

Research Article

# Neutralization of either IL-17A or IL-17F is sufficient to inhibit house dust mite induced allergic asthma in mice

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T helper (Th)17 immune response participates in allergic lung inflammation and asthma is reduced in the absence of interleukin (IL)-17 in mice. Since IL-17A and IL-17F are induced and bind the shared receptor IL-17RA, we asked whether both IL-17A and IL-17F contribute to house dust mite (HDM) induced asthma. We report that allergic lung inflammation is attenuated in absence of either IL-17A or IL-17F with reduced airway hyperreactivity, eosinophilic inflammation, goblet cell hyperplasia, cytokine and chemokine production as found in absence of IL-17RA. Furthermore, specific antibody neutralization of either IL-17A or IL-17F given during the sensitization phase attenuated allergic lung inflammation and airway hyperreactivity. *In vitro* activation by HDM of primary dendritic cells revealed a comparable induction of CXCL1 and IL-6 expression and the response to IL-17A and IL-17F relied on IL-17RA signaling via the adaptor protein act1 in fibroblasts. Therefore, HDM-induced allergic respiratory response depends on IL-17RA via act1 signaling and inactivation of either IL-17A or IL-17F is sufficient to attenuate allergic asthma in mice.

## Introduction

Allergic asthma is a chronic inflammatory disorder of the lung with a prevailing T helper (Th) 2 immune response to inhaled allergens leading to airway hyperreactivity, recruitment of eosinophils, mast cells, and lymphocytes, and hyperplasia of smooth muscle and goblet cells, often associated with increased serum IgE concentrations [1]. Th2 cells are recruited along concentration gradients of the thymus- and activation-regulated chemokine (TARC)/CCL17 and produce the Th2 cytokines interleukin (IL)-4, IL-5, and IL-13 [2]. Recently, we and others reported that Th17 cells are involved in the sensitization and challenge phases of allergic asthma. IL-17A and IL-17F and their heterodimer IL-17AF are produced by Th17 cells, a Th-cell lineage distinct from Th1 and Th2 cells, which is negatively regulated by IFN- $\gamma$  and IL-4 [3]. However, IL-17A and in some instances IL17F and IL-17AF have been shown to be produced by other adaptive and innate cell types including CD8<sup>+</sup> cytotoxic T cells,  $\gamma\delta$  T cells, mucosa-associated invariant T (MAIT) cells, invariant natural killer T (iNKT) cells, NK cells, B cells, neutrophils, macrophages, mast cells, and eosinophils [4,5]. IL-17 is critical for the clearance of several pathogens [6] and is involved in chronic and autoimmune inflammation such as encephalomyelitis, type II collagen induced arthritis, and chronic enterocolitis as reviewed [5].

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We have reported before that allergic asthma is reduced in the absence of IL-17RA, in the ovalbumin (OVA) model [7]. Since IL-17A and its homolog IL-17F activate the IL-17RA/C receptor complex either as homo- or heterodimers [8], we asked whether IL-17A and F have differential effects *in vivo* in regulating the Th2 response to inhaled allergens.

House dust mite (HDM) is a common and major source of allergens in more than 85% of asthmatic patients and contains both cysteine proteases and serine proteases [9]. HDM has been shown to induce epithelial barrier dysfunction [10], the production of proinflammatory cytokines, and cleavage of various tight junctions, and to modulate function of various cell types including Th2 response [11].

Here, we demonstrate that IL-17A or IL-17F are required and sufficient for a full allergic response to mucosal-administered HDM and the response is largely abrogated in either IL-17A or IL-17F deficient mice. Both cytokines induce a Th2 cell response, airway hyperreactivity, neutrophil infiltration, and mucous production. Importantly, the inhibition is not further augmented in the absence of shared IL-17RA. Preventive administration of neutralizing antibodies recapitulates the inhibitory effect. The data therefore suggest that the blockade of either IL-17A or IL-17F dimer protein has comparable inhibitory effect and is not augmented by the blockade of both ligands. A potential involvement of the heteromeric IL-17AF in this model is also possible.

## Methods

### Animals

IL-17A, IL-17F, and IL-17R1 deficient mice on C57BL/6 background (7–8 weeks old) were generated by (3,6) were bred in our animal facility at the Transgenose Institute (TAAM, CNRS, Orleans). Mice were on C57BL/6J genetic background (back-crossed at least 15 times) and wild-type C57BL/6J (B6 or WT) mice were used as control. Mice were maintained under specific pathogen-free conditions in our specific animal facility at CNRS, Orléans, France. Mice were maintained in a temperature controlled (23°C) facility with a strict 12-h light/dark cycle with food and water provided *ad libitum*. Experiments were performed using female mice aged 8–10 weeks with six mice/group and performed twice. All protocols were approved by the CNRS institutional animal research ethical committee and complied with the French Government's ethical and animal experiment regulations.

### HDM induced allergic airway inflammation induction

To induce HDM-driven eosinophilic airway inflammation, isoflurane anesthetized mice were administered intranasally twice at days 0 and 7 with 25 µg HDM extracts (ALK-Abello, Denmark) without adjuvant, control mice were challenged with saline alone and challenged on days 14, 15, and 16 with 5 µg of HDM extracts.

Twenty four hours after the last challenge, mice were killed; bronchoalveolar lavage (BAL) and lung were collected for EPO enzyme, cytokines, chemokines, histology, qPCR and airway hyperreactivity and Western blot analysis.

Neutralizing mouse anti-mouse IL-17A and anti-IL-17F antibodies (clones MM17A and MM17F) from J.v.S., Brussels) are specific for rmIL-17A and rmIL-17F, respectively [12,13] as tested by ELISA (Supplementary Figure S1). The antibodies were injected i.p. into B6 mice weekly at 250 µg at days 0 and 7 during sensitization and with the allergen challenge on days 14, 15, and 16 or only during the challenge phase.

### BAL

BAL was performed by washing the lungs four times with 0.5 ml saline solution at room temperature. After centrifugation at 400×g for 10 min at 4°C, the supernatant (cell-free BALF) was stored at –20°C for cytokine analysis. FACS on BAL and lung mononuclear cells was performed [14].

### EPO activity

EPO activity was determined in order to estimate the recruitment of eosinophils to the lung parenchyma as described [15].

### Lung mononuclear cells isolation

Lung mononuclear cells were isolated from WT and IL-17A and IL-17F deficient mice 24 h after the last challenge as described previously [16]. Briefly, the aorta and the inferior vena cava were sectioned and the lungs were perfused with saline. The lobes of the lungs were sliced into small cubes and then incubated for 45 min in 2 ml RPMI 1640 solution containing DNase I (1 mg/ml) and collagenase IV (2 mg/ml) (Sigma–Aldrich). Lung mononuclear cells were separated by centrifugation on discontinuous Percoll (Amersham Biosciences) gradients (35/70%). Isolated lung mononuclear single cells were plated in round bottom 96-well plates ( $2 \times 10^6$ /ml) and restimulated for 4 h *in vitro* with PMA (50 ng/ml) and ionomycin (750 ng/ml; both from Sigma–Aldrich) in complete medium (IMDM supplemented with

5% (v/v) FBS, L-glutamine (2  $\mu$ M), penicillin (100 U/ml), streptomycin (100  $\mu$ g/ml), and  $\beta$ -mercaptoethanol (50 nM) (all from Invitrogen).

## Flow cytometry analysis of lung mononuclear cells

Lung mononuclear cells were stained as described before and labeled with PerCP-conjugated CD45 (clone 30F11), FITC-conjugated CD3 $\epsilon$  (clone 145-2C11), and v450-conjugated CD4 (clone RM4-5) [14]. After washing, cells were permeabilized for 20 min with cytofix/cytoperm kit (BD Biosciences, France) and stained with PE-conjugated APC-conjugated IL-5 (clone TRFK5), APC-conjugated or APC-conjugated FoxP3 (clone FJK-16s, eBioscience). All antibodies used in the present study were from BD Biosciences, unless otherwise specified. Data were acquired using FACS Canto II flow cytometer and analyzed using Diva and FlowJo software [14].

## Determination of airway bronchial hyperresponsiveness

For invasive measurement of dynamic resistance, mice were anesthetized with intraperitoneal injection of solution containing ketamine (100 mg/kg, Merial) and xylazine (10 mg/kg, Bayer), paralyzed using D-tubocurarine (0.125%, Sigma), and intubated with an 18-gauge catheter. Respiratory frequency was set at 140 breaths per min with a tidal volume of 0.2 ml and a positive end expiratory pressure of 2 ml H<sub>2</sub>O. Increasing concentrations of aerosolized methacholine were administered. Resistance was recorded using an invasive plethysmograph (Buxco, London, U.K.). Baseline resistance was restored before administering the subsequent doses of methacholine as described [14].

