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The Nonantibiotic Macrolide EM900 Attenuates House Dust Mite-Induced Airway Inflammation in a Mouse Model of Obesity-Associated Asthma

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Keywords
Asthma · Obesity · EM900 · House dust mite · Macrolide

Abstract
Introduction: Obesity-associated asthma is characterized by type 2-low airway inflammation. We previously showed that EM900, which is a 12-membered nonantibiotic macrolide, suppressed airway inflammation in a mouse model of asthma exacerbation. The aim of this study was to clarify the effects of EM900 in obesity-associated asthma. ***Methods:*** BALB/c mice were fed a low-fat diet (LFD) or high-fat diet (HFD). Mice were intranasally sensitized and challenged with house dust mites (HDMs) and were orally administered EM900. Airway inflammation was assessed using inflammatory cells in bronchoalveolar lavage (BALF). Cytokines were examined by ELISA in lung tissues. Lung interstitial macrophages ($CD45^+$, $CD11c^{low}$, $CD11b^+$, and $Ly6c^-$) were counted by flow cytometry in single cells from lung tissues. ***Results:*** Body weight increased significantly in the HFD compared with the LFD group. The total cell count and numbers of neutrophils and eosinophils in BALF were significantly suppressed by EM900 administration in the HFD-HDM group. The levels of interleukin (IL)-17A were increased in the HFD-HDM group compared with the LFD-HDM group, although the difference did not reach statistical significance. The levels of IL-17A, macrophage inflammatory protein 2, IL-1β, IL-5, and regulated on activation, normal T cell expressed and secreted in lung tissue were significantly suppressed by EM900 administration in the HFD-HDM group. The percentage of interstitial macrophages in lungs was significantly decreased by EM900 administration in the HFD-HDM group. ***Conclusion:*** Both type 2 and type 2-low airway inflammation were attenuated by EM900 in this obesity-associated asthma model. These results show that EM900 might be a candidate agent for the treatment of obesity-associated asthma.

Introduction

Asthma is characterized by airway inflammation and airway hyperresponsiveness [1]. Airway inflammation is mainly associated with type 2 cytokines, such as interleu-

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kin (IL)-4, IL-5, and IL-13 [2]. Inhaled corticosteroids and/or long-acting beta 2 agonists have contributed to disease control in asthmatics in recent times [3]. However, approximately 10% of patients with asthma are reported to have severe asthma, which is defined as frequent exacerbations or poor asthma control in spite of high doses of inhaled corticosteroids [4, 5]. The factors related to severe asthma are current smoking, complicating eosinophilic rhinosinusitis, gastroesophageal reflux, and obesity [6].

Obesity is a common comorbidity in patients with severe asthma. A previous study showed that 57% of patients with severe asthma are obese, compared to obesity rates of 35% in the general population in the USA [7]. Gibson et al. also reported that 48% of severe asthmatics are obese compared to an obesity rate of 25% in the general population in England [8]. Furthermore, obesity is associated with a higher risk of hospitalization, higher risk of mechanical ventilation, and longer hospital stays in patients with severe asthma [9–11]. Severe asthma in obese individuals is related to poor asthma control, frequent exacerbations, and greater likelihood of 30-day readmission compared to normal-weight asthmatic patients [12, 13]. Several studies have shown that obesity seems to polarize asthma patients toward a neutrophil dominant rather than eosinophil-dominant inflammatory phenotype. Previous studies have shown that sputum neutrophils and IL-17A, but not eosinophils, were higher in obese than nonobese asthmatics [14, 15].

Although molecular targeted biologic therapies, including immunoglobulin E, IL-5, and IL-4/IL-13, have been developed for clinical use in asthma [16–19], the treatment for type 2-low inflammation is not yet adequately known. Several studies reported the efficacy of macrolides in patients with asthma. For example, clarithromycin contributed to an increase in symptom-free days and reduction of days with loss of control in pediatric patients with asthma [20]. Azithromycin decreased the frequency of asthma exacerbations among patients with adult asthma and improved quality of life (QoL) in both type 2 and type 2-low asthma [21]. However, it remains unclear whether macrolides are effective in obesity-associated asthma. Additionally, long-term use of macrolides for asthma might induce antibiotic resistance in bacteria. EM900 is a 12-membered nonantibiotic macrolide derived from erythromycin that has been found to exert potent anti-inflammatory and immunomodulatory effects [22]. We previously reported that the nonantibiotic macrolide EM900 has the potential to inhibit virus-associated asthma exacerbation in mice [23]. The present study investigated the effects and mechanisms of activity of EM900 in type 2-low airway inflammation related to obesity in mice.

Materials and Methods

Allergens and Chemicals

House dust mite (HDM) extracts of *Dermatophagoides farinae* were purchased from Institute of Tokyo Environmental Allergy (ITEA, Tokyo, Japan) and (8R,9S)-8,9-dihydro-6,9-epoxy-8,9-anhydropseudoerythromycin A (EM900), provided by Kitasato University, was dissolved in dimethyl sulfoxide and diluted in PBS. Palmitic acid (PA) (Sigma-Aldrich, Saint Louis, MO, USA) was dissolved in 50% ethanol at 60°C to yield a 50 mm stock concentration. PA was diluted to the appropriate concentration using 1% fatty acid-free bovine serum albumin (BSA) at 37°C. LPS (Sigma-Aldrich, Saint Louis, MO, USA) was dissolved in PBS.

Mice

Six-week-old female BALB/c mice (Japan SLC, Hamamatsu, Japan) were kept at the Saga University Animal Facility under specific pathogen-free conditions.

Feeding of a Low-Fat Diet or High-Fat Diet

Starting at 6 weeks of age, female mice were fed either a low-fat diet (LFD) or high-fat diet (HFD) for 11 weeks. The LFD (D12450J; Research Diets Inc., New Brunswick, NJ, USA) provided 10% of energy in the form of fat and the HFD (D12492; Research Diets Inc.) provided 60% of energy in the form of fat. Body weight was measured every week.

