BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther 29(5), 492-497 (2021)

Suppressive Effect of CYM50358 $S1P_4$ Antagonist on Mast Cell Degranulation and Allergic Asthma in Mice

Wi-Jin Jeon[1], Ki Wung Chung[1], Joon-Hee Lee[1] and Dong-Soon Im[1,2,*]

[1]College of Pharmacy, Pusan National University, Busan 46241,
[2]Laboratory of Pharmacology, College of Pharmacy, and Department of Biomedical and Pharmaceutical Sciences, Graduate School, Kyung Hee University, Seoul 02447, Republic of Korea

Abstract

Levels of sphingosine 1-phosphate (S1P), an intercellular signaling molecule, reportedly increase in the bronchoalveolar lavage fluids of patients with asthma. Although the type 4 S1P receptor, $S1P_4$ has been detected in mast cells, its functions have been poorly investigated in an allergic asthma model *in vivo*. $S1P_4$ functions were evaluated following treatment of CYM50358, a selective antagonist of $S1P_4$, in an ovalbumin-induced allergic asthma model, and antigen-induced degranulation of mast cells. CYM50358 inhibited antigen-induced degranulation in RBL-2H3 mast cells. Eosinophil accumulation and an increase of Th2 cytokine levels were measured in the bronchoalveolar lavage fluid and via the inflammation of the lungs in ovalbumin-induced allergic asthma mice. CYM50358 administration before ovalbumin sensitization and before the antigen challenge strongly inhibited the increase of eosinophils and lymphocytes in the bronchoalveolar lavage fluid. CYM50358 administration inhibited the increase of IL-4 cytokines and serum IgE levels. Histological studies revealed that CYM50358 reduced inflammatory scores and PAS (periodic acid–Schiff)-stained cells in the lungs. The pro-allergic functions of $S1P_4$ were elucidated using *in vitro* mast cells and *in vivo* ovalbumin-induced allergic asthma model experiments. These results suggest that $S1P_4$ antagonist CYM50358 may have therapeutic potential in the treatment of allergic asthma.

Key Words: $S1P_4$, Sphingosine 1-phosphate, Anti-allergic, Anti-asthmatic, Degranulation, Mast cell

INTRODUCTION

Initial sensitization and subsequent repeated exposure to antigens are known to compose the pathogenesis of asthma development (Khalaf *et al*., 2019). During the antigen sensitization process, antigen-presenting dendritic cells are known to play a pivotal role (Van Rijt and Lambrecht, 2005). The activation of eosinophils and mast cells leads to inflammatory reactions in the airways during the antigen exposures process (Gilfillan *et al*., 2009). The importance of sphingolipid synthesis and sphingosine 1-phosphate (S1P) signaling cascade has been reported in bronchial asthma and allergic diseases (Moffatt *et al*., 2007; Saluja *et al*., 2017; Worgall, 2017). Reportedly, S1P levels are elevated after antigen exposure in patients with asthma (Ammit *et al*., 2001). Antigen-induced crosslinking of the immunoglobulin E (IgE) antibody on mast cells induces sphingosine kinase activation, generating S1P in mast cells (Choi *et al*., 1996; Prieschl *et al*., 1999; Jolly *et al*., 2004). The secreted S1P, the ligand of specific five S1P receptors, $S1P_{1\text{-}5}$, induces a variety of pathophysiological responses (Graler *et al*., 1998; Park and Im, 2017).

In asthma, $S1P_1$ and $S1P_2$ has been extensively studied (Park and Im, 2019). In particular, S1P-induced degranulation of mast cells is mediated through $S1P_2$ receptors (Jolly *et al*., 2004; Oskeritzian *et al*., 2010). Previously, the involvement of $S1P_2$ in allergic asthma and atopic dermatitis has been studied in murine models (Park and Im, 2019, 2020). In contrast, $S1P_4$ has been found in mast cells, but its functions have been poorly investigated in an allergic asthma model *in vivo* (Kulinski *et al*., 2018). Therefore, first, RBL-2H3 mast cells were used to determine whether $S1P_4$ is involved in antigen-induced degranulation by treatment with CYM50358, a selective antagonist of $S1P_4$ (Guerrero *et al*., 2012). Second, using an ovalbumin (OVA)-induced allergic asthma model, the effects of $S1P_4$ suppression was assessed.

