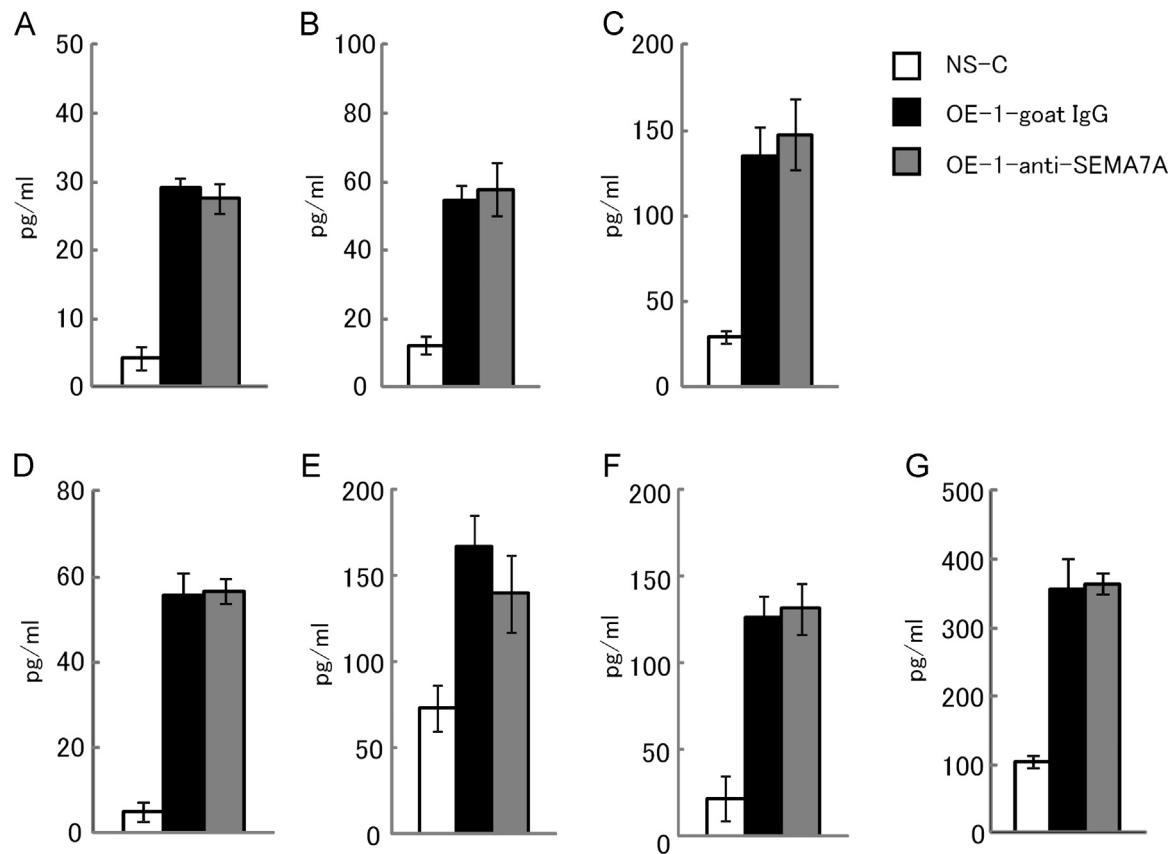


Fig. 3. Effect of treatment with anti-SEMA7A antibody on IgE-mediated cytokine production in the lungs of IgE-sensitized mice. Anti-SEMA7A antibody was intratracheally administered on days 8, 9, 10, and 15 (OE-1-anti-SEMA7A). Negative and positive controls were nonsensitized-challenged (NS-C) and OE-1-sensitized-challenged, control goat IgG-treated mice (OE-1-goat IgG), respectively. Effect of treatment with anti-SEMA7A antibody on the production of IL-4 (A), IL-5 (B), IL-13 (C), IL-33 (D), IL-17A (E), IL-6 (F), and CXCL1 (G) in the lung homogenate supernatants 24 h after the seventh challenge. Each value is the mean $\pm$ S.E.M. of four or five animals.

