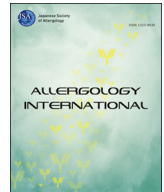




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Letter to the Editor

Dectin-1 plays a critical role in HDM-induced PGE₂ production in macrophages

Dear Editor,

Asthma is a chronic allergic inflammatory disease characterized by intense eosinophil and lymphocyte infiltration, mucus hyperproduction, and airway hyperresponsiveness, which is caused by a variety of stimuli. These pathological features are mainly mediated by antigen-specific Th2 cells and their cytokines, including IL-4, IL-5, and IL-13.¹ In the majority of patients, disease activities are controlled by inhaled corticosteroids with or without long acting β_2 agonist; however, in patients with severe asthma, the symptoms are refractory to these standard treatments, and often lead to emergency visits and hospitalization.

Although the mechanisms underlying the development of severe asthma are poorly understood, it has been shown that severity of asthma is positively correlated with the sensitization to fungi.² Furthermore, it has been shown that the number of neutrophils in sputum is associated with the severity of asthma.³ Considering that neutrophilia is a characteristic feature of Th17 cell-mediated inflammation, which is essential for the efficient protection against fungi, these findings suggest that Th17 cells may be involved in the pathogenesis of severe asthma.

Dectin-1 is a C-type lectin receptor that recognizes fungal cell wall component β -glucan, and is involved in the development of Th17 cells.⁴ Recently, we have shown that, among dendritic cell (DC) subsets in the lung, dectin-1 is expressed on CD11b⁺ DCs,⁵ an essential DC subset for the development of allergic airway inflammation. Moreover, we have shown that dectin-1 expressed on CD11b⁺ DCs is involved in the induction of Th2 cells as well as Th17 cells in the lung in house dust mite (HDM)-induced asthma models.⁵ Furthermore, we found that dectin-1 signaling enhances the migration of DCs from the lung to draining lymph nodes by inducing the expression of chemokine receptors.⁵ Although these findings highlight the importance of dectin-1 expressed on CD11b⁺ DCs in HDM-induced allergic airway inflammation, little is known about the roles of dectin-1 expressed on other cell types in the lung.

To identify the cell types that express dectin-1 in the lung, we analyzed single cell suspensions of the lung by flow cytometry using anti-dectin-1 antibody together with a series of cell lineage markers. In accordance with a previous study,⁶ dectin-1 was expressed on CD11b⁺ myeloid cells but not on lymphocytes including CD4⁺ T cells (Fig. 1). Among myeloid cell populations in the lung, dectin-1 was expressed at high levels on interstitial macrophages (IMs) (defined as MHC II⁺ CD11c⁺ CD11b⁺ CD64⁺ CD24⁻

cells) and at moderate levels on CD11b⁺ DCs (CD11b⁺ CD11c⁺ MHC class II⁺ CD24⁺ cells), neutrophils (CD11b⁺ Ly6G⁺ cells), and alveolar macrophages (AMs) (CD11c⁺ Siglec-F⁺ cells), irrespective of HDM stimulation (Fig. 1). These results suggest that dectin-1 expressed on macrophages may have a role in the development of allergic airway inflammation.

Recently, Kim *et al.* have reported that antibiotic administration induces overgrowth of *Candida* species in the gut, resulting in the exacerbation of allergic airway inflammation through the production of prostaglandin E₂ (PGE₂).⁷ In addition, it has been shown that dectin-1 is involved in PGE₂ production from macrophages upon β -glucan stimulation.⁸ To determine whether dectin-1 is involved in PGE₂ production from HDM-stimulated macrophages, we generated bone marrow-derived macrophages (BMDMs) from wild type (WT) mice and dectin-1-deficient (*Clec7a*^{-/-}) mice, stimulated them with HDM, and analyzed the levels of PGE₂ in the supernatants by ELISA. As shown in Figure 2A, HDM induced PGE₂ production in BMDMs in WT mice. Importantly, HDM-induced PGE₂ production was less significant in *Clec7a*^{-/-} BMDMs than that in WT BMDMs, suggesting that dectin-1 is involved in HDM-induced PGE₂ production in macrophages.

