

Interleukin-18-deficient mice exhibit diminished chronic inflammation and airway remodelling in ovalbumin-induced asthma model

S. Yamagata,* K. Tomita,* R. Sato,*
A. Niwa,[†] H. Higashino[†] and
Y. Tohda*

Departments of *Respiratory Medicine and
Allergology and [†]Pharmacology, Kinki University
School of Medicine, Osaka-Sayama, Osaka, Japan

Summary

Interleukin (IL)-18, which is produced by activated monocytes/macrophages and airway epithelial cells, is suggested to contribute to the pathophysiology of asthma by modulating airway inflammation. However, the involvement of IL-18 on modulating chronic airway inflammation and airway remodelling, which are characterized in a refractory asthma model exposed to long-term antigen, has not been investigated sufficiently. We examined the role of IL-18 in chronic airway inflammation and airway remodelling by long-term antigen exposure. IL-18-deficient and C57BL/6-wild-type mice were sensitized by ovalbumin (OVA) and were then exposed to aerosolized OVA twice a week for 12 weeks. We assessed airway inflammation by assessing the infiltration of cells into the airspace and lung tissues, and airway remodelling by airway mucus expression, peribronchial fibrosis and smooth muscle thickness. In IL-18-deficient mice, when exposed to OVA, the total cells and neutrophils of the bronchoalveolar lavage fluid (BALF) were diminished, as were the number of infiltrated cells in the lung tissues. IL-18-deficient mice exposed to OVA after 12 weeks showed significantly decreased levels of interferon (IFN)- γ , IL-13 and transforming growth factor (TGF)- β 1 in the BALF. The airway hyperresponsiveness to acetyl- β -methacholine chloride was inhibited in IL-18-deficient mice in comparison with wild-type mice. In addition, IL-18-deficient mice exposed to OVA had fewer significant features of airway remodelling. These findings suggest that IL-18 may enhance chronic airway inflammation and airway remodelling through the production of IFN- γ , IL-13 and TGF- β 1 in the OVA-induced asthma mouse model.

Keywords: airway remodelling, chronic airway inflammation, interleukin-18, interleukin-18-deficient mouse, refractory asthma

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Correspondence: S. Yamagata, Department of
Respiratory Medicine and Allergology, Kinki
University School of Medicine, 377-2, Ohno-
Higashi, Osaka-Sayama, Osaka, 589-8511 Japan.
E-mail: yamagata@ko-arena.med.kindai.ac.jp

Introduction

Bronchial asthma is characterized as an immunoreactive disease with chronic airway inflammation, airway hyperresponsiveness (AHR) and elevated serum immunoglobulin E (IgE). Patients with asthma exhibit structural changes in the lungs, termed 'airway remodelling', which is associated with airway mucus expression, peribronchial fibrosis and smooth muscle hypertrophy. Airway remodelling is a characteristic feature of chronic asthma, which is seen particularly in patients with refractory asthma and a progressive decline in lung function [1].

Interleukin (IL)-18 is released from activated monocytes/macrophages and airway epithelial cells and contributes to proinflammatory actions in asthma [2]. IL-18 is a cytokine

with potent interferon (IFN)- γ -inducing activity and can increase serum IgE levels and promote allergen-induced eosinophil influx into the airways of mice in asthma models [3–5]. In the ovalbumin (OVA)-induced asthma model, IL-18 expression was up-regulated in the macrophages obtained from bronchoalveolar lavage fluid (BALF) and airway epithelial cells [2]. The combination of antigen plus IL-18-stimulated T helper (Th) type 1 cells can result in overproduction of IFN- γ , IL-9, IL-13, regulated upon activation normal T-lymphocyte expressed and secreted protein, macrophage inflammatory protein-1 α and granulocyte-macrophage colony-stimulating factor, and may thereby induce acute airway inflammation in asthma [6,7]. However, these findings were taken from experiments with short-term antigen exposure, not from a sufficiently long-term antigen

exposure protocol to result in chronic airway inflammation and airway remodelling. After allergic sensitization, repeated allergen exposure leads to chronic allergic airway inflammation, characterized by an influx of eosinophils, mast cells and allergen-specific T cells, a Th2-type cytokine pattern and airway remodelling [8]. Chronic airway inflammation in asthma is characterized not only by these inflammatory cells, but also by neutrophils [9–11].

Little is known about the pathophysiological features of chronic airway inflammation and the airway remodelling of asthma, which might be modulated by IL-18. Therefore, we have examined the role of IL-18 in chronic airway inflammation and airway remodelling in IL-18-deficient mice, chronically exposed to OVA.

Materials and methods

Animals and reagents

Specific pathogen-free (SPF) 7-week-old female C57BL/6-wild type (IL-18^{+/+}) mice and C57BL/6-background 6–7-week-old female IL-18-deficient (IL-18^{-/-}) mice were purchased from SLC Japan, Inc. (Shizuoka, Japan) and Jackson Laboratory (Bar Harbor, ME, USA) respectively. All mice were raised under SPF conditions at the animal facilities of the Kinki University School of Medicine (Osaka, Japan).

Experimental design

The mice were divided randomly into four groups, as described below.

Phosphate-buffered saline (PBS) (Wako, Osaka, Japan)-sensitized and PBS-exposed group (six wild-type mice and six IL-18^{-/-} mice). The mice were sensitized by intraperitoneal (i.p.) injections of 200 µl of PBS absorbed to 100 µg of aluminium hydroxide gel (Alum) (Wako) on days 0 and 14. Exposure with aerosolized PBS for 20 min using a nebulizer (Ultra A-I-R®; Omron, Kyoto, Japan) was then applied twice each week for 12 weeks.

The PBS-sensitized and OVA (purified ≥98%; Wako)-exposed group (six wild-type mice). The mice were sensitized by i.p. injections of 200 µl of PBS absorbed to 100 µg of Alum in 200 µl of PBS on days 0 and 14. Exposure with aerosolized OVA (1% wt/vol diluted in PBS) for 20 min was repeated twice each week for 12 weeks.