Open Access **https://doi.org/10.4062/biomolther.2020.206**

Received Nov 12, 2020 **Revised** Jan 4, 2021 **Accepted** Jan 6, 2021
Published Online Jan 27, 2021

***Corresponding Author**
E-mail: imds@khu.ac.kr
Tel: +82-2-961-9377, Fax: +82-2-961-9580

MATERIALS AND METHODS

Materials

CYM50358 was purchased from Tocris (Bristol, UK). Other materials were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell culture

Rat RBL-2H3 mast cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). RBL-2H3 cells were cultured at 37°C in a 5% CO_2-humidified incubator, and maintained in 10% (v/v) heat-inactivated fetal bovine serum containing high-glucose Dulbecco's modified Eagle medium (DMEM) with 2 mM glutamine, 100 U/mL penicillin, 1 mM sodium pyruvate, and 50 μg/mL streptomycin (Huang *et al*., 2018).

Animals

Female five-week-old BALB/c mice were purchased from Daehan Biolink (Seoul, Korea). They were housed in the laboratory animal facility at Kyung Hee University (Seoul, Korea) and provided *ad libitum* water and food. The Kyung Hee University Institutional Animal Care Committee reviewed and approved the protocol with respect to ethical issues and scientific care (Approval Number, KHSASP-20-197).

Assessment of degranulation

By measuring β-hexosaminidase activity in the medium, degranulation of RBL-2H3 cells was assessed. Monoclonal anti-dinitrophenyl mouse immunoglobulin E and human dinitrophenyl albumin were used to induce degranulation (Huang *et al*., 2018).

Asthma induction in mice and administration of CYM50358

Following a simple randomization procedure, 6-week-old female BALB/c mice (22 g) were randomly assigned to one of four treatment groups (n=5): phosphate-buffered saline (PBS)-injected control group, OVA-injected asthma group, CYM50358-treatment before sensitization plus OVA-injected group, and CYM50358-treatment before challenge plus OVA-injected group. Asthma was induced by intraperitoneal injection of 50 μg OVA and 1 mg aluminum hydroxide on D0 and D14 (sensitization). Mice were challenged by exposing to nebulized OVA for D28, D29, and D30 (challenge) (Kim and Im, 2019; Park and Im, 2019). CYM50358 was administered via intraperitoneal injection 30 min before OVA sensitization or OVA challenge. Bronchoalveolar lavage fluids (BALF) were collected from the lungs on D32, and cell population of BALF cells was analyzed after staining.

Cell counting and analysis in BALF

Using a Cellspin® centrifuge (Hanil Electric, Seoul, Korea), immune cells in BALF were adhered to a glass slide and fixed in methanol for 30 s. Staining with May-Grünwald solution was conducted in the cells on slides for 8 min and subsequently by Giemsa solution for 12 min.

Histological examination of the lungs

Tissue sections of lungs from mice of each group were prepared. Hematoxylin and Eosin (H&E) staining and periodic acid-Schiff (PAS) staining were conducted to find mucus-secreting goblet cells and eosinophil infiltration, respectively. For PAS staining, Schiff's regent was used and for H&E staining, hematoxylin and eosin regents were used (Heo and Im, 2019).

Degree of lung inflammation was measured using a subjective scale of 0-3 by a treatment-blind observer. Mucin-secreting cells stained with PAS in the airways were counted from two lung sections per mouse. At the same time we also measured the length of the bronchi basal lamina using ImageJ software (National Institutes of Health, Bethesta, MD, USA). Mucous production was expressed by the number of PAS-positive cells per mm of bronchiole (Kim and Im, 2019).

Measurement of total serum IgE levels and IL-4 cytokine levels

Mouse IgE levels in the serum were determined using ELISA kits (eBioscience, San Diego, CA, USA). IL-4 and IL-13 levels in BALF were quantitated using ELISA kits (eBioscience). Capture antibodies and biotinylated detection antibodies specific for IL-4 and IL-13 were obtained from eBioscience (IL-4: cat. 14-7041-68 and cat. 33-7042-68C or IL-13: cat no. 14-7043-68 and 33-7135-68B). Avidin-horseradish peroxidase was used and the absorbance was measured at 450 nm (Lee *et al*., 2018).

Statistical analysis

Results are expressed as means ± standard errors (SEs). For statistical significance analysis of variance (ANOVA) was used, and followed by Turkey's *post hoc* test using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). p values<0.05 indicated statistical significance.