## Lung histology

Lungs were fixed in 4% buffered formaldehyde and 3- $\mu$ m sections were stained with PAS and examined with Leica DM2500 microscope ( $\times$ 40 magnification). Peribronchial infiltrates and mucus hypersecretion were assessed by a semiquantitative severity score (0–5) by two observers independently as described before [17].

## In vitro culture of dendritic and MEFs

Murine bone marrow cells were isolated from femurs and differentiated into dendritic cells. Cells ( $1 \times 10^6$ /ml) were cultured for 10 days in DMEM (Sigma) supplemented with 20% horse serum (Gibco) with GM-CSF. After 7 days, cells were washed and recultured in fresh medium for another 3 days. For the experiment, the cells were resuspended in medium containing 0.2% FBS and plated in 48-well microtiter plates ( $1 \times 10^6$  cells/well), and stimulated upon adherence with HDM (10  $\mu$ g/ml, Stallergenes). Cell supernatants lysates were harvested after 6 or 24 h and analyzed directly for cytokine quantitation.

MEFs from BL6 and Act1 deficient mice were obtained from Professor X. Li (Immunology, Cleveland, U.S.A.) and cultured as described [18].

## Cytokines measurement by ELISA

IL-1 $\beta$ , IL-5, IL-6, IL-13, IFN $\gamma$ , CCL11 (eotaxin 1) concentrations in lung homogenate were determined by ELISA, using commercial kits from R&D (Abingdon, U.K.) according to the manufacturer's recommendations or by multiplex (MAGPIX, Bio-Rad).

## Statistical analysis

Data were analyzed using Prism version 5 (Graphpad Software, San Diego, CA). The non-parametric Kruskal–Wallis test with Dunn's multiple comparison test or the parametric one-way ANOVA test with multiple Bonferroni's comparison test were used. Values are expressed as mean  $\pm$  SEM. Statistical significance was defined at a *P*-value < 0.05.

## Results

### Expression of IL-17A and IL-17F expression in the lung upon HDM challenge

To investigate the pulmonary expression of IL-17A and IL-17F activating the IL-17RA/RC receptor [8,19], BL6 mice were immunized and challenged by intranasal instillation of HDM to induce allergic lung inflammation (Figure 1a). Upon HDM exposure, both *IL-17A* and *IL-17F* mRNA and proteins are expressed in lung homogenate of BL6 mice (Figure 1b,c). IL-17A and IL-17F were increased in the reciprocal single KO mice likely to be due to compensatory mechanisms as well as in 17-RA KO mice (Figure 1c).

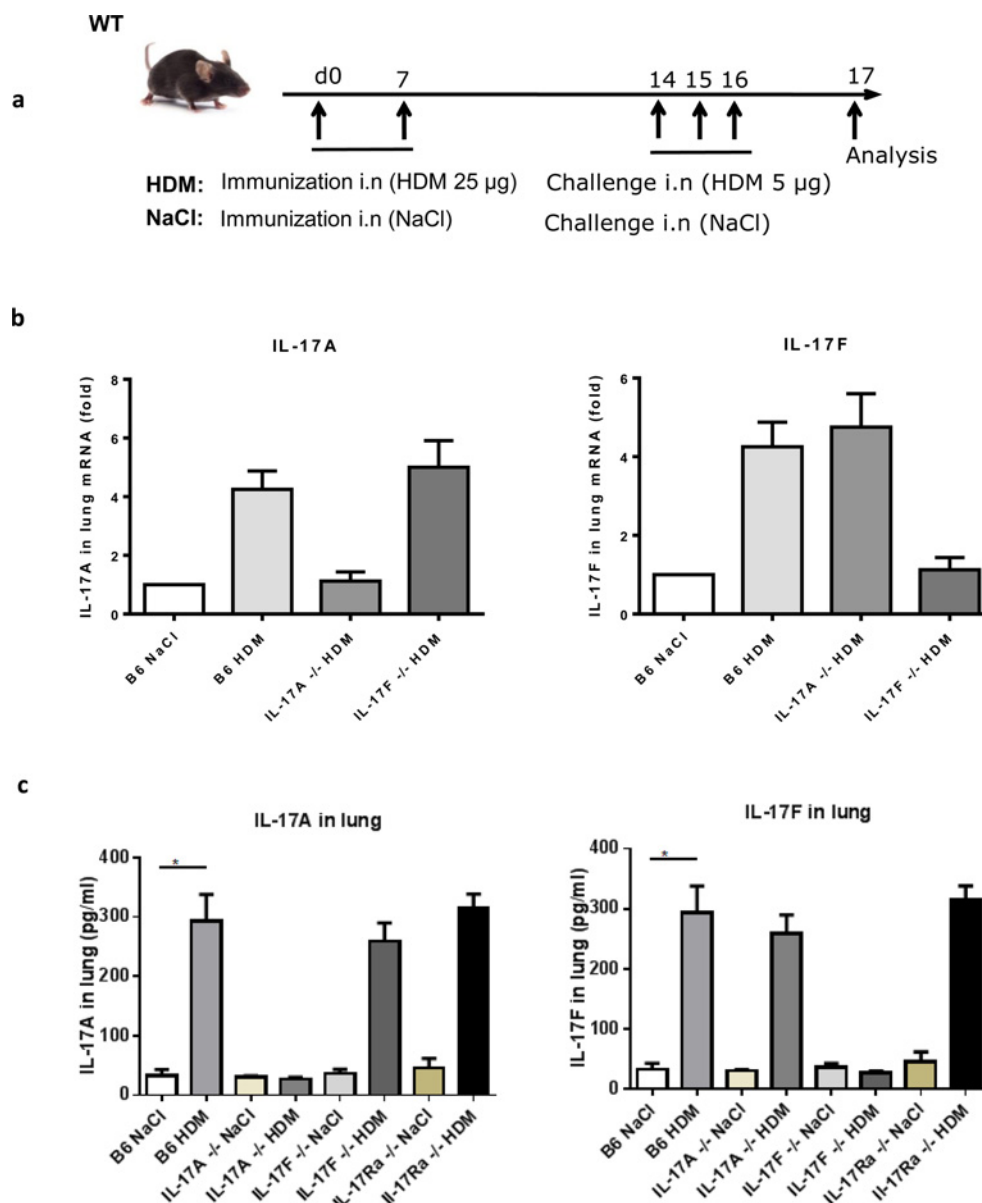


**Figure 1. Expression of IL-17A and IL-17F in the lung upon HDM challenge**

Experimental protocol (a). *IL-17A* and *IL-17F* mRNA expression in lung from HDM sensitized and challenged BL6, *IL-17A*, or *IL-17F* KO mice (b). *IL-17A* or *IL-17F* protein expression in BL6, *IL-17A*, *IL-17F*, and *IL-17R1A* KO mice (c). Lung homogenates were analyzed on day 17 in HDM sensitized and challenged mice. The data are from two experiments ( $n=6$  mice per group) and expressed as mean values  $\pm$  S.E.M. (\* $P \leq 0.05$ ).

Since *IL-17A* and *IL-17F* are up-regulated in HDM-induced allergic asthma, both may have similar biological functions. To understand the role of *IL-17A* and *IL-17F*, we conducted the HDM-induced allergic asthma model in single *IL-17A*, *IL-17F*, and in the shared *IL-17RA* KO mice. Finally, we used specific *IL-17A* and *IL-17F* neutralizing antibodies in BL6 wild-type mice.

## Absence of *IL-17A* or *IL-17F* prevents HDM allergic inflammation

Mice immunized and challenged with HDM developed a robust allergic response, while mice deficient for either *IL-17A* or *IL-17F* had reduced total cell recruitment into bronchoalveolar lavage (BAL) compared with the wild-type BL6 littermate controls (Figure 2a). In particular, a decrease in eosinophil numbers in BAL fluid (BALF) (Figure 2b)

with diminished eosinophil peroxidase (EPO) activity in lung (Figure 2c), and reduced lymphocyte and neutrophil recruitment in the BAL were found (Figure 2d,e), whereas macrophage numbers were not significantly different (Figure 2f).