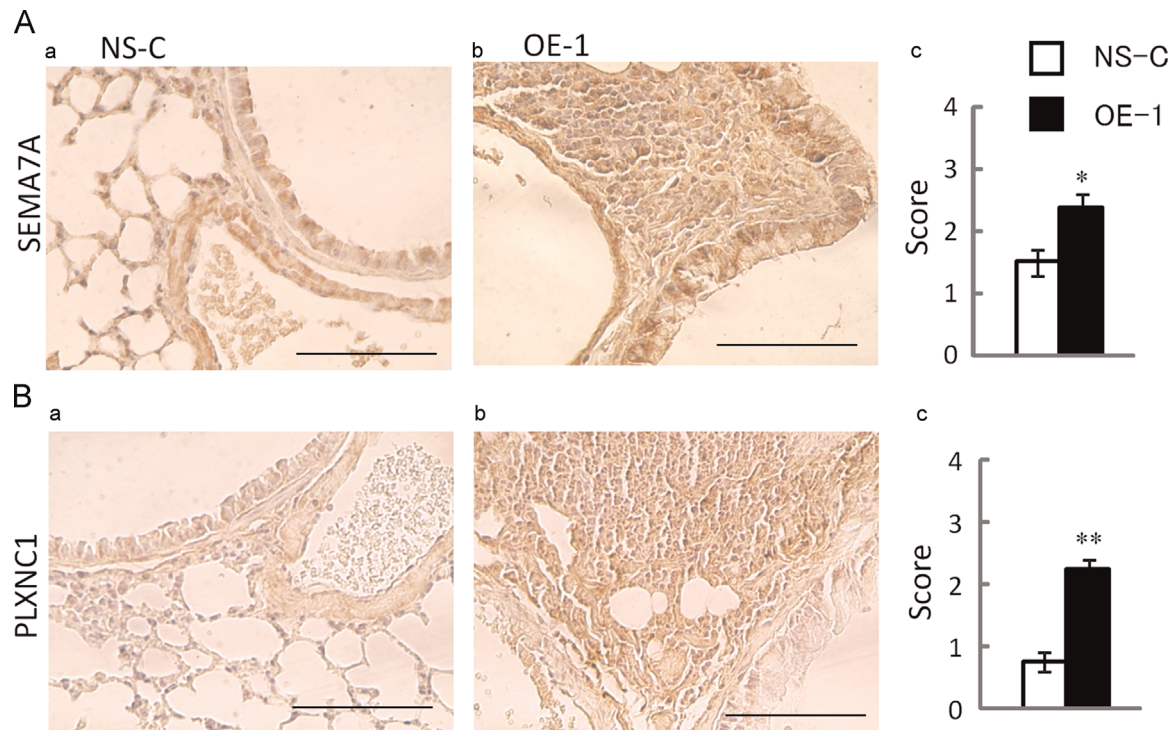


Fig. 4. Expression of SEMA7A and PLXNC1 in the lungs of IgE-sensitized mice. (A and B) Expression of SEMA7A (A) and PLXNC1 (B) in the lungs 24 h after the seventh challenge in nonsensitized-challenged (a, NS-C) and OE-1-sensitized mice (b, OE-1). Scale bars, 100 μ m. Histological appearance was scored for SEMA7A (Ac) and PLXNC1 (Bc). Each value is the mean $\pm$ S.E.M. of four animals. * P < 0.05 and ** P < 0.01 compared with the NS-C group (Student's t -test (two-tailed)).