RESULTS

CYM50358 repressed degranulation of mast cells

Mast cells play a pivotal role in asthma episodes (Prussin and Metcalfe, 2003). Antigen exposure induces cross-linking of IgEs on mast cell membranes, resulting in degranulation (Gilfillan *et al*., 2009). The degranulation of the mast cells releases mediators of allergic responses such as histamine, leukotrienes, and prostaglandins (Brown *et al*., 2008). RBL-2H3 rat basophilic leukemia cells were used to measure degranulation responses. The β-hexosaminidase activity in the medium was increased following antigen exposure (Fig. 1). Treatment of CYM50358 suppressed the release of β-hexosaminidase in a concentration-dependent manner (Fig. 1). The inhibition induced by CYM50358 was significant at a concentration of 10 μM (Fig. 1).

CYM50358 repressed the increase of eosinophils and lymphocytes in the bronchoalveolar lavage fluid (BALF)

Next, an OVA-induced mouse model of asthma was employed to verify the inhibitory effect of CYM50358. The total cell number was assessed in the BALF, and the distribution of immune cell populations was calculated. In the BALF, the total cell number increased to 293.1% in the OVA-induced asthma group when compared with that in the PBS-treated control group (Fig. 2). CYM50358 treatment before antigen sensitization or before antigen challenge significantly inhibited the OVA-induced increase in the total cell number by 56.6 and 41.7%, respectively (Fig. 2B). Immune cell populations in the BALF were also assessed, with the eosinophil number

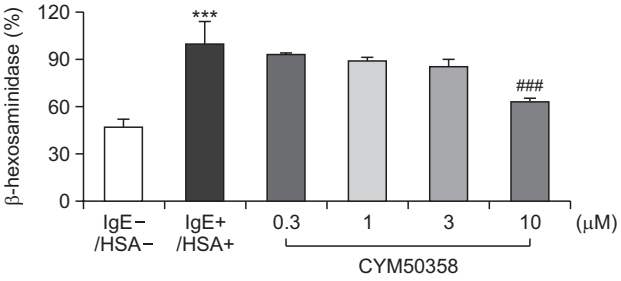

Fig. 1. CYM50358 represses antigen-induced degranulation in RBL-2H3 mast cells. After sensitization with anti-DNP IgE for 18 h, RBL-2H3 cells were challenged with DNP human serum albumin (HSA). CYM50358 was treated at the indicated concentrations 30 min before antigen challenge. Basal degranulation shows samples without IgE and HSA, and the positive control of antigen-induced degranulation is shown in the samples with IgE and HSA. The results are presented as the means ± standard error (SE) of three independent experiments. ***p<0.001 *vs.* the HSA-untreated group. ###p<0.001 *vs.* the HSA-treated group.

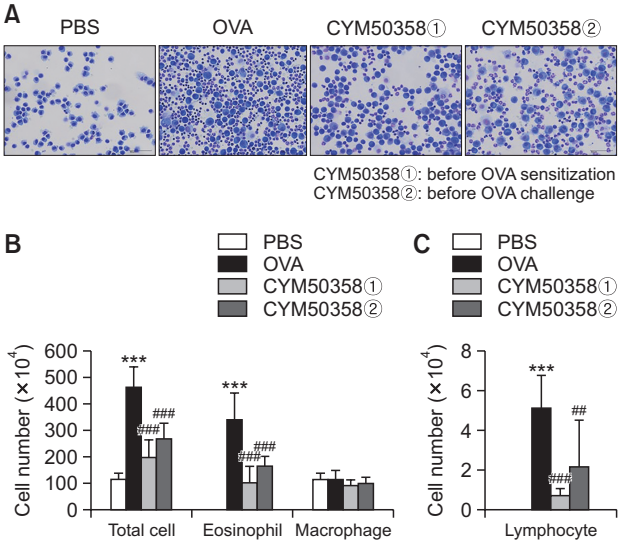

Fig. 2. CYM50358 represses OVA-induced immune cell accumulation in BALF. (A) Mice were sensitized with OVA twice by i.p. injection on day 0 (D0) and D14, and later challenged on D28, D29, and D30 with nebulized OVA. CYM50358 was administrated intraperitoneally at the dose of 10 mg/kg, 30 min before OVA sensitization or before OVA challenge. BALF cells were stained using May-Grünwald stain and counted. (B) Total cell counts, eosinophils, and macrophages in BALF. (C) Lymphocytes counts in the BALF. The results are presented as the mean ± SE cell count values (n=5). ***p<0.001 *vs.* the PBS-treated group, ##p<0.01, ###p<0.001 *vs.* the OVA-treated group.

increased by the OVA treatment and significantly decreased by CYM50358 treatment before sensitization or before challenge, by 69.3 and 48.5%, respectively (Fig. 2B). Although the lymphocyte number was lower than the eosinophil number, OVA induced an increase and CYM50358 treatment decreased the lymphocyte counts by both treatments (Fig. 2C). Macrophage numbers were not significantly altered by OVA or CYM50358 (Fig. 2B).