Protocol for HDM-Induced Airway Inflammation in Mice with LFD or HFD

After 8 weeks of LFD or HFD intake, airway sensitization was achieved by intranasal administration of 25 μg HDMs, or PBS was administered as a control, on days 1, 8, and 15. Exposure was achieved by intranasal administration of 10 μg HDMs or PBS on days 22, 23, and 24. Next, the mice were orally administered either a placebo (PBS containing dimethyl sulfoxide) or 25 mg/kg EM900 on days 21, 22, 23, and 24. The placebo or EM900 was administered 2 h before administration of PBS or HDMs on days 22, 23, and 24. Finally, the mice were divided into 6 groups: LFD-PBS-placebo (LFD-control), LFD-HDM-placebo (LFD-HDM), LFD-HDM-EM900 (LFD-EM900), HFD-PBS-placebo (HFD-control), HFD-HDM-placebo (HFD-HDM), and HFD-HDM-EM900 (HFD-EM900). Mice in all these models were euthanized by intraperitoneal injection of midazolam, medetomidine, and butorphanol 24 h after the final HDM exposure on day 25, and their bronchoalveolar lavage fluid (BALF), and lung tissues were collected for further analyses (Figs. 1a, 2a).

Collection of BALF

BALF samples were collected as described previously [24]. Briefly, a 23-G tube was inserted into the trachea, and lung lavage was performed twice, each with 1 mL of saline. The acquired cell suspension was centrifuged at 100 *g* for 5 min at 4°C. The total number of cells was counted using a hemocytometer. Cytospin

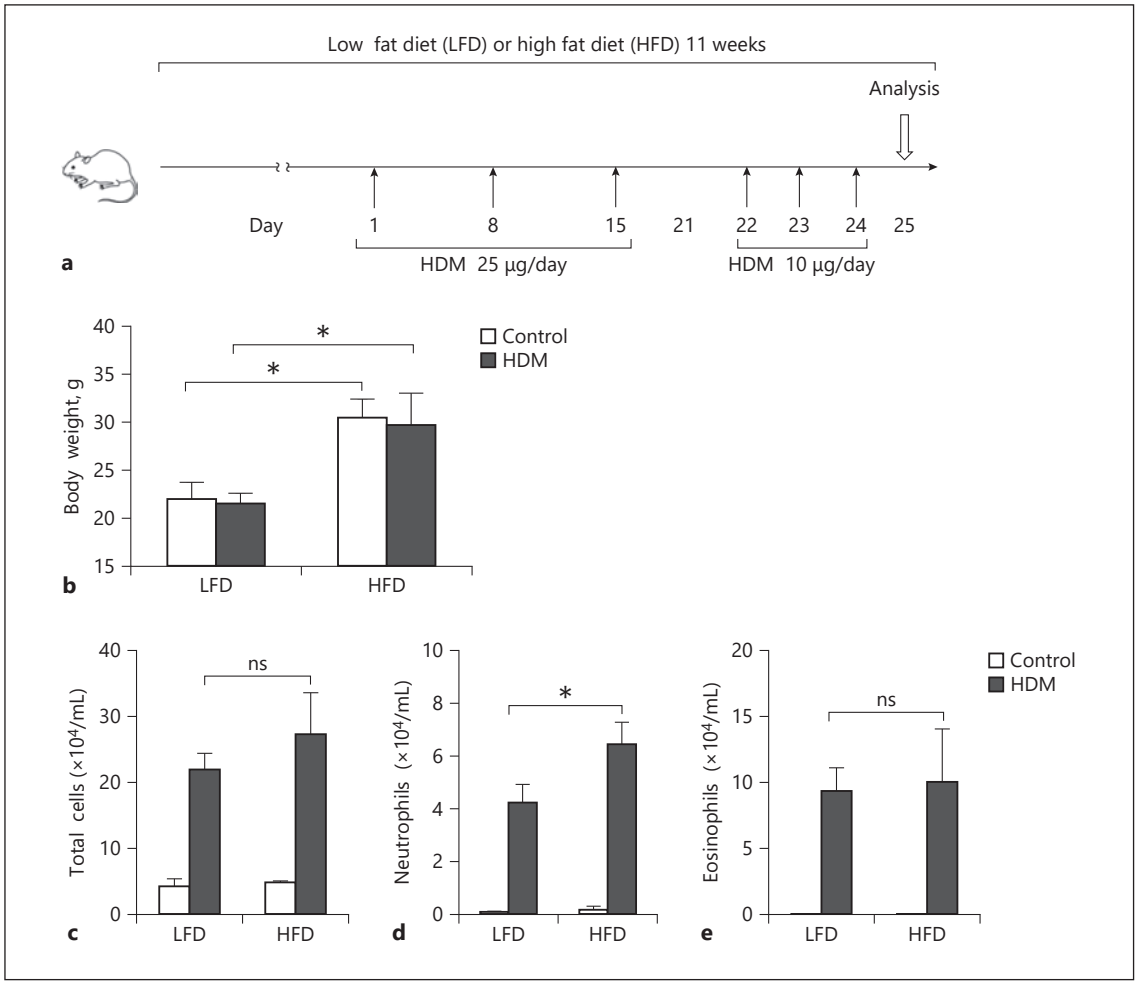


Fig. 1. HDM-induced neutrophilic airway inflammation in a mouse model of HFD-induced obesity. **a** Protocol for HDM-induced airway inflammation in mice with an LFD or HFD. **b** Body weight after 11 weeks of an LFD or HFD ($n = 6$ in each group). BALF fluid analysis for counts of total cells (**c**), neutrophils (**d**), and eosinophils (**e**) among LFD-control, LFD-HDM, HFD-control, and HFD-HDM mice ($n = 6$ in each group). The bar graphs present the mean ± SD of 4 independent experiments. *$p < 0.05$. ns, not significant; BALF, bronchoalveolar lavage; HDM, house dust mite; HFD, high-fat diet; LFD, low-fat diet.

samples were prepared from the cell suspension. Cell differentiation was determined by counting at least 300 leukocytes in samples stained with Diff-Quik (Siemens, Munich, Germany).