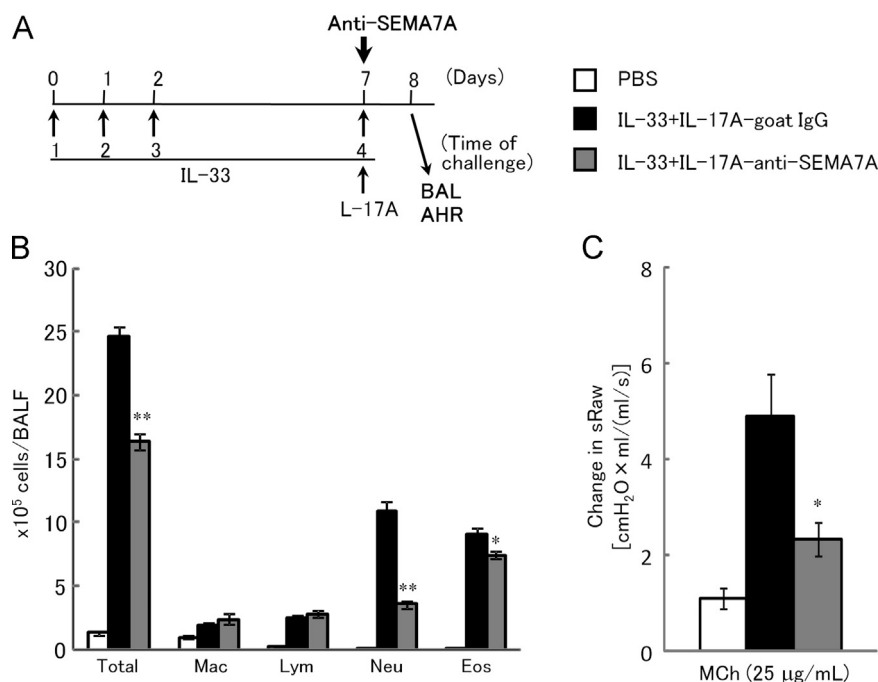


Fig. 5. Effect of treatment with anti-SEMA7A antibody on the exacerbation of IL-33-induced neutrophilic inflammation and AHR by IL-17A in mice. (A) Experimental protocol for intratracheal administration of IL-33 and IL-17A in BALB/c mice. IL-33 was administered intratracheally to normal mice on days 0, 1, and 2; furthermore, on day 7, IL-33+IL-17A was administered intratracheally. Control goat IgG (IL-33+IL-17A-goat IgG) or anti-SEMA7A antibody (IL-33+IL-17A-anti-SEMA7A) was administered intratracheally 30 min before the instillation of IL-33+IL-17A on day 7. In the negative control group, PBS was administered intratracheally to normal mice on days 0, 1, 2, and 7 (PBS). (B and C) Effect of treatment with anti-SEMA7A antibody on inflammatory cells in BALF (B) and AHR to MCh (C). Each value is the mean $\pm$ S.E.M. of four to six animals. * $P < 0.05$ and ** $P < 0.01$ compared with the IL-33+IL-17A-goat IgG group (Student's *t*-test (two-tailed)). Total, all cells; Mac, macrophages; Lym, lymphocytes; Neu, neutrophils; Eos, eosinophils.

indeed increased compared with that in nonsensitized-challenged mice ($P < 0.01$); additionally, its expression was observed in the inflammatory cells in the lungs of IgE-sensitized mice, although it was not observed in the lungs of nonsensitized-challenged mice (Fig. 4B).

3.4. Effect of anti-SEMA7A antibody on the exacerbation of IL-33-induced neutrophilic inflammation and AHR by IL-17A

We have reported that IL-33 and IL-17A contribute to the development of AHR through neutrophilic airway inflammation in IgE-sensitized mice (Mizutani et al., 2012a, 2013, 2014); furthermore, IL-33 cooperates with IL-17A to exacerbate AHR by enhancing neutrophilic inflammation in mice (Mizutani et al., 2014). Therefore, we examined whether treatment with anti-SEMA7A antibody inhibits neutrophilic inflammation and AHR induced by the coadministration of IL-33 and IL-17A. Treatment with anti-SEMA7A antibody upon the coadministration of IL-33 and IL-17A inhibited neutrophilic inflammation (67% inhibition, $P < 0.01$) and AHR to MCh (68% inhibition, $P < 0.05$) (Fig. 5B and C). Furthermore, the infiltration by eosinophils (18% inhibition, $P < 0.05$), but not macrophages ($P > 0.3$) and lymphocytes ($P > 0.4$), was reduced by it (Fig. 5B).

4. Discussion

Neutrophilic airway inflammation is a hallmark of patients with severe asthma (Jatakanon et al., 1999; Louis et al., 2000). We have reported that IgE-mediated neutrophilic inflammation contributes to the production of IL-33, IL-17A, IL-6, or CXCL1 in the lungs (Mizutani et al., 2012a, 2013, 2014). Furthermore, the increased levels of pro-inflammatory cytokines such as IL-6 and CXCL1 in a murine ventilator-induced lung injury model were

inhibited by treatment with anti-PLXNC1 antibody (Granja et al., 2014). However, in the present study, we showed that treatment with anti-SEMA7A antibody failed to suppress the production of IL-33, IL-17A, IL-6, and CXCL1 in the lungs of IgE-sensitized mice; furthermore, the increased levels of Th2 cytokines including IL-4, IL-5, and IL-13 were not inhibited. Thus, treatment with anti-SEMA7A antibody inhibits neutrophilic inflammation through a mechanism not affecting the production of these cytokines and chemokines in IgE-sensitized mice.

