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Dectin-1 Plays an Important Role in House Dust Mite–Induced Allergic Airway Inflammation through the Activation of CD11b⁺ Dendritic Cells

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It is well known that sensitization against fungi is closely associated with severity of asthma. Dectin-1 (gene symbol *Clec7a*), a C-type lectin receptor, recognizes the fungal cell wall component β -glucan, as well as some component(s) in house dust mite (HDM) extract. However, the roles of Dectin-1 in HDM-induced allergic airway inflammation remain unclear. In this study, we used Dectin-1-deficient (*Clec7a*^{−/−}) mice to examine whether Dectin-1 is involved in HDM-induced allergic airway inflammation. We found that HDM-induced eosinophil and neutrophil recruitment into the airways was significantly attenuated in *Clec7a*^{−/−} mice compared with that in wild-type mice. In addition, HDM-induced IL-5, IL-13, and IL-17 production from mediastinum lymph node cells was reduced in HDM-sensitized *Clec7a*^{−/−} mice. Dectin-1 was expressed on CD11b⁺ dendritic cells (DCs), an essential DC subset for the development of allergic inflammation, but not on CD103⁺ DCs, plasmacytoid DCs, or lung epithelial cells. Transcriptome analysis revealed that the expression of chemokine/chemokine receptors, including CCR7, which is indispensable for DC migration to draining lymph nodes, was decreased in *Clec7a*^{−/−} DCs. In accordance with these results, the number of HDM-labeled CD11b⁺ DCs in mediastinum lymph nodes was significantly reduced in *Clec7a*^{−/−} mice compared with wild-type mice. Taken together, these results suggest that Dectin-1 expressed on CD11b⁺ DCs senses some molecule(s) in HDM extract and plays a critical role in the induction of HDM-induced allergic airway inflammation by inducing the expression of chemokine/chemokine receptors in DCs. *The Journal of Immunology*, 2017, 198: 000–000.

Asthma is a chronic lung disease that is characterized by intense eosinophilic infiltration into the airway, mucus hyperproduction, and airway hyperresponsiveness (1, 2). Although it is well established that these characteristics are mainly caused by CD4⁺ Th cells that produce IL-4, IL-5, and IL-13, recent studies showed that asthma is a complex heterogeneous disease with different phenotypes and variable clinical manifestations. By using a cluster analysis, Moore et al. (3) demonstrated

that there are five clusters in asthmatics, and that patients in Cluster 5, which is characterized by severe airway obstruction and poor responsiveness to bronchodilators, have the most severe airflow limitation. Notably, patients in Cluster 5 showed increased numbers of neutrophils in the sputum. Moreover, a recent multivariate analysis using clinical phenotypes and sputum cellular patterns revealed that sputum neutrophilia is associated with a severe phenotype (4). These findings suggest that neutrophilic inflammation, as well as eosinophilic inflammation, is associated with the pathogenesis of severe asthma.

Neutrophilic inflammation is a hallmark of Th17 cell-mediated inflammatory diseases. A series of clinical studies indicated that IL-17 is involved in the pathogenesis of neutrophilic severe asthma. The level of IL-17 was increased in induced sputum obtained from severe asthma and correlated with sputum neutrophilia (5). It also was shown that the level of IL-17 in bronchoalveolar lavage fluid (BALF) positively correlates with severity of asthma (6, 7). In addition, IL-17 was shown to be associated with structural alteration of lung epithelial cells and smooth muscle contraction (6, 8). The recent identification of a steroid-insensitive Th17 cell pathway further supports the involvement of Th17 cells in severe asthma (9). However, it remains to be elucidated how Th17 cells are induced at the site of allergic airway inflammation.

Dectin-1 and Dectin-2, which are C-type lectin receptors, are pattern recognition receptors (PRRs) that recognize the fungal cell wall components β -glucan and α -mannan, respectively (10, 11). Importantly, recent studies showed that these C-type lectin receptors are involved in the development of Th17 cells, which are essential for protection against fungi (11, 12). With regard to the relationship between C-type lectin receptors and asthma, we

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Abbreviations used in this article: BALF, bronchoalveolar lavage fluid; BMDC, bone marrow-derived DC; DC, dendritic cell; HDM, house dust mite; LN, lymph node; MLN, mediastinal lymph node; PAS, periodic acid–Schiff; PRR, pattern recognition receptor; qPCR, quantitative real-time PCR; Treg, regulatory T cell; WT, wild-type.

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showed that Dectin-2 signaling promotes the development of Th2 cells and Th17 cells and enhances allergic airway inflammation (13). However, in contrast to Dectin-2, studies investigating the roles of Dectin-1 in allergic airway inflammation reached conflicting conclusions. Lilly et al. (14) reported that Dectin-1 signaling enhances *Aspergillus*-induced allergic inflammation via IL-22 production, whereas another group reported that Dectin-1 is irrelevant (15). Interestingly, house dust mite (HDM), a representative allergen in allergic asthma, was shown to contain some Dectin-1 ligands, including β -glucan (16, 17), raising the possibility that Dectin-1 is involved in the pathogenesis of HDM-induced allergic airway inflammation.

In this study, we examined the role of Dectin-1 in HDM-induced allergic airway inflammation by using Dectin-1-deficient (*Clec7a*^{-/-}) mice; we found that eosinophilic and neutrophilic inflammation, as well as Th2 and Th17 differentiation, was markedly decreased in *Clec7a*^{-/-} mice. In the lung, Dectin-1 was expressed on CD11b⁺ dendritic cells (DCs), which have been implicated in the induction of allergic airway inflammation (18). We also found that chemokine/chemokine receptor expression and migration of DCs were impaired in *Clec7a*^{-/-} mice. These results indicate that Dectin-1 plays important roles in the development of HDM-induced allergic airway inflammation by activating CD11b⁺ DCs.

Materials and Methods

Mice

C57BL/6 mice (Charles River Laboratories, Kanagawa, Japan) and *Clec7a*^{-/-} mice on a C57BL/6 background (10) were housed in micro-isolator cages under specific pathogen-free conditions. The Chiba University Animal Care and Use Committee approved the animal procedures used in this study.

HDM-induced allergic airway inflammation

Mice were sensitized and challenged with intratracheal administration of HDM extracts (Greer Laboratories, Lenoir, NC), as described previously (13). In brief, mice were anesthetized with pentobarbital and sensitized with 100 μ g of HDM in 50 μ l of PBS twice at a 7-d interval. Seven days after the last sensitization, mice were challenged with 10 μ g of HDM for five consecutive days. In some experiments, mice were sensitized by intratracheal administration of HDM-sensitized DCs, as described previously (18), and challenged with HDM, as described above. Forty-eight hours after the last HDM administration, the numbers of eosinophils, neutrophils, and lymphocytes recovered in BALF were evaluated, as described previously (18). Blood was taken to examine the levels of total IgE and HDM-specific Abs. Mediastinal lymph nodes (MLNs) were taken to examine cytokine-expression profiles by ELISA and flow cytometry. Airway resistance was measured by a FlexiVent system (SCIREQ, Montreal, QC, Canada), as described previously (19).

OVA-induced allergic airway inflammation

Mice were sensitized and challenged with OVA (Sigma, St Louis, MO), and airway inflammation was evaluated as described previously (19).

DC migration assay

DC migration from lung to MLN was analyzed as described previously (18). In brief, HDM extract was labeled with a DyLight 650 Microscale Antibody Labeling kit (Thermo Scientific, Waltham, MA). *Clec7a*^{-/-} mice and wild-type (WT) mice were sensitized once with unlabeled HDM and were challenged with the labeled HDM 7 d later. In some experiments, non-sensitized *Clec7a*^{-/-} mice and WT mice were challenged with the labeled HDM. Forty-eight hours after the challenge, cells harvested from the lung and MLNs were analyzed by flow cytometry. The viability of DCs was assessed by measuring the capacity of cells to exclude DAPI (Thermo Scientific).