Histological Examination of Lung Sections

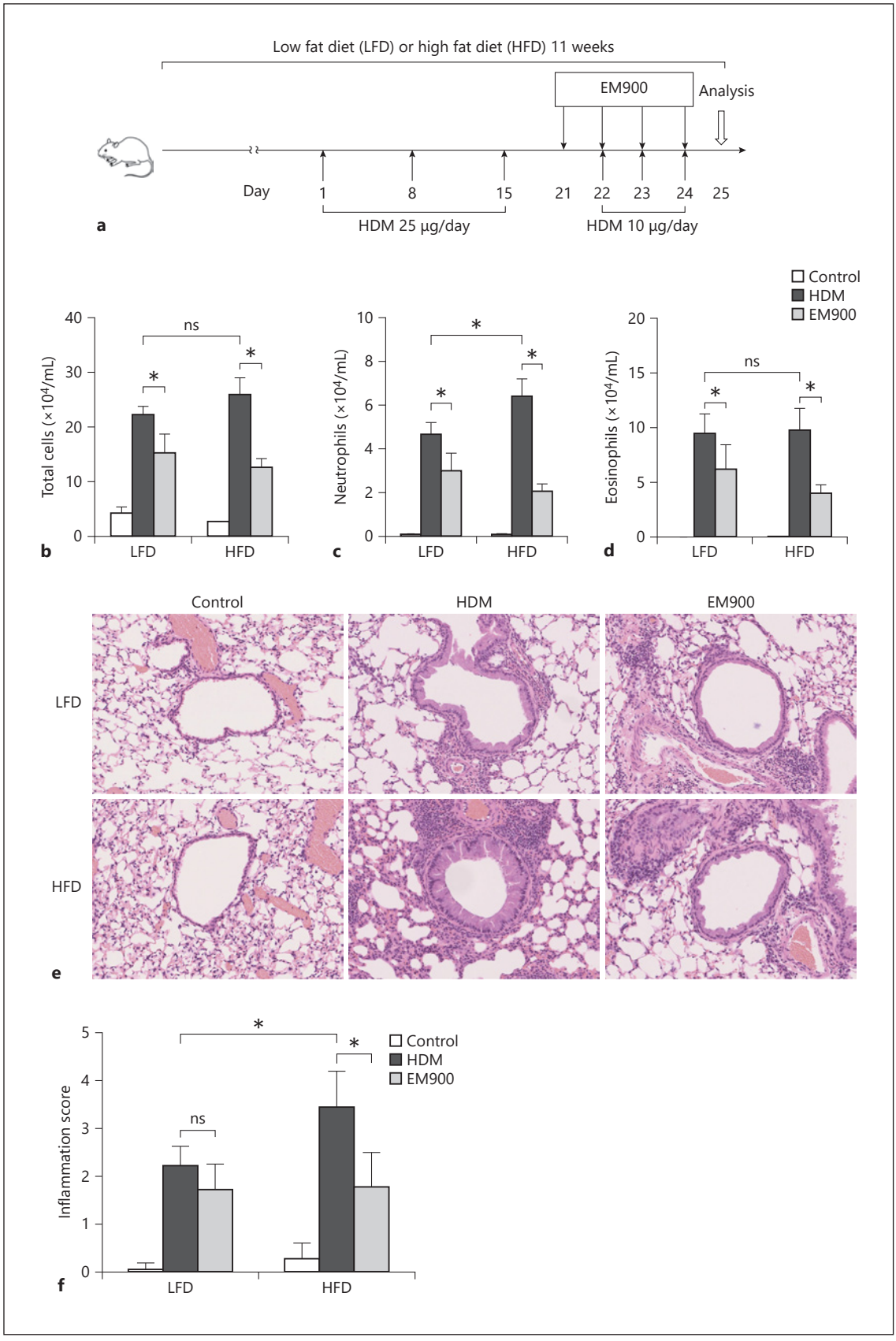
Histological examinations were performed as previously reported [25]. Lungs were fixed in 10% neutral-buffered formalin (Wako Chemicals, Osaka, Japan) and embedded in paraffin. Lung sections were stained with hematoxylin and eosin. Slides were examined in a blinded fashion by 3 experienced observers, as described previously [26]. For each slide, 6 randomly chosen areas were scored. Peribronchial and perivascular inflammation was scored in a semiquantitative fashion on hematoxylin and eosin slides. Scoring of inflammation was as follows: 0 = none; 1 = minimal; 2 = slight; 3 = moderate; and 4 = severe inflammation.

Preparation of Lung Homogenates

After BAL, the lung was isolated and homogenized in 50 mM Tris-buffered saline (pH 7.4) containing 1.0% Triton X-100, 0.1% sodium dodecyl sulfate, 150 mM sodium chloride, 0.5% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, 1 µg/mL aprotinin, 1 µg/mL leupeptin, and 1 mM Na_3VO_4 [27]. Lung homogenates were centrifuged at 10,000 *g* for 15 min, and supernatants were collected and stored at −80°C until needed.

Isolation of Single Cells from Lung Tissue

Lung tissue was cut into small pieces that were transferred through a 70-µm mesh before processing in a digestion buffer that included 0.02 mg/mL deoxyribonuclease I (Invitrogen, Waltham, MA, USA) and 0.7 mg/mL collagenase type 2 (Worthington, Lakewood, NJ, USA). The remaining red cells were lysed using BD



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Pharm Lysis (BD Biosciences, San Jose, CA, USA) to obtain single-cell suspensions [28].

Flow Cytometry

Single-cell suspensions were preincubated with FcγR-specific blocking antibody and washed before staining. Cells were stained with CD45 (clone: 30-F11), CD11c (clone: N418), CD11b (clone: M1/70), and Ly6c (clone: HK1.4) (eBioscience, San Diego, CA, USA) before collection on a flow cytometer (FACS Verse; BD Bioscience, Franklin Lakes, NJ, USA) and analyzed using FlowJo 8.3.3 software (Tree Star, Ashland, OR, USA).

Cell Culture of Peritoneal Macrophages (Peritoneal Exudate Cells)

To obtain peritoneal exudate cells (PECs), mice were injected intraperitoneally with 2 mL thioglycollate (3%) [29]. After 4 days, peritoneal fluid was obtained by lavage with 10 mL PBS. The fluid was centrifuged to isolate peritoneal macrophages, which were resuspended in RPMI 1640 medium. These cells were cultured at a density of 1×10^6 cells in RPMI 1640 containing fetal calf serum, and the macrophages were analyzed by ELISA.

Quantification of Cytokines Using ELISA

IL-17A, macrophage inflammatory protein 2 (MIP-2), IL-1β, regulated on activation, normal T cell expressed and secreted (RANTES), IL-5, and IL-13 were measured from lung homogenates using ELISA kits (R&D Systems, Minneapolis, MN, USA), according to the manufacturers' instructions. PECs were stimulated with 500 μm PA and 2 ng/mL LPS, and 50 μg/mL EM900 was added. After 24 h of stimulation, monocyte chemoattractant protein 1 (MCP-1), IL-1β, tumor necrosis factor α (TNF-α), MIP-2, and RANTES were measured from PEC culture supernatants using ELISA.