The OVA-sensitized and PBS-exposed group (six wild-type mice). The mice were sensitized by i.p. injections of 50 µg of OVA absorbed to 100 µg of Alum in 200 µl of PBS on days 0 and 14. Exposure with aerosolized PBS for 20 min was repeated twice each week for 12 weeks.

OVA-sensitized and OVA-exposed group (six wild-type mice and six IL-18^{-/-} mice). The mice were sensitized by i.p. injections of 50 µg of OVA absorbed to 100 µg of Alum in 200 µl of PBS on days 0 and 14. Exposure with aerosolized OVA (1% wt/vol diluted in PBS) for 20 min was repeated twice each week for 12 weeks.

Twenty-four hours after the final exposure, the mice were anaesthetized with an i.p. injection of sodium pentobarbital; plasma samples and BALF were collected and the lungs were harvested. The animal protocols were approved by the Kinki University Animal Care Committee and were performed in accordance with the Kinki University Animal Care Guidelines.

Bronchoalveolar lavage

The trachea of each animal was surgically exposed and cannulated with 27-gauge silicon tubing attached to a 23-gauge needle. Bronchoalveolar lavage was performed three times with 0.5 ml of PBS (37°C) per mouse. The BALF from each animal was pooled in plastic tubes on ice and centrifuged (250 g) at 4°C for 5 min. The total cell counts in the BALF were analysed using a haemocytometer (Erma Inc., Tokyo, Japan). Thin-layer preparations of BALF provided by a centrifugal machine (Cytospin®; Shandon Inc., Cheshire, UK) were stained with Diff-Quik (Kokusai Shiyaku, Kobe, Japan), and differential counts were obtained based on the morphology and staining characteristics of at least 200 cells. The supernatants of the BALF were stored at -80°C for the cytokine determinations.

Plasma levels of OVA-specific IgE

At the time the animals were killed, blood samples were drawn from the heart. The plasma levels of OVA-specific IgE were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Dainippon Sumitomo Pharma Co., Osaka, Japan). The limit of detection was 8.0 ng/ml.

Cytokine quantification

Cytokine levels in the supernatants of the BALF were measured using ELISA kits (IL-5, IL-12 and IFN-γ: Endogen, Woburn, MA, USA; IL-4, IL-13 and transforming growth factor-β1 (TGF-β1): R&D Systems Inc., Minneapolis, MN, USA). The detection limits were 2 pg/ml for IL-4, 5 pg/ml for each of IL-5 and IL-12, 10 pg/ml for IFN-γ, 1.5 pg/ml for IL-13 and 4.61 pg/ml for TGF-β1.

Measurement of AHR

The AHR was assessed before sensitization by OVA and 24 h after the final OVA exposure at the end of 12 weeks. We measured the AHR to acetyl-β-methacholine chloride

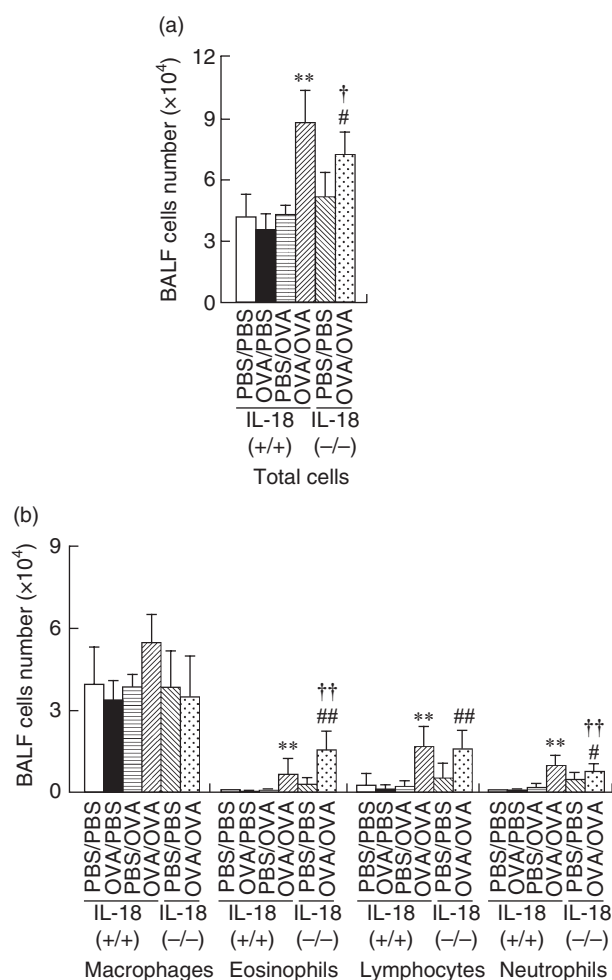


Fig. 1. Bronchoalveolar lavage fluid (BALF) cells in the airway in wild-type [interleukin (IL)-18^{+/+}] and IL-18-deficient (IL-18^{-/-}) mice sensitized and exposed to phosphate-buffered saline (PBS) or ovalbumin (OVA) and wild-type control groups. The total number of cells (a) and those of eosinophils, lymphocytes and neutrophils (b) in the BALF were indicated. As described in Materials and methods, the mice were sensitized and exposed to PBS or OVA for 12 weeks. Twenty-four hours after the final exposure, Bronchoalveolar lavage was performed three times with 0.5 ml of PBS per mouse. The total cell counts in the BALF were analysed and differential cell counts were determined based on the morphology and staining characteristics of at least 200 cells. The data are expressed as means $\pm$ standard deviation. ** P < 0.01 versus IL-18^{+/+} mice sensitized and exposed to PBS; * P < 0.05; ## P < 0.01 versus IL-18^{-/-} mice sensitized and exposed to PBS; † P < 0.05; †† P < 0.01 versus IL-18^{+/+} mice sensitized and exposed to OVA.