To determine whether dectin-1 is involved in PGE₂ production in HDM-induced asthma models, we next measured the levels of PGE₂ in the bronchoalveolar lavage fluid (BALF) in HDM-sensitized and -challenged WT mice and *Clec7a*^{-/-} mice. As shown in Figure 2B, the levels of PGE₂ were significantly increased in the BALF by HDM challenge in WT mice. Consistent with the *in vitro* findings, HDM-induced PGE₂ production in the BALF was significantly decreased in *Clec7a*^{-/-} mice as compared with that in WT mice (Fig. 2B). These results suggest that dectin-1 is involved in PGE₂ production in HDM-induced asthma models.

In this study, we found that dectin-1 is expressed on AMs and IMs (Fig. 1), which are abundant in the lung in a steady-state condition. We also found that HDM-induced PGE₂ production is diminished in *Clec7a*^{-/-} mice (Fig. 2). Taken together with previous studies in humans and animal models, our findings suggest that the main producers of PGE₂ in HDM-induced asthma models are either AMs or IMs and its production depends on dectin-1 signaling. This notion is consistent with previous *in vitro* and *in vivo* studies showing that β -glucan, a ligand of dectin-1, induces the production of PGE₂ from macrophages, and then PGE₂ enhances allergic airway inflammation through the induction of M2 macrophages.⁷ Our findings are in good agreement with a previous finding that dectin-1 signaling, in cooperation with DC-SIGN and

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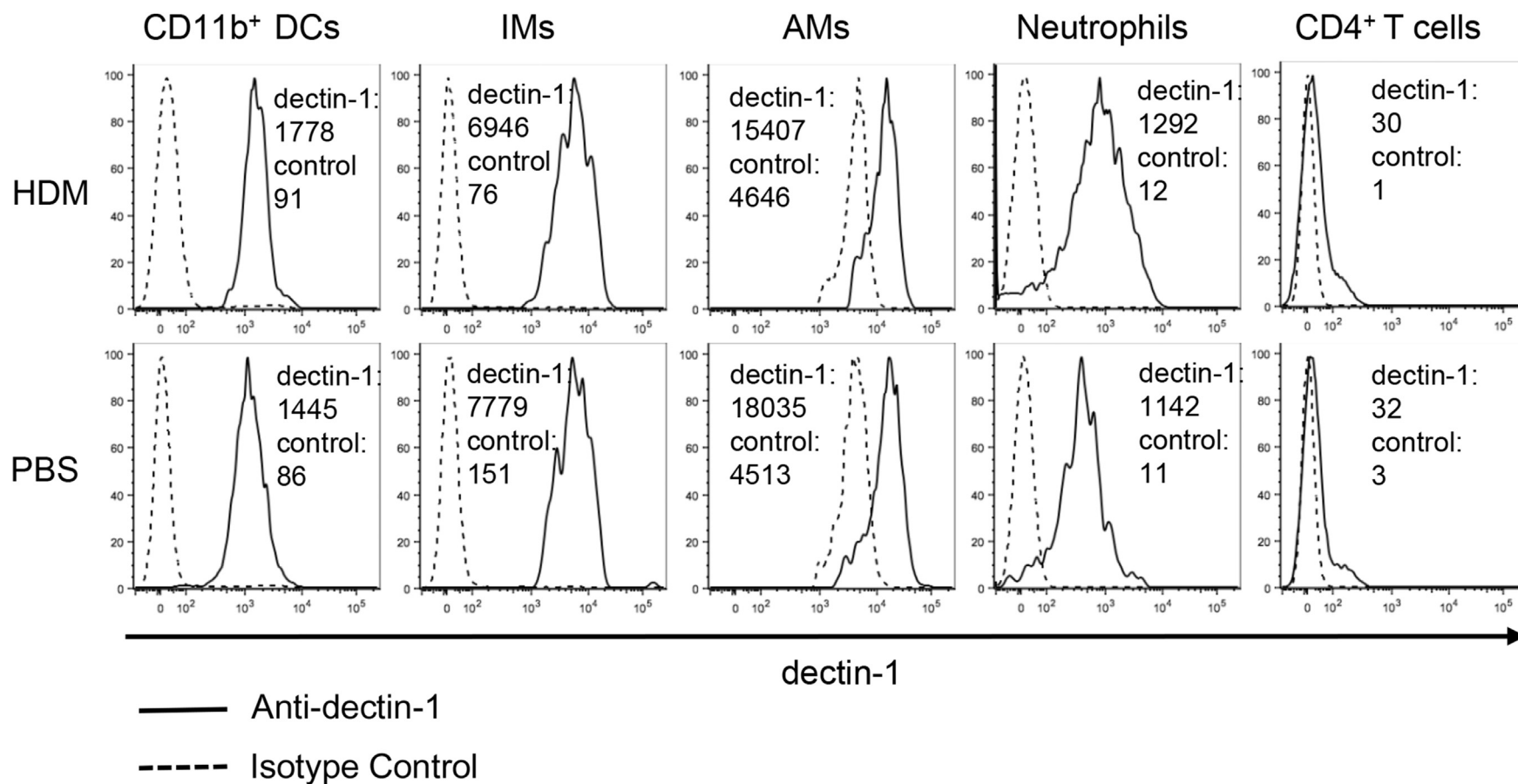