Statistical Analysis

ANOVA was used for multiple comparisons of continuous variables. When a significant difference was identified, the difference between each group was tested using the nonparametric Mann-Whitney *U* test. All tests were 2-sided, and significance was set at the level of $p < 0.05$. Data were analyzed using JMP Pro version 14 (SAS Institute Japan, Tokyo, Japan).

Fig. 2. EM900 suppressed HDM-induced airway inflammation. **a** Protocol for HDM-induced airway inflammation treated with EM900 in mice receiving an LFD or HFD. **b–d** BALF fluid analysis for counts of total cells (**b**), neutrophils (**c**), and eosinophils (**d**) among LFD-control, LFD-HDM, LFD-EM900, HFD-control, HFD-HDM, and HFD-EM900 mice ($n = 6$ in each group). **e** Histological examination for airway inflammation. Sections were stained with HE. Original magnification, ×200. **f** Slides were scored for peribronchial inflammation and airway mucosal hyperplasia, using a semiquantitative score from 0 to 4. The HE-stained slides were histologically scored as follows: 0 = none; 1 = minimal; 2 = slight; 3 = moderate; or 4 = severe inflammation. The bar graphs present the mean ± SD of 6 independent experiments. $*p < 0.05$. ns, not significant; BALF, bronchoalveolar lavage; HDM, house dust mite; LFD, low-fat diet; HFD, high-fat diet; HE, hematoxylin and eosin.

Results

HDM-Induced Neutrophilic Airway Inflammation Increased in HFD Mice

Body weight was significantly greater in HFD groups compared with LFD groups (Fig. 1b). The total number of cells, and numbers of neutrophils and eosinophils in BALF were significantly increased by HDMs in both HFD and LFD mice, with the number of neutrophils showing a significantly greater increase in the HFD-HDM group than the LFD-HDM group, although the total number of cells and number of eosinophils were not significantly different between HFD-HDM and LFD-HDM groups (Fig. 1c–e).

EM900 Suppressed HDM-Induced Airway Inflammation

We investigated the effects of EM900 in a mouse model of HDM-induced airway inflammation with LFD or HFD. The total number of cells and number of neutrophils and eosinophils in BALF were significantly suppressed by EM900 administration in both LFD and HFD groups (Fig. 2b–d). Pathologic examination of the lungs revealed marked infiltration of inflammatory cells with HDM exposure in both LFD and HFD groups. Inflammatory cell infiltration was significantly augmented by an HFD and was attenuated by EM900 (Fig. 2e–f).

EM900 Suppressed Both Type 2 and Type 2-Low Airway Inflammation

Levels of cytokines in lung tissues, measured using ELISA, showed significantly increased concentrations of IL-17A, MIP-2, IL-1β, IL-13, IL-5, and RANTES with HDM exposure in both LFD and HFD groups (Fig. 3a–f). The concentrations of IL-17A showed a greater increase in the HFD-HDM group compared with the LFD-HDM group, although the difference was not statistically significant. Concentrations of IL-17A, MIP-2, IL-1β, IL-5, and RANTES were significantly suppressed by EM900 administration in the HFD-HDM group. On the other hand, concentrations of only IL-1β and RANTES were significantly suppressed by EM900 administration in the LFD-HDM group (Fig. 3a–f). These results suggested that EM900 suppressed not only type 2-low neutrophilic airway inflammation, but also type 2 eosinophilic airway inflammation. Concentrations of both type 2 and type 2-low cytokines in BALF were significantly increased by HDM administration. The levels of these cytokines in BALF were not suppressed by EM900 administration, except for CXCL5 and RANTES in the LFD-HDM group

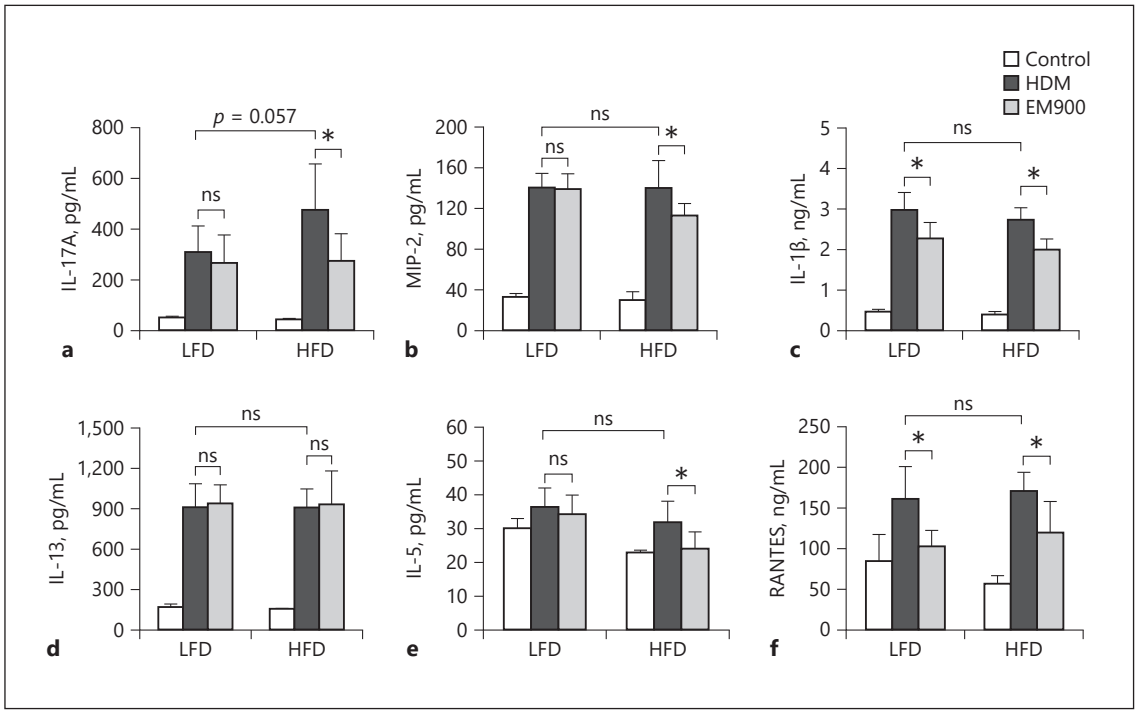

Fig. 3. EM900 suppressed both type 2 and type 2-low cytokines in lung tissues. Concentrations of IL-17A (**a**), MIP-2 (**b**), IL-1β (**c**), IL-13 (**d**), IL-5 (**e**), and RANTES (**f**) in lung tissues were measured by ELISA (n = 6–7 in each group). The bar graphs present the mean ± SD of 6 independent experiments. *$p < 0.05$. ns, not significant; HDM, house dust mite; LFD, low-fat diet; HFD, high-fat diet; IL, interleukin; MIP-2, macrophage inflammatory protein 2; RANTES, regulated on activation, normal T cell expressed and secreted.

and CXCL1 in the HFD-HDM group (see online suppl. Table 1; see www.karger.com/doi/10.1159/000508709 for all online suppl. material).