Histological analysis revealed that HDM-challenged BL6 wild-type mice developed a robust lung inflammatory response characterized by perivascular and peribronchiolar infiltration of eosinophils and mononuclear cells (Figure

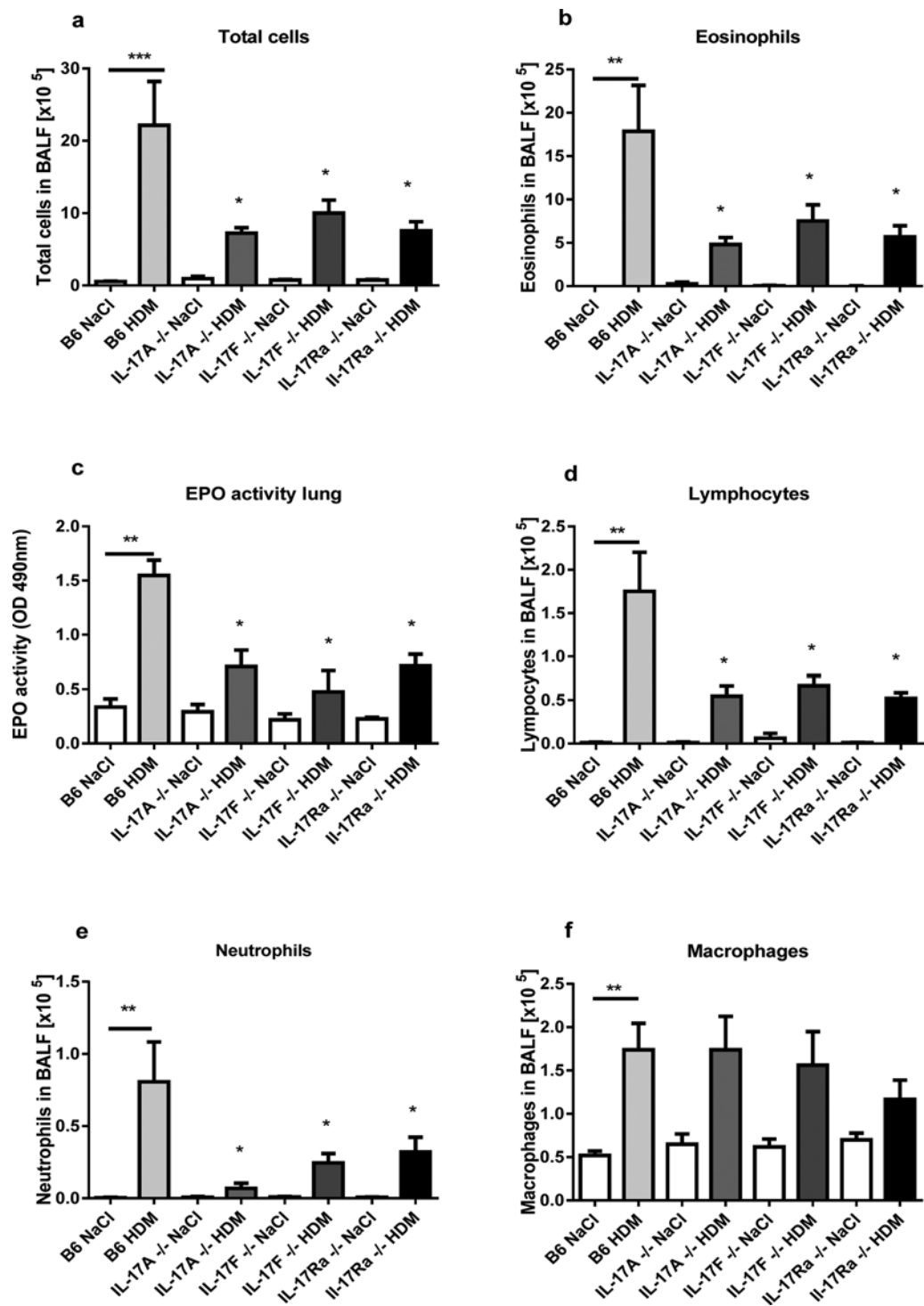


Figure 2. Reduced allergic asthma in IL-17A, IL-17F, and IL-17RA deficient mice (Continues on next page)

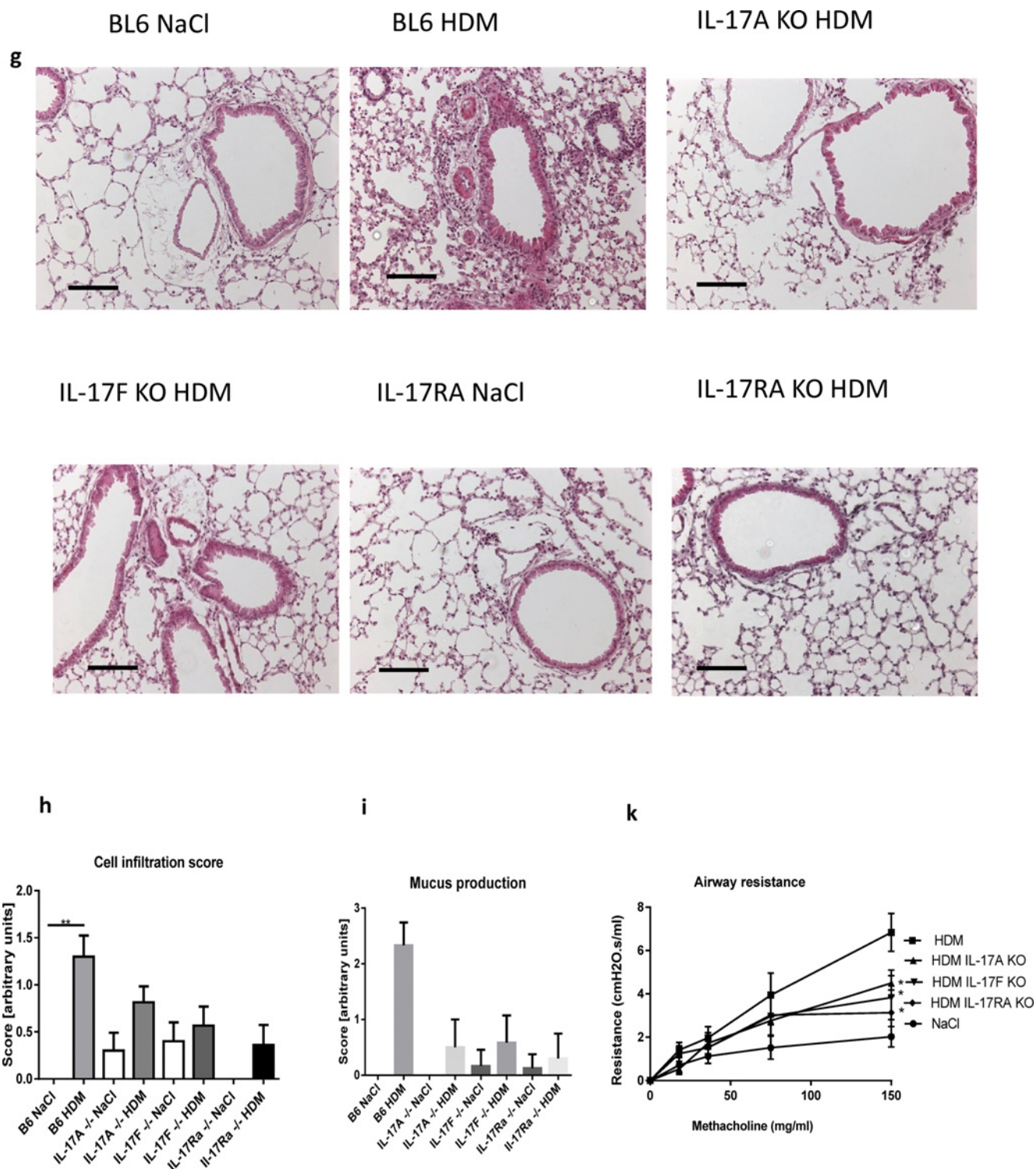


**Figure 2. Reduced allergic asthma in IL-17A, IL-17F, and IL-17RA deficient mice**

Reduced total cell recruitment in BALF with diminished eosinophils (a,b) and EPO activity in lung (c), lymphocytes and neutrophil counts in IL-17A, IL-17F, and IL-17RA KO mice (d,e), while macrophage counts were not affected (f). Reduced lung inflammation by microscopy (scale bar: 200  $\mu$ m) in the tested gene-deficient mice (g,h) with reduced mucus production (i), and airway resistance (RL) (k). The data are representative of two independent experiments ( $n=6$  mice per group) and expressed as mean  $\pm$  S.E.M. (\* $P \leq 0.05$ , \*\* $P \leq 0.01$ ).

2g). In contrast, IL-17A and IL-17F KO mice displayed a significant reduction in inflammatory cell infiltration in lung consistent with the reduced cell recruitment in the BALF (Figure 2h). Additionally, the extensive hyperplasia of goblet cells and mucus production observed in challenged WT mice was drastically reduced in gene-deficient mice after HDM challenge at day 17 (Figure 2i).

A hallmark of allergic lung inflammation is increased airway resistance (RL), which was measured by invasive plethysmography in WT and gene-deficient mice. We found significant reduction in RL in IL-17A, IL-17F, and IL-17RA deficient mice challenged with HDM as compared with WT mice (Figure 2k).

In summary, absence of either 17A, IL-17F, or IL-17RA impaired inflammatory cell recruitment in the BAL and lung associated with reduced airway hyperreactivity in HDM induced allergic lung inflammation.