The expression of SEMA7A in the lungs of IgE-sensitized mice was increased compared with that in nonsensitized mice; consistent with previous data (Holmes et al., 2002; Sato and Takahashi, 1998; Mine et al., 2000; Esnault et al., 2014), the epithelial cells, blood vessels, and inflammatory cells in the lungs of IgE-sensitized mice showed this expression. Furthermore, it has been reported that SEMA7A facilitates increased transendothelial migration of neutrophils depending on the PLXNC1 for the receptor (Morote-Garcia et al., 2012); PLXNC1 is expressed in neutrophils in peripheral blood of normal mice (Konig et al., 2014), and is associated with adhesion in the inflammatory cell transmigration process (Holmes et al., 2002; Lazova et al., 2009). On the basis of these findings, we hypothesized that, in the first step for the infiltration of neutrophils into the lungs of IgE-sensitized mice, the expression of SEMA7A in the endothelial cells of blood vessels may be important for catching neutrophils expressing PLXNC1. Therefore, we examined whether PLXNC1 was expressed in the inflammatory cells including neutrophils in the lungs of IgE-sensitized mice; the results showed its strong expression in the inflammatory cells of the lungs. Thus, the expression of SEMA7A in the blood vessels may contribute to the transmigration of neutrophils expressing PLXNC1 into the lungs.

In our previous studies, IL-33 and IL-17A were shown to contribute to IgE-mediated AHR via neutrophilic inflammation (Mizutani et al., 2012a, 2013); furthermore, IL-17A was found to

promote IL-33-induced AHR by enhancing neutrophilic inflammation in normal mice (Mizutani et al., 2014). These findings prompted us to examine whether treatment with anti-SEMA7A antibody inhibits the exacerbation of neutrophilic inflammation and AHR induced by the co-administration of IL-33 and IL-17A, resulting in the inhibition of neutrophilic inflammation and AHR by the treatment. Furthermore, we have reported that depletion by treatment with anti-Gr-1 mAb inhibited the development of AHR induced by the coadministration of IL-33 and IL-17A (Mizutani et al., 2014). Thus, SEMA7A contributes to IL-33- and IL-17A-induced exacerbation of AHR through enhancing neutrophilic airway inflammation.

In the present study, treatment with anti-SEMA7A antibody inhibited the infiltration by not only neutrophils but also macrophages, lymphocytes, and eosinophils in IgE-sensitized mice. It has been reported that the inflammatory cells including macrophages, lymphocytes, and eosinophils express PLXNC1 (Esnault et al., 2014; König et al., 2014), suggesting that SEMA7A contributes to the infiltration by not only neutrophils but also macrophages, lymphocytes, and eosinophils in the lungs of IgE-sensitized mice. However, in the airway inflammation by the coadministration of IL-33 and IL-17A, the treatment with anti-SEMA7A antibody did not suppress the infiltration by macrophages and lymphocytes in BALF, although neutrophils and eosinophils were inhibited. This difference of inhibitory effects of anti-SEMA7A antibody between IgE-sensitized and IL-33+IL-17A-treated mice may be related to the timing of administration of anti-SEMA7A antibody because, in the coadministration of IL-33 and IL-17A, the antibody was only administered at the last instillation.

Airway remodeling including goblet cell hyperplasia and sub-epithelial fibrosis in the lungs is characteristic of severe and/or chronic asthma (Ebina et al., 1993; Hossain and Heard, 1970; Jeffery et al., 1992; Roche et al., 1989). Therefore, we examined the role of SEMA7A in the development of airway remodeling in IgE-sensitized mice. Treatment with anti-SEMA7A antibody failed to inhibit goblet cell hyperplasia in IgE-sensitized mice. In our previous and other studies (Mizutani et al., 2013; Lanone et al., 2002), IL-33 and/or IL-13 were shown to contribute to goblet cell hyperplasia in allergic asthma mouse models; however, in the present study, treatment with anti-SEMA7A antibody did not inhibit the production of these cytokines in the lungs of IgE-sensitized mice, indicating that SEMA7A does not contribute to goblet cell hyperplasia in IgE-sensitized mice. Meanwhile, treatment with anti-SEMA7A antibody suppressed sub-epithelial fibrosis in the lungs of IgE-sensitized mice, although the inhibitory effect was weak. It has been reported that SEMA7A contributes to transforming growth factor (TGF)- β 1-induced sub-epithelial fibrosis in the lungs of mice (Kang et al., 2007); furthermore, TGF- β 1 contributes to sub-epithelial fibrosis in experiment allergic asthma (Tanaka et al., 2004). On the basis of these findings, we suggest that SEMA7A may contribute to the development of sub-epithelial fibrosis induced by TGF- β 1 in the lungs of IgE-sensitized mice.

5. Conclusion

Our results highlight the importance of SEMA7A for neutrophilic airway inflammation, which is related to the development of AHR, in IgE-sensitized mice, indicating critical steps of leukocyte infiltration in vivo. The efficacy of functional blockage of SEMA7A may offer a new therapeutic approach in the treatment of severe and/or chronic allergic asthma associated with neutrophilic airway inflammation.

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