European Journal of Pharmacology 764 (2015) 149–156

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Immunopharmacology and inflammation

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

Nobuaki Mizutani [a,*], Takeshi Nabe [b,c], Shin Yoshino [a]

[a] Department of Pharmacology, Kobe Pharmaceutical University, 4-19-1 Motoyamakita, Higashinada, Kobe 658-8558, Japan
[b] Department of Pharmacology, Kyoto Pharmaceutical University, 5 Nakauchi, Misasagi, Yamashina, Kyoto 607-8414, Japan
[c] Department of Immunopharmacology, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka 573-0101, Japan

ARTICLE INFO

Article history:
Received 10 April 2015
Received in revised form
29 June 2015
Accepted 1 July 2015
Available online 2 July 2015

Keywords:
Semaphorin 7A
Plexin C1
Asthma
IgE
Neutrophil
Airway hyperresponsiveness

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.

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

* Corresponding author. Fax: +81 78 441 7573.
E-mail address: mizutani@kobepharma-u.ac.jp (N. Mizutani).

http://dx.doi.org/10.1016/j.ejphar.2015.07.004
0014-2999/

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 CELLine 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

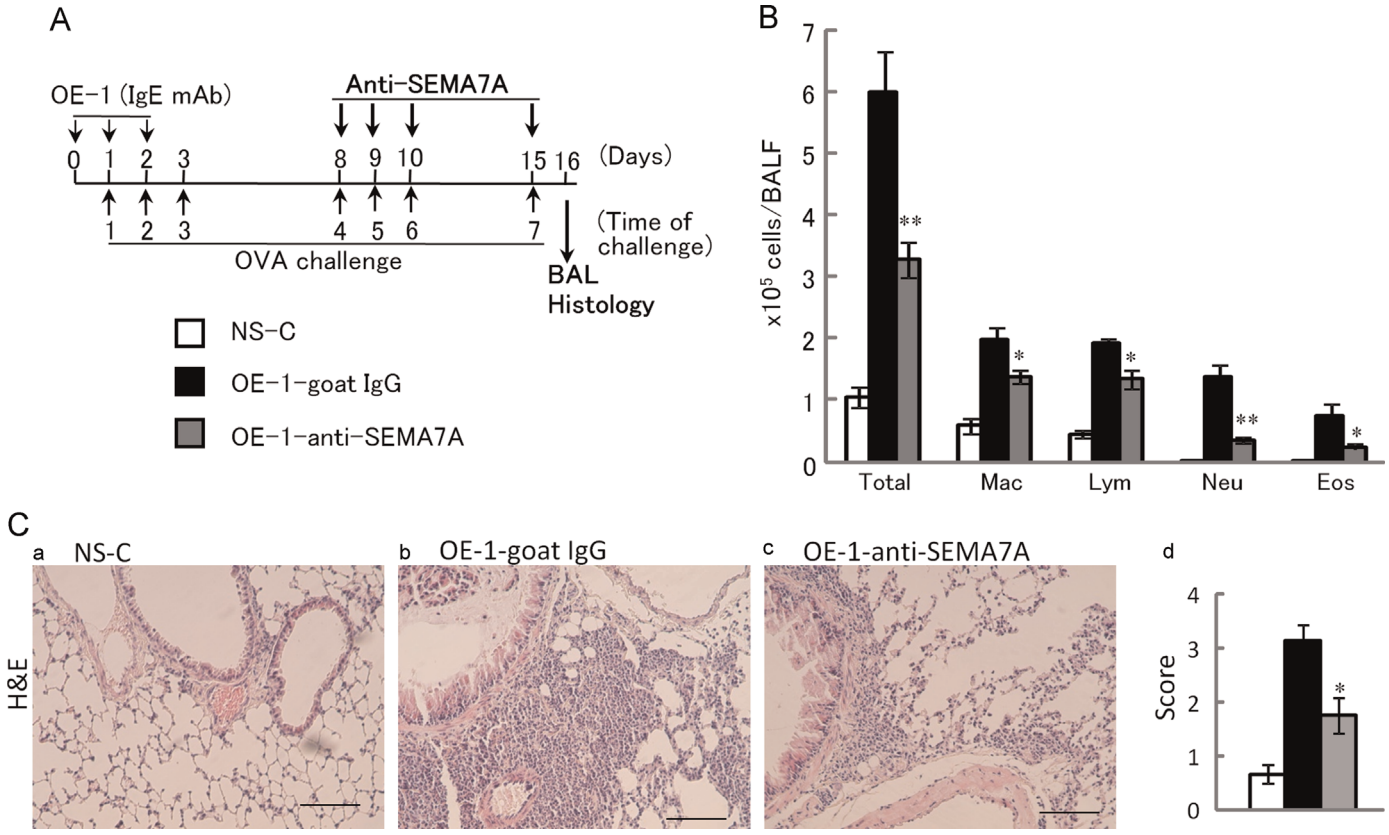

Fig. 1. Effect of treatment with anti-SEMA7A antibody on IgE-mediated airway inflammation. (A) Experimental protocol for sensitization with OE-1 and challenge with antigen, and treatment with anti-SEMA7A antibody. Anti-SEMA7A antibody was intratracheally administered on days 8, 9, 10, and 15 (OE-1-anti-SEMA7A). 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.