## **Comparable reduction in inflammatory mediators and RL in IL-17A and IL-17F deficient mice**

In response to HDM challenge protein and MPO in BALF and pulmonary IL-1 $\beta$ , IL-5, IL-13, and CCL-11 were increased (Figure 3a–f). In accordance with reduced allergic inflammation, total protein and MPO were reduced in BALF (Figure 3a,b), and IL-1 $\beta$ , IL-5, IL-13, and CCL-11 were diminished in the lung homogenate of HDM-sensitized and challenged IL-17A, IL-17F, and IL-17RA deficient mice compared with BL6 control mice (Figure 3c–f). Therefore, the data demonstrate that the absence of either IL-17A or IL-17F reduced HDM induced allergic lung inflammation.

## **Absence of IL-17RA prevents HDM-induced asthma as in IL-17A or IL-17F deficient mice**

Since IL-17A and F signal through IL-17RA, we asked whether there is an additive inhibition in IL-17RA-deficient mice. We find that the cell recruitment, airway hyperreactivity and lung tissue inflammation, and RL are inhibited to the same extent in the absence of 17RA as in mice deficient for either IL-17A or IL-17F (Figures 2 and 3). Therefore, the data suggest that either IL-17A or IL-17F are sufficient to activate the IL-17RA, and absence of either ligand or IL-17RA abrogates the allergic response.

## **Antibody neutralization of either IL-17A or IL-17F prevents HDM-induced asthma**

To ascertain that the observed effects are not due to an artifact of the genetic model, we used neutralizing mouse anti-mouse IL-17A and anti-IL-17F antibodies with high specificity [12,13]. Antibodies were injected into BL6 mice weekly at 250  $\mu$ g during sensitization and allergen challenge (Figure 4a) and an IgG1 isotype antibody was used as control. We found that both neutralizing antibodies drastically reduced cell recruitment and lung tissue inflammation to the same extent observed in the genetic absence of 17RA, IL-17A, or IL-17F, while the isotype control antibody had no effect (Figure 4a–f). Moreover, the combination of the antibodies had no additive inhibitory effect (results not shown). Microscopic assessment of lungs from HDM-challenged mice treated with neutralizing IL-17A and IL-17F antibodies revealed diminished inflammation (Figure 4g,h) and mucus hypersecretion as assessed by periodic acid Schiff reagent (PAS) staining (Figure 4i). We further investigated RL by invasive plethysmography and found reduced RL in mice given neutralizing IL-17A or IL-17F antibodies during HDM sensitization and challenge (Figure 4k), but not when the antibodies were given only during the antigen challenge phase (Figure 4l). The data demonstrate that either IL-17A or IL-17F antibodies prevented allergic asthma as found in the gene-deficient mice. Moreover, the proinflammatory cytokines such as IL-1 $\beta$ , IL-5, IL-13, and CCL11/eotaxin were significantly reduced in the lung homogenate from HDM-challenged BL6 mice receiving either IL-17A or IL-17F neutralizing antibodies during the sensitizing phase to a comparable extent as in IL-17RA-deficient mice (Figure 5a–f).

Therefore, preventive antibody therapy had a major impact on airway hyperreactivity and allergic lung inflammation consistent with our previous data in the OVA model [20]. Importantly, IL-17A or IL-17F antibody neutralization during HDM challenge had no effect on the cytokine/chemokine responses and airway hyperreactivity.

In conclusion, preventive administration of IL-17A and IL-17F neutralizing antibodies reduced allergic lung inflammation in response to HDM to the same extent as found in the corresponding gene-deficient mice.

## **Reduced lymphocytes recruitment to the lung upon HDM challenge in the absence of IL-17A, IL-17F, and IL-17RA**

T lymphocytes from HDM immunized and sensitized mice were isolated from the lung, restimulated with PMA and ionomycin, and analyzed by flow cytometry for IL-5, ROR $\gamma$ t and FoxP3 expression (Supplementary Figure S2).

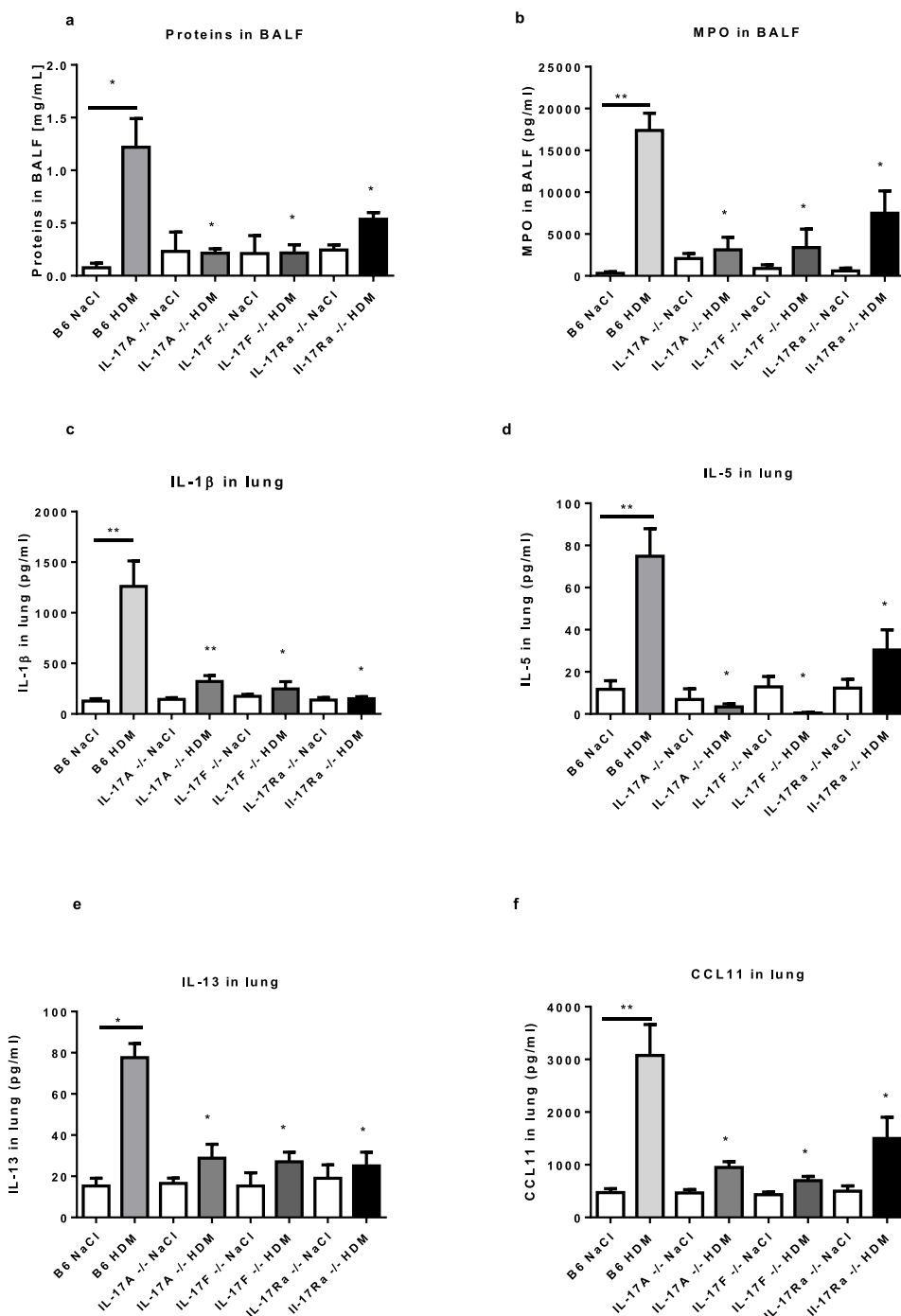


**Figure 3. Reduced inflammatory mediators in IL-17A, IL-17F, and IL-17RA deficient mice**

Reduced total protein and MPO in BALF (a,b) and IL-1 $\beta$ , IL-5, IL-13, and CCL11 in lung homogenate of IL-17A, IL-17F, and IL-17RA KO mice at day 17 after immunization and challenge with HDM (c–f). Representative data of two independent experiments ( $n=6$  mice per group) showing mean values  $\pm$  S.E.M. (\* $P \leq 0.05$ , \*\* $P \leq 0.01$ ).

