

Letter to the Editor

Involvement of apoptosis signal-regulating kinase-1 in house dust mite-induced allergic asthma in mice



Dear Editor,

Allergic asthma is a complex disease characterized by eosinophilic airway inflammation, mucus hyperproduction, and reversible airway obstruction.¹ The presence of house dust mites (HDM) is a major environmental factor associated with the pathogenesis of allergic asthma. A previous study reported that HDM allergens can be recognized by Toll-like receptor 4 (TLR4) on airway epithelial cells, thereby triggering the production of epithelial cell-derived cytokines, such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), which induce the cardinal features of asthma.² The HDM allergen Der p2 structurally and functionally mimics myeloid differentiation-2 (MD-2), which is a lipopolysaccharide (LPS)-binding component of the TLR4 signaling complex.³ Furthermore, we have previously reported that MD-2 is highly expressed in lung vascular endothelial cells and increases in the serum during the amplification process of HDM sensitization in an animal model of asthma.⁴ Thus, the TLR4/MD-2 complex might be critical for HDM-induced asthma. However, little is known about the downstream regulators of TLR4, which are involved in HDM-induced allergic asthma.

Apoptosis signal-regulating kinase 1 (ASK1) is a conserved mitogen-activated protein 3 kinase that activates both Jnk and p38 mitogen-activated protein kinases and is involved in cytokine- and oxidative stress-induced apoptosis, differentiation, and inflammation.⁵ A previous study reported that TLR4 ligand (LPS)-mediated intracellular reactive oxygen species (ROS) activate the ASK1/p38 pathway and induce septic shock.⁶ Based on this report, we hypothesized that ASK1 is a downstream molecule of TLR4 involved in the regulation of HDM-induced allergic asthma.

To investigate whether ASK1 is involved in HDM-induced allergic asthma, we used ASK1-deficient (*ASK1*^{−/−}) mice (C57BL/6 background) and assessed asthma-like phenotypes, such as eosinophilic recruitment and mucus hyperproduction in the airway. We used 6 to 8-week-old male C57BL/6J wild-type (WT) mice (Charles River Laboratories Japan, Yokohama, Japan) as controls. All protocols were approved by the Animal Care and Use Committee at Nihon University School of Medicine. The WT and *ASK1*^{−/−} mice were intratracheally exposed to HDM [*Dermatophagoides pteronyssinus* at 100 µg/mouse/treatment (GREER Laboratories, Lenoir, NC, USA)] or phosphate-buffered saline (PBS) as

control on days 1, 8, and 15. We collected bronchoalveolar lavage fluid (BALF) with 1 ml of PBS and lung tissues on day 18. We evaluated the presence of eosinophils in the BALF after HDM exposure. Lungs were fixed in formalin and embedded in paraffin. Sections were obtained and were stained with hematoxylin and eosin to determine airway infiltration of inflammatory cells and with Alcian blue/periodic acid Schiff to determine HDM-induced mucus hyperproduction (Fig. 1A). The number of eosinophils in the BALF was lower in the HDM-exposed *ASK1*^{−/−} mice than in the WT mice (Fig. 1B). Similarly, the infiltration of inflammatory cells in the lung was lower in the HDM-exposed *ASK1*^{−/−} mice than in the WT mice (Fig. 1C, E). Moreover, mucus hyperproduction in the airway was lower in the HDM-exposed *ASK1*^{−/−} mice than in the WT mice (Fig. 1D, F). IL-4, IL-5, IL-13, interferon gamma (IFNγ), IL-33, and TSLP mRNA expression in lung tissues was also measured using the quantitative reverse transcription polymerase chain reaction (qRT-PCR) (Fig. 1G). Airway hyperresponsiveness (AHR) was measured according to the method of Koyama *et al.*⁴ (Fig. 1H). There were some trends but no significant differences in cytokine expression and AHR between the HDM-exposed *ASK1*^{−/−} and WT mice.

In this study, we found that HDM-induced allergic inflammation and mucus hyperproduction in the airway were lower in *ASK1*^{−/−} mice than in WT mice. Therefore, ASK1 might, at least in part, play a role in HDM-induced asthma-like responses.

Research on innate immunity has shed light on the pathogenesis of asthma owing to the discovery of type 2 innate lymphoid cells (ILC2) and production of epithelial cell-derived cytokines.¹ The HDM-exposed animal model of asthma, which we used in this study, is innate immunity-dependent because HDM contain Der p2, LPS, and other allergens.⁷ Ovalbumin (OVA)-induced allergic responses are also partly innate immunity-dependent because classically used OVA is contaminated with LPS, which can act as an adjuvant and prime allergic sensitization in the airway via TLR4 to promote an asthma-like phenotype.⁸ Takeda *et al.* reported that ASK1 is required for inducing allergic asthma sensitized by intraperitoneal administration of OVA-containing LPS.⁹ These findings suggest that the TLR4/ASK1 axis may be involved in promoting asthma by priming allergic sensitization to antigens in the airway.