(MCh) inhalation in mice using whole body plethysmography (Respiromax®; Columbus Inc., Columbus, OH, USA) and an analysis software program (PowerLab®; ADI Inc., Castle Hill, Australia) for 5 min (20 periods of 15 s each) before inhalation, after exposure to aerosolized PBS, and after exposure to each concentration of aerosolized MCh (ranging from 0.33 to 27 mg/ml). We used tidal mid-expiratory flow (EF₅₀) as a parameter of AHR, because it correlates closely

with pulmonary resistance [12]. The minimal EF₅₀ values are expressed as the % change from the baseline value.

Histochemical analyses

The harvested lung tissues were fixed in 10% formalin in buffered PBS, embedded in paraffin, cut into 3- μ m thick sections and analysed histologically. The sections were stained with haematoxylin and eosin to determine the infiltration of cells which reflect the chronic airway inflammation into the lung tissues. Mucus expression was determined by staining the sections with periodic acid Schiff's reagent (PAS). Peribronchial fibrosis was assessed by staining with elastica-Masson's trichrome [13–15]. Airway smooth muscle thickness was determined by staining the sections with anti- α -smooth muscle actin antibody [13–15]. A computer-assisted morphometric analysis was performed using a digital camera (FX380®; Olympus Inc., Tokyo, Japan) and a software program for image analysis (Flovel Inc., Tokyo, Japan). Trans-sectional images of bronchioles measuring from 150 to 200 μ m in diameter were examined. The number of inflammatory cells in the bronchioles was counted in the submucosal area at a depth of 50 μ m beneath the epithelial basement membrane. Thereafter, the final

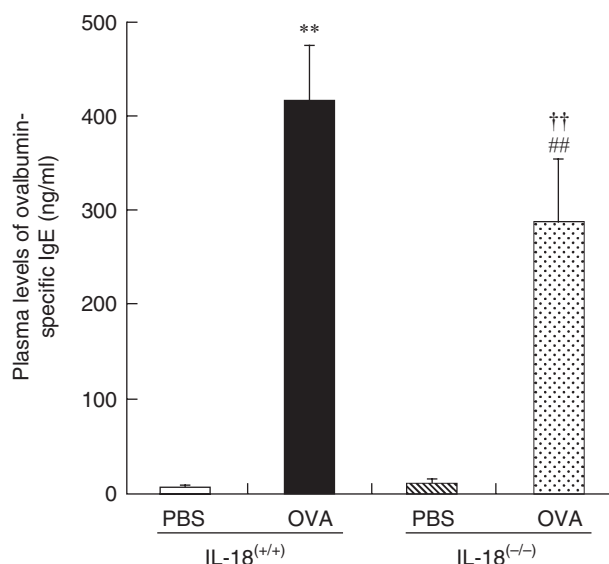


Fig. 2. Plasma levels of ovalbumin (OVA)-specific immunoglobulin E (IgE) in wild-type [interleukin (IL)-18^{+/+}] and IL-18-deficient (IL-18^{-/-}) mice sensitized and exposed to OVA. As described in Materials and methods, the mice were sensitized and exposed to phosphate-buffered saline (PBS) or OVA for 12 weeks. After the final exposure, the plasma levels of OVA-specific serum IgE were measured using enzyme-linked immunosorbent assay using plasma from blood samples drawn from the heart. The data are expressed as means $\pm$ standard deviation. ** P < 0.01 versus IL-18^{+/+} mice sensitized and exposed to PBS; ## P < 0.01 versus IL-18^{-/-} mice sensitized and exposed to PBS; †† P < 0.01 versus IL-18^{+/+} mice sensitized and exposed to OVA.

result was calculated as the number of cells equivalent to per 10 000 μm^2 area in at least 10 bronchioles per slide [16]. Mucus expression was quantified by counting the number of PAS-positive and -negative cells in the epithelium of each bronchiole in at least 10 bronchioles per slide. Peribronchial fibrosis was quantified as the stained area divided by the length of the basement membrane in at least 10 bronchioles per slide. Airway smooth muscle thickness was quantified by measuring the thickness in at least 10 bronchioles per slide.

Statistical analysis

Results in the different groups of mice were compared using one-way analysis of variance, followed by the Dunnett *post hoc* test. All results are presented as means $\pm$ standard deviation. *P* values of <0.05 were considered to be statistically significant.

Results

Chronic airway inflammation in IL-18-deficient mice

Airway inflammation was evaluated by assessing the cell numbers of the BALF and the infiltration of cells into the lung. First, in order to confirm that no contamination of the endotoxins occurred in OVA, three controls were set as mice sensitized by PBS and exposed to PBS (PBS/PBS), those sensitized by OVA and exposed to PBS (OVA/PBS) and those sensitized by PBS and exposed to OVA (PBS/OVA). The total number of cells, eosinophils, lymphocytes and neutrophils in the BALF did not differ among the three controls (Fig. 1a and b). In wild-type mice, the absolute numbers of total cells, eosinophils, lymphocytes and neutrophils in the BALF were significantly greater in the OVA-exposed group than in the PBS-exposed group ($P < 0.01$) (Fig. 1a and b). In