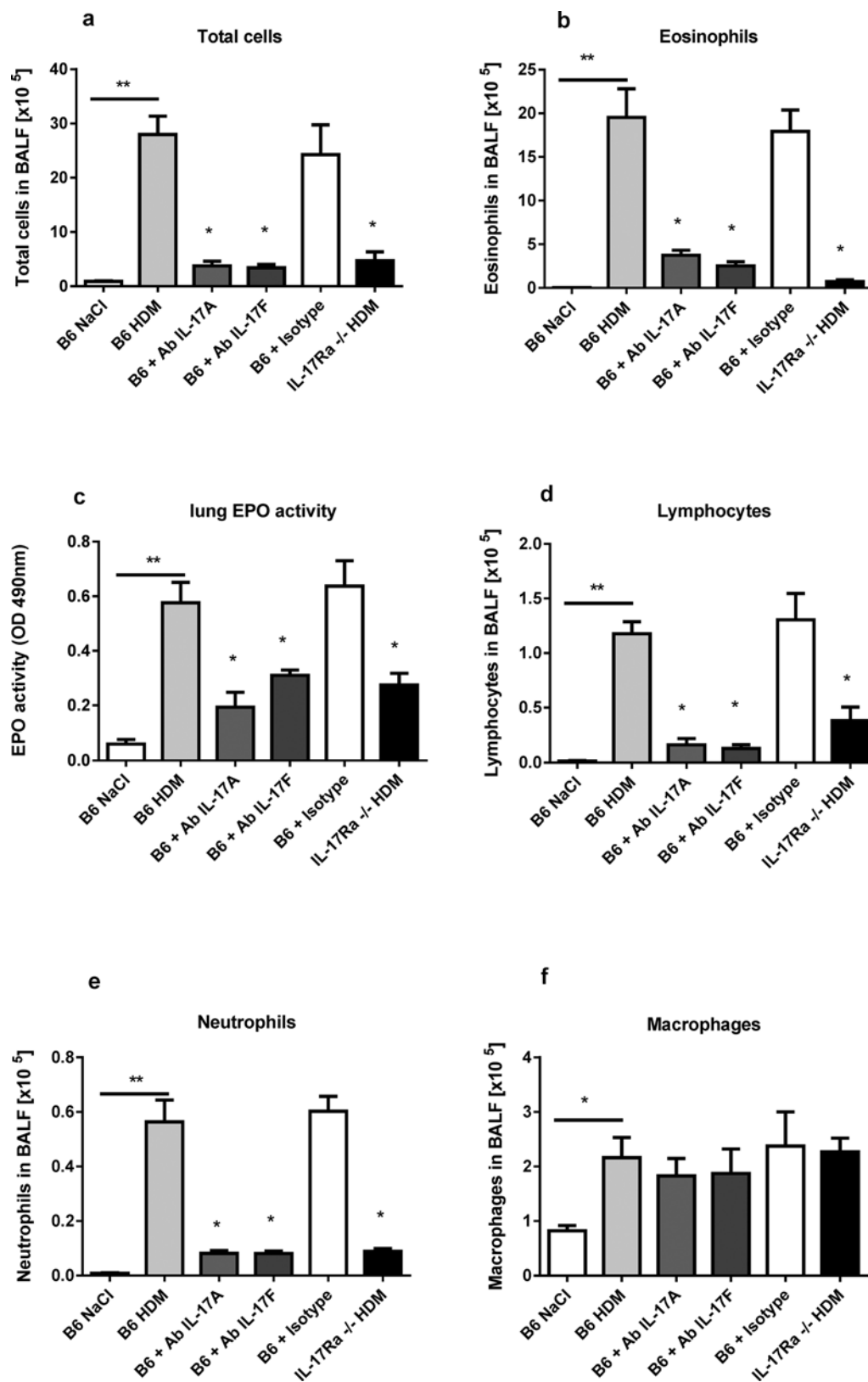


Figure 4. Antibody neutralization of either IL-17A or IL-17F prevents HDM-induced asthma (Continues on next page)

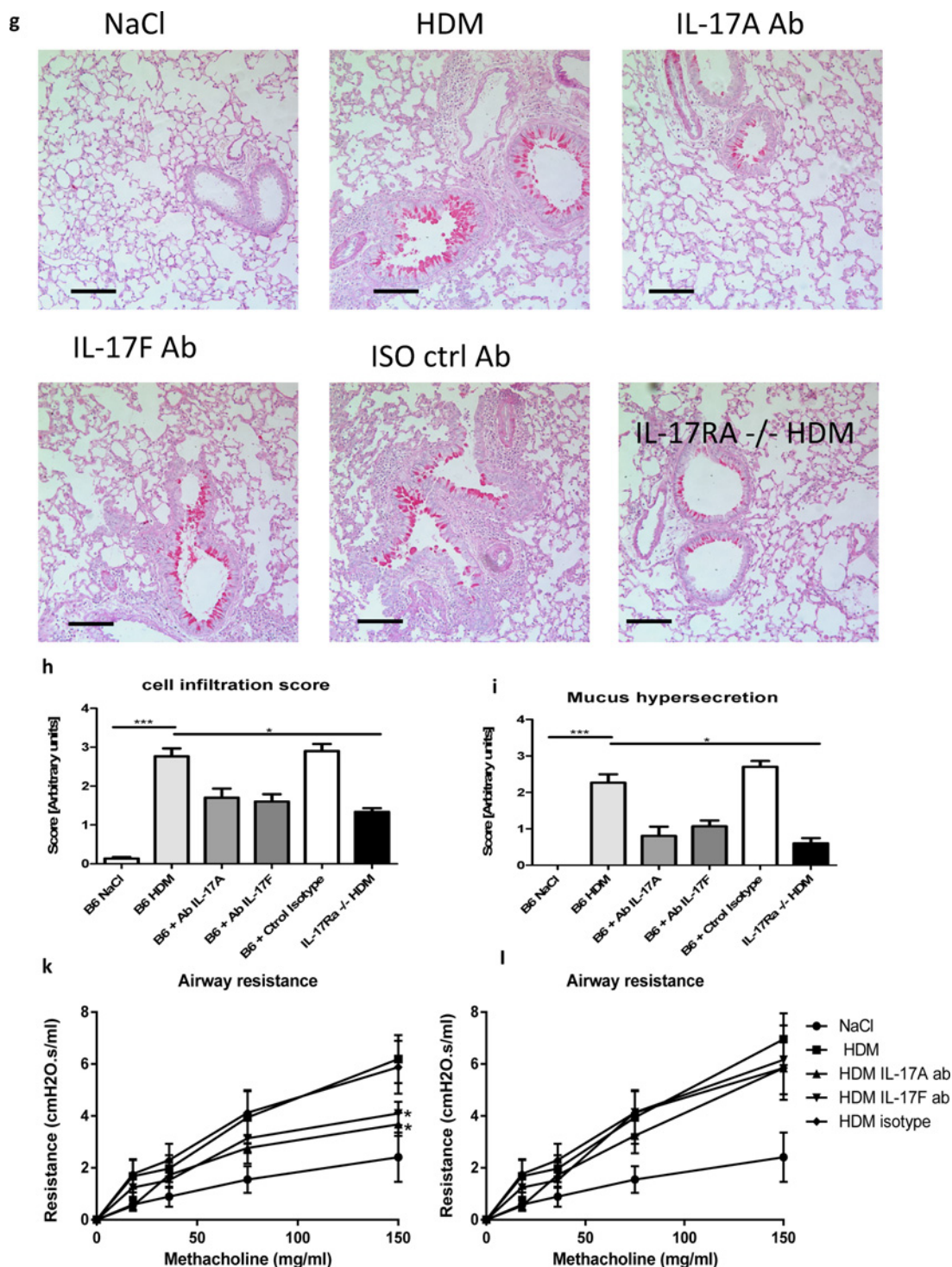


**Figure 4. Antibody neutralization of either IL-17A or IL-17F prevents HDM-induced asthma**

Preventive antibody administration diminished allergic lung inflammation with reduced total cell and eosinophil recruitment into BALF (a,b), diminished EPO activity in lung (c), lymphocytes, and neutrophil counts in BAL (d,e), but not macrophages (Figure 2f) in BL6 mice at day 17 after antibody neutralization compared with IL-17RA KO control mice. Diminished lung inflammation and mucus overexpression assessed by microscopy (scale bar: 200  $\mu$ m) on H&E and PAS stained lung tissue slides (g–i). Reduced RL upon preventive (k), but not therapeutic antibody administration (l). The data are representative of two independent experiments ( $n=6$  mice per group) and mean values  $\pm$  S.E.M. (\* $P\leq 0.05$ , \*\* $P\leq 0.01$ ) are shown.