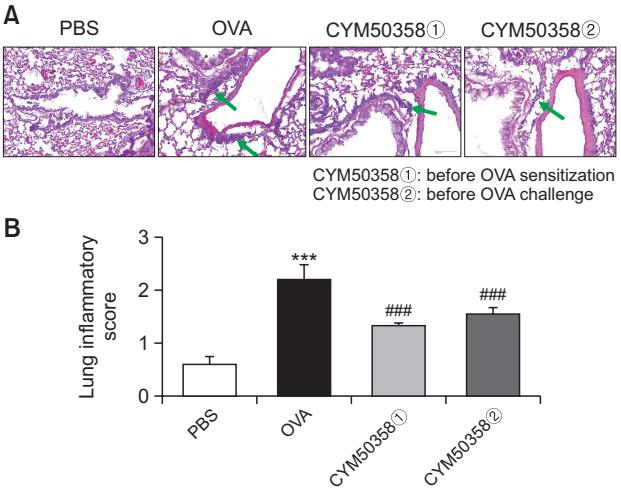

Fig. 3. CYM50358 protects against airway inflammation. (A) Panels show H&E-stained sections of lung tissues from the PBS group, OVA group, and CYM50358-treated OVA groups (before sensitization or before challenge). Small navy blue dots around the bronchioles indicates eosinophils. Eosinophils are scarcely observed in the PBS group, whereas they are densely accumulated around bronchioles in the OVA group (green arrows). However, eosinophil accumulation was less obvious in the OVA+CYM50358 groups than in the OVA group. (B) Lung inflammation was semi-quantitatively evaluated; histological findings were scored as described in the Materials and methods section. Values represent the means ± SEs (n=5). ***p<0.001 *vs.* the PBS-treated group, ###p<0.001 *vs.* the OVA-treated group.

CYM50358 repressed the mucin secretion and inflammation in the lungs

Additionally, histological analysis of the lung samples was performed. In the H&E staining, eosinophils in the lung sections were present as small, navy-blue dots (Fig. 3). Although substantially few eosinophils were detected in the PBS control group, numerous eosinophils densely surrounded bronchioles in the OVA group (Fig. 3). CYM50358 treatment before sensitization or before challenge reduced eosinophil numbers (Fig. 3). On using a subjective scale of 0-3, semi-quantitative evaluation of lung inflammation indicated an average inflammation score of 2.2 in the OVA-treated group, and CYM50358 treatment before sensitization or before challenge significantly reduced the score (Fig. 3).

PAS staining was also performed to reveal mucins and mucous glycoproteins produced by goblet cells. As shown in Fig. 4, secreted or stored mucins appeared as dark violet. Mucins were stained in cells surrounding bronchioles in the OVA group. However, mucin production was suppressed following CYM50358 treatment before sensitization or before challenge (Fig. 4). Furthermore, a semi-quantitative analysis of mucin production was performed by counting PAS-positive cells in bronchioles (Fig. 4). Stained cells were scarce in the PBS-treated group. However, in the OVA-treated group, approximately 100 PAS-positive cells/mm were detected, and CYM50358 treatment before sensitization or before challenge significantly suppressed the number of PAS-positive cells (Fig. 4).

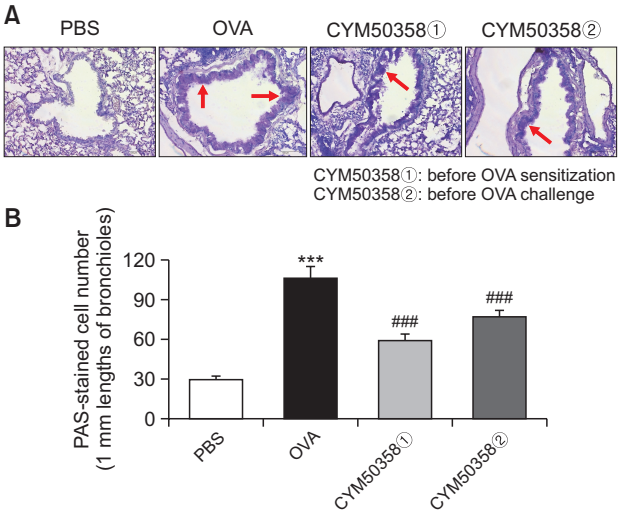

Fig. 4. CYM50358 protects against mucin production. (A) Panels show PAS/hematoxylin-stained sections of lung tissues from the PBS group, OVA group, and CYM50358-treated OVA groups (before sensitization or before challenge). In PAS staining, mucin is stained as purple color. Darker and thicker purple color is observed surrounding the bronchiole in the OVA group compared to the PBS group. (B) Mucous production was measured by counting the number of PAS-positive cells (red arrows) per mm of bronchiole (n=5 per group). ***p<0.001 *vs.* the PBS-treated group, $^{###}p$<0.001 *vs.* the OVA-treated group.