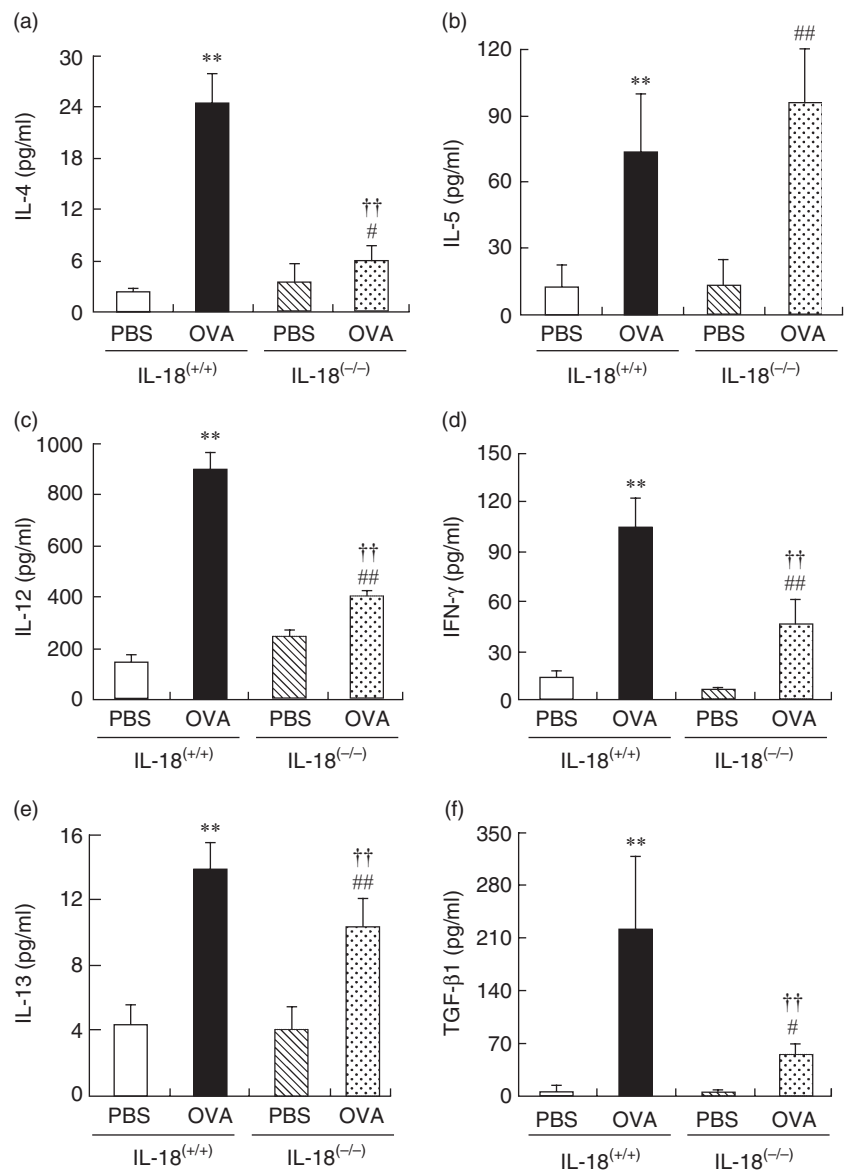


Fig. 3. Bronchoalveolar lavage fluid (BALF) levels of interleukin (IL)-4, IL-5, IL-12, interferon (IFN)- γ , IL-13, and transforming growth factor (TGF)- β 1 in wild-type IL-18^{+/+} and IL-18-deficient (IL-18^{-/-}) mice sensitized and exposed to ovalbumin (OVA). The levels of IL-4 (a), IL-5 (b), IL-12 (c), IFN- γ (d), IL-13 (e) or TGF- β 1 (f) in the supernatants from the BALF were measured using enzyme-linked immunosorbent assay kits. Data are expressed as means $\pm$ standard deviation. ** $P < 0.01$ versus IL-18^{+/+} mice sensitized and exposed to phosphate-buffered saline (PBS); * $P < 0.05$; ## $P < 0.01$ versus IL-18^{-/-} mice sensitized and exposed to PBS; †† $P < 0.01$ versus IL-18^{+/+} mice sensitized and exposed to OVA.

Table 1. Infiltrated cells number in the lung tissues in wild-type [interleukin (IL)-18^{+/+}] and IL-18-deficient (IL-18^{-/-}) mice.

	IL-18 ^{+/+}	IL-18 ^{-/-}
PBS-sensitized and PBS-exposed group	40 ± 12	42 ± 14
OVA-sensitized and OVA-exposed group	90 ± 20**	70 ± 18 ^{##,††}

The number of cells was calculated equivalent to per 10 000 µm² area in at least 10 bronchioles per slide. ***P* < 0.01 versus IL-18^{+/+} mice sensitized and exposed to phosphate-buffered saline (PBS); ^{##}*P* < 0.01 versus IL-18^{-/-} mice sensitized and exposed to PBS; ^{††}*P* < 0.01 versus IL-18^{+/+} mice sensitized and exposed to ovalbumin (OVA).

IL-18-deficient mice, the absolute numbers of total cells, eosinophils, lymphocytes and neutrophils in the BALF were significantly greater in the OVA-exposed group than in the PBS-exposed group (*P* < 0.05). IL-18-deficient OVA mice had a significantly greater number of eosinophils in the BALF in comparison with wild-type mice exposed to OVA (*P* < 0.01), but fewer total cells and neutrophils (*P* < 0.01).

In the histochemical study, the cells which infiltrated into the lung tissues of the OVA-exposed group showed a marked increase (*P* < 0.01) in comparison with that of the PBS-exposed group in wild-type mice (Table 1). In contrast, in IL-18-deficient mice, the cells which infiltrated into the lung tissues of the OVA-exposed group decreased significantly in comparison with wild-type mice exposed to OVA (*P* < 0.01); however, they were significantly higher than those in IL-18-deficient mice exposed to PBS (*P* < 0.01).

Plasma levels of OVA-specific IgE in IL-18-deficient mice

In wild-type mice, the plasma levels of OVA-specific IgE in the OVA-exposed group were significantly higher than those in the PBS-exposed group after 12 weeks (*P* < 0.01) (Fig. 2). In IL-18-deficient mice, the plasma levels of OVA-specific IgE in the OVA-exposed group were significantly lower than those in wild-type mice exposed to OVA after 12 weeks (*P* < 0.01), but were significantly higher than those in IL-18-deficient mice exposed to PBS (*P* < 0.01).