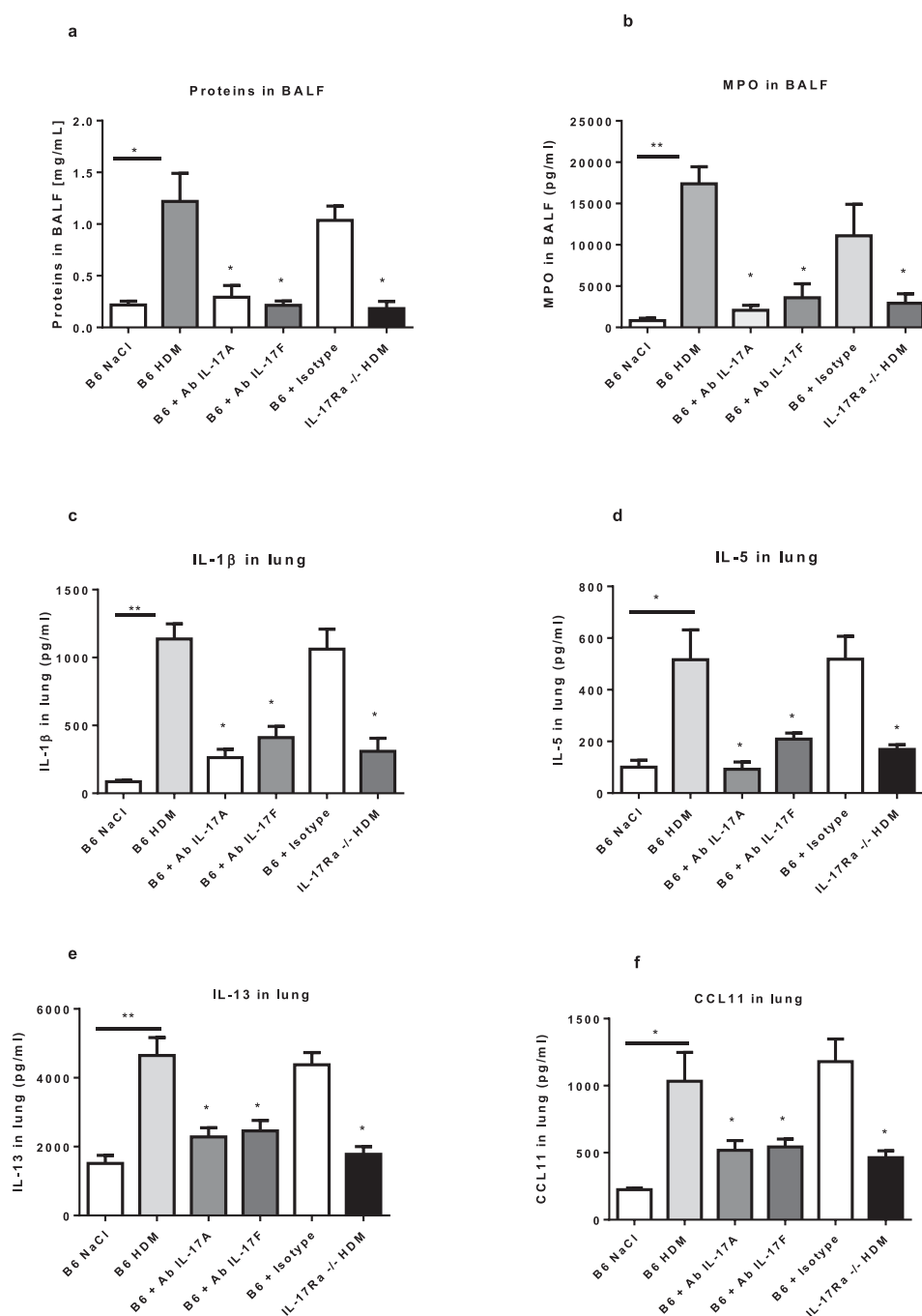


**Figure 5. Reduced inflammatory mediators upon blockade with either IL-17A or IL-17F antibody**

Reduced protein and MPO in BALF, and IL-1 $\beta$ , IL-5, IL-13, and CCL17/TARC levels in lung homogenate (a–f) at day 17 in BL6 mice after antibody neutralization compared with IL-17RA KO control mice. The data are representative of two independent experiments and expressed as mean values  $\pm$  S.E.M. (\* $P \leq 0.05$ , \*\* $P \leq 0.01$ ).

HDM induced the recruitment of CD4<sup>+</sup> T lymphocytes expressing IL-5, ROR $\gamma$ t, and FoxP3 in the lung (Figure 6). The recruitment of CD4<sup>+</sup>IL-5<sup>+</sup> lymphocytes cells expressing IL-5 was reduced to a comparable extent in IL-17A, IL-17F, and IL-17RA deficient mice (Figure 6). Furthermore, the recruitment of Th17 cells expressing ROR $\gamma$ t was only slightly, but not significantly diminished, while no increase in FoxP3<sup>+</sup> cells was found. The data suggest that

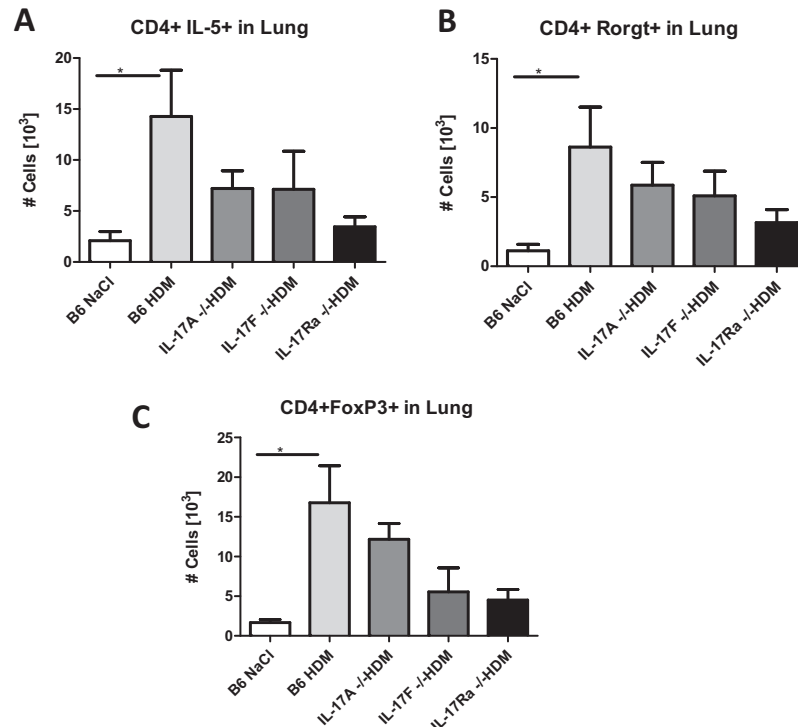


**Figure 6. Reduced recruitment of IL-17A and IL-17F expressing cells in the lung upon HDM challenge in IL-17A, IL-17F, and IL-17RA deficient mice**

T lymphocytes from HDM immunized and sensitized mice were isolated on day 17 from the lung of BL6, IL-17A, IL-17F, and IL-17RA deficient mice, and were restimulated with PMA and ionomycin for 4 h, stained with either IL-17A or IL-17F antibody and analyzed by flow cytometry. While HDM induced an increase in CD4<sup>+</sup>IL-5<sup>+</sup> (A), CD4<sup>+</sup>RORγt<sup>+</sup> (B) and CD4<sup>+</sup>FoxP3<sup>+</sup> (C) cells in lung, the IL-17A, IL-17F, and IL-17RA KO mice showed a trend of reduced cell recruitment ( $n=6$  mice/group).

IL-17A or IL-17F blockade inhibits Th2 differentiation, while the data on Th17 and Treg cell recruitment are less conclusive and need further investigations.

## IL-17A and IL-17F engage the IL-17RA leading to Act1-dependent cell activation *in vitro*

To further explore the mechanisms of the inflammatory response we conducted *in vitro* primary cultures studies on murine bone marrow dendritic cells (BMDCs). In cultured BMDCs stimulated with HDM, we found reduced CXCL1/KC and IL-6 expression in dendritic cells derived from IL-17RA KO mice (Figure 7a); IL-17A or IL-17F induced comparable CXCL1/KC and IL-6 expression in dendritic cells (Figure 7b), which was enhanced by TNF.

We then investigated whether the proximal adaptor protein Act1 of the IL-17RA is required for signaling by either ligand [19] using mouse embryonic fibroblasts (MEFs) [21]. We found that Act1 is required for IL-17A or IL-17F induced act1 activation in MEF, since IL-17A or IL-17F induced CXCL1/KC and IL-6 expression were abrogated in act1-deficient fibroblasts (Figure 7c), while IL-1 $\beta$  stimulation was act1 independent (not shown).

Therefore the act1 adaptor is essential for 17A or IL-17F induced IL-17RA signaling with inflammatory cytokine production. Blockade with either antibody abrogated the response to the same extent as in IL-17RA-deficient fibroblasts and act1 is critically involved. Therefore, either IL-17A or IL-17F is sufficient to engage IL-17RA activation via act1.