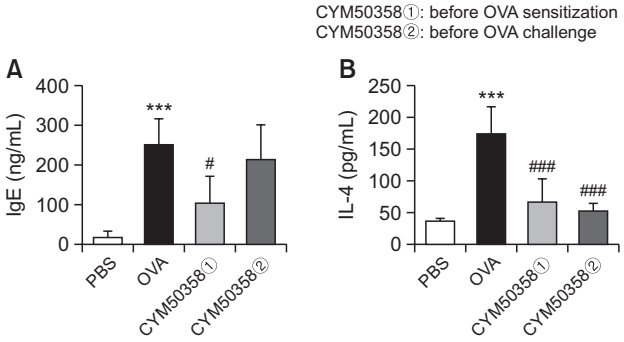

Fig. 5. Effect of CYM50358 on IgE levels in serum and IL-4 levels in BALF. (A) Serum IgE levels. Results are presented as means ± SEM (n=5). ***p<0.001 *vs.* the PBS-treated group, $^{#}p$<0.05, $^{###}p$<0.001 *vs.* the OVA-treated group. (B) ELISA was used to measure the protein levels of IL-4 in BALF. The results represent the mean ± SE of protein levels (n=5). ***p<0.001 *vs.* the PBS-treated group, $^{###}p$<0.001 *vs.* the OVA-treated group.

CYM50358 suppressed OVA-induced increase in serum IgE and BALF IL-4 levels

Serum IgE levels were assessed to confirm the immunological effects of OVA and CYM50358. IgE production was increased in the sera of OVA-treated mice (Fig. 5A). An OVA-induced increase in serum IgE levels was significantly repressed by CYM50358 treatment, both before antigen sensitization and challenge.

Th2 cytokines, such as IL-4, play major roles in the progression of allergic asthma (Romagnani, 2002). Th2 cytokines induce eosinophil recruitment and activation, hypersecretion of mucus in epithelial cells, metaplasia of goblet cells, and proliferation of smooth muscle cells (Tagaya and Tamaoki, 2007). The protein levels of Th2 cytokines, IL-4 and IL-13 in BALF were measured by ELISA. The IL-4 levels were increased in the OVA-induced group compared to the vehicle-treated control group, and the increase in IL-4 levels was significantly suppressed by both treatments of CYM50358 (Fig. 5B). We were not able to detect IL-13 levels in the BALF, because they were below the detectable ranges.

DISCUSSION

In the present study, two new findings were revealed using the $S1P_4$ antagonist, CYM50358. First, the $S1P_4$ receptor was involved in mast cell degranulation in RBL-2H3 cells and OVA-induced allergic asthma. Following the administration of CYM50358 before antigen challenges, immunological responses such as increase of IgE levels, immune cell accumulation in BALF, and increased mucin-secreting cells in the lungs were significantly suppressed. CYM50358-mediated inhibition of mast cell degranulation might contribute to the suppressive actions of the CYM50358 *in vivo*. In particular, effect of CYM50358 treatment before antigen challenge imply that suppression of mast cell degranulation plays an important role in the *in vivo* efficacy. Second, blockage of the $S1P_4$ receptor suppressed exacerbation of allergic asthma responses *in vivo*, which was demonstrated by both CYM50358 treatments, that is, before sensitization and before antigen challenge. The effect of CYM50358 treatment before sensitization may imply that $S1P_4$ functions in dendritic cells. Reportedly, $S1P_4$ expression is found to be high in lymphoid tissues and hematopoietic cells, including dendritic cells and neutrophils (Graler *et al*., 1998; Schulze *et al*., 2011; Kulinski *et al*., 2018). Previously, $S1P_4$ deficiency was found to affect dendritic cell migration and cytokine secretion and reduce Th17 differentiation of T cells in a murine model (Schulze *et al*., 2011). Also $S1P_4$ is suggested to be required for plasmacytoid dendritic cell differentiation and CYM50358 prevented $S1P_4$-dependent reduction of IFN-α production in human plasmacytoid dendritic cells (Dillmann *et al*., 2015, 2016). S1P accumulation in S1P lyase deficient mice caused neutrophilia and deletion of $S1P_4$ partially rescued the neutrophil recruitment (Allende *et al*., 2011). Therefore, further researches on other cell types expressing $S1P_4$ need to be conducted to elucidate their contribution on anti-inflammatory and anti-allergic effects.