Recruitment of Interstitial Macrophages into the Lungs Was Attenuated by EM900

To clarify the mechanisms underlying the effects of EM900, we examined lung single cells by flow cytometry. Lung-resident macrophages were classified as interstitial macrophages and alveolar macrophages [30]. We defined $CD45^+$, $CD11c^{low}$, $CD11b^+$, and $Ly6c^-$ cells as lung interstitial macrophages, with reference to previous reports [31, 32]. The percentage of interstitial macrophages in the lungs was significantly increased by HDM administration and was significantly decreased by EM900 administration in the HFD-HDM group (Fig. 4a, b). We examined the production of MCP-1 from macrophages in vitro. PA was used as it was the prevalent saturated fatty acid in the HFD. Concentrations of MCP-1 were significantly increased by LPS with PA stimulation and were significantly suppressed by addition of EM900 in PECs (Fig. 4c). These results suggested that EM900 attenuated the recruitment of interstitial macrophages into the lungs in the HFD-HDM group via suppression of MCP-1 production from macrophages.

EM900 Suppressed the Production of LPS-Induced Proinflammatory Cytokines in vitro

To clarify the interaction between EM900 and macrophages, we used in vitro assays of EM900 and macrophages using PA and LPS as above. Concentrations of IL-1β, TNF-α, MIP-2, and RANTES in PECs were significantly increased by LPS stimulation and were significantly suppressed by EM900. Concentrations of IL-1β, TNF-α, and MIP-2 were significantly increased by LPS with PA compared with LPS without PA (Fig. 5a–d).

Discussion

The present study demonstrated that both type 2 and type 2-low airway inflammation was attenuated by EM900 in an obesity-associated asthma model. EM900 decreased the number of eosinophils and neutrophils in

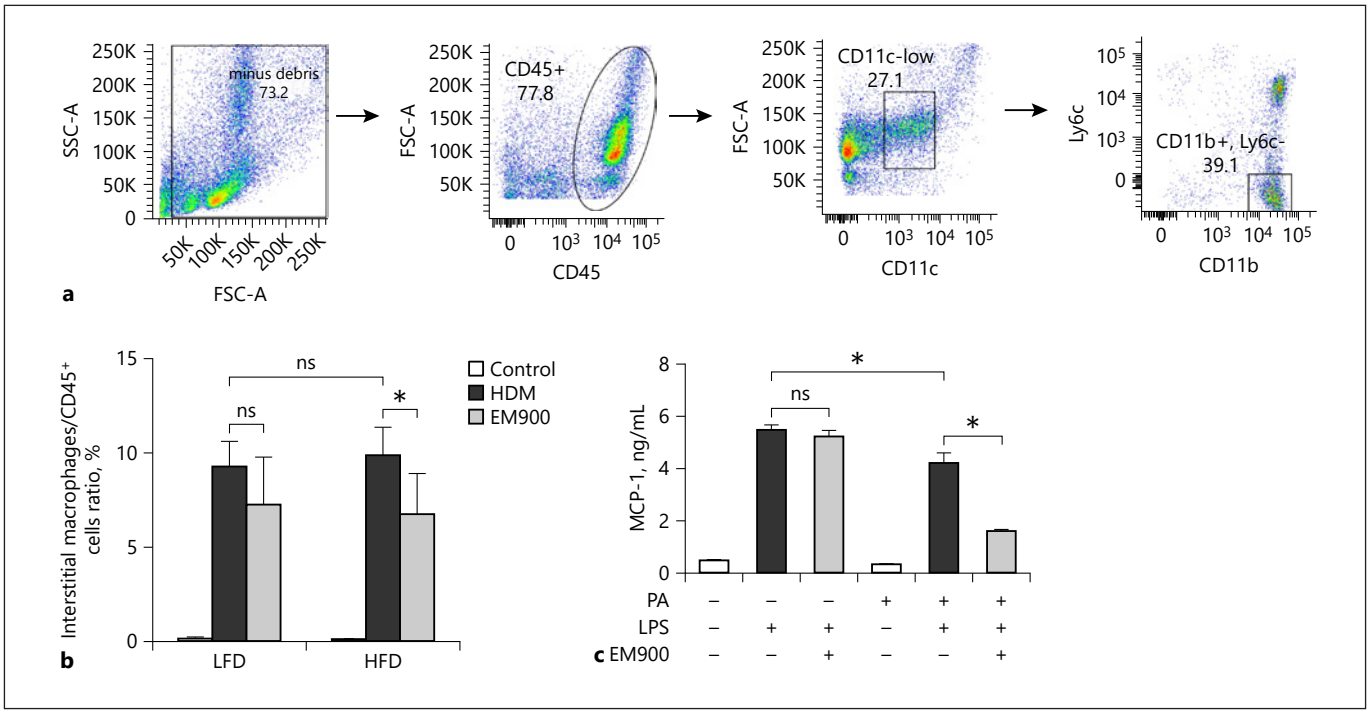

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Fig. 4. EM900 decreased the number of lung macrophages in mice with HFD-induced obesity sensitized to HDMs. **a** Cells isolated from digested lungs after exclusion of doublets and debris, and in which leukocytes were separated by CD45 staining. CD11c-low, CD11b-positive, and Ly6c-negative cells were classified as interstitial macrophages. **b** Percentages of $CD45^+$, $CD11c^{low}$, $CD11b^+$, and $Ly6c^-$ cells in LFD-control, LFD-HDM, LFD-EM900, HFD-control, HFD-HDM, and HFD-EM900 mice ($n = 6$ in each group). PECs were stimulated for 24 h by PA (500 μM), LPS (2 ng/mL), and EM900 (50 μg/mL). For cells cultured with both PA and LPS, PA was preincubated for 1 h before stimulation by LPS. **c** Concentrations of MCP-1 in the supernatant of PECs that were stimulated with LPS ± PA ± EM900 for 24 h were measured by ELISA. The bar graphs present the mean ± SD of 6 independent experiments. *$p < 0.05$. ns, not significant; HDM, house dust mite; LFD, low-fat diet; HFD, high-fat diet; MCP-1, monocyte chemoattractant protein 1; PECs, peritoneal exudate cells; PA, palmitic acid.