## Discussion

We demonstrate that HDM-induced allergic lung inflammation is dependent on IL-17A and IL-17F signaling via IL-17RA. The cardinal features of asthma include airway hyperreactivity, inflammation, remodeling and cytokines, and chemokine release. This report demonstrates that either IL-17A or IL-17F blockade is sufficient to block allergic

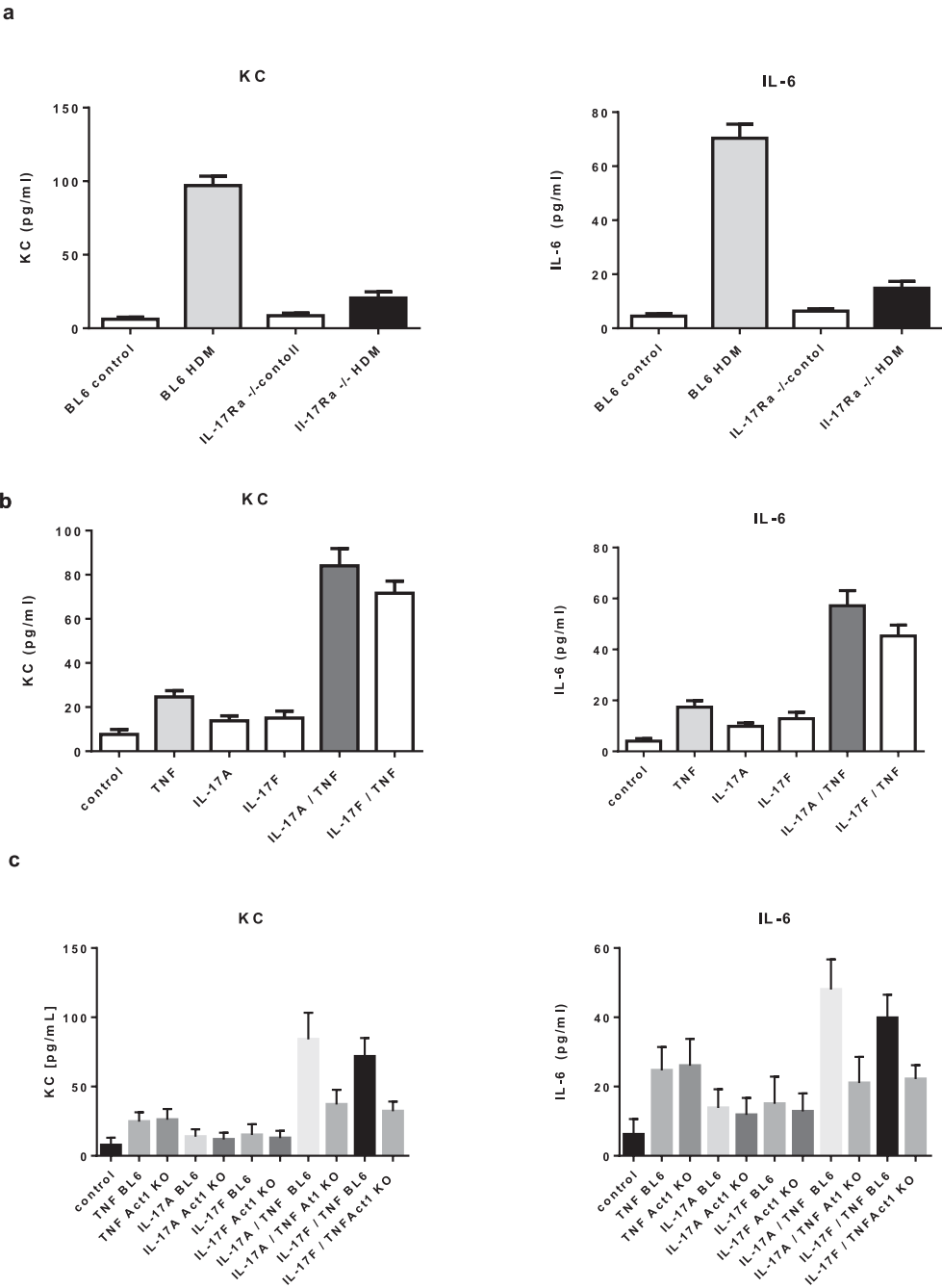


**Figure 7. IL-17A and IL-17F engage the IL-17 RA on DC and fibroblasts to produce KC and IL-6**

BMDs from WT and IL-17RA mice were cultured with either HDM, IL-17A, or IL-17F and the production of KC/CXCL1 and IL-6 was assessed at 10 h in the supernatant by ELISA. (a) BMDs produce KC and IL-6 upon HDM stimulation, which is IL17RA dependent (b); Act1-deficient mouse embryonic fibroblasts (MEFs) respond to IL-17A and IL-17F with the production of KC and IL-6, which is enhanced by TNF; (c) IL-17A and IL-17F activate BL6, but not act1-deficient DC to produce KC and IL-6 which is augmented by TNF. The data are representative of two independent experiments ( $n=4$ /group) and expressed as mean  $\pm$  S.E.M.

lung inflammation induced by HDM. They both activate the shared IL-17RA/C pathways, suggesting a complementary activity of the two ligands.

The homo- and heterodimer of IL-17, i.e. IL-17AA, IL-17AF, and IL-17FF are all recognized by the IL17RA/IL17RC complex [19,22]. Now, we show that IL-17A and IL-17F contribute equally to HDM induced lung inflammation, which is IL-17RA dependent. The potential contribution of IL-17AF cannot be excluded as this molecule is absent from IL17A or IL17F KO mice.

Recently in asthma models induced by *A. fumigatus* antigens or OVA, eosinophils were shown to be a source of the heterodimer IL-17AF [4]. However, in the present study, no data were provided on the presence of IL17A and IL17F homodimers. Therefore, it does not allow defining the relative contributions of the three molecules.

Previous studies using gene-deficient mice suggested some functional specialization IL-17A and IL-17F in host resistance, autoimmunity, and inflammation [3,23]. Th17 cells coexpress IL-17A and IL-17F [23,24], while a subset expresses IL-22, which may have a protective effect [25]. A role of IL-17F has been reported in experimental asthma models [26,27], using either *in vitro* or OVA-induced asthma models [23], which differ from the protease allergen used in the present study.

We find that IL-17A or IL-17F antibody blockade diminished allergic inflammation when given during the immunization, but was ineffective during the HDM challenge. This supports the notion that IL-17 is required for the generation of a Th17 response confirming our previous data, as we showed that rmIL-17A instilled during the effector phase inhibits asthma [20]. By contrast, intranasal administration of 25 µg IL-17A antibody during the challenge phase only in HDM (*Dermatophagoides farinae*) induced asthma reduced neutrophil recruitment in BAL and airway hyperreactivity, but had no effect on IL-5 levels and eosinophil recruitment [28], which differ from our study using MM antibodies and i.p. injections without any effect of IL-17A compared with IL-17F blockade during the challenge phase.

In another model such as collagen-induced arthritis (CIA), only IL-17A neutralizing antibody reduced the severity of arthritis while IL-17F neutralization had no effect [29].

In the present study, we showed that both IL-17A and IL-17F neutralization by antibodies or deficiency of these cytokines diminishes IL-5 and IL-13 production and Th2 cell generation. In a different setting, it has been shown that neutralization of IL-17A in the RSV-infected mice results in a significant decrease in the mucus production and inhibition of the Th2 cytokines IL-5, IL-4, and IL-13, but also of the Th17 response. In addition, a decrease in activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells and neutrophils was observed [30,31].

Important question to be addressed in the future is the role of IL-17A and IL-17F on the development of type 2 and 3 innate lymphoid cells preceding the adaptive immune response [32] and the potential role of PKC-θ as recently reported [14].

Our data indicate a relevant role not only of IL-17A, but also IL-17F in regulation of lung Th2 responses. Moreover, if the mouse data can be translated to human patients, both IL-17A or IL-17F neutralizing antibodies might have comparable effects.

Mechanistically, we found that the response to HDM of dendritic cells depends on IL-17RA to produce CXCL1 and IL-6. Further, CXCL1 and IL-6 expression induced by IL-17A or IL-17F were indistinguishable. The proximal adaptor protein Act1 of the IL-17RA complex is required for signaling by either ligand [19,21]. Using MEFs or BMDCs, we show that IL-17A or IL-17F induced act1-dependent CXCL1 and IL-6 production. Therefore, the Act1 adaptor is essential for 17A or IL-17F induced IL-17RA signaling with inflammatory cytokine production.

In conclusion, inactivation of either IL-17A or IL-17F is sufficient to prevent allergic asthma in mice. Therefore, the data suggest that IL-17A and IL-17F homodimer or the IL-17A/IL-17F heterodimer activate the IL-1RA/C via act1 signaling conveying a comparable inflammatory response.

## Clinical perspectives

- IL-17 produced by Th17 cells is an important factor in severe neutrophilic allergic lung inflammation contributing to Th2 driven allergic asthma by HDM in mice. Both IL-17A and IL-17F are induced activating the shared receptor IL-17RA.
- The present study reveals that severe allergic lung inflammation is attenuated in absence of either IL-17A or IL-17F or IL-17RA in mice. Furthermore, IL-17A or IL-17F antibody blockade prevented lung allergic inflammation. The experimental data demonstrate that either IL-17A or IL-17F blockade is sufficient to attenuate severe allergic asthma in mice.

