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The role of CCR7 in allergic airway inflammation induced by house dust mite exposure

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ABSTRACT

House dust mite (HDM), the most common allergen, activate both the IgE-associated and innate immune responses. To clarify the process of sensitization, we investigated the role of the CCL21, CCL19, and CCR7 axis in a mouse model of HDM-induced allergic asthma. HDM inhalation without systemic immunization resulted in a HDM-specific IgE response. CCR7-knockout (CCR7KO) mice exhibited greater airway inflammation and IgE responses compared to wild-type mice. We examined FoxP3 expression in these mice to clarify the contribution of regulatory cells to the responses. FoxP3 expression was higher in the lungs but not in the lymph nodes of CCR7KO mice compared to wild-type mice. In CCR7KO mice, FoxP3-positive cells were found in lung, but we observed higher release of IL-13, IL-5, TGF- β , IL-17, and HMGB1 in bronchoalveolar lavage fluid. We demonstrate here that immuno-regulation through CCR7 expression in T cells plays a role in HDM-specific sensitization in the airway.

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1. Introduction

The inhalation of allergens induces airway inflammation, which is a prominent feature of asthma [1–4]. Dendritic cells are antigen-presenting cells that initiate several immune responses [5]. After dendritic cells take up allergens, they enter afferent lymphatic vessels through a process promoted by CCL21 [6,7]. Antigen-laden antigen-presenting cells then induce activation, expansion, and differentiation of antigen-specific T cells within the draining lymph nodes. We previously showed that paucity of lymph node T cells (plt) mice, which are deficient in CCL21 and CCL19, resulted in reduced but still substantial airway inflammation, suggesting that allergen sensitization and airway inflammation can be induced without secondary lymph nodes [8]. In contrast, CCL21 and

CCL19 play a critical role in the resolution phase of airway inflammation, which is similar to their role in the mucosal immune system [8–10].

In animal asthmatic models, systemic immunization using allergens formulated with alum is used to induce IgE. In plt mice we used ovalbumin in alum to clarify the role of CCL21 [8]. Administration of house dust mite (HDM) by inhalation was recently shown to induce airway inflammation without systemic immunization [11,12]. In many countries, HDM is one of the most common allergens triggering asthma [13–15]. HDM allergens have proteinase activity, which is critical for sensitization [16,17]. HDM allergens react with toll-like receptor 4 (TLR4), which is a receptor for lipopolysaccharide (LPS) [18,19]. In addition, Derf 2, a major HDM allergen, possesses structural homology to myeloid differentiation factor (MD) 2, the LPS-binding component of the TLR4 signaling complex [20,21]. Taken together, these findings indicate that allergen sensitization involves complex interactions between antigen-specific and innate immune responses that have not yet been clarified.

CCR7 is the receptor for CCL19 and CCL21, and CCR7-knockout (CCR7KO) mice possess similar impairment of the secondary lymph nodes as plt mice [22]. The impairment of contact sensitivity in CCR7KO mice is greater than that in plt mice [22].

Abbreviations: HDM, house dust mite; CCR7KO, CCR7-knockout; LPS, lipopolysaccharide; BALF, bronchoalveolar lavage fluid; HE, haematoxylin-eosin; PAS, periodic acid-Schiff; iBAL, inducible bronchus-associated lymphoid tissue; HMGB, high-mobility group box; Treg, regulatory T; ACh, acetylcholine; Raw, airway resistance.

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Furthermore, CCR7 is important in maintaining immune responses and immune tolerance [23]. CCR7 is also essential to migrate and exhibit regulatory function of regulatory T (Treg) cells [24–26]. To clarify the process of allergen sensitization and the role of CCR7 expression in this process, we investigated the ability of CCR7KO mice to exhibit HDM-specific airway inflammation. We found that airway inflammation was significantly augmented and lymphocyte accumulation was prominent in CCR7KO mice. Although FoxP3-positive cells were present in the lung, they did not function effectively in CCR7KO mice. This finding suggests that allergen sensitization is controlled by the expression of CCR7. The targeted control of CCR7 expression has potential as an immunotherapy for asthma.

2. Methods

2.1. Animals

C57BL/6 mice were purchased from SLC (Shizuoka, Japan). CCR7^{−/−} C57BL/6 mice were established as previously reported [22] and bred in the animal facilities of Musashino University School of Medicine under specific pathogen-free conditions under approval of Institutional Animal experiments Committee of Musashino University. Care and use of the animals followed the guidelines of the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research. To genotype the pups, tail DNA was extracted by using a DNA Isolation Kit (GENTRA, Minneapolis, MN, USA) and examined by PCR analysis to detect the wild-type and knockout alleles. The sense and antisense primers to amplify the 600-bp CCR7 wild-type allele were 5′-CCCCGGCAATGTCTGA-3′ and 5′-CGTGTCTCGCCGCTGTT-3′, respectively. The sense and antisense alleles to amplify the 400-bp knockout allele were 5′-CTCTCGTGGGATCATTGTTTTCT-3′ and 5′-GTCTCCGCCTCATGCTTACC-3′, respectively.

2.2. Treatment

Mice were treated by intranasal administration with 40 µg of HDM extract dissolved in saline once per day for 7–10 days. The HDM extract was kindly provided by ITEA Inc., Tokyo, Japan. Mice treated with saline were used as controls.

2.3. Measurements of serum and HDM-specific IgE levels

For total IgE detection, total IgE ELISA kits (Morinaga Institute of Biological Science, Yokohama, Japan) were used following the manufacturer's instructions. For mite-specific IgE detection, plates were coated with 20 µg/ml HDM extract in 50 mM carbonate buffer (pH 9.0). After blocking the coated plates with SuperBlock (Pierce, Rockford, In.), samples were diluted 20-fold and added to the plates. Antibodies were detected with rat anti-mouse IgE specific monoclonal antibody (YAMASA, Chiba, Japan), followed by the addition of Streptavidin-HRP (Dako, Corp., Glodtrup, Denmark). The color was developed with tetramethylbenzidine + substrate (BioFz Labo., MD, USA) and the reaction stopped by addition of 1 N H₂SO₄. Optical density at 450 nm (OD₄₅₀) was measured using a microplate reader. Antibody titers were calculated by comparison with internal standards.