Production of cytokines in BALF obtained from IL-18-deficient mice

The BALF levels of IL-4, IL-5, IL-12, IFN-γ, IL-13 and TGF-β1 in wild-type mice exposed to OVA were significantly higher than those in wild-type mice exposed to PBS at 12 weeks (*P* < 0.01) (Fig. 3a–f). BALF levels of IL-4, IL-5, IL-12, IFN-γ, IL-13 and TGF-β1 in the IL-18-deficient mice exposed to OVA were significantly higher than those in IL-18-deficient mice exposed to PBS after 12 weeks (*P* < 0.05). In addition, the BALF levels of IL-4, IL-12, IFN-γ, IL-13 and TGF-β1 in IL-18-deficient mice exposed to OVA were significantly lower than those in wild-type mice exposed to OVA (*P* < 0.01), but the BALF level of IL-5 was not changed.

Airway responsiveness in IL-18-deficient mice

The AHR did not differ among the groups at the baseline (Fig. 4a). Wild-type mice exposed to OVA developed sustained increases in AHR to MCh in comparison with wild-type mice exposed to PBS after the final exposure at the end of 12 weeks (MCh: 9 and 27 mg/ml for *P* < 0.05 and 0.01 respectively) (Fig. 4b). IL-18-deficient mice exposed to OVA developed sustained increases in AHR to MCh in comparison with IL-18-deficient mice exposed to PBS after 12 weeks (MCh: 27 mg/ml, *P* < 0.05), but developed decreases in AHR to MCh in comparison with wild-type mice exposed to OVA after 12 weeks (MCh: 27 mg/ml, *P* < 0.05).

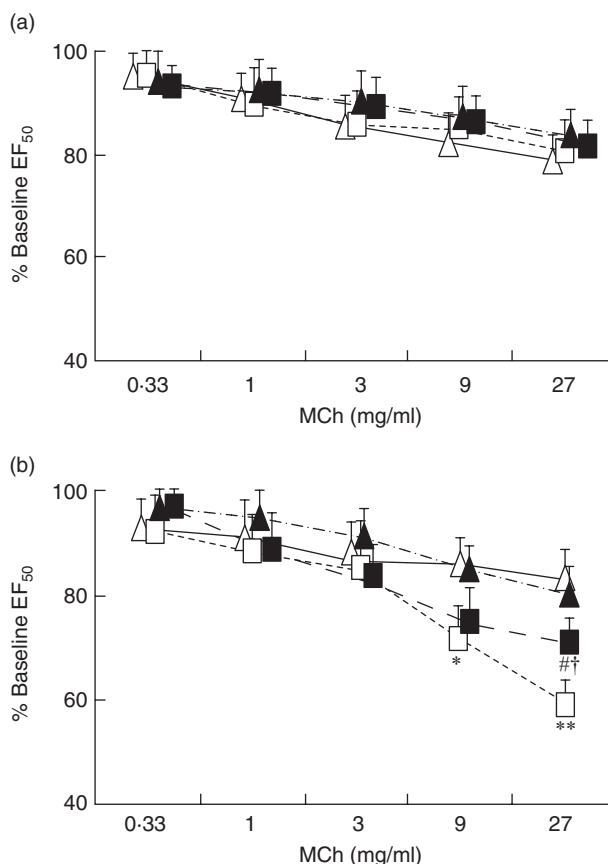


Fig. 4. Airway hyperresponsiveness (AHR) in wild-type [interleukin (IL)-18^{+/+}] and IL-18-deficient (IL-18^{-/-}) mice sensitized and exposed to ovalbumin (OVA). As described in Materials and methods, mice were sensitized and exposed to phosphate-buffered saline (PBS) or OVA for 12 weeks. AHR was assessed before OVA sensitization (a) and 24 h after the final OVA exposure (b). We measured AHR to acetyl-β-methacholine chloride (MCh) (range: 0.3–27 mg/ml) inhalation in mice. The results are shown as % baseline tidal mid-expiratory flow (EF₅₀) in each group of mice. Data are expressed as means ± standard deviation. **P* < 0.05, ***P* < 0.01 versus IL-18^{+/+} mice sensitized and exposed to PBS; ^{##}*P* < 0.05 versus IL-18^{-/-} mice sensitized and exposed to PBS; ^{††}*P* < 0.05 versus IL-18^{+/+} mice sensitized and exposed to OVA.