HDM-induced cytokine production

Cells isolated from MLNs (1×10^6 cells) were stimulated with HDM (30 μ g/ml) for 5 d in a round-bottom 96-well plate. The levels of cytokines

in the culture supernatants were measured by ELISA kits, according to the manufacturer's instructions.

Intracellular staining

Cells isolated from MLNs were stimulated with PMA (20 ng/ml) and ionomycin (1 μ g/ml; both from Calbiochem, San Diego, CA) for 4 h in the presence of brefeldin A (10 μ M; BD Bioscience, San Diego, CA). Cytokine profiles (IFN- γ , IL-13, IL-17A, IL-9, and IL-22) of CD4⁺ T cells were evaluated by flow cytometry, as described previously (20). Intracellular staining for Foxp3 was performed as described previously (21).

Histological analysis of the lung

Lung sections (3 μ m thick) were stained with H&E or periodic acid-Schiff (PAS), according to standard protocols. The number of goblet cells was counted on PAS-stained lung sections, as described elsewhere (22).

Quantitative real-time PCR analysis

Quantitative real-time PCR (qPCR) was performed with a standard protocol on an ABI PRISM 7300 instrument using Power SYBR Green PCR Master Mix (both from Applied Biosystems, Foster City, CA).

Dectin-1 staining

Lung cell suspensions were prepared as described previously (13). Expression levels of Dectin-1 on each DC population were analyzed using anti-Dectin-1 Ab (clone 2A11; kindly provided by Dr. G.D. Brown, University of Aberdeen, Aberdeen, U.K.) labeled with a DyLight 650 Microscale Antibody Labeling kit (Thermo Scientific). As controls, isotype-matched IgG (rat IgG2b) was labeled with the same kit.

Preparation of bone marrow-derived DCs

Bone marrow-derived DCs (BMDCs) were prepared as described previously (10). In brief, single-cell suspensions of bone marrow cells were obtained from 8-wk-old *Clec7a*^{-/-} mice and WT mice and cultured in complete DMEM containing GM-CSF (20 ng/ml) for 7 d, with replacement of medium containing GM-CSF every 3 d. CD11c⁺ cells were purified using CD11c MicroBeads (Miltenyi Biotec, Sunnyvale, CA), according to the manufacturer's instructions.

RNA-sequencing analysis

BMDCs of WT mice and *Clec7a*^{-/-} mice were stimulated with HDM (100 μ g/ml) for 4 h, and total RNA was isolated from these cells using a PureLink RNA Mini Kit (Invitrogen, Carlsbad, CA). Sequencing was performed on an Illumina HiSeq2500 using a TruSeq Rapid SBS Kit (Illumina, San Diego, CA) in a 50 base single-end mode. mRNA profiles were calculated with Cufflinks software and expressed as fragments per kilobase exon model per million mapped fragments). An M-A plot was obtained based on log ratios and mean of all transcripts in BMDCs of WT mice and *Clec7a*^{-/-} mice. Differentially expressed genes were determined by a weighted average difference method using the TCC package in R software (23).

β -glucanase treatment of HDM

HDM was treated with β -glucanase, as described previously (17). In brief, HDM (2 μ g/ml) was incubated with β -glucanase (4 μ g/ml; Sigma) or vehicle (maleic acid, 0.1 mM [pH 5.2]; Wako, Osaka, Japan) at 50°C for 1 h.

Data analysis

Data are summarized as mean $\pm$ SEM. The statistical analyses of the results were performed using the unpaired *t* test or ANOVA. The *p* values < 0.05 were considered significant.

Results

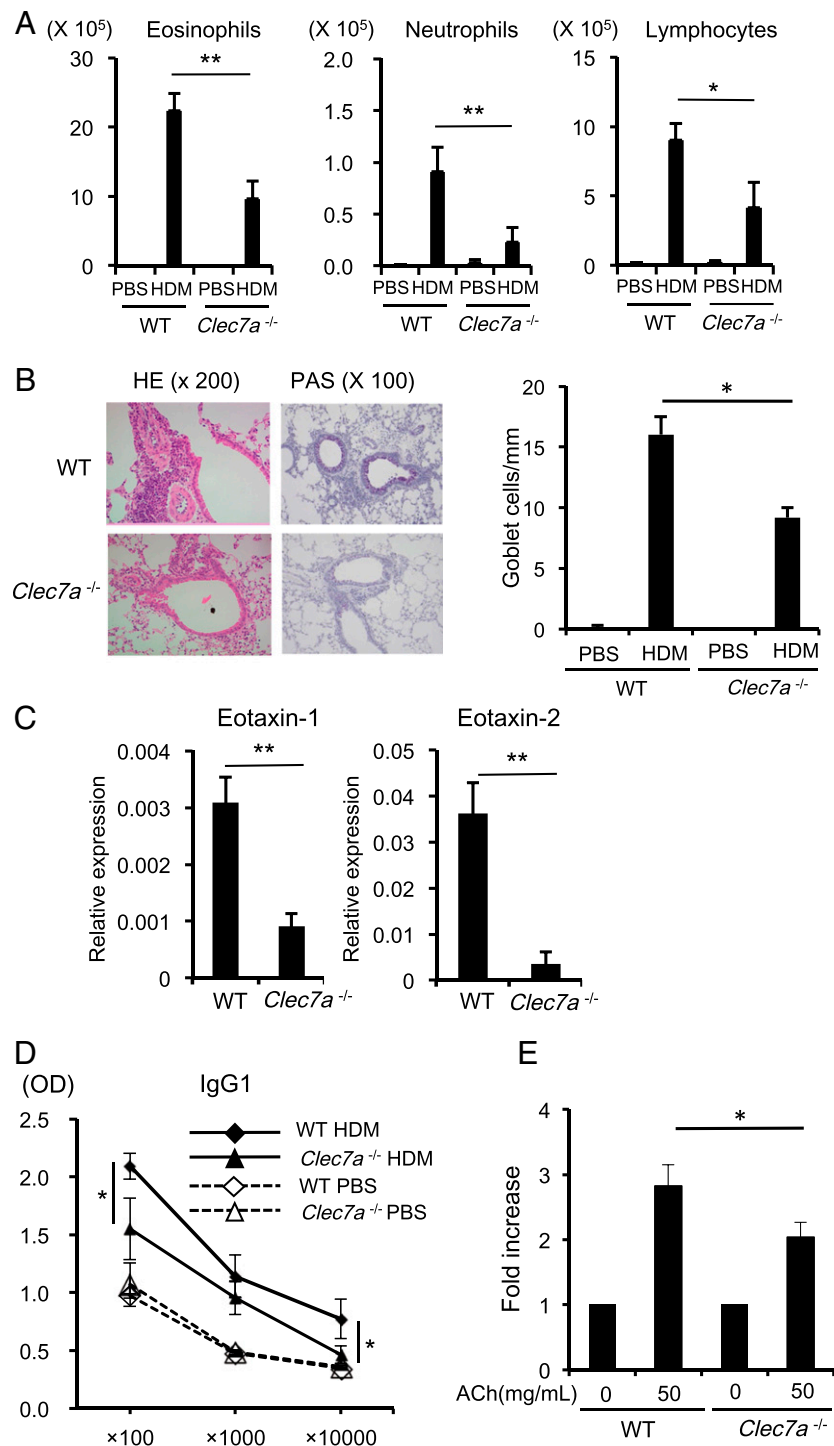
Dectin-1 plays an important role in the development of HDM-induced allergic airway inflammation in mice