- Therefore, the results are relevant for clinical use of either IL-17A or IL-17F antibodies or related inhibitors in IL-17RA-dependent severe asthma, suggesting equal efficacy using either agonist or receptor blockade to treat severe asthma crisis.

## Author contribution

P.C., F.d.P., D.T. and B.R. designed the experiments. P.C., L.F., F.M., T.M., N.R. and A.L. conducted the study. P.M. and R.L. performed the mouse embryo transfer. C.U. and J.v.S. provided the mouse monoclonal antibodies. Y.I. provided the IL-17-deficient mice. B.R., V.Q., J.v.S. and F.d.P. wrote and corrected the manuscript.

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## Competing interests

The authors declare that there are no competing interests associated with the manuscript.

## Abbreviations

BAL, bronchoalveolar lavage; BALF, BAL fluid; BMDC, bone marrow dendritic cell; EPO, eosinophil peroxidase; HDM, house dust mite; IL, interleukin; MEF, mouse embryonic fibroblast; OVA, ovalbumin; PAS, periodic acid Schiff reagent; RL, airway resistance; Th, T helper.

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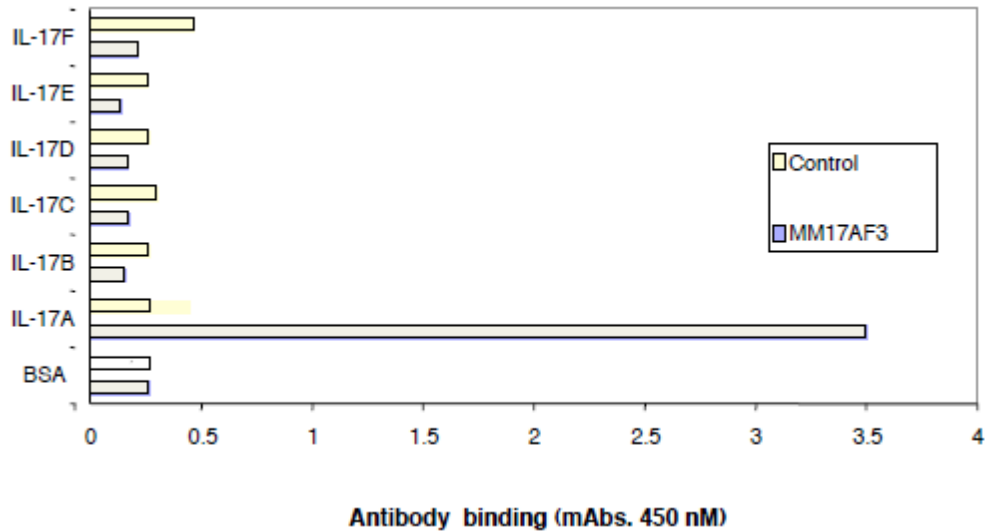
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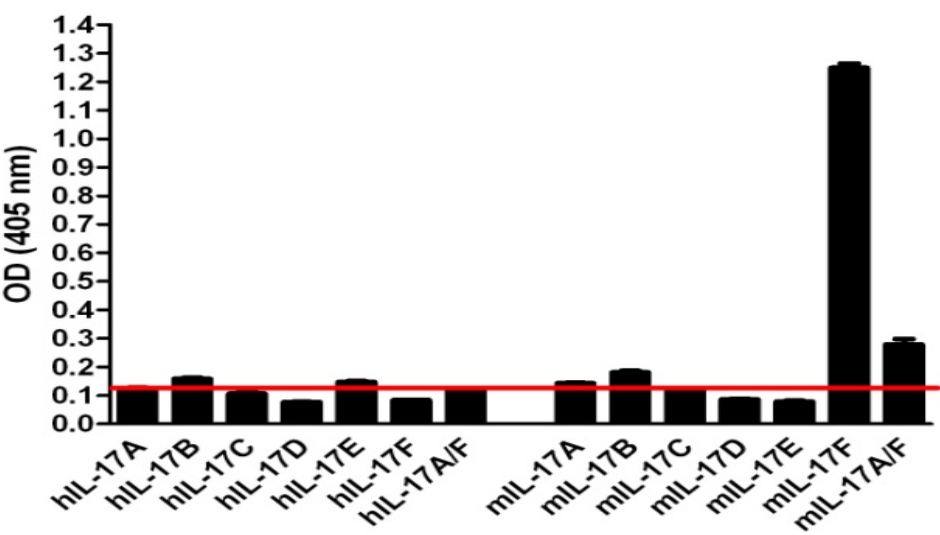
Supplemental Figure 1

Specificity of IL-17A and IL-17F MM antibodies

a IL-17A mab                      MM17 AF3

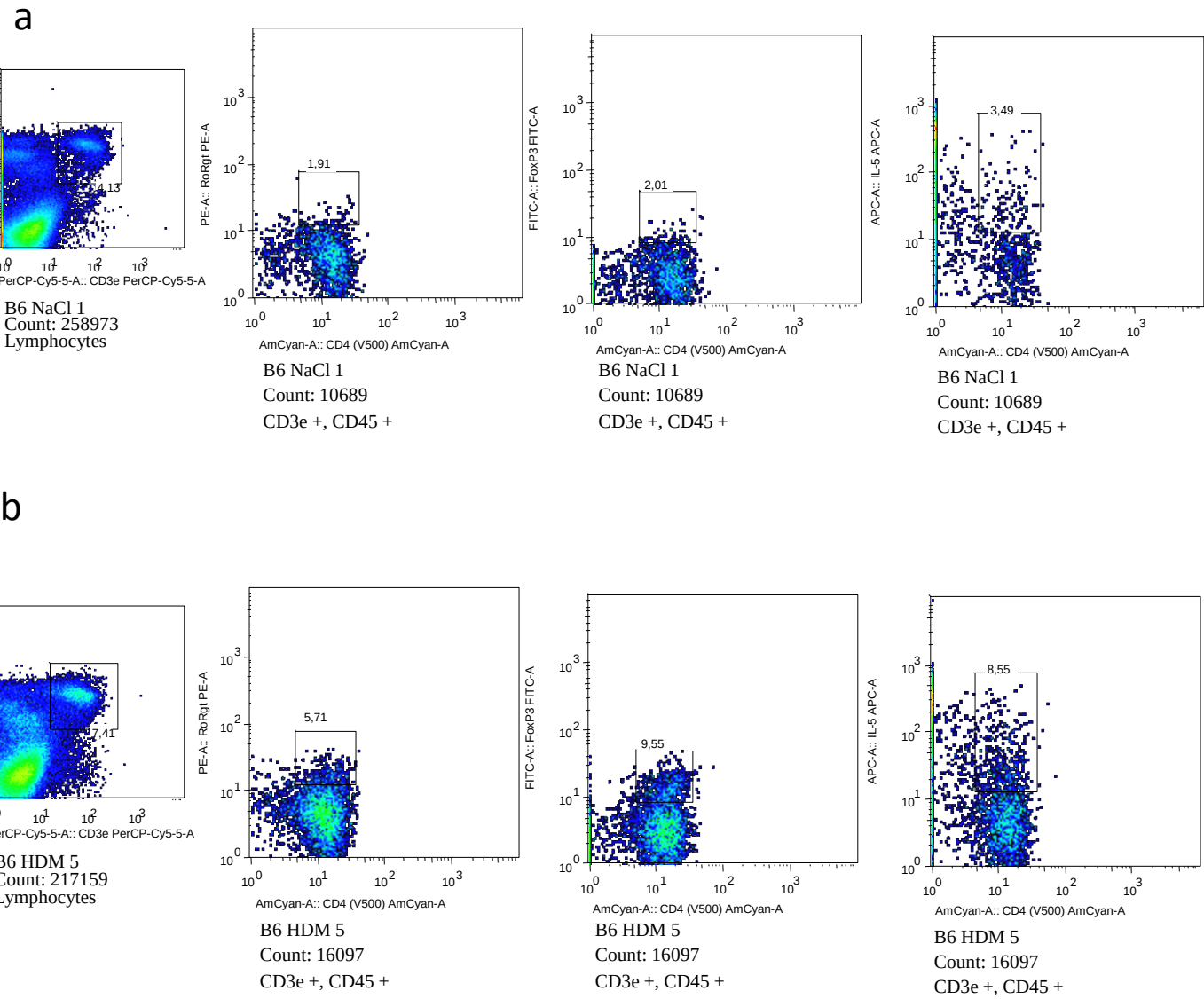


b IL-17F mab                      MM17F 8F5



Supplemental Figure 2

Flow cytometric analysis of lung infiltrating cells



**c**