2.4. Bronchoalveolar lavage fluid (BALF) cell analysis

BALF cell analysis was performed 24 h after the last HDM administration, as previously reported [27]. BALF was obtained by incubating and repeatedly washing the lung with 1 ml of saline until the recovered volume was 2 ml. The isolated BALF was centri-

fuged at 1500 rpm for 10 min at 4 °C. Pellets were dissolved in 1 ml PBS and the number of cells counted. Cytospin preparations were obtained by spinning at 640 rpm for 2 min, stained with Diff Quik (International Reagents Corporation, Osaka, Japan), and then examined by microscopy.

The content of cytokine in the BALF was measured using the ELISA kits; IL-13, IL-5 and TGF-β by Quantising kit (R&D System, Inc. (Minneapolis, MN), IL-35 by specific ELISA kit (Cusabio Biotech, P.R., China). HMGB1 was measured by using an HMGB1 ELISA Kit II (Shino-test Co., Tokyo, Japan), which specifically detects HMGB1 [28].

2.5. Assessment of airway responsiveness

The assessment of airway responsiveness was undertaken 24 h after the last mite administration, as previously reported [27]. Briefly, the anesthetized mice were tracheostomized and injected with pancuronium bromide. The animals were connected to a Harvard ventilator with 0.25 ml tidal volume and a respiratory frequency of 150/min. Next, they were placed in the whole-body plethysmograph (Buxco Electronics) to measure airway resistance (Raw). To assess airway responsiveness, increasing dose of acetylcholine (ACh) (3.12–25 mg/mL) was administered by nebulization. Data are expressed as [Raw after inhalation of ACh/Raw before inhalation] × 100 (%).

2.6. Histological examination

Mice were perfused transcardially with PBS and then 4% paraformaldehyde in 100 mM phosphate buffer (pH 7.4). The lungs were removed and then immersed in 4% paraformaldehyde for 2 h at 4 °C. After washing with PBS, the specimens were soaked overnight in 30% sucrose in PBS, embedded in optimal cutting temperature (OCT) compound (Miles, Elkhart, IN, USA), and then quickly frozen. Cryostat sections were cut at a thickness of 10 µm by using a Leica CM3050 cryostat (Leica Microsystems, Wetzlar, Germany), and thaw-mounted on poly-L-lysine-coated glass slides (Matsunami Glass, Osaka, Japan). The samples were analyzed by Haematoxylin-eosin (HE) and periodic acid-Schiff (PAS) staining.

For immunohistological analysis, lung sections were preincubated overnight with anti-FoxP3 (1:1000; eBioscience, San Diego, CA) and then incubated with Alexa Fluor 488-coupled donkey anti-IgG (Molecular Probes, Eugene, OR, USA). Rat anti-mouse CD4, CD8 and CD3 antibodies (BD Pharmingen) were used for CD4, CD8 and CD3 positive cell detection. Goat anti-mouse CD4 antibody (R&D Systems) was used for double staining experiments. Sections were counterstained with 4′,6′-diamidino-2-phenylindole (DAPI; Roche Diagnostics, GmbH, Mannheim, Germany) and mounted with Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA). Control tissues were prepared by omitting primary antibodies from the incubation solutions; no specific immunoreactivity was observed. Immunofluorescence images were examined with a Leica TCS-SP2 confocal microscope (Leica Microsystems) with excitation wavelengths of 350, 488, and 543 nm. Images were collected and analyzed in Leica Confocal Software (Leica Microsystems).

2.7. CD4+CD25+ and dendritic cell isolation

Lung was homogenized in 1% penicillin/streptomycin/RPMI1640 using cell strainers with 70 µm pores (BD Falcon, NJ, USA). Specimens were then centrifuged at 1500 rpm for 10 min at 4 °C, and the cell pellets were washed twice. Lung cells were purified using Nycoprep (AXIS-SHIELD plc, Dundee, Scotland) followed by separation using CD4+CD25+ regulatory T cell isolation kits (Miltenyi Biotech GmbH, Nordrhein-Westfalen, Germany). In the