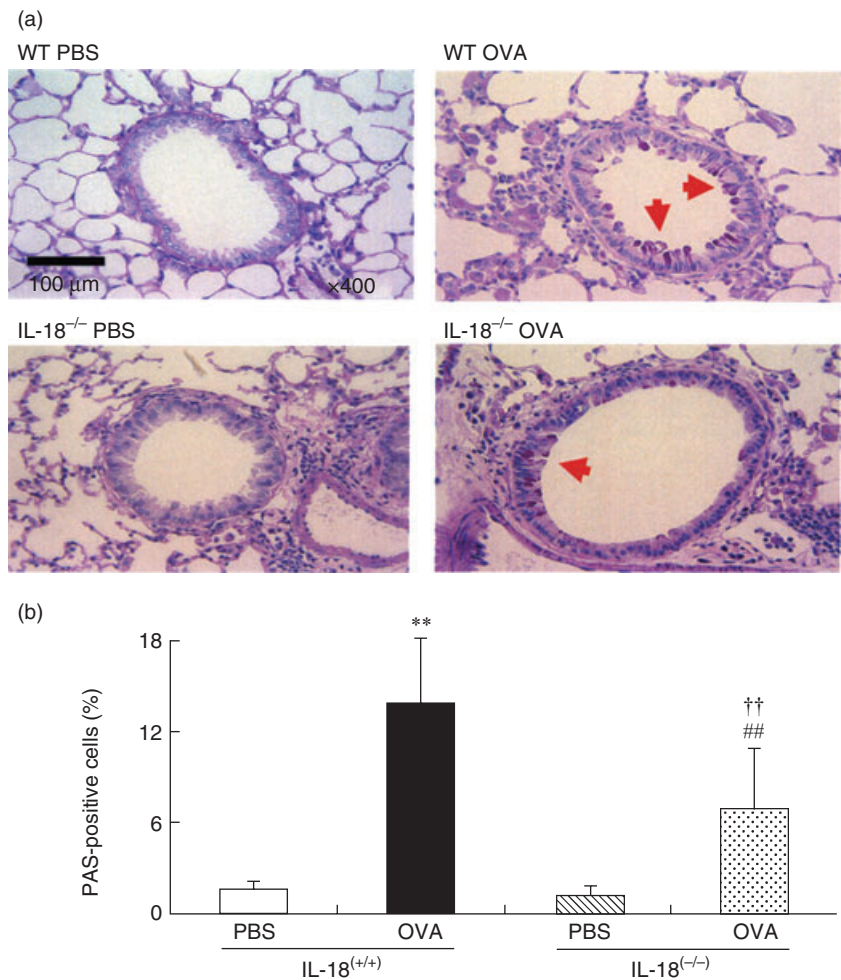


Fig. 5. Mucus expression in lung tissues in wild-type (WT) [interleukin (IL)-18^{+/+}] and IL-18-deficient (IL-18^{-/-}) mice sensitized and exposed to ovalbumin (OVA). As described in Materials and methods, the mice were sensitized and exposed to phosphate-buffered saline (PBS) or OVA for 12 weeks. Airway remodelling in lung tissues was determined by staining with periodic acid Schiff's reagent (PAS) (a). Trans-sectional images of bronchioles with diameters of 150–200 μm were examined. The mucus expression was quantified by counting the numbers of PAS-positive and -negative cells in the epithelium of each bronchiole (b) in at least 10 bronchioles per slide. Data are expressed as means ± standard deviation. ***P* < 0.01 versus IL-18^{+/+} mice sensitized and exposed to PBS; ##*P* < 0.01 versus IL-18^{-/-} mice sensitized and exposed to PBS; ††*P* < 0.01 versus IL-18^{+/+} mice sensitized and exposed to OVA.

Levels of mucus expression in the airways

In wild-type mice, the percentage of PAS-positive cells in the airway epithelium of the OVA-exposed group was significantly greater than that of the PBS-exposed group after 12 weeks (*P* < 0.01) (Fig. 5a and b). The percentage of PAS-positive cells in the airway epithelium of the OVA-exposed group in IL-18-deficient mice was significantly less than that of the OVA-exposed group in wild-type mice after 12 weeks (*P* < 0.01), but the percentage was significantly greater than that of the PBS-exposed group in IL-18-deficient mice (*P* < 0.01).

Levels of peribronchial fibrosis in the airways

In wild-type mice, the area of peribronchial fibrosis determined by elastica-Masson's trichrome staining in the OVA-exposed group was significantly larger than that in the PBS-exposed group after 12 weeks (*P* < 0.01) (Fig. 6a and b). In IL-18-deficient mice exposed to OVA the area of peribronchial fibrosis was significantly less than that in wild-type mice exposed to OVA after 12 weeks (*P* < 0.01), but signifi-

cantly larger than that in the PBS-exposed group in IL-18-deficient mice (*P* < 0.01).

Levels of smooth muscle thickness in the airways

In wild-type mice, the airway smooth muscle thickness in the OVA-exposed group was significantly greater than that in the PBS-exposed group after 12 weeks (*P* < 0.01) (Fig. 7a and b). In IL-18-deficient mice, the OVA-exposed group showed significantly less airway smooth muscle thickness in wild-type mice exposed to OVA after 12 weeks of treatment (*P* < 0.01), but significantly greater than that in the PBS-exposed group in IL-18-deficient mice (*P* < 0.01).

Discussion

In the present study, we have examined the role of IL-18 on chronic airway inflammation and airway remodelling in IL-18-deficient mice subjected to long-term exposure to OVA. The number of total cells and neutrophils, but not eosinophils, in the BALF in IL-18-deficient mice exposed to OVA was significantly lower in comparison with wild-type