Chan *et al.* reported that HDM exposure induced ROS-mediated oxidative damage of epithelial cells and allergic airway inflammation.¹⁰ We speculate that inhaled HDM, which have Der p2 and LPS, are identified by TLR4 on the surface of innate immune cells, such as airway epithelial cells, airway macrophages, and dendritic cells, resulting in the production of ROS, which

Peer review under responsibility of Japanese Society of Allergy.

<http://dx.doi.org/10.1016/j.alit.2017.05.007>1323-8930/2017, Japanese Society of Allergy. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

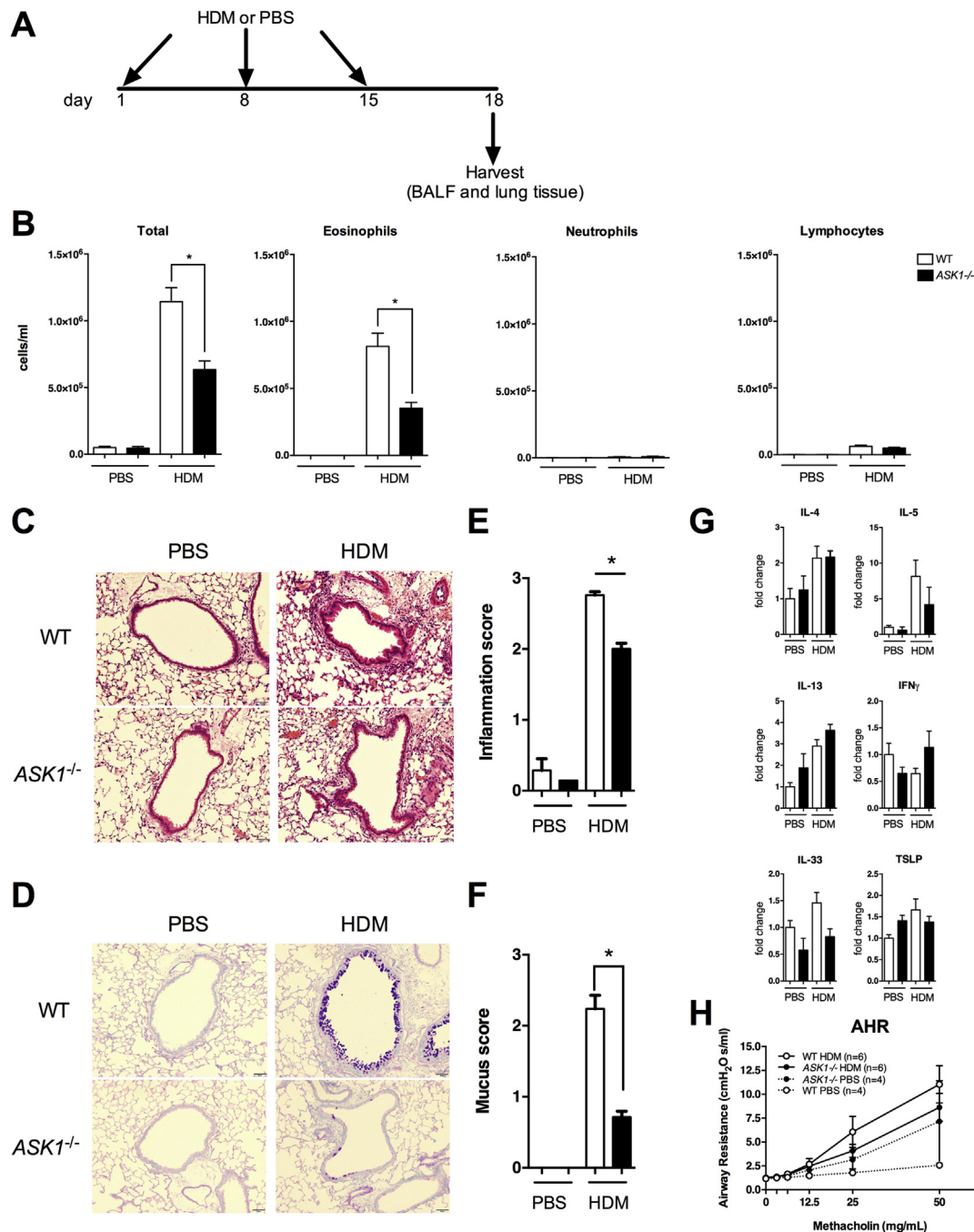


Fig. 1. ASK1 is involved in HDM-induced allergic airway inflammation and mucus hyperproduction. (A) Timeline for the animal model of asthma involving exposure to HDM. C57BL/6J WT and ASK1-deficient mice (ASK1^{-/-}) were intratracheally exposed to HDM or PBS (as control) on days 1, 8, and 15. BALF and lung tissues were harvested on day 18. (B) Leukocyte count in the BALF. Values shown are the mean $\pm$ SEM of 14–19 mice per group from three independent experiments. * $P < 0.05$. Total cells ($P = 0.002$) or eosinophils ($P = 0.0001$) from HDM-exposed ASK1^{-/-} compared with those from HDM-exposed WT mice. The experimental groups were analyzed using the Mann–Whitney U test. (C) Representative images ($\times 200$) of lung sections stained with hematoxylin and eosin showing airway inflammation. (D) Representative images ($\times 200$) of lung sections stained with Alcian blue/periodic acid Schiff showing mucus-producing cells. Data were representative of three independent experiments. Airway inflammation score (E) and mucus score (F) are shown. Scoring was performed in seven different fields for each lung section from three animals according to the method of Koyama *et al.*⁴ * $P < 0.05$. (G) Cytokine mRNA expression in the lungs was measured using qRT-PCR according to the method of Koyama *et al.*⁴ (mean $\pm$ SEM of 8 mice per group; not significant, Mann–Whitney U test). (H) AHR was measured. (mean $\pm$ SEM of 4–6 mice per group; not significant, ANOVA). All data were analyzed with GraphPad Prism version 6 (GraphPad Software, La Jolla, CA, USA). Statistical significance was set at a P value of < 0.05 . AHR, airway hyperresponsiveness; ASK1, apoptosis signal-regulating kinase-1; BALF, bronchoalveolar lavage fluid; HDM, house dust mite; PBS, phosphate-buffered saline; WT, wild-type.

subsequently activate the ASK1/p38 axis and induce asthma-like phenotypes. Further studies are needed to clarify the molecular mechanisms involved in the activation of ASK1 by HDM and determine which cell types mainly respond to HDM via the TLR4/ASK1 axis.