BALF and decreased the levels of IL-17A, MIP-2, IL-1β, IL-5, and RANTES. These results suggest that EM900 suppressed both type 2 and type 2-low airway inflammation. To our knowledge, this is the first report to demonstrate the effects of EM900 in an obesity-associated asthma model.

Compared to asthmatic patients with normal BMI, it is difficult to achieve good asthma control in obese asthmatic patients [33]. Corticosteroids also seem to be less effective in obese asthmatics [34]. The characteristics of asthma with obesity reported type 2-low inflammation and less eosinophilic airway inflammation [35]. In a previous study, an increase in plasma IL-17A levels was associated with airway neutrophilia in asthmatic patients with obesity [36]. The present study also showed increases in neutrophils and IL-17A in mice with obesity-associated asthma, which is consistent with previous reports.

Treatments for type 2 predominant asthma involve several biologic targets, such as IL-4, IL-5, IL-13, and immunoglobulin E, while treatments for type 2-low asthma are still being investigated. Currently, there is no specific molecular target treatment for obesity-associated asthma. Decrease in body weight, by diet and exercise or by surgical intervention, has been shown to improve asthma control and asthma-related QOL [37]. In the present study, EM900 showed more remarkable anti-inflammatory effects in the HFD group than in the LFD group. Therefore, we focused on the differences in neutrophilic airway inflammation between the HFD and LFD groups. Inhibitory effects of macrolides on neutrophilic inflammation have been observed in human neutrophils in blood and sputum [38, 39]. Clarithromycin contributes to improved asthma control through the suppression of sputum IL-8 and neutrophil accumulation in severe non-eosinophilic asthma [40]. In the present study, the number of neutrophils in BALF and concentrations of IL-17A in lung tissues showed greater increases in the HFD-HDM group than in the LFD-HDM group. We attrib-

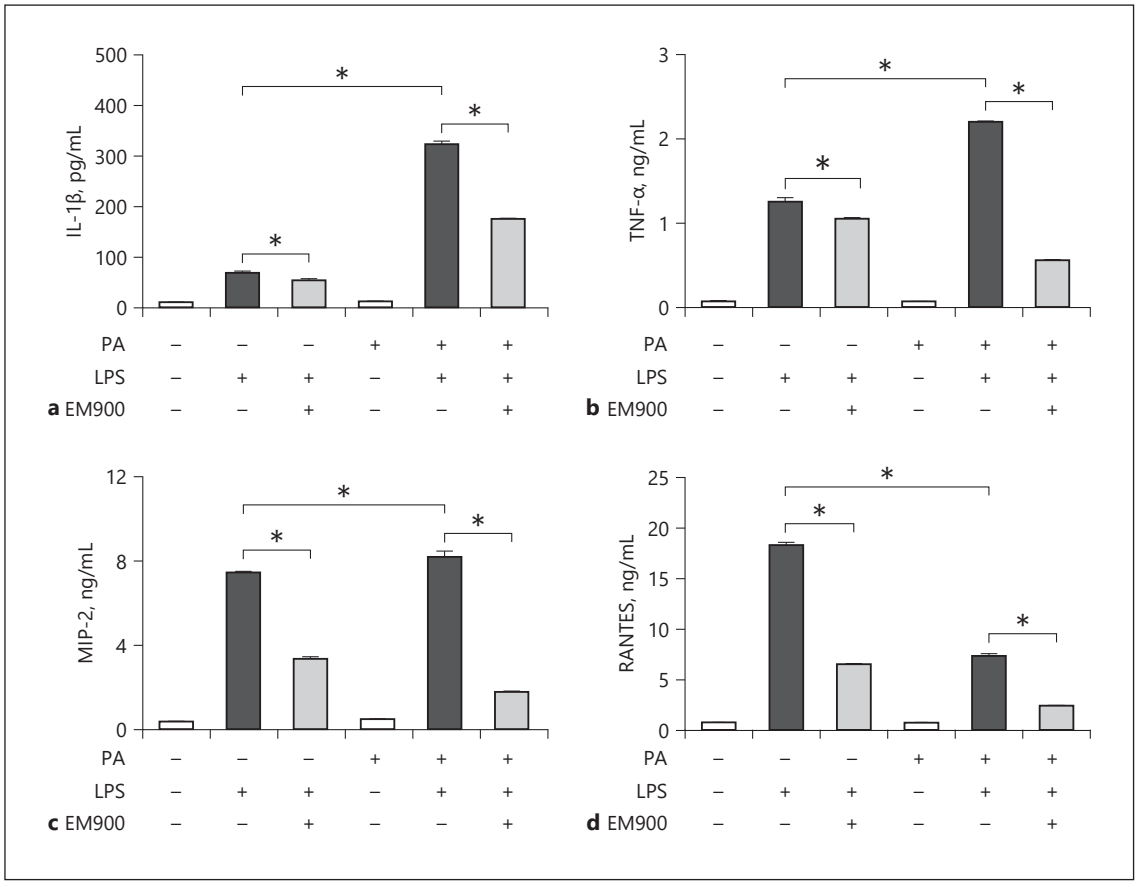

Fig. 5. EM900 suppressed cytokine production in PECs. PECs were stimulated for 24 h by PA (500 μM), LPS (2 ng/mL), and EM900 (50 μg/mL). For cells cultured with both PA and LPS, PA was preincubated for 1 h before stimulation by LPS. Concentrations of IL-1β (**a**), TNF-α (**b**), MIP-2 (**c**), and RANTES (**d**) in supernatant from PECs stimulated with LPS ± PA ± EM900 for 24 h were measured by ELISA. The bar graphs present the mean ± SD of 6 independent experiments. *$p < 0.05$. ns, not significant; IL, interleukin; TNF-α, tumor necrosis factor α; MIP-2, macrophage inflammatory protein 2; RANTES, regulated on activation, normal T cell expressed and secreted; PECs, peritoneal exudate cells; PA, palmitic acid.

uted the greater efficacy of EM900 in the HFD group to increased neutrophilic airway inflammation in this group. The results of the present study show that EM900 might also be an effective treatment option for obese patients with asthma.