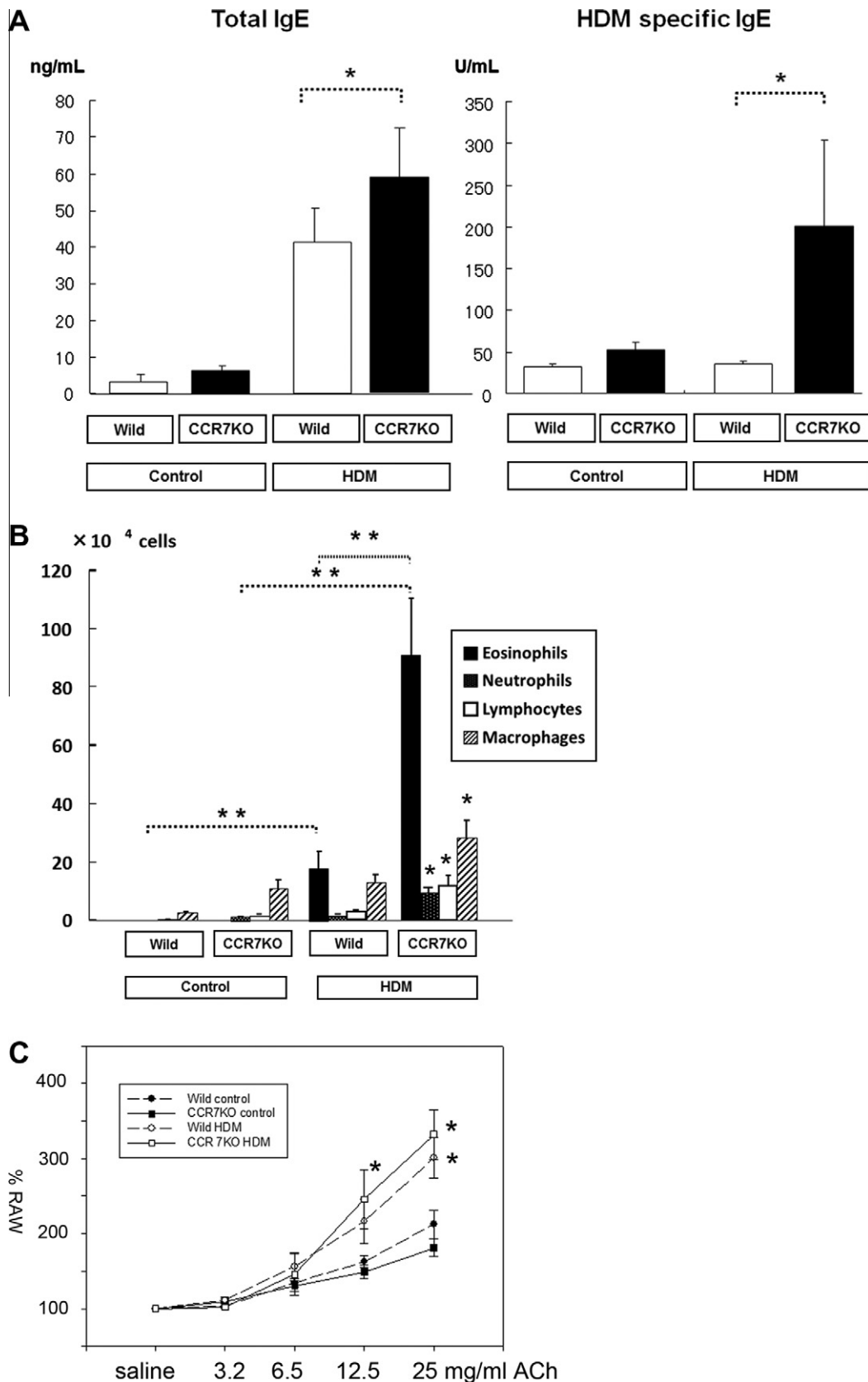


Fig. 1. Airway inflammation and airway response. (A) IgE and HDM-specific IgE synthesis. Each bar represents the mean $\pm$ SEM of values obtained from 10–12 different mice. Control indicated mice treated with saline. HDM indicated mice treated with house dust mite (HDM). * $p < 0.05$. (B) BALF cell analysis BALF cells were placed on glass slides using Cytospin. Slides were then stained with Diff Quik, and cell differentiation was assessed microscopically. Control indicated mice treated with saline. HDM indicated mice treated with HDM. The Y axis shows the number of cells in total BALF (2 mL). Each bar indicates mean $\pm$ SEM of 5–7 different mice. ** $p < 0.01$. * $p < 0.05$, CCR7KO compared with wild type mice. (C) Airway response to acetylcholine. The indicated concentrations of ACh were inhaled for 3 min. Airway resistance (Raw) was measured using whole body plethysmographs as described in the Methods. ACh-evoked changes in Raw are expressed as a percentage of the Raw observed before ACh inhalation (100%). The data shown are means $\pm$ SEM for six mice. * $p < 0.05$, HDM-treated vs control mice.