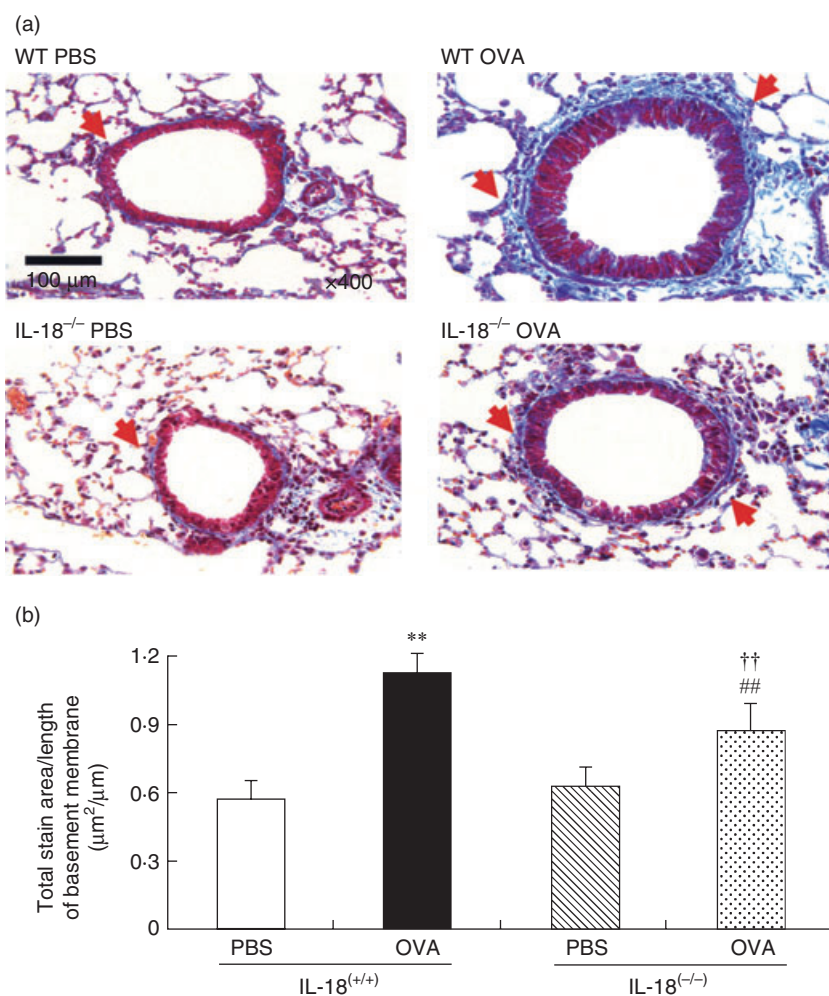


Fig. 6. Peribronchial fibrosis in lung tissues in wild-type (WT) [interleukin (IL)-18^{+/+}] and IL-18-deficient (IL-18^{-/-}) mice sensitized and exposed to ovalbumin (OVA). As described in Materials and methods, the mice were sensitized and exposed to phosphate-buffered saline (PBS) or OVA for 12 weeks. Airway remodelling in the lung tissues was determined by staining with elastica-Masson's trichrome (a). Trans-sectional images of bronchioles with diameters of 150–200 μm were examined. Quantitation of peribronchial fibrosis is expressed as stained area divided by length of basement membrane (b) in at least 10 bronchioles per slide. Data are expressed as means ± standard deviation. ***P* < 0.01 versus IL-18^{+/+} mice sensitized and exposed to PBS; ##*P* < 0.01 versus IL-18^{-/-} mice sensitized and exposed to PBS; ††*P* < 0.01 versus IL-18^{+/+} mice sensitized and exposed to OVA.

mice exposed to OVA (Fig. 1a and b). IL-18-deficient mice showed reduced AHR in comparison with wild-type mice (Fig. 4b). We also demonstrated that remodelling in IL-18-deficient mice was reduced in comparison with wild-type mice exposed to OVA (Figs 5–7).

Chronic airway inflammation in asthma is characterized by T cells, mast cells, eosinophils and neutrophils [9–11]. Our results were consistent with this finding, as our models of long-term antigen exposure for 12 weeks induced typical chronic inflammation, with T cells, eosinophils and neutrophils infiltrating into the airways in wild-type mice. In this study, we recognized no contamination of endotoxin in OVA, because the mice sensitized by PBS and exposed to OVA showed no accumulation of neutrophils into the airways. In addition, our results show that chronic airway inflammation in IL-18-deficient mice exposed to OVA was mainly inhibited, as shown by a diminished number of neutrophils. A previous report suggested that airway inflammation in asthma is composed mainly of eosinophils [17]. On the other hand, patients with refractory asthma show an increase in the infiltration of neutrophils into the airways [18]. The role of neutrophils in asthma remains unclear. The infiltration of neutrophils in the

asthmatic airway may be related to IFN-γ [19], and this feature was consistent with our results. Wenzel *et al.* reported that patients with refractory asthma show an increase in airway neutrophils, but not eosinophils [18]. IL-18 might relate to chronic airway inflammation through neutrophils in our chronic inflammation model.

Our data demonstrated that IL-18-deficient mice had many eosinophils in their BALF after exposure to OVA. IL-18 may regulate the eosinophilic infiltration in the airway induced by antigen exposure [6,20]. Our results were in agreement with the report by Kodama and colleagues, that IL-18 deficiency enhanced pulmonary eosinophilia in an acute inflammation model. The mechanism of the enhanced eosinophilic infiltration in IL-18-deficient mice may be that of an IL-18-dependent release of IFN-γ or eosinophil-specific chemokines. IFN-γ production was defective in IL-18-deficient mice, and this might lead to enhanced antigen-induced pulmonary eosinophilia [20].

Several factors, including cytokines, effect chronic airway inflammation [21,22]. Our study examined those factors which related to the production of IL-18. The number of OVA-specific IgE is reflected partially in allergic reaction

