Published in final edited form as:

J Allergy Clin Immunol. 2009 October ; 124(4): 761–70.e1. doi:10.1016/j.jaci.2009.07.040.

Exaggerated IL-17 response to epicutaneous sensitization mediates airway inflammation in the absence of IL-4 and IL-13

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Abstract

Background—Atopic dermatitis (AD) is characterized by local and systemic Th2 responses to cutaneously introduced allergens and is a risk factor for asthma. Blockade of Th2 cytokines has been suggested as therapy for AD.

Objectives—To examine the effect of the absence of IL-4 and IL-13 on the Th-17 response to epicutaneous (EC) sensitization in a mouse model