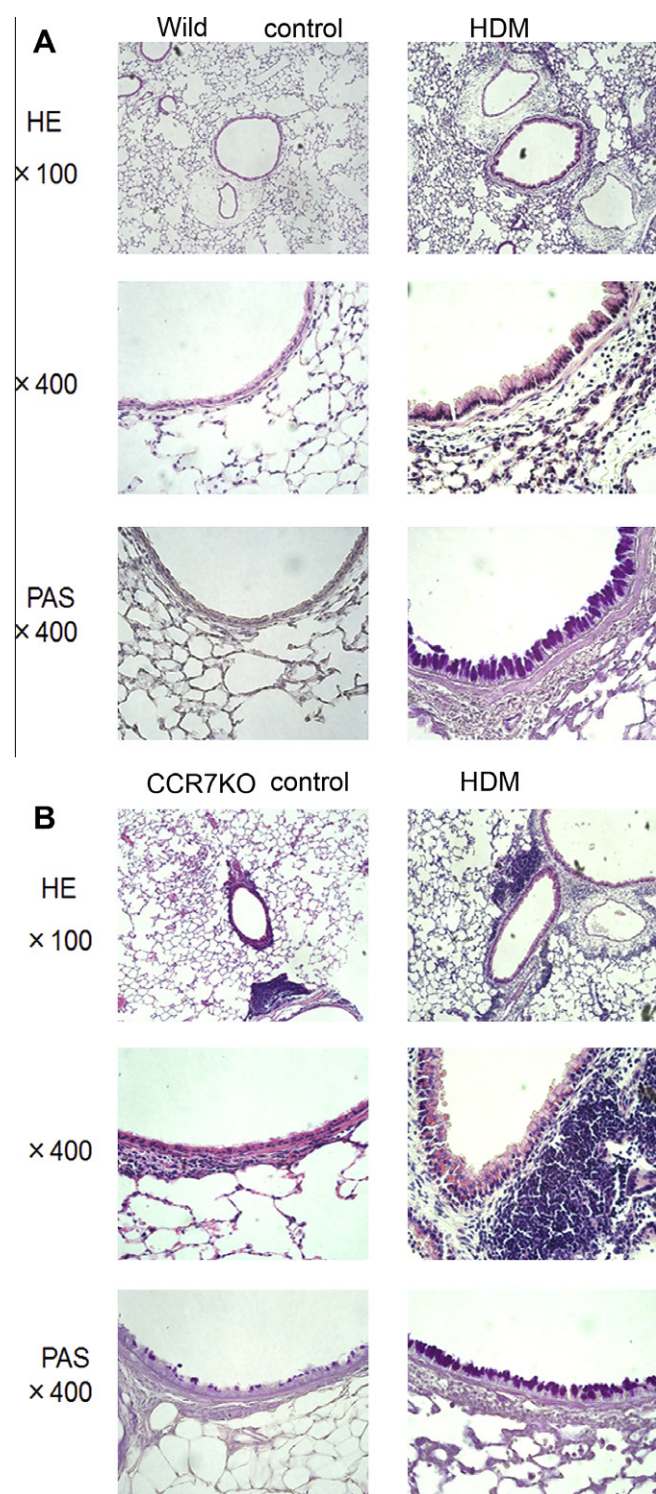


Fig. 2. Histological analysis. Histological analysis was performed with HE and PAS staining. (A) Wild type mice and (B) CCR7KO mice. Control indicated mice treated with saline. HDM indicated mice treated with house dust mite (HDM). Figures are representative data from histological examination of five mice.

first step, CD4⁺ cells were separated from all non-CD4 cells, and in the second step CD25⁺ cells were positively selected by using mAb-coated microbeads and magnetic separation columns. The purity of the resultant CD4⁺CD25⁺ cell populations was 95% (data not shown). After 2 days of culture, the supernatant was harvested.

For dendritic cells isolation, the lung cells were purified using NycoPrep (AXIS-SHIELD), followed by incubation with anti-CD11c

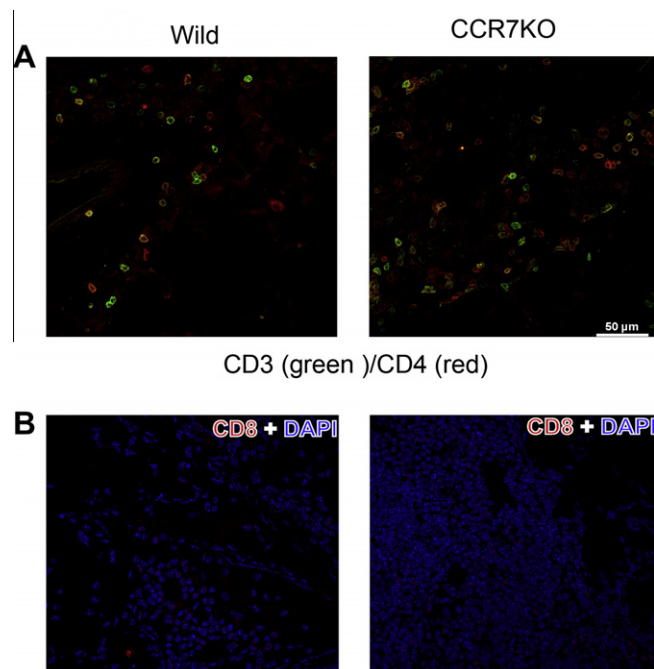


Fig. 3. Confocal micrographs. CD3 (green) and CD4 (red) (A) or CD8 (red) (B) were stained using the specific antibodies. DNA was counterstained with DAPI (blue). original magnification, 400 $\times$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mAb-coated microbeads (Miltenyi Biotec). Bead-bound cells were then isolated using magnetic separation columns. The purities of the CD11c⁺ cell populations were 90% as shown in Fig. 4.

For flow cytometric analysis, anti-mouse CD16/CD32(Fc block), CD11c, CD40, CD80, CD86, CD83 antibodies (BD Pharmingen) were used. Data were collected using FACSaria (B D Biosciences).

2.8. Examination of mRNA expression using real-time PCR

Total RNA was extracted with ISOGEN (Nippon Gene Co., Ltd., Tokyo, Japan) by a modified acid-guanidium thiocyanate-phenol-chloroform method. RNA purity was established by means of a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The RNA had an OD260/280 ratio of 1.8 indicating high purity. After measuring the amount of total RNA, cDNA synthesis was performed with a cDNA Synthesis Kit (Bio-Rad Laboratories Inc., Hercules, California, USA). The levels of mRNA were examined by real-time PCR using a Light Cycler-Fast Start DNA Master Syber Green I kit (Roche Diagnostics, Mannheim, Germany). In this system, double-stranded DNA labeled with Syber Green I is detected by real-time PCR. Quantification was performed by comparison to a standard curve generated from a serial dilution of specific PCR products. mRNA levels were standardized according to β -actin mRNA levels and were expressed as ratios of control values. The primers used were as follows: β -actin, 5'-CCTGTATGCCT-CTGGTCGTA-3' (sense) and 5'-CCATCTCCTGCTCGAAGTCT-3' (anti-sense) to amplify a 260 bp product; FoxP3, 5'-TCTTCGAGGAGCCA-GAAGAG-3' and 5'-TACTGGTGGCTACGATGCAG-3 to amplify a 240 bp product.

2.9. Statistics

For comparisons of multiple parameters, we used multiple analysis of variance (MANOVA). The level of statistical significance was set at $p < 0.05$. Data were expressed as the mean $\pm$ SEM.