The results with $S1P_4$ deficient mice are in contrast with the present results. In the present study, inhibition of $S1P_4$ using CYM50358 suppressed allergic responses, while $S1P_4$ gene deficiency increased the magnitude of Th2-dominated immune responses, including the aggravation of passive systemic anaphylaxis to IgE/anti-IgE in mice (Schulze *et al*., 2011; Kulinski *et al*., 2018). Conversely, Th1-dominated mechanisms were diminished in $S1P_4$ deficient mice (Schulze *et al*., 2011). S1P suppresses collagen-induced activation of human platelets and induces anti-inflammatory effects *in vitro* and *in vivo* via the $S1P_4$ receptor (Onuma *et al*., 2017; Fettel *et al*., 2019). The discrepancy of phenotypes between $S1P_4$ deficient mice and CYM50358-treated mice may be attributed to the compensatory adaptation in $S1P_4$ deficient mice as $S1P_4$ gene deficiency was maintained from the embryonic stage. Instead, the present study temporarily suppressed the $S1P_4$

functions during antigen sensitization or during antigen challenge. Therefore, the phenotypes observed with CYM50358 treatments could be the more relevant outcomes when $S1P_4$ is temporarily suppressed.

An abundant expression of $S1P_4$ has been observed in cultured mouse mast cells (Kulinski *et al*., 2018). However, $S1P_4$ gene deficiency did not affect mast cell proliferation in culture or the differentiation of bone marrow progenitors into mast cells (Kulinski *et al*., 2018). Therefore, $S1P_4$ was suggested to be dispensable for cytokine/chemokine production, degranulation, and FcεRI-mediated chemotaxis in mast cells *in vitro* (Kulinski *et al*., 2018). However, in the present study, CYM50358 suppressed antigen-induced degranulation of RBL-2H3 mast cells as well as OVA-induced allergic responses. Additionally, enhancement of IgE-induced degranulation by IL-33 is reportedly suppressed in peritoneal mast cells from $S1P_4$ deficient mice, suggesting a negative regulatory role of $S1P_4$ in mast cells (Kulinski *et al*., 2018). Therefore, the functions of $S1P_4$ in mast cells might be more complicated depending on the circumstances.

Previously, the significance of S1P and its receptors has been reported in animal models, such as antigen-induced allergic asthma and airway inflammation (Roviezzo *et al*., 2007; Chiba *et al*., 2010; Park and Im, 2019). $S1P_4$ expression and functions in mast cells and dendritic cells have been evaluated in $S1P_4$ deficient mice (Schulze *et al*., 2011; Kulinski *et al*., 2018). However, direct chemical modification of $S1P_4$ has not been attempted. In the present study, the *in vitro* efficacy of CYM50358 was tested in the RBL-2H3 cells for the first time, revealing the stimulatory role of $S1P_4$ on mast cell degranulation. Additionally, the *in vivo* efficacy of CYM50358 was investigated for the first time using an OVA-induced asthma model, demonstrating significant suppression of allergic asthma in both CYM50358 treatments, before sensitization and before challenge. Significant suppression was observed in immune cell accumulation, lung inflammation, IL-4 secretion, and mucin production. In summary, in the present study, the efficacy of CYM50358, a chemical antagonist of $S1P_4$, was revealed on allergic responses, demonstrating the suppression of mast cell degranulation, as well as reduced OVA-induced allergic responses, and thus providing evidence for the potential therapeutic applications of $S1P_4$ in allergic asthma.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENTS

This research was supported by the Basic Research Laboratory Program (BRL) and the Basic Science Research Program of the Korean National Research Foundation funded by the Korean Ministry of Science, ICT and Future Planning (NRF-2020R1A4A1016142 and NRF-2019R1A2C1005523).

REFERENCES

Allende, M. L., Bektas, M., Lee, B. G., Bonifacino, E., Kang, J., Tuymetova, G., Chen, W., Saba, J. D. and Proia, R. L. (2011) Sphingosine-1-phosphate lyase deficiency produces a pro-inflammatory response while impairing neutrophil trafficking. *J. Biol. Chem.* **286**, 7348-7358.