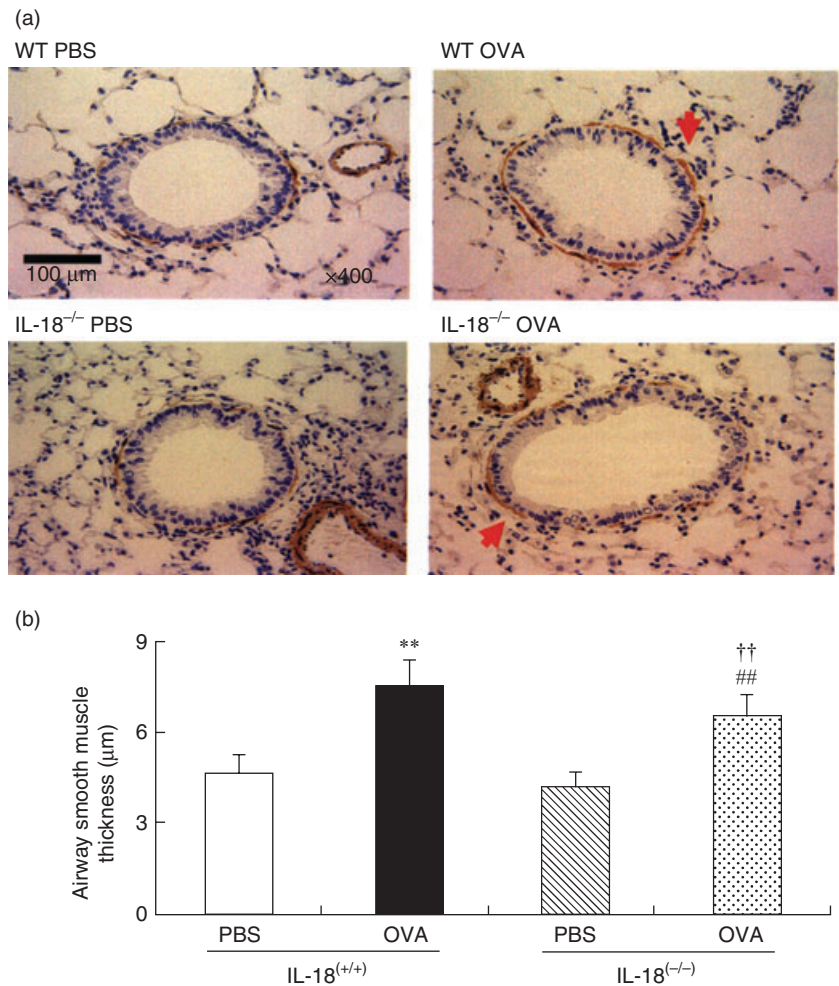


Fig. 7. Smooth muscle thickness of lung tissues in wild-type (WT) [interleukin (IL)-18^{+/+}] and IL-18-deficient (IL-18^{-/-}) mice sensitized and exposed to ovalbumin (OVA). As described in Materials and methods, mice were sensitized and exposed to phosphate-buffered saline (PBS) or OVA for 12 weeks. Airway remodelling in lung tissues was determined by staining with anti- α -smooth muscle actin antibody (a). Trans-sectional images of bronchioles with diameters of 150–200 μ m were examined. Quantitation of airway smooth muscle thickness was determined in at least 10 bronchioles per slide (b) in at least 10 bronchioles per slide. The data are expressed as means $\pm$ standard deviation. ** P < 0.01 versus IL-18^{+/+} mice sensitized and exposed to PBS; ## P < 0.01 versus IL-18^{-/-} mice sensitized and exposed to PBS; †† P < 0.01 versus IL-18^{+/+} mice sensitized and exposed to OVA.

[23]. The allergic reaction induced by OVA might be inhibited through the IL-18 pathway in IL-18-deficient mice. Previous reports showed that production of IgE was related to IL-4 and IL-13 [24–26]. In our results, diminished levels of serum OVA-specific IgE in IL-18-deficient mice might be due to lower levels of IL-4 and IL-13 in the BALF (Fig. 2). Each of the cytokines interacts with each other to modulate chronic airway inflammation. Levels of IL-13 and TGF- β 1 in the BALF were reduced significantly in IL-18-deficient mice exposed to OVA, in comparison with wild-type mice exposed to OVA (Fig. 3e and f). IL-18 stimulated the production of IFN- γ and IL-13 [6,7]. IL-13 also stimulated the production and release of TGF- β 1 from bronchial epithelial cells [27,28]. Therefore, the production of IFN- γ , IL-13 and TGF- β 1 might be inhibited in IL-18-deficient mice exposed to OVA.

In our results, airway remodelling, induction of airway mucus expression, peribronchial fibrosis and airway smooth muscle thickness in IL-18-deficient mice exposed to OVA were inhibited significantly in comparison with wild-type mice exposed to OVA (Figs 5–7). AHR in IL-18-deficient mice exposed to OVA was decreased significantly in com-

parison with wild-type mice exposed to OVA after long-term OVA exposure (Fig. 4b). Several studies have suggested that airway remodelling interacts with AHR [29–35]. Long-term continuous airway inflammation might induce airway remodelling and AHR. Previous studies have highlighted the effects of IL-13 in asthma, such as the direct effect on smooth muscle and airway epithelial cells, as well as allergic inflammation [36–43] and the effects from developing airway remodelling after chronic allergen exposure [44–46]. TGF- β 1 has been suggested to promote fibrotic changes in the airway [47]. IFN- γ also effects airway remodelling in the asthma model [48,49]. The cytokines may be related not only to chronic airway inflammation, but also airway remodelling. It was reported that IL-18 induced IFN- γ , IL-13 and TGF- β 1 directly or indirectly [6,7,27,28].

We made two hypotheses concerning our results with regard to the fact that IL-18-deficiency diminished airway inflammation and remodelling in chronic exposure model. First, IL-18-deficient mice which were exposed to antigen long-term demonstrated that IL-18 modulated chronic airway inflammation through enhanced production of various factors (IFN- γ , IL-13 and TGF- β 1) following infil-

tration of neutrophils into the lung. In addition, these interactions might inhibit airway remodelling as well as AHR in IL-18-deficient mice. Secondly, the tolerance of the OVA-specific response might diminish airway inflammation and remodelling in IL-18-deficient mice. Chronic exposure of OVA leads to the development of tolerance and down-regulation of the OVA-specific inflammatory response [50]. The response, including IgE production, was better tolerated in the IL-18-deficient mice [51]. In future studies, in order to confirm these hypotheses, it will be necessary to examine whether transfer of T cells from IL-18-sufficient mice into IL-18-deficient mice could deteriorate airway inflammation in a chronic model.

Refractory asthma comprises about 10% of the asthmatic population, and the disease remains difficult to control [10]. Refractory asthma is any form of severe asthma that is poorly controlled despite the use of high doses of medications, particularly inhaled corticosteroids [52]. Importantly, levels of IL-18 were increased in pulmonary macrophages from smokers and patients with chronic obstructive lung disease. These studies demonstrate that IL-18 and the IL-18 pathway are activated in cigarette smoke-exposed patients [53]. Smokers with asthma are known to have more severe disease in comparison with never-smokers [52,54]. Pathological studies of refractory asthma have suggested recently that neutrophils are present in higher quantities in the airways of these patients than in the airways of patients with mild asthma or in normal control subjects [10,55]. Our results might suggest that IL-18 contributes to the pathophysiology of refractory asthma through an eosinophil-independent pathway. We also suggest that IL-18 might be one of the important key cytokines in refractory asthma, which was characterized by an enhanced infiltration of neutrophils into the airways and the remodelling of airways. Therefore, it is necessary to investigate these relationships further.

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