Clinically available macrolides, such as erythromycin, clarithromycin, and azithromycin, have both anti-inflammatory and antibacterial effects [41]. Azithromycin decreases the frequency of asthma exacerbations among patients with asthma and improves QOL in both type 2 and type 2-low asthma [21]. However, prolonged antibiotic usage has the risk of inducing drug resistance in bacteria [42]. Previous studies have shown that overall antibiotic usage correlated with penicillin-non-susceptible *Streptococcus pneumoniae* and macrolide-resistant *S. pneumoniae* in various countries [43]. Resistant streptococci were found to have increased for 6 months after administration of clarithromycin for 7 days [44]. In contrast, EM900, a nonantibiotic macrolide, inhibits LPS-induced mucus production by rat nasal epithelium and inhibits MUC5AC secretion [45]. EM900 also inhibits invasive pneumococcal infections by clearance of nasopharyngeal pneumococcal colonization in mice [46]. Therefore, we considered that EM900 would attenuate airway inflammation without inducing bacterial drug resistance.

We previously reported the mechanisms underlying the involvement of monocytes or macrophages in a mouse model of airway inflammation. In the previous studies, saturated fatty acids increased the recruitment of lung

macrophages and augmented HDM-induced airway inflammation in obese mice, and EM900 suppressed the recruitment of lung macrophages in a virus-induced asthma exacerbation model [23, 28]. In the present study, the number of interstitial macrophages (defined as $CD45^+$, $CD11c^{low}$, $CD11b^+$, and $Ly6c^-$ cells) was significantly decreased by EM900 in an obesity-associated mouse asthma model. A previous study also showed that interstitial macrophages play a role in immune responses, such as in allergic airway inflammation [31]. In the present study, cytokine production of IL-1β, TNF-α, MIP-2, and RANTES induced by LPS and PA were significantly suppressed by EM900 in vitro. These data suggest that EM900 might partially suppress airway inflammation in obesity-associated asthma through suppression of recruitment of lung macrophages and inflammatory cytokine production from macrophages.

In conclusion, this study showed that the nonantibiotic macrolide, EM900, attenuated airway inflammation in a mouse model of obesity-associated asthma. Clinical use of EM900 is anticipated because EM900 has therapeutic effects on asthma with obesity.

Statement of Ethics

All animal experiments were performed in accordance with the guidelines for the care and use of experimental animals by the Japanese Association for Laboratory Animals Science (1987) and the ARRIVE guidelines and were approved by the Saga University Animal Care and Use Committee.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions Statement

H.S., H.T., G.K., Y.K., and K.T. designed and performed the study. K.K. contributed to the production of HDM. Y.N., S.O., and T.S. developed and provided EM900. N.S.A. and S.K. supervised the study. H.S. and K.T. drafted the manuscript. All authors read and approved the final manuscript.

References

1 Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. The Lancet. 2018;391(10122):783–800.
2 Lambrecht BN, Hammad H. The immunology of asthma. Nat Immunol. 2015;16(1):45–56.
3 Rddel HK, Bateman ED, Becker A, Boulet LP, Cruz AA, Drazen JM, et al. A summary of the new GINA strategy: a roadmap to asthma control. Eur Respir J. 2015;46(3):622–39.
4 Ray A, Raundhal M, Oriss TB, Ray P, Wenzel SE. Current concepts of severe asthma. J Clin Invest. 2016;126(7):2394–403.
5 Wenzel S. Severe asthma: from characteristics to phenotypes to endotypes. Clin Exp Allergy. 2012;42(5):650–8.
6 Bousquet J, Mantzouranis E, Cruz AA, Aït-Khaled N, Baena-Cagnani CE, Bleecker ER, et al. Uniform definition of asthma severity, control, and exacerbations: document presented for the World Health Organization Consultation on Severe Asthma. J Allergy Clin Immunol. 2010;126(5):926–38.
7 Schatz M, Hsu JW, Zeiger RS, Chen W, Dorenbaum A, Chipps BE, et al. Phenotypes determined by cluster analysis in severe or difficult-to-treat asthma. J Allergy Clin Immunol. 2014;133(6):1549–56.
8 Gibeon D, Batuwita K, Osmond M, Heaney LG, Brightling CE, Niven R, et al. Obesity-associated severe asthma represents a distinct clinical phenotype: analysis of the British Thoracic Society Difficult Asthma Registry Patient cohort according to BMI. Chest. 2013;143(2):406–14.
9 Shore SA, Johnston RA. Obesity and asthma. Pharmacol Ther. 2019;110(1):83–02.
10 Luthe SK, Hirayama A, Goto T, Faridi MK, Camargo CA Jr, Hasegawa K. Association between obesity and acute severity among patients hospitalized for asthma exacerbation. J Allergy Clin Immunol Pract. 2018;6(6):1936–e4.
11 Rodrigo GJ, Plaza V. Body mass index and response to emergency department treatment in adults with severe asthma exacerbations: a prospective cohort study. Chest. 2007;132(5):1513–9.
12 Okubo Y, Michihata N, Yoshida K, Morisaki N, Matsui H, Fushimi K, et al. Impact of pediatric obesity on acute asthma exacerbation in Japan. Pediatr Allergy Immunol. 2017;28(8):763–7.
13 To M, Hitani A, Kono Y, Honda N, Kano I, Haruki K, et al. Obesity-associated severe asthma in an adult Japanese population. Respir Investig. 2018;56(6):440–7.
14 Scott HA, Gibson PG, Garg ML, Wood LG. Airway inflammation is augmented by obesity and fatty acids in asthma. Eur Respir J. 2011;38(3):594–602.
15 Marijsse GS, Seys SF, Schelpe AS, Dilissen E, Goeminne P, Dupont LJ, et al. Obese individuals with asthma preferentially have a high IL-5/IL-17A/IL-25 sputum inflammatory pattern. Am J Respir Crit Care Med. 2014;189(10):1284–5.
16 Holgate S, Smith N, Massanari M, Jimenez P. Effects of omalizumab on markers of inflammation in patients with allergic asthma. Allergy. 2009;64(12):1728–36.
17 Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. N Engl J Med. 2014;371(13):1198–207.
18 Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. N Engl J Med. 2018;378(26):2486–96.
19 FitzGerald JM, Bleecker ER, Nair P, Korn S, Ohta K, Lommatzsch M, et al. Benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2016;388(10056):2128–41.
20 Koutsoubari I, Papaevangelou V, Konstantinou GN, Makrinioti H, Xepapadaki P, Kafetzis D, et al. Effect of clarithromycin on acute asthma exacerbations in children: an open randomized study. Pediatr Allergy Immunol. 2012;23(4):385–90.
21 Gibson PG, Yang IA, Upham JW, Reynolds PN, Hodge S, James AL, et al. Effect of azithromycin on asthma exacerbations and quality of life in adults with persistent uncontrolled asthma (AMAZES): a randomised, double-blind, placebo-controlled trial. Lancet. 2017;390(10095):659–68.