Fig. 1. Dectin-1 is highly expressed in lung macrophages. The expression levels of dectin-1 on indicated cell populations were analyzed as described in the [Supplementary Methods](#). Shown are representative histograms of the dectin-1 (solid line) and control IgG staining (dotted line) on CD11b⁺ DCs (CD11b⁺ CD11c⁺ MHC class II⁺ CD24⁺ cells), interstitial macrophages (IMs) (MHC II⁺ CD11c⁺ CD11b⁺ CD64⁺ CD24⁺ cells), alveolar macrophages (AMs) (CD11c⁺ Siglec-F⁺ cells), neutrophils (CD11b⁺ Ly6G⁺ cells), and CD4⁺ T cells (CD4⁺ CD3⁺ cells) in the lung with mean fluorescent intensity. Data are representative of 3 independent experiments.

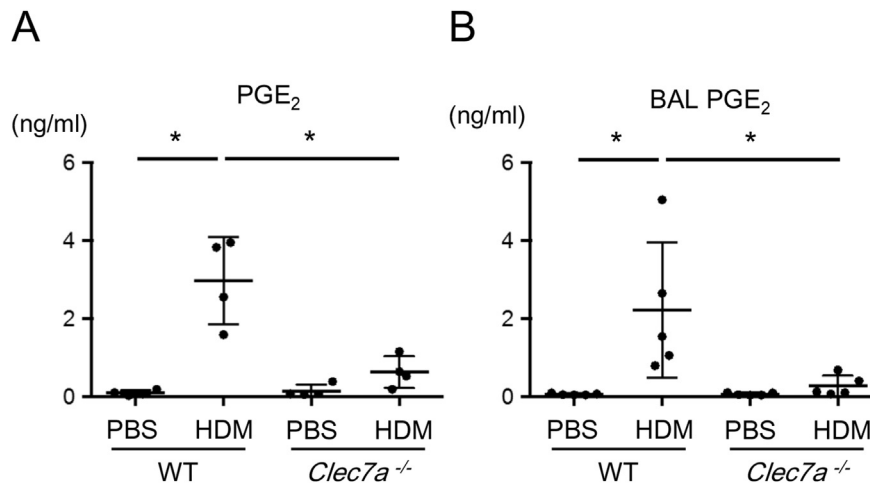


Fig. 2. Dectin-1 is involved in PGE₂ production upon HDM stimulation. **(A)** BMDMs were generated from WT mice and *Clec7a*^{-/-} mice, and then incubated with or without HDM as described in the [Supplementary Methods](#). The levels of PGE₂ in the supernatants were measured by ELISA. Results are shown as individual data with means ± SEM for 4 mice in each group. **p* < 0.05. **(B)** WT mice and *Clec7a*^{-/-} mice were sensitized and challenged with HDM as described in the [Supplementary Methods](#). Forty-eight hours after the last HDM inhalation, the levels of PGE₂ in the BALF were measured by ELISA. Results are shown as individual data with means ± SEM for 5 mice in each group. **p* < 0.05.

TLR2, is capable of inducing the expression of enzymes involved in PGE₂ production such as cytosolic phospholipase A₂ (cPLA₂) and cyclooxygenase 2 (COX-2).⁹ In addition, PGE₂ has been shown to possess an ability to enhance the differentiation and function of Th17 cells,¹⁰ which is implicated in the pathogenesis of severe asthma. Therefore, it is suggested that PGE₂ accelerates the development of allergic airway inflammation in at least two distinct mechanisms.

In conclusion, our results indicate that dectin-1 signaling plays a pivotal role in PGE₂ production in HDM-induced asthma models and suggest that this pathway could be a therapeutic target for the treatment of severe asthma.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.alit.2017.05.001>.

Conflict of interest

The authors have no conflict of interest to declare.

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