To determine the roles of Dectin-1 in HDM-mediated immune responses, we first compare HDM-induced allergic airway inflammation in Dectin-1-deficient (*Clec7a*^{-/-}) mice and WT mice. In these experiments, HDM-sensitized *Clec7a*^{-/-} mice and WT

mice were challenged with intratracheal administration of HDM for five consecutive days; 48 h after the last administration, the numbers of eosinophils, neutrophils, and lymphocytes in BALF were examined. As shown in Fig. 1A, the numbers of eosinophils and lymphocytes in BALF were significantly reduced in *Clec7a*^{-/-} mice compared with WT mice. Consistent with the ability of Dectin-1 to promote Th17-type immune responses (12), the numbers of neutrophils in the BALF were also reduced in *Clec7a*^{-/-} mice (Fig. 1A). Histological analysis of the lung revealed that HDM-induced peribronchial and perivascular inflammation, as well as goblet cell hyperplasia, was attenuated in *Clec7a*^{-/-} mice compared with WT mice (Fig. 1B). In addition,

Clec7a^{-/-} mice exhibited a significant reduction in mRNA expression of eotaxin-1 and eotaxin-2, potent chemokines for the recruitment of eosinophils (24, 25), in the lung (Fig. 1C). Moreover, compared with WT mice, HDM-specific IgG1 was reduced in *Clec7a*^{-/-} mice (Fig. 1D). In contrast, HDM-specific IgE and IgG2c were below the limits of detection, and the levels of total IgE were similar between HDM-sensitized WT mice and *Clec7a*^{-/-} mice in this experimental condition (data not shown). Furthermore, airway responsiveness to acetylcholine was reduced in HDM-sensitized and -challenged *Clec7a*^{-/-} mice (Fig. 1E). In contrast to the attenuated HDM-induced eosinophilic inflammation in *Clec7a*^{-/-} mice (Fig. 1A), OVA-induced eosinophilic

FIGURE 1. HDM-induced allergic airway inflammation is attenuated in *Clec7a*^{-/-} mice. *Clec7a*^{-/-} mice and WT mice were sensitized and challenged with HDM or PBS (as a control), as described in *Materials and Methods*. **(A)** The numbers of eosinophils, neutrophils, and lymphocytes in the BALF were evaluated at 48 h after the last HDM challenge. Data are mean $\pm$ SEM for 10 mice in each group. **(B)** Representative photomicrographs (H&E and PAS staining) of lung sections (left panels) and the numbers of goblet cells (right panels) at 48 h after the last HDM challenge. Data are mean $\pm$ SEM for eight mice in each group. **(C)** The expression levels of eotaxin-1 and eotaxin-2 in the lung were analyzed by qPCR at 48 h after the last HDM challenge. Data are mean $\pm$ SEM of three independent experiments. **(D)** Serum levels of HDM-specific IgG1. Data are mean $\pm$ SEM for five mice in each group. **(E)** Airway resistance to acetylcholine was measured at 48 h after the last HDM challenge. Data are mean $\pm$ SEM of fold increase over baseline for six mice in each group. * p < 0.05, ** p < 0.01.



inflammation was comparable between WT mice and *Clec7a*^{-/-} mice (Supplemental Fig. 1). Taken together, these results indicate that Dectin-1 plays an important role in the development of HDM-induced airway inflammation, presumably by sensing some ligand(s) in HDM extract.

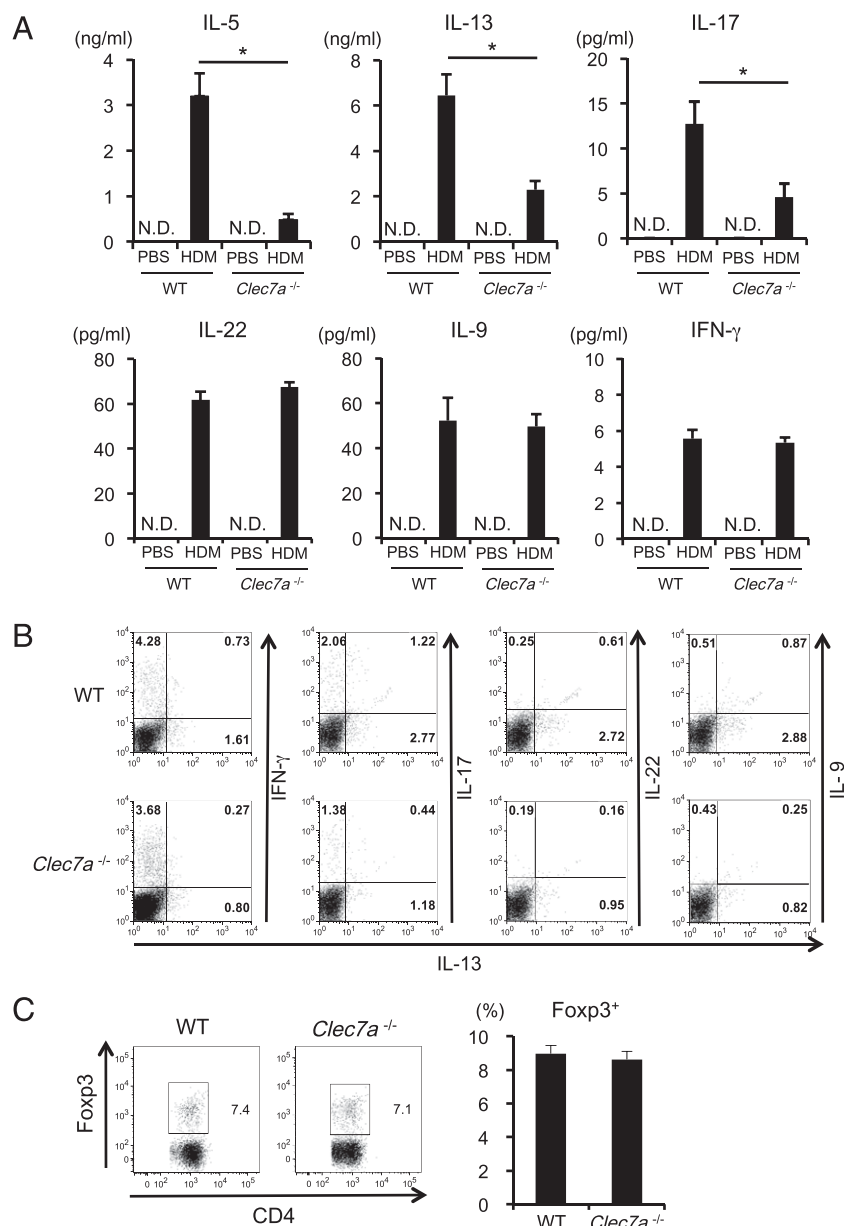
Dectin-1 is involved in HDM-mediated Th2 and Th17 cell induction

To clarify the mechanism underlying the attenuated allergic inflammation upon HDM administration in *Clec7a*^{-/-} mice, we next examined cytokine production of MLN cells of HDM-sensitized and -challenged mice. As shown in Fig. 2A, HDM-induced IL-5 and IL-13 production by MLN cells was reduced significantly in *Clec7a*^{-/-} mice. In accordance with the reduced neutrophil recruitment in *Clec7a*^{-/-} mice, MLN cells of *Clec7a*^{-/-} mice produced less IL-17A than did those of WT mice (Fig. 2A). Although recent studies suggested the involvement of IL-9 and IFN- γ in the pathogenesis of severe asthma (26, 27), the levels of IL-9 and IFN- γ were comparable between WT mice and

Clec7a^{-/-} mice (Fig. 2A). Although a previous report showed that Dectin-1 signaling induces IL-22 production in *Aspergillus*-induced allergic inflammation (15), the levels of IL-22 were not significantly different between WT mice and *Clec7a*^{-/-} mice in this experimental setting (Fig. 2A).

Consistently, flow cytometric analysis of MLN cells revealed that CD4⁺ T cells in *Clec7a*^{-/-} mice produced less IL-13 and IL-17A than did those in WT mice, whereas IFN- γ production of CD4⁺ T cells was comparable between *Clec7a*^{-/-} mice and WT mice (Fig. 2B). In addition, the proportions of Th9 cells and Th22 cells, recently described CD4⁺ T cell subsets that are implicated in asthma (27, 28), were comparable between *Clec7a*^{-/-} mice and WT mice (Fig. 2B). Although Tang et al. (29) showed that Dectin-1 is involved in the expansion of regulatory T cells (Tregs) through a commensal-host homeostasis, the frequency of Foxp3⁺ Tregs in MLNs was similar between WT mice and *Clec7a*^{-/-} mice (Fig. 2C). These results suggest that Dectin-1 is involved in the development of Th2 cells and Th17 cells, but not Tregs, in HDM-induced allergic airway inflammation.