22 Sugawara A, Sueki A, Hirose T, Nagai K, Gouda H, Hirono S, et al. Novel 12-membered non-antibiotic macrolides from erythromycin A; EM900 series as novel leads for anti-inflammatory and/or immunomodulatory agents. Bioorg Med Chem Lett. 2011;21(11):3373–6.
23 Sadamatsu H, Takahashi K, Tashiro H, Kato G, Noguchi Y, Kurata K, et al. The non-antibiotic macrolide EM900 attenuates HDM and poly(I:C)-induced airway inflammation with inhibition of macrophages in a mouse model. Inflamm Res. 2020;69(1):139–51.
24 Kato G, Takahashi K, Tashiro H, Kurata K, Shirai H, Kimura S, et al. β2 adrenergic agonist attenuates house dust mite-induced allergic airway inflammation through dendritic cells. BMC Immunol. 2014;15:39.
25 Tashiro H, Takahashi K, Hayashi S, Kato G, Kurata K, Kimura S, et al. Interleukin-33 from monocytes recruited to the lung contributes to house dust mite-induced airway inflammation in a mouse model. PLoS One. 2016;11(6):e0157571.
26 Bopp T, Dehzad N, Reuter S, Klein M, Ullrich N, Stassen M, et al. Inhibition of cAMP degradation improves regulatory T cell-mediated suppression. J Immunol. 2009;182(7):4017–24.
27 Takahashi K, Koga K, Linge HM, Zhang Y, Lin X, Metz CN, et al. Macrophage CD74 contributes to MIF-induced pulmonary inflammation. Respir Res. 2009;10:33.
28 Tashiro H, Takahashi K, Sadamatsu H, Kato G, Kurata K, Kimura S, et al. Saturated fatty acid increases lung macrophages and augments house dust mite-induced airway inflammation in mice fed with high-fat diet. Inflammation. 2017;40(3):1072–86.
29 Takahashi K, Shibata T, Akashi-Takamura S, Kiyokawa T, Wakabayashi Y, Tanimura N, et al. A protein associated with Toll-like receptor (TLR) 4 (PRAT4A) is required for TLR-dependent immune responses. J Exp Med. 2007;204(12):2963–76.
30 Misharin AV, Morales-Nebreda L, Mutlu GM, Budinger GR, Perlman H. Flow cytometric analysis of macrophages and dendritic cell subsets in the mouse lung. Am J Respir Cell Mol Biol. 2013;49(4):503–10.
31 Schyns J, Bureau F, Marichal T. Lung interstitial macrophages: past, present, and future. J Immunol Res. 2018;2018:1–10.
32 Zaynagetdinov R, Sherrill TP, Kendall PL, Segal BH, Weller KP, Tighe RM, et al. Identification of myeloid cell subsets in murine lungs using flow cytometry. Am J Respir Cell Mol Biol. 2013;49(2):180–9.
33 Boulet LP, Franssen E. Influence of obesity on response to fluticasone with or without salmeterol in moderate asthma. Respir Med. 2007;101(11):2240–7.
34 Pradeepan S, Garrison G, Dixon AE. Obesity in asthma: approaches to treatment. Curr Allergy Asthma Rep. 2013;13(5):434–42.
35 Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med. 2010;181(4):315–23.
36 Chen JH, Qin L, Shi YY, Feng JT, Zheng YL, Wan YF, et al. IL-17 protein levels in both induced sputum and plasma are increased in stable but not acute asthma individuals with obesity. Respir Med. 2016;121:48–8.
37 Peters U, Dixon AE, Forno E. Obesity and asthma. J Allergy Clin Immunol. 2018;141(4):1169–79.
38 Marjanović N, Bosnar M, Michielin F, Willé DR, Anić-Milić T, Culić O, et al. Macrolide antibiotics broadly and distinctively inhibit cytokine and chemokine production by COPD sputum cells in vitro. Pharmacol Res. 2011;63(5):389–97.
39 Anderson R, Theron AJ, Feldman C. Membrane-stabilizing, anti-inflammatory interactions of macrolides with human neutrophils. Inflammation. 1996;20(6):693–705.
40 Simpson JL, Powell H, Boyle MJ, Scott RJ, Gibson PG. Clarithromycin targets neutrophilic airway inflammation in refractory asthma. Am J Respir Crit Care Med. 2008;177(2):148–55.
41 Tamaoki J, Kadota J, Takizawa H. Clinical implications of the immunomodulatory effects of macrolides. Am J Med. 2004;117(Suppl 9A):5S–11S.
42 Livermore DM. Bacterial resistance: origins, epidemiology, and impact. Clin Infect Dis. 2003;36(Suppl 1):S11–23.
43 Albrich WC, Monnet DL, Harbarth S. Antibiotic selection pressure and resistance in Streptococcus pneumoniae and Streptococcus pyogenes. Emerging Infect Dis. 2004;10(3):514–7.
44 Malhotra-Kumar S, Lammens C, Coenen S, Van Herck K, Goossens H. Effect of azithromycin and clarithromycin therapy on pharyngeal carriage of macrolide-resistant streptococci in healthy volunteers: a randomised, double-blind, placebo-controlled study. Lancet. 2007;369(9560):482–90.
45 Tojima I, Shimizu S, Ogawa T, Kouzaki H, Omura S, Sunazuka T, et al. Anti-inflammatory effects of a novel non-antibiotic macrolide, EM900, on mucus secretion of airway epithelium. Auris Nasus Larynx. 2015;42(4):332–6.
46 Iwanaga N, Nakamura S, Oshima K, Kajihara T, Takazono T, Miyazaki T, et al. Macrolides promote CCL2-mediated macrophage recruitment and clearance of nasopharyngeal pneumococcal colonization in mice. J Infect Dis. 2015;212(7):1150–9.