In conclusion, we have demonstrated for the first time that ASK1 is involved in HDM-induced asthma-like phenotypes, such as eosinophilic inflammation and mucus hyperproduction in the airway of mice. ASK1 might be a therapeutic target for HDM-induced allergic asthma.

Acknowledgments

This work was supported in part by the Strategic Research Foundation at Private Universities from the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government (S1511014).

Conflict of interest

The authors have no conflict of interest to declare.

Shuichiro Maruoka^a, Yasuhiro Gon^{a,*}, Kenji Mizumura^a, Shinichi Okamoto^a, Kota Tsuya^a, Sotaro Shikano^a, Kaori Soda^a, Isao Naguro^b, Hidenori Ichijo^b, Shu Hashimoto^a

^a Division of Respiratory Medicine, Department of Internal Medicine, Nihon University School of Medicine, Tokyo, Japan

^b Laboratory of Cell Signaling, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan

* Corresponding author. Division of Respiratory Medicine, Department of Internal Medicine, Nihon University School of Medicine, 30-1 Oiyagutikami-cho, Itabashi-ku, Tokyo 173-8610, Japan.

E-mail address: gon.yasuhiro@nihon-u.ac.jp (Y. Gon).

References

1. Lambrecht BN, Hammad H. The immunology of asthma. *Nat Immunol* 2015;**16**:45–56.
2. Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009;**15**:410–6.
3. Trompette A, Divanovic S, Visintin A, Blanchard C, Hegde RS, Madan R, et al. Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 2009;**457**:585–8.
4. Koyama D, Maruoka S, Gon Y, Shintani Y, Sekiyama T, Hiranuma H, et al. Myeloid differentiation-2 is a potential biomarker for the amplification process of allergic airway sensitization in mice. *Allergol Int* 2015;**64**(Suppl):S37–45.
5. Soga M, Matsuzawa A, Ichijo H. Oxidative stress-induced diseases via the ASK1 signaling pathway. *Int J Cell Biol* 2012;**2012**:439587.
6. Matsuzawa A, Saegusa K, Noguchi T, Sadamitsu C, Nishitoh H, Nagai S, et al. ROS-dependent activation of the TRAF6-ASK1-p38 pathway is selectively required for TLR4-mediated innate immunity. *Nat Immunol* 2005;**6**:587–92.
7. Lambrecht BN, Hammad H. Allergens and the airway epithelium response: gateway to allergic sensitization. *J Allergy Clin Immunol* 2014;**134**:499–507.
8. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, Toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002;**196**:1645–51.
9. Takada E, Furuhata M, Nakae S, Ichijo H, Sudo K, Mizuguchi J. Requirement of apoptosis-inducing kinase 1 for the induction of bronchial asthma following stimulation with ovalbumin. *Int Arch Allergy Immunol* 2013;**162**:104–14.
10. Chan TK, Loh XY, Peh HY, Tan WN, Tan WS, Li N, et al. House dust mite-induced asthma causes oxidative damage and DNA double-strand breaks in the lungs. *J Allergy Clin Immunol* 2016;**138**:84–96.e1.

Received 1 February 2017

Received in revised form 9 May 2017

Accepted 11 May 2017

Available online 16 June 2017