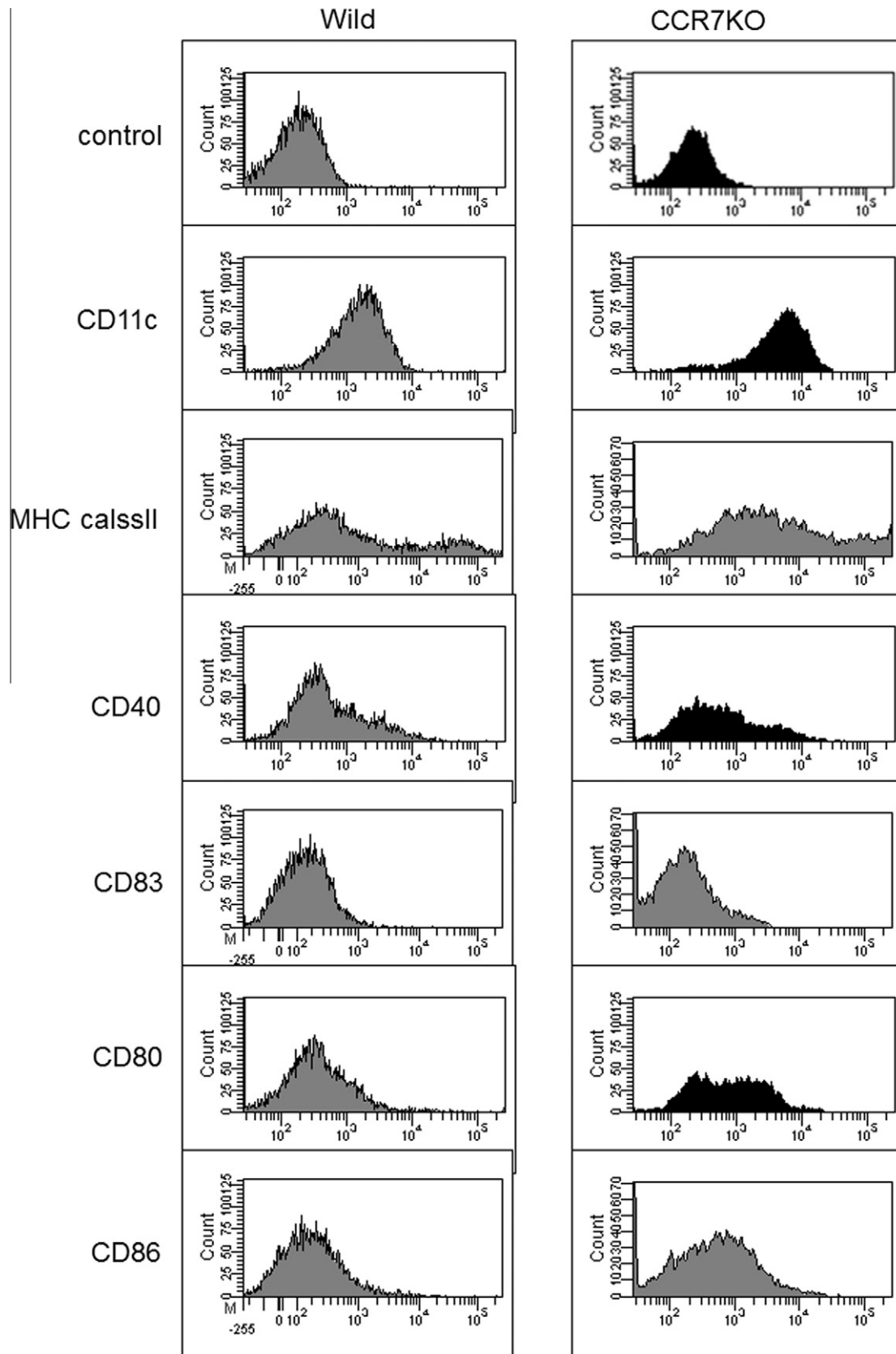
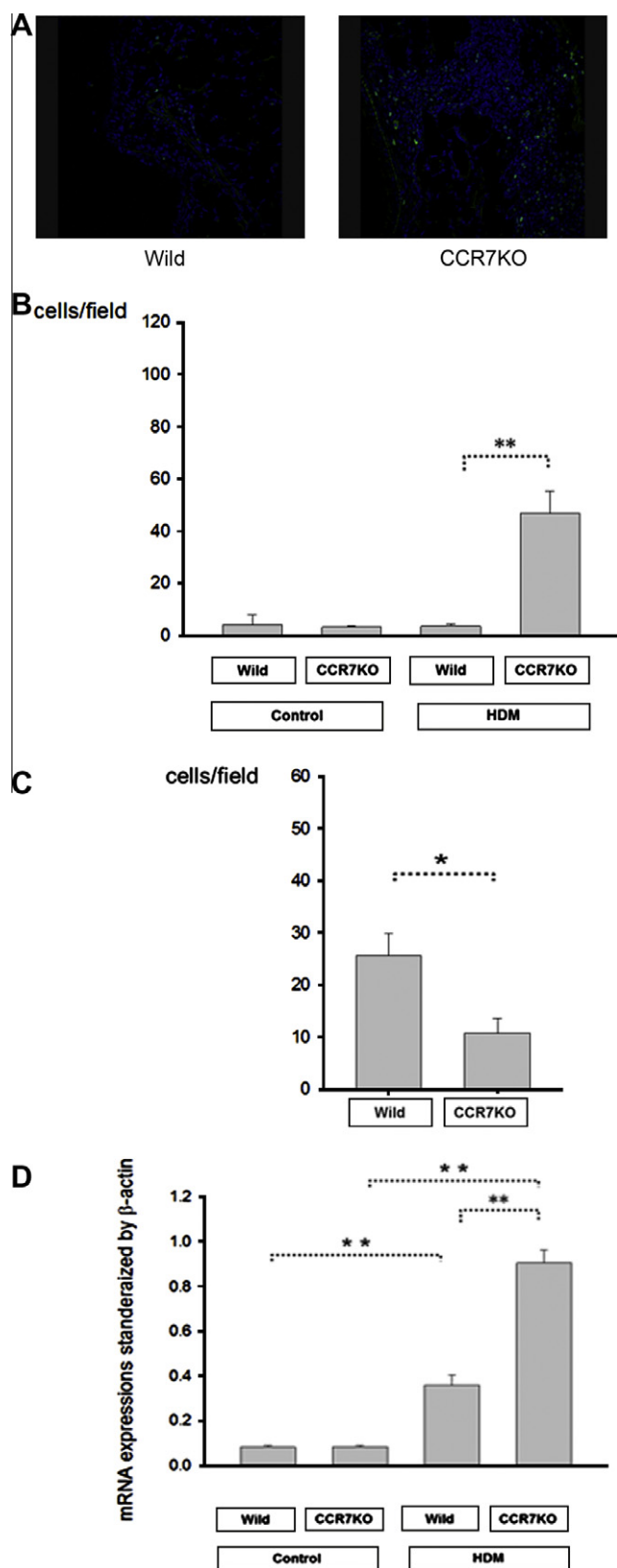