Ammit, A. J., Hastie, A. T., Edsall, L. C., Hoffman, R. K., Amrani, Y., Krymskaya, V. P., Kane, S. A., Peters, S. P., Penn, R. B., Spiegel, S. and Panettieri, R. A., Jr. (2001) Sphingosine 1-phosphate modulates human airway smooth muscle cell functions that promote inflammation and airway remodeling in asthma. *FASEB J.* **15**, 1212-1214.

Brown, J., Wilson, T. and Metcalfe, D. (2008) The mast cell and allergic diseases: role in pathogenesis and implications for therapy. *Clin. Exp. Allergy* **38**, 4-18.

Chiba, Y., Suzuki, K., Kurihara, E., Uechi, M., Sakai, H. and Misawa, M. (2010) Sphingosine-1-phosphate aggravates antigen-induced airway inflammation in mice. *Open Respir. Med. J.* **4**, 82-85.

Choi, O. H., Kim, J.-H. and Kinet, J.-P. (1996) Calcium mobilization via sphingosine kinase in signalling by the FcεRI antigen receptor. *Nature* **380**, 634-636.

Dillmann, C., Mora, J., Olesch, C., Brune, B. and Weigert, A. (2015) S1PR4 is required for plasmacytoid dendritic cell differentiation. *Biol. Chem.* **396**, 775-782.

Dillmann, C., Ringel, C., Ringleb, J., Mora, J., Olesch, C., Fink, A. F., Roberts, E., Brune, B. and Weigert, A. (2016) S1PR4 signaling attenuates ILT 7 internalization to limit IFN-alpha production by human plasmacytoid dendritic cells. *J. Immunol.* **196**, 1579-1590.

Fettel, J., Kuhn, B., Guillen, N. A., Surun, D., Peters, M., Bauer, R., Angioni, C., Geisslinger, G., Schnutgen, F., Meyer Zu Heringdorf, D., Werz, O., Meybohm, P., Zacharowski, K., Steinhilber, D., Roos, J. and Maier, T. J. (2019) Sphingosine-1-phosphate (S1P) induces potent anti-inflammatory effects *in vitro* and *in vivo* by S1P receptor 4-mediated suppression of 5-lipoxygenase activity. *FASEB J.* **33**, 1711-1726.

Gilfillan, A. M., Peavy, R. D. and Metcalfe, D. D. (2009) Amplification mechanisms for the enhancement of antigen-mediated mast cell activation. *Immunol. Res.* **43**, 15-24.

Graler, M. H., Bernhardt, G. and Lipp, M. (1998) EDG6, a novel G-protein-coupled receptor related to receptors for bioactive lysophospholipids, is specifically expressed in lymphoid tissue. *Genomics* **53**, 164-169.

Guerrero, M., Urbano, M., Zhao, J., Crisp, M., Chase, P., Hodder, P., Schaeffer, M. T., Brown, S., Rosen, H. and Roberts, E. (2012) Discovery, design and synthesis of novel potent and selective sphingosine-1-phosphate 4 receptor ($S1P_4$-R) agonists. *Bioorg. Med. Chem. Lett.* **22**, 537-542.

Heo, J. Y. and Im, D. S. (2019) Anti-allergic effects of salvianolic acid A and tanshinone IIA from *Salvia miltiorrhiza* determined using *in vivo* and *in vitro* experiments. *Int. Immunopharmacol.* **67**, 69-77.

Huang, J., Su, M., Lee, B. K., Kim, M. J., Jung, J. H. and Im, D. S. (2018) Suppressive effect of 4-hydroxy-2-(4-hydroxyphenethyl) isoindoline-1,3-dione on ovalbumin-induced allergic asthma. *Biomol. Ther. (Seoul)* **26**, 539-545.

Jolly, P. S., Bektas, M., Olivera, A., Gonzalez-Espinosa, C., Proia, R. L., Rivera, J., Milstien, S. and Spiegel, S. (2004) Transactivation of sphingosine-1–phosphate receptors by FcεRI triggering is required for normal mast cell degranulation and chemotaxis. *J. Exp. Med.* **199**, 959-970.

Khalaf, K., Paoletti, G., Puggioni, F., Racca, F., De Luca, F., Giorgis, V., Canonica, G. W. and Heffler, E. (2019) Asthma from immune pathogenesis to precision medicine. *Semin. Immunol.* **46**, 101294.

Kim, M. J. and Im, D. S. (2019) Suppressive effects of type I angiotensin receptor antagonists, candesartan and irbesartan on allergic asthma. *Eur. J. Pharmacol.* **852**, 25-33.