FIGURE 2. Dectin-1 plays key roles in Th2 and Th17 cell differentiation in HDM-induced allergic airway inflammation. **(A)** MLN cells were harvested from HDM-sensitized and -challenged *Clec7a*^{-/-} mice and WT mice and were stimulated with HDM for 5 d. The levels of IL-5, IL-13, IL-17A, IL-22, IL-9, and IFN- γ in the culture supernatants were measured by ELISA. Data are mean $\pm$ SEM for five mice in each group. **(B)** Single-cell suspensions of MLN cells from HDM-sensitized and -challenged *Clec7a*^{-/-} mice and WT mice were stimulated with PMA + ionomycin for 4 h. Shown are representative FACS profiles of intracellular staining for IL-13, IFN- γ , IL-17A, IL-22, and IL-9. **(C)** Representative CD4 versus Foxp3 staining and the frequency of Foxp3⁺ cells in CD4⁺ cells of MLNs from HDM-sensitized and -challenged *Clec7a*^{-/-} mice and WT mice. Data are mean $\pm$ SEM for three mice in each group. **p* < 0.05. N.D., not detected.



Dectin-1 is expressed on CD11b⁺ DCs but not on CD103⁺ DCs, plasmacytoid DCs, or lung epithelial cells

To address the mechanisms underlying the impaired Th2 and Th17 responses in *Clec7a*^{-/-} mice, we next examined Dectin-1-expressing populations in the lung. A previous study demonstrated that Dectin-1 is expressed on myeloid cells, including DCs (30). Therefore, we analyzed the expression of Dectin-1 on three major lung DC subsets: CD11b⁺ DCs, CD103⁺ DCs, and plasmacytoid DCs (31, 32). As shown in Fig. 3A, flow cytometric analysis of cells isolated from the lung showed that Dectin-1 was expressed on CD11b⁺ DCs but not on CD103⁺ DCs or plasmacytoid DCs. Although it was shown that Dectin-1 is expressed on a lung epithelial cell line and regulates chemokine expression (17), Dectin-1 was barely detected on freshly isolated murine lung epithelial cells (Fig. 3A). Although the *Clec7a* locus is located near the *Clec4n* (gene symbol for Dectin-2) locus in the NK cluster (33), the expression levels of Dectin-2 mRNA in CD11b⁺ DCs were not affected by gene targeting of *Clec7a* (Supplemental Fig. 2).

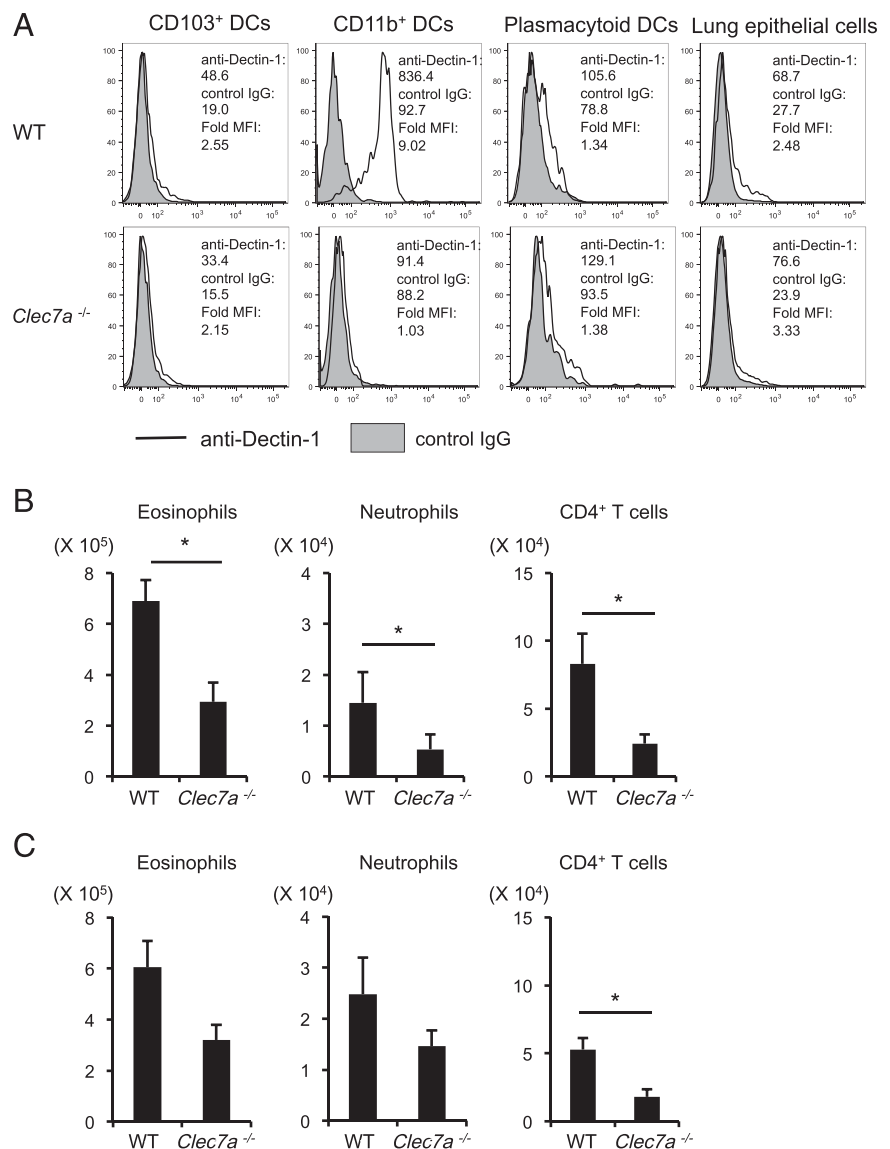
To determine the role of Dectin-1 expressed on CD11b⁺ DCs in HDM-induced allergic airway inflammation, CD11b⁺ DCs isolated from the lung of HDM-challenged WT mice or *Clec7a*^{-/-} mice were transplanted intratracheally to WT mice, and these mice were challenged with HDM for 5 d. Importantly, the

numbers of eosinophils, neutrophils, and CD4⁺ T cells in the BALF were reduced significantly in the mice transplanted with *Clec7a*^{-/-} CD11b⁺ DCs versus WT CD11b⁺ DCs (Fig. 3B). In addition, although the difference did not reach statistical significance, similar results were obtained when *Clec7a*^{-/-} mice were used as recipient mice (Fig. 3C). Taken together, these results suggest that Dectin-1 expressed on CD11b⁺ DCs is likely to be involved in the induction of HDM-induced allergic airway inflammation, although we could not exclude the possibility that Dectin-1 expressed on other cell type(s) is also involved.