Fig. 4. Flow cytometric analysis of dendritic cells. Dendritic cells positively separated from lung were analyzed for activation marker and co-stimulatory molecules.

**Table 1**

Characteristics of CD11c positive dendritic cells in the lung.

Percentage of positive cells(%)	Wild type	CCR7KO	
MHC class II positive	43.8 (8.9)	42.1(13.9)	NS
CD40	12.6 (4.9)	11.5 (8.1)	NS
CD83	4.7 (2.3)	5.8 (3.3)	NS
CD80	15.4 (6.5)	16.4 (11.4)	NS
CD86	7.3 (4.0)	17.1 (14.1)	NS

NS: not statistically significant. The number is mean (SE), $n = 3-4$.**Table 2**

Characteristics of CD4+CD25+ cells in the lung.

	Wild type	CCR7KO	
Number of cells ($\times 10^5$)	3.4 (1.3)	11.9 (3.4)	$p < 0.001$
IL-10 production (pg/ml)	1453 (134)	1714 (6)	NS
IL-35 production (pg/ml)	190 (23)	256 (24)	NS

Number of cells was number of CD4+CD25+ cells separated from one lung. Cytokine production was from 1×10^4 cells. NS: not statistically significant. The number is Mean (SE), $n = 3-4$.

3. Results

3.1. Total and HDM-specific IgE synthesis after HDM exposure

Total and HDM-specific IgE levels were measured in serum by ELISA to assess the allergic sensitization induced by inhalation of HDM. Inhaled HDM induced IgE synthesis ($p < 0.01$ compared to control) and that IgE synthesis was significantly augmented in CCR7KO mice compared to the wild type ($p < 0.01$) (Fig. 1A). HDM-specific IgE was also elevated in CCR7KO mice treated with HDM. These results indicate that continuous HDM inhalation induced systemic allergic sensitization in both the wild type and CCR7KO mice and that the level of sensitization was higher in CCR7KO mice.

3.2. Role of CCR7 in HDM-induced airway inflammation

We analyzed the infiltration of inflammatory cells into the BALF to examine the role of CCR7 in HDM-induced airway inflammation (Fig. 1B). Significant infiltration of eosinophils and macrophages was observed in mice exposed to HDM compared to controls a (Fig. 1B, $p < 0.01$). The number of eosinophils, neutrophils, lymphocytes and macrophages were significantly higher in CCR7KO mice than in the wild type ($p < 0.01$ for eosinophils, $p < 0.05$ for neutrophils, lymphocytes and macrophages).

Airway responsiveness was also analyzed. CCR7KO mice exhibited significant higher response compared with untreated mice at 12.5 and 25 mg/ml ACh. Wild type mice showed significant response at 25 mg/ml ACh (Fig. 1C).

Histological analysis by HE staining revealed that HDM exposure caused more severe airway inflammation in CCR7KO mice than in the wild type (Fig. 2). As shown by PAS staining, there were significantly more mucus-producing cells in the airways of CCR7KO mice than in the wild type (Fig. 2). These data indicate that HDM induced severe airway inflammation in CCR7KO mice. We also observed prominent lymphoid aggregates in the bronchioles of CCR7KO mice (Fig. 2). Immuno-histochemical examination revealed that these aggregates were composed predominantly of CD4+CD3+ cells and contained a few CD4 single positive cells and few CD8+ cells (Fig. 3).

Fig. 5. FoxP3 expression. (A) Confocal micrographs. FoxP3 (green) was stained using the specific antibodies. DNA was counterstained with DAPI (blue). (B) Number of FoxP3 positive cells in lung sections examined by confocal microscopy. (C) Number of FoxP3 positive cells in lymph node. (D) FoxP3 mRNA expression in the lung. * $p < 0.05$, ** $p < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Dendritic cells play a key role for cell differentiation and antigen presentation [5]. When dendritic cell immobilize antigen, their phenotype changes to mature type and express CCR7 to migrate secondary lymph node [23,29,30]. We examined the mature status of dendritic cells in the lung by flow cytometric analysis. As shown in the Fig. 4, dendritic cells with wild type and those from CCR7KO mice expressed some amount of MHC class II, CD40, CD80, CD83 and CD86 molecules. The amount of expression varied but there were no significant difference between dendritic cells with wild type and those from CCR7KO mice (Table 1). These data suggested that dendritic cells acquired some activation without CCR7 expression.

CCR7 expression is critical for T cells to exit peripheral tissues [31,32]. FoxP3 positive T cells, which are known to suppress airway inflammation, express CCR7 and are activated by pulmonary dendritic cells within secondary lymph nodes [26,33]. We next examined FoxP3 expression in the lung. The results from immuno-histochemical analyses using Foxp3 antibodies are shown in Fig. 5A. In lung sections, we observed significantly more FoxP3-positive cells in CCR7KO mice than in the wild type (Fig. 5B). In the lymph node, however, there were significantly more FoxP3-positive cells in the wild type than in CCR7KO mice (Fig. 5C). FoxP3 mRNA expression was also significantly higher in CCR7KO mice than in the wild type in the lung (Fig. 5D).