Kulinski, J. M., Proia, R. L., Larson, E. M., Metcalfe, D. D. and Olivera, A. (2018) S1P(4) regulates passive systemic anaphylaxis in mice but is dispensable for canonical IgE-mediated responses in mast cells. *Int. J. Mol. Sci.* **19**, 1279.

Lee, B. K., Park, S. J., Nam, S. Y., Kang, S., Hwang, J., Lee, S. J. and Im, D. S. (2018) Anti-allergic effects of sesquiterpene lactones from *Saussurea costus* (Falc.) Lipsch. determined using *in vivo* and *in vitro* experiments. *J. Ethnopharmacol.* **213**, 256-261.

Moffatt, M. F., Kabesch, M., Liang, L., Dixon, A. L., Strachan, D., Heath, S., Depner, M., von Berg, A., Bufe, A. and Rietschel, E. (2007) Ge-

netic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* **448**, 470-473.
Onuma, T., Tanabe, K., Kito, Y., Tsujimoto, M., Uematsu, K., Enomoto, Y., Matsushima-Nishiwaki, R., Doi, T., Nagase, K., Akamatsu, S., Tokuda, H., Ogura, S., Iwama, T., Kozawa, O. and Iida, H. (2017) Sphingosine 1-phosphate (S1P) suppresses the collagen-induced activation of human platelets via $S1P_4$ receptor. *Thromb. Res.* **156**, 91-100.
Oskeritzian, C. A., Price, M. M., Hait, N. C., Kapitonov, D., Falanga, Y. T., Morales, J. K., Ryan, J. J., Milstien, S. and Spiegel, S. (2010) Essential roles of sphingosine-1–phosphate receptor 2 in human mast cell activation, anaphylaxis, and pulmonary edema $S1P_2$ axis in anaphylaxis and pulmonary edema. *J. Exp. Med.* **207**, 465-474.
Park, S. J. and Im, D. S. (2017) Sphingosine 1-phosphate receptor modulators and drug discovery. *Biomol. Ther. (Seoul)* **25**, 80-90.
Park, S. J. and Im, D. S. (2019) Blockage of sphingosine-1-phosphate receptor 2 attenuates allergic asthma in mice. *Br. J. Pharmacol.* **176**, 938-949.
Park, S. J. and Im, D. S. (2020) Blockage of sphingosine-1-phosphate receptor 2 attenuates **2**,4-dinitrochlorobenzene-induced atopic dermatitis in mice. *Acta Pharmacol. Sin.* **41**, 1487-1496.
Prieschl, E. E., Csonga, R., Novotny, V., Kikuchi, G. E. and Baumruker, T. (1999) The balance between sphingosine and sphingosine-1-phosphate is decisive for mast cell activation after Fcε receptor I triggering. *J. Exp. Med.* **190**, 1-8.
Prussin, C. and Metcalfe, D. D. (2003) 4. IgE, mast cells, basophils, and eosinophils. *J. Allergy Clin. Immunol.* **111**, S486-S494.
Romagnani, S. (2002) Cytokines and chemoattractants in allergic inflammation. *Mol. Immunol.* **38**, 881-885.
Roviezzo, F., Di Lorenzo, A., Bucci, M., Brancaleone, V., Vellecco, V., De Nardo, M., Orlotti, D., De Palma, R., Rossi, F., D'Agostino, B. and Cirino, G. (2007) Sphingosine-1-phosphate/sphingosine kinase pathway is involved in mouse airway hyperresponsiveness. *Am. J. Respir. Cell Mol. Biol.* **36**, 757-762.
Saluja, R., Kumar, A., Jain, M., Goel, S. K. and Jain, A. (2017) Role of sphingosine-1-phosphate in mast cell functions and asthma and its regulation by non-coding RNA. *Front. Immunol.* **8**, 587.
Schulze, T., Golfier, S., Tabeling, C., Rabel, K., Graler, M. H., Witzenrath, M. and Lipp, M. (2011) Sphingosine-1-phospate receptor 4 ($S1P_4$) deficiency profoundly affects dendritic cell function and TH17-cell differentiation in a murine model. *FASEB J.* **25**, 4024-4036.
Tagaya, E. and Tamaoki, J. (2007) Mechanisms of airway remodeling in asthma. *Allergol. Int.* **56**, 331-340.
Van Rijt, L. S. and Lambrecht, B. (2005) Dendritic cells in asthma: a function beyond sensitization. *Clin. Exp. Allergy* **35**, 1125-1134.
Worgall, T. S. (2017) Sphingolipids, ORMDL3 and asthma: what is the evidence? *Curr. Opin. Clin. Nutr. Metab. Care* **20**, 99-103.