HDM induces chemokine and chemokine receptor expression in BMDCs through a Dectin-1-dependent mechanism

To gain further insight into the roles of Dectin-1 in HDM-induced allergic airway inflammation, we performed an unbiased assessment of HDM-induced mRNA induction of *Clec7a*^{-/-} BMDCs and WT BMDCs by RNA-sequencing analysis. This analysis revealed that a series of genes, including costimulatory molecules and cytokines that have roles in T cell differentiation and activation, were decreased significantly in *Clec7a*^{-/-} BMDCs (Fig. 4A, 4B). We also found that the expression of a number of chemokines/chemokine receptors was decreased in *Clec7a*^{-/-} BMDCs (Fig. 4A, 4B). Importantly, HDM-induced expression of

FIGURE 3. Dectin-1 is expressed on lung CD11b⁺ DCs but not on CD103⁺ DCs, plasmacytoid DCs, or lung epithelial cells. **(A)** Expression levels of Dectin-1 on CD11b⁺ DCs (MHC II⁺ CD11c⁺ CD11b⁺ CD24⁺ cells), CD103⁺ DCs (MHC II⁺ CD11c⁺ CD103⁺ cells), plasmacytoid DCs (MHC II⁺ CD11c⁺ PDCA-1⁺ cells), and epithelial cells (CD45⁺ EpCAM-1⁺ cells) in the lung were analyzed by flow cytometry. Shown are representative graphs of anti-Dectin-1 staining of WT mice and *Clec7a*^{-/-} mice and of isotype-matched control IgG staining (filled gray area) of three independent experiments. Mean fluorescent intensity (MFI) of anti-Dectin-1 staining and isotype-matched control IgG staining and fold MFI are shown within each panel. CD11b⁺ DCs were isolated from the lung of WT mice or *Clec7a*^{-/-} mice at 48 h after HDM administration and were intratracheally transplanted to WT mice **(B)** or to *Clec7a*^{-/-} mice **(C)**. These mice were then challenged with HDM for five consecutive days, and the numbers of eosinophils, neutrophils, and CD4⁺ T cells in the BALF were evaluated at 48 h after the last HDM challenge. Data are mean ± SEM for seven mice (B) or six mice (C) in each group. **p* < 0.05.



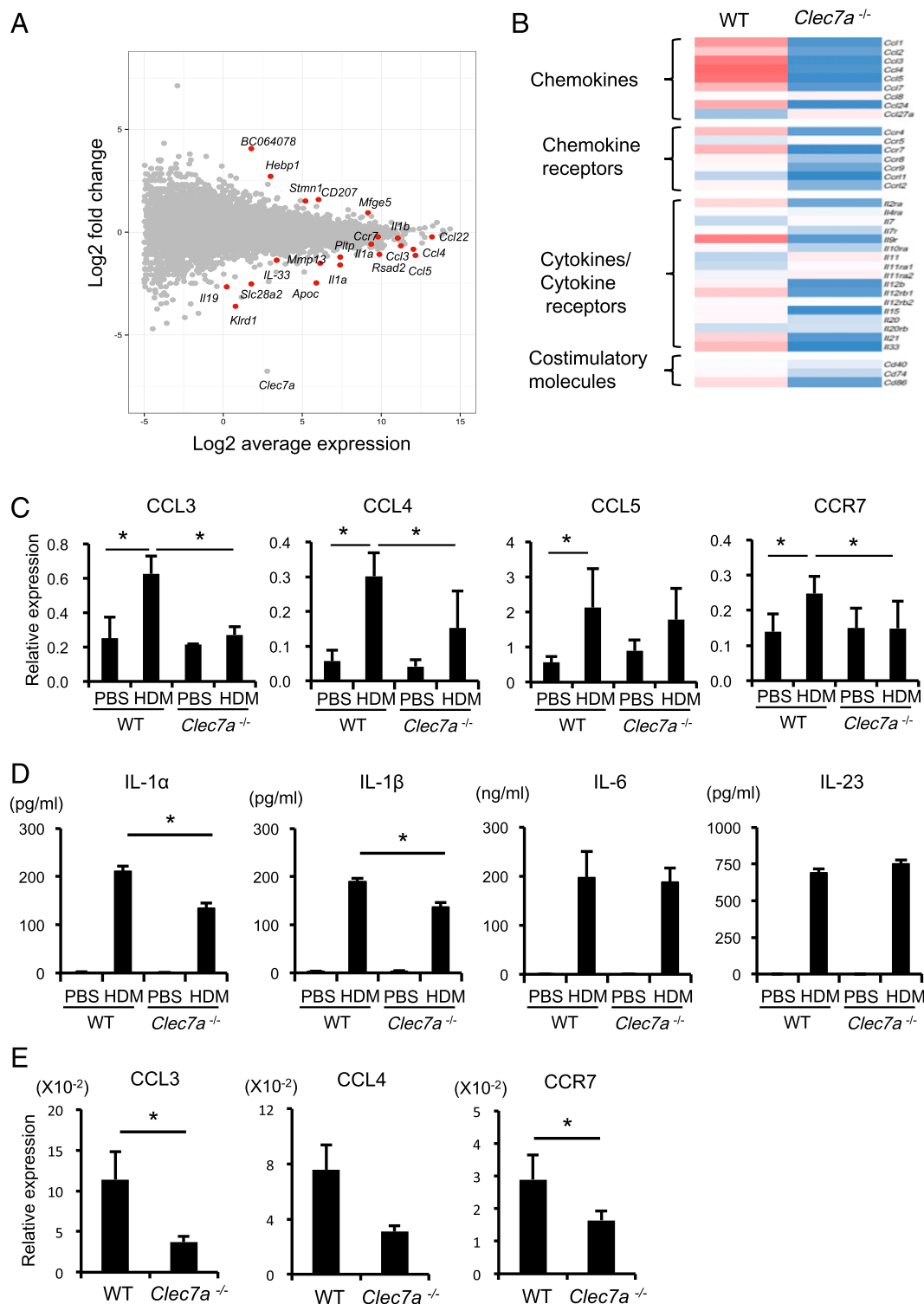


FIGURE 4. Dectin-1 is involved in HDM-induced expression of chemokines and chemokine receptors in DCs. **(A–C)** BMDCs of WT mice and *Clec7a*^{-/-} mice were stimulated with HDM for 4 h. Total RNAs were prepared and subjected to RNA-sequencing analysis. **(A)** M-A plot based on log ratios [*Clec7a*^{-/-} BMDCs – WT BMDCs (log2)] and means [(*Clec7a*^{-/-} BMDCs + WT BMDCs)/2(log2)] of all transcripts in the BMDCs of *Clec7a*^{-/-} mice and WT mice. **(B)** Heat map of the levels of chemokines/chemokine receptors, cytokines/cytokine receptors, and costimulatory molecules. **(C)** The expression levels of CCL3, CCL4, CCL5, and CCR7 were evaluated by qPCR analysis. Data are mean ± SEM for five independent experiments. **(D)** BMDCs of WT mice and *Clec7a*^{-/-} mice were stimulated with HDM for 24 h. The levels of IL-1α, IL-1β, IL-6, and IL-23 in the supernatants were evaluated by ELISA. Data are mean ± SEM for four independent experiments. **(E)** CD11b⁺ DCs were isolated from the lung of HDM-sensitized and -challenged WT mice and *Clec7a*^{-/-} mice. The expression levels of CCL3, CCL4, and CCR7 were evaluated by qPCR analysis. Data are mean ± SEM for four mice in each group. **p* < 0.05.

CCR7, which is essential for the migration of DCs (34), was downregulated in *Clec7a*^{-/-} BMDCs (Fig. 4B, 4C). *Clec7a*^{-/-} BMDCs also expressed significantly lower levels of CCL3 and CCL4 (Fig. 4B, 4C), which are involved in the recruitment of inflammatory cells (35). In contrast, the expression of CCL5, an inflammatory chemokine regulating the trafficking of Th1 cells, DCs, NK cells, eosinophils, and macrophages, was comparable between WT BMDCs and *Clec7a*^{-/-} BMDCs (Fig. 4C). Intriguingly, the ability of HDM to induce the expression of CCL3, CCL4, and CCR7 was reduced significantly by the pretreatment of HDM extract with β -glucanase (Supplemental Fig. 3), suggesting that the β -glucan-like substance(s) included in HDM extract is involved in the induction.

We also examined the roles of Dectin-1 in HDM-induced cytokine production of BMDCs at protein levels. As shown in Fig. 4D, *Clec7a*^{-/-} BMDCs produced lower levels of IL-1 α and IL-1 β than did WT BMDCs. In contrast, the levels of IL-6 and IL-23 were not significantly different between them (Fig. 4D).

Consistent with the results using BMDCs (Fig. 4C), freshly isolated CD11b⁺ DCs from HDM-sensitized and -challenged *Clec7a*^{-/-} mice expressed significantly lower levels of mRNAs for CCL3 and CCR7 than did those from HDM-sensitized and -challenged WT mice (Fig. 4E). Moreover, CD11b⁺ DCs from *Clec7a*^{-/-} mice tended to express decreased levels of CCR4 mRNA (Fig. 4E), although the difference did not reach statistical significance. These results suggest that Dectin-1 signaling induces the expression of CCL3 and CCR7 in CD11b⁺ DCs.

Dectin-1 is involved in the HDM-induced activation and migration of CD11b⁺ DCs

Given that Dectin-1 is involved in the induction of CCR7 (Fig. 4), which is essential for the migration of DCs from peripheral tissue to draining lymph nodes (LNs) (18), we examined whether Dectin-1 is involved in the migration of DCs upon HDM stimulation. In this experiment, WT mice and *Clec7a*^{-/-} mice were sensitized by intratracheal administration of unlabeled HDM; 7 d later, these mice were challenged intratracheally with fluorescent-labeled HDM. Consistent with the previous report (18), the majority of fluorescent-labeled cells were CD11b⁺ CD103⁻ DCs, representative migratory DCs, in MLNs (Fig. 5A). Importantly, although the number of fluorescent-labeled CD11b⁺ DCs in the lung was comparable between WT mice and *Clec7a*^{-/-} mice, the number of these cells in MLNs was decreased significantly in *Clec7a*^{-/-} mice (Fig. 5A, 5B). Meanwhile, there was no significant difference in the viability of CD11b⁺ DCs in MLNs between WT mice and *Clec7a*^{-/-} mice (data not shown). Furthermore, FACS analysis revealed that, although the expression of CD80, CD86, and CCR7 on fluorescent-labeled CD11b⁺ DCs in the lung was comparable between WT mice and *Clec7a*^{-/-} mice, the expression of these molecules on CD11b⁺ DCs in MLNs was reduced in *Clec7a*^{-/-} mice (Fig. 5C). Even in the initial phase of HDM sensitization, reduced numbers of fluorescent-labeled CD11b⁺ DCs and reduced expression of CD80, CD86, and CCR7 on CD11b⁺ DCs in MLNs were observed in *Clec7a*^{-/-} mice (Supplemental Fig. 4), although the magnitude of DC responses were relatively small. Taken together, these results suggest that Dectin-1 is involved in HDM-induced activation and migration of lung DCs and, thus, exacerbates HDM-induced allergic airway inflammation.

Discussion

In this study, we show that Dectin-1 plays an important role in the induction of HDM-induced allergic airway inflammation (Fig. 1). We also show that Dectin-1 is critical for the development of Th2

cells, as well as Th17 cells, upon HDM stimulation (Fig. 2). In addition, we found that Dectin-1 is expressed on CD11b⁺ DCs, but not on CD103⁺ DCs, plasmacytoid DCs, or lung epithelial cells (Fig. 3A), that CD11b⁺ DCs from *Clec7a*^{-/-} mice are less efficient in the induction of HDM-induced airway inflammation than those from WT mice (Fig. 3B), and that Dectin-1 is involved in the expression of chemokine/chemokine receptors that are critical for DC migration (Fig. 4). Indeed, in *Clec7a*^{-/-} mice, the migration of CD11b⁺ DCs to draining LNs is impaired upon intratracheal administration of HDM (Fig. 5). Taken together, these results suggest that Dectin-1 expressed on CD11b⁺ DCs senses some molecule(s) in HDM extract and plays a critical role in the induction of HDM-induced allergic airway inflammation, presumably by inducing the expression of chemokine/chemokine receptors.

Although allergic asthma has been regarded as a typical allergic disease associated with the development of adaptive Th2-type immune responses, recent studies also suggested the involvement of innate immunity to inhaled allergens (17, 36, 37). Among PRRs, C-type lectin receptors, which recognize fungal cell wall components, are of interest because of the strong relationship between fungal sensitization and severe asthma (38, 39). However, in contrast to another C-type lectin receptor, Dectin-2, which is implicated in the induction of HDM-induced allergic airway inflammation (13, 40), previous studies investigating the roles of Dectin-1 in allergic inflammation reached conflicting conclusions (14, 15, 41).

Albacker et al. (15) showed that Dectin-1 is irrelevant to the development of airway hyperreactivity induced by fungal Ags. They showed that airway hyperreactivity induced by intranasal administration of *A. fumigatus* extract for 3 d was not affected by pretreatment with a blocking Ab against Dectin-1 (15). In contrast, using *Clec7a*^{-/-} mice, we clearly showed a role for Dectin-1 in the induction of HDM-induced allergic airway inflammation (Fig. 1). We also found a significant reduction in HDM-induced Th2 and Th17 cell development in *Clec7a*^{-/-} mice (Fig. 2). We speculate that the differences in the experimental protocols between the previous study and ours may explain the discrepancy. In this regard, Cheng et al. (42) recently showed that pretreatment of monocytes with β -glucan enhances the responsiveness to subsequent stimuli, suggesting the involvement of Dectin-1 in trained innate immunity. Therefore, it is possible that repeated administration of HDM extract in our sensitization/challenge protocol enhances the function of Dectin-1-expressing DCs in the lung and promotes Dectin-1-dependent allergic airway inflammation. Further studies are needed to clarify whether trained innate immunity is involved in Dectin-1-mediated enhancement of HDM-induced allergic airway inflammation.

Transcriptome analysis of BMDCs revealed that Dectin-1 signaling enhances the expression of CCL3 and CCL4 (Fig. 4), which induce the recruitment of monocytes, NK cells, and memory T cells (43). The importance of CCL3 and CCL4 in the pathophysiology of asthma is underscored by the fact that the expression levels of these chemokines are increased in patients with steroid-resistant asthma (44). Moreover, the levels of CCL3 and CCL4 were shown to be associated with airflow obstruction (45). Although we do not have direct evidence, the induction of CCL3 and CCL4 in DCs is suggested to be involved in HDM-induced Dectin-1-mediated allergic airway inflammation.

The transcriptome analysis also revealed that Dectin-1 is involved in the induction of CCR7, which is essential for DC migration from peripheral tissue to draining LNs upon HDM inhalation (18). Consistent with these results, we found that, although the numbers of HDM-labeled CD11b⁺ DCs in the lung were comparable between WT mice and *Clec7a*^{-/-} mice, those in the draining LNs were reduced significantly in *Clec7a*^{-/-} mice

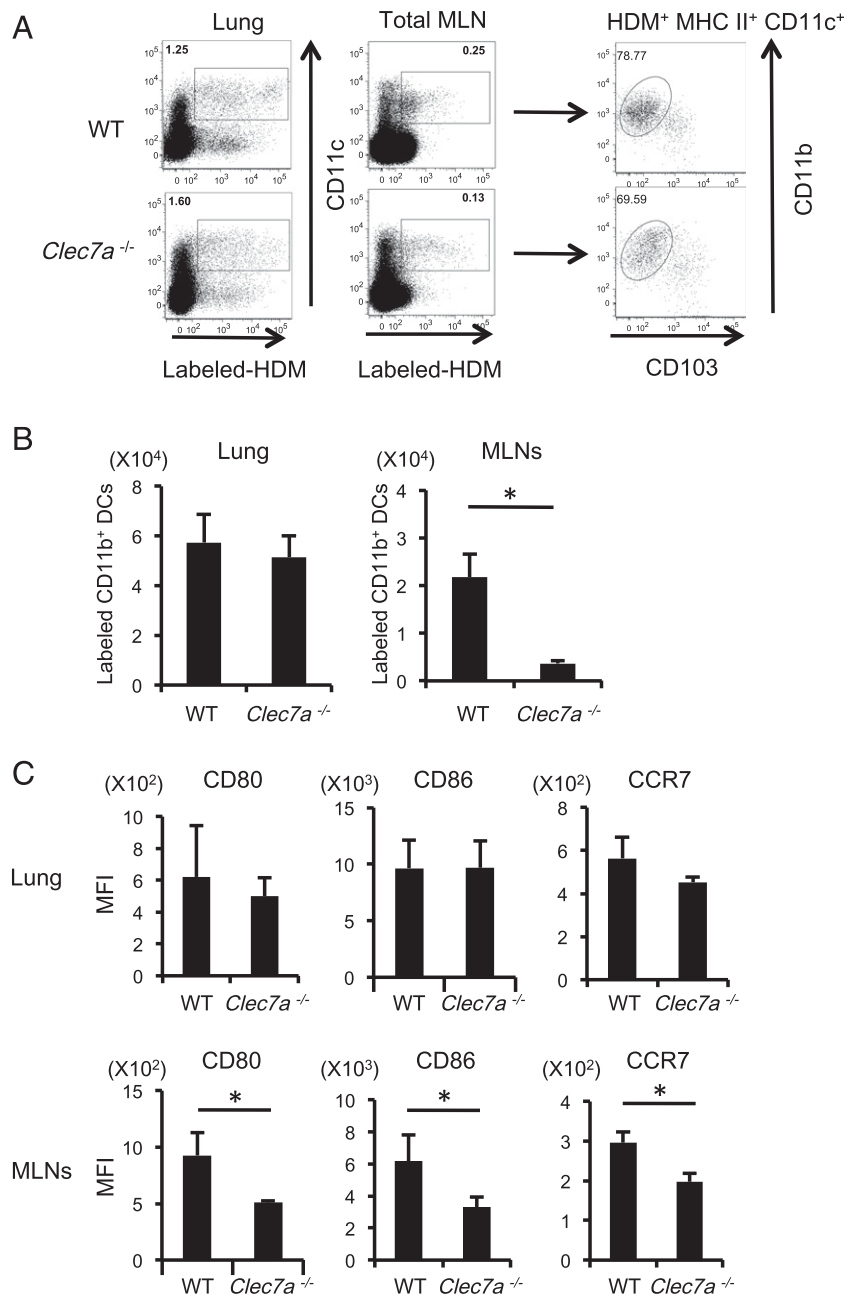


FIGURE 5. Dectin-1 signaling enhances the migration of CD11b⁺ DCs to draining LNs upon HDM stimulation. (A–C) *Clec7a*^{-/-} mice and WT mice were sensitized once with unlabeled HDM; 7 d later, these mice were challenged with fluorescent-labeled HDM. Forty-eight hours after the challenge, cells harvested from the lung and MLNs were analyzed by flow cytometry. Shown are representative FACS profiles (A) and absolute numbers of fluorescent-labeled CD11b⁺ cells (B) in the lung and MLNs. Data are mean ± SEM for four mice in each group. (C) The expression levels of CD80, CD86, and CCR7 on fluorescent-labeled CD11b⁺ DCs in the lung (upper panels) and MLNs (lower panels) were evaluated by flow cytometry. Data are mean ± SEM of mean fluorescent intensity (MFI) for four mice in each group.

(Fig. 5). Although the role of CCR7 in allergic asthma remains unclear, it was shown that the inhibition of CCR7 significantly inhibits the development of HDM-induced allergic inflammation in humanized SCID mice (46). These results suggest that Dectin-1 signaling promotes HDM-induced allergic airway inflammation by enhancing DC migration to draining LNs through CCR7 expression and subsequent T cell priming.

In addition to CCR7, we found that CD11b⁺ DCs in MLNs from *Clec7a*^{-/-} mice expressed lower levels of CD80 than did those from WT mice (Fig. 5). Because CD80 is well known to be required for Th2 cell differentiation (47), its reduced expression might be involved in the decreased HDM-induced Th2 cell differentiation in *Clec7a*^{-/-} mice. This hypothesis should be tested in experiments using naive CD4⁺ T cells from HDM-specific TCR-transgenic mice as responder cells.

We found that Dectin-1 was expressed on CD11b⁺ DCs but not on CD103⁺ DCs or plasmacytoid DCs (Fig. 3A). In addition, adoptive-transfer experiments using CD11b⁺ DCs revealed the

importance of Dectin-1 expressed on CD11b⁺ DCs in HDM-induced allergic airway inflammation (Fig. 3B). These results are consistent with a previous finding that CD11b⁺ DCs, but not CD103⁺ DCs, are essential for the induction of HDM-induced allergic airway inflammation (18). Interestingly, recent studies demonstrated that these CD11b⁺ DCs are highly heterogeneous. Within CD11b⁺ DCs, the IRF4-dependent population is reported to be essential for Th17 cell responses in skin and lung (48, 49). IRF4-expressing DCs also were shown to promote Th2-type inflammation in the lung (50). Furthermore, Tussiwand et al. (51) demonstrated that the IRF4-expressing, KLF4-dependent DC subset is required for HDM-induced allergic inflammation. Further studies are needed to clarify the expression levels of Dectin-1 on each CD11b⁺ DC subset, as well as to clarify the role of Dectin-1 expressed on each CD11b⁺ DC subset in HDM-induced allergic inflammation.

It is possible that PRRs other than Dectin-1 are also involved in HDM-induced Th2 and Th17 cell differentiation, because

HDM-induced Th2 and Th17 cell differentiation was reduced, but still observed, in *Clec7a*^{-/-} mice (Fig. 2B). Indeed, we reported that Dectin-2, another C-type lectin receptor that recognizes α -mannan in fungal cell walls, is able to enhance Th2 and Th17 cell differentiation upon HDM administration (13). Like Dectin-1, Dectin-2 is preferentially expressed on CD11b⁺ DCs and uses signaling pathways that largely overlap with Dectin-1 (52). Therefore, the inhibition of signaling pathways shared between Dectin-1 and Dectin-2 may be an efficient therapeutic strategy, especially in asthmatic patients who exhibit characteristics of Th17-type immune responses, such as neutrophilic airway inflammation.

In conclusion, we showed that Dectin-1 is involved in the development of HDM-induced allergic airway inflammation, presumably via the activation of CD11b⁺ DCs. Although further studies are needed, our results should add a new insight into the pathophysiological roles of Dectin-1 expressed in CD11b⁺ DCs in asthma and suggest that Dectin-1 or its downstream signaling molecule could be a therapeutic target in the treatment of severe asthma.

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Disclosures

The authors have no financial conflicts of interest.

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Fig. S1

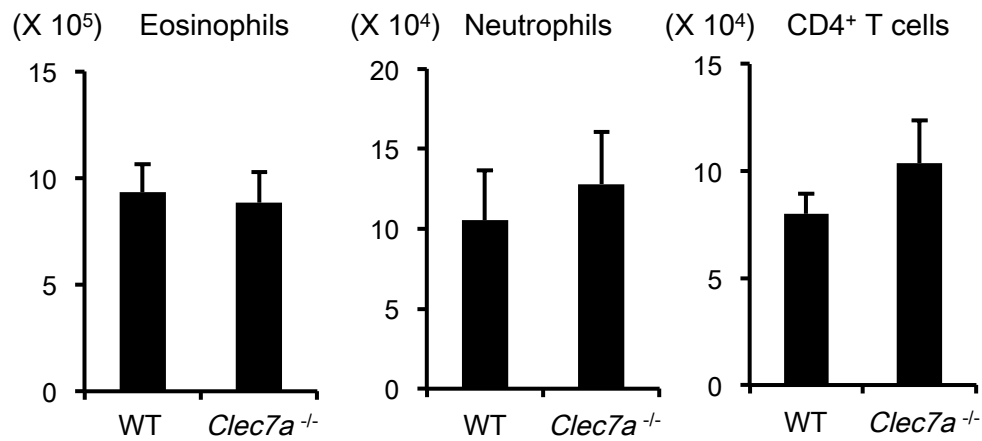


Figure S1: OVA-induced allergic airway inflammation is similarly induced in WT mice and *Clec7a*^{-/-} mice

Clec7a^{-/-} mice and WT mice were sensitized and challenged with OVA as described in the Materials and Methods. The numbers of eosinophils, neutrophils, and CD4⁺ T cells in the BALF were evaluated at 48 hours after the OVA challenge. Data are means $\pm$ SEM for 4 mice in each group.

Fig. S2

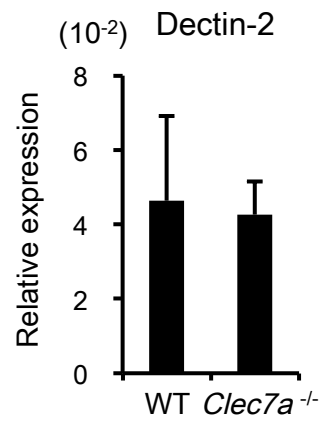


Figure S2: The expression levels of Dectin-2 in lung CD11b⁺ DCs are comparable between WT mice and *Clec7a*^{-/-} mice

CD11b⁺ DCs were isolated from the lung of HDM-sensitized and -challenged WT mice and *Clec7a*^{-/-} mice at 48 hours after the last HDM challenge. The expression levels of Dectin-2 were evaluated by qPCR analysis. Shown are mean ± SEM of 5 independent experiments.

Fig. S3

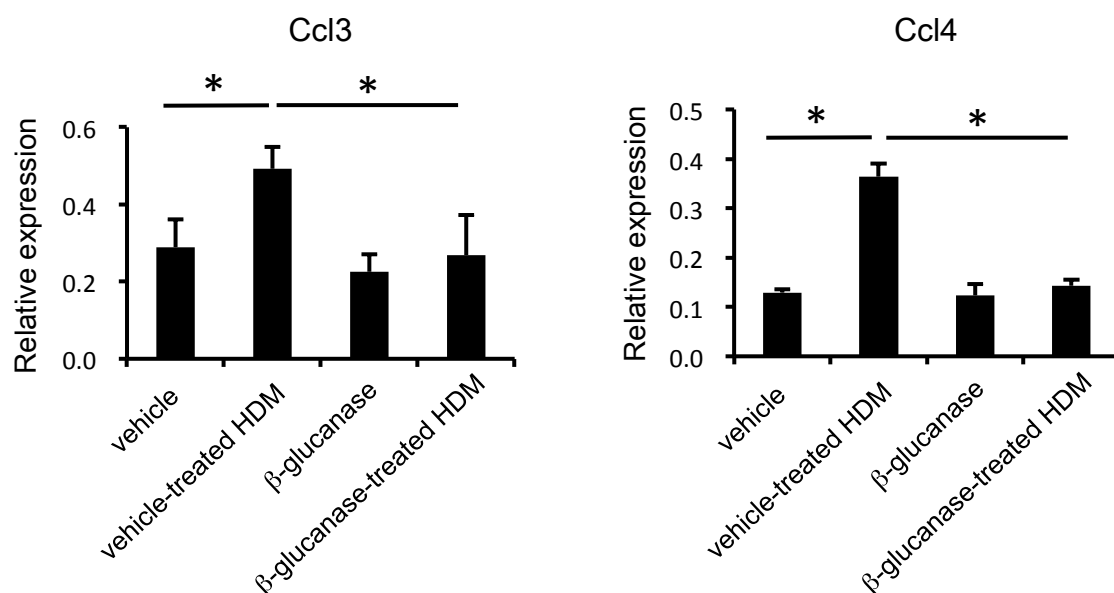


Figure S3: The effect of β-glucanase treatment on HDM-induced CCL3 and CCL4 induction in BMDCs

WT BMDCs were stimulated with either vehicle, vehicle-treated HDM, β-glucanase, or β-glucanase-treated HDM for 4 hours. The expression levels of CCL3 and CCL4 were evaluated by qPCR. Shown are mean ± SEM for 4 independent experiments. * $p < 0.05$.

Fig. S4

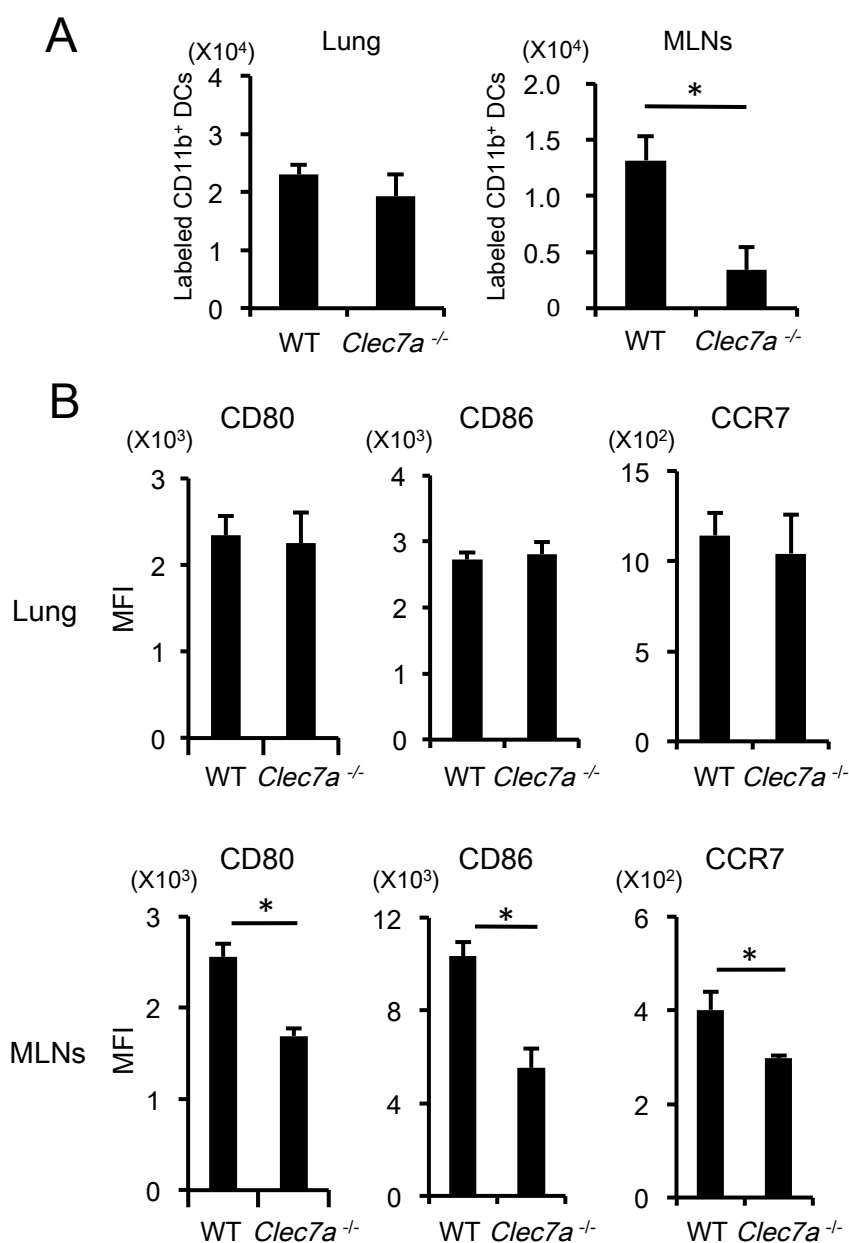


Figure S4: Dectin-1 signaling enhances the migration of CD11b⁺ DCs to draining LNs during the initial phase

Non-sensitized *Clec7a*^{-/-} mice and WT mice were challenged with fluorescent-labeled HDM. Forty-eight hours after the challenge, the numbers of fluorescent-labeled CD11b⁺ DCs (**A**) and the expression levels of CD80, CD86, and CCR7 on fluorescent-labeled CD11b⁺ DCs in the lung and MLNs (**B**) were evaluated by flow cytometry. Shown are means $\pm$ SEM for 4 mice in each group. * $p < 0.05$.