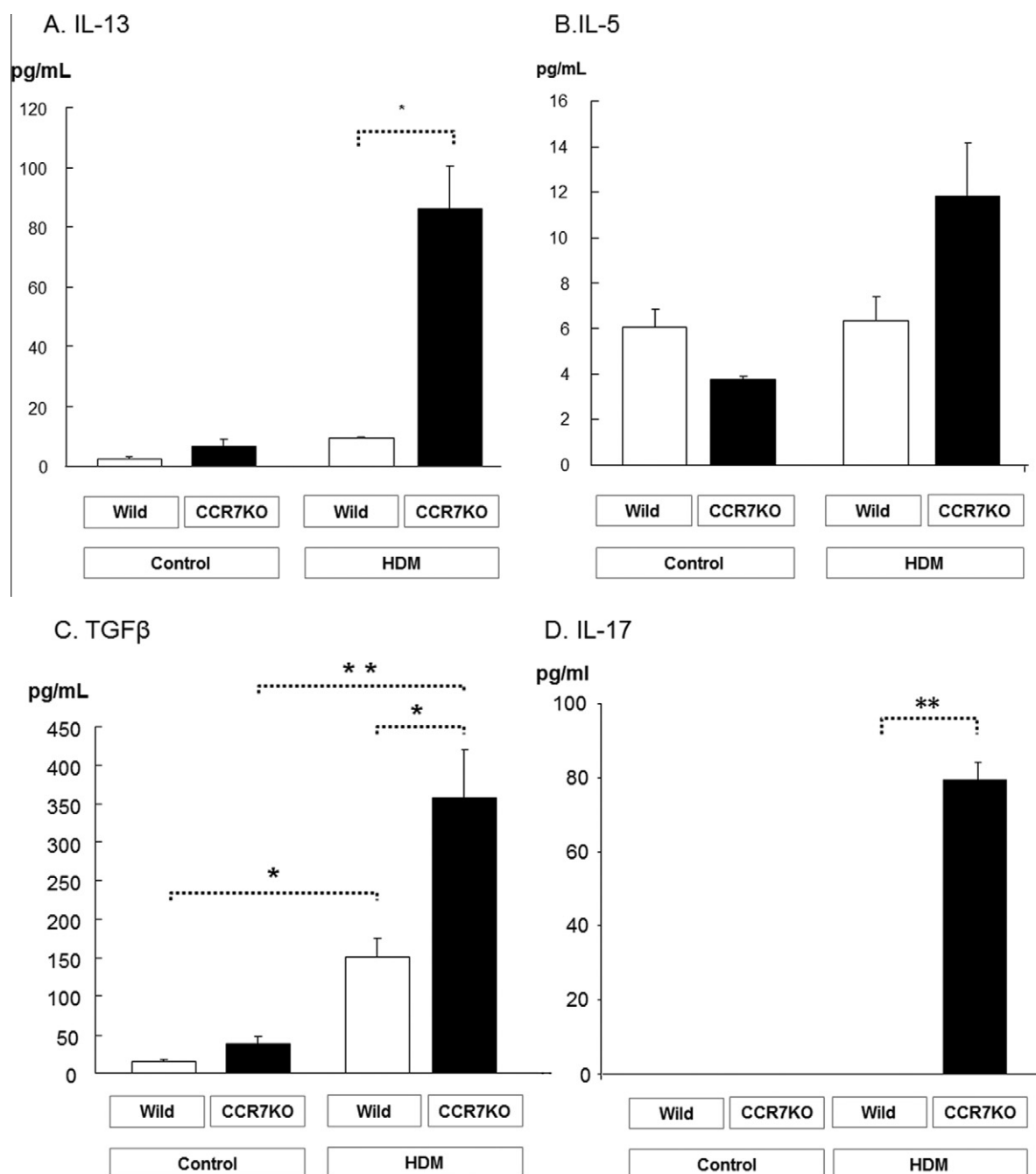


Fig. 6. Analysis of cytokine production in the BALF. BALF supernatants were analyzed for cytokine production by cytokine-specific ELISA. Each bar indicates mean $\pm$ SEM of 5–8 different mice. * $p < 0.05$, ** $p < 0.01$.

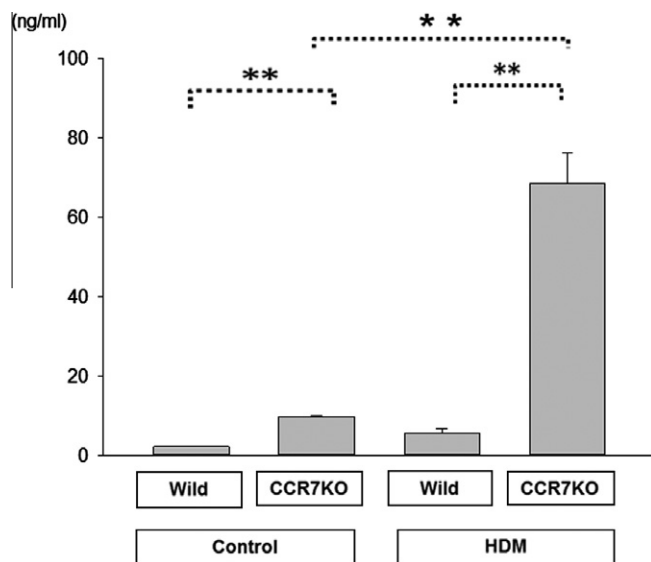


Fig. 7. HMGB1 production in the BALF. BALF supernatants were analyzed for HMGB1 production by specific ELISA. Each bar indicates mean $\pm$ SEM of four different mice. ** $p < 0.01$.

To further examine the Treg cell function in the lung, we separated Treg cell using CD4+CD25+ cell selection kits. The number of CD4+CD25+ cells was significantly increased in CCR7KO mice compared to wild type mice (Table 2). To examine the regulatory function of CD4+CD25+ cells, we examined the synthesis of IL-10 and IL-35, which is reported the one of the key cytokine of regulatory function [34,35]. When we examined the cytokine production, CD4+CD25+ cells produced equal amount of IL-10 and IL-35 in wild type and CCR7KO mice (Table 2).

3.3. Role of CCR7 in HDM-induced cytokine production

We examined cytokine production in BALF in CCR7KO and wild-type mice to explore the type of airway inflammation induced by HDM in the absence of CCR7. Levels of the Th2 cytokines, IL-13 and IL-5, were significantly higher in CCR7KO mice than in the wild type (Fig. 6A and B). By comparison, levels of the Th1 cytokine, IFN- γ , were below the detection limit (9.4 pg/ml, $n = 6$ for each group), suggesting that the inflammation in CCR7KO mice is Th2-dominant allergic inflammation. We also analyzed the expression levels of the regulatory cytokines TGF- β and IL-17 [36] and found that both were expressed at higher levels in CCR7KO mice than in the wild type (Fig. 6C and D). The formation of lymphocyte aggregates in the CCR7KO mice is consistent with the increased expression of IL-17 in these mice compared to the wild type and with the role of IL-17 in the development of inducible bronchus-associated lymphoid tissue (iBALT) [37].

Next, we examined HMGB1 production in the BALF of CCR7KO and wild-type mice. HMGB1 is a critical mediator of the innate immune response to inflammation and infection [38–40]. HMGB1 is also a regulator of Treg cells [41,42]. We found that HMGB1 production is significantly higher in CCR7KO mice than in the wild type, suggesting that innate immune response is augmented and Treg cell function is depressed in CCR7KO mice ($p < 0.01$, Fig. 7).

4. Discussion

In the present study, we showed that continuous HDM exposure induced allergic airway inflammation, which was augmented in CCR7KO mice. The prominent lymphoid aggregates observed in the bronchioles of CCR7 KO mice were predominantly composed of

CD4+ cells, but also contained a few CD8+ cells. We also observed FoxP3-positive cells. Although there was an increased number of FoxP3-positive cells in the lung of CCR7KO mice compared to wild-type mice, the levels of Th2 cytokines as well as IL-17 and TGF- β were also increased. In addition, HMGB1 production was also increased in CCR7KO mice. These results suggest that CCR7-deficient FoxP3-positive cells, which down-regulate allergic airway inflammation [43,44], did not function efficiently in CCR7KO mice.

Intra-tracheal continuous HDM exposure to mice induces the asthmatic phenotype [11,12,45]. In the current study, we also found that HDM exposure by inhalation induced IgE responses. These responses were increased in CCR7KO mice, which was consistent with the increase in systemic immunization seen in CCR7KO and plt mice [8,23,46]. CCR7 is a receptor of CCL21 and CCL19 that plays a critical role in the development of secondary lymph nodes [22,47]. CCR7, which is expressed by dendritic cells and T cells, plays a critical role in the movement of lymphocyte and dendritic cells from peripheral tissue into afferent lymphatics [32,47]. Disruption of this process in CCR7KO mice causes lymphocytes to accumulate in the tissue of these mice [32] and is the reason why HDM exposure caused lymphoid aggregates to form in their bronchioles.

Another reason for T cell accumulation in CCR7KO mice may be related to an impairment of Treg cells in these mice [33]. CCR7KO mice accumulate BALT from day 5 after birth [33]. Treg cells suppress effector T cell function by homing to lymphoid organs, which is confirmed by the fact that Treg cells from wild-type mice work perfectly in CCR7KO mice [33]. Consistent with the previous report that CCR7-deficient T reg cells work normally in vitro [10], we found that Treg cells, present in the BALT, produced IL-10 and IL-35 in vitro. We also found that dendritic cells in the lung of CCR7KO mice expressed activation makers such as MHC class II and co-stimulatory molecules. It is possible that the structure of BALT may not be sufficient for FoxP3 positive cell to possess suppressive function with CCR7 deficient condition. In the case of influenza infection, iBALT, which is essential for local immunity to influenza, is not efficiently formed in the absence of CCL21 and CCL19 [48]. Recently, Ishimaru et al. have reported that CCR7 negative FoxP3-positive cells have defects in the c-Jun and FoxP3 interaction [24]. Thus, it is also possible that FoxP3-positive cells in CCR7KO mice possess defects in local migration to exhibit regulatory function in iBALT. To confirm the Treg function of CCR7KO mice in allergen sensitization, we should precede the experiments by transfusion of Treg cells in vivo and precise functional analysis in vitro, which is continuing subject to be solved.

HDM is known to accelerate not only antigen-specific immunity but also innate immunity through the TLRs [19,20]. TLR, TLR2, and TLR4 are thought to be required to induce allergic inflammation [19,20]. A recent finding of homology between Derf2 and MD2, the relation to TLR4 has been stressed, because MD2 is critical for signal transduction through TLR4 [20]. HMGB1 was produced in CCR7KO mice as an accelerated innate immune response. Beyond its role in maintaining chromosomal integrity, HMGB1 is secreted as a cytokine and is considered an alarmin [40].

In conclusion, we provide results here to highlight CCR7 expression as a promising target for regulation of HDM-induced innate immunity and antigen responses. Further studies are needed to develop the targeted control of CCR7 expression as immunotherapy for asthma.

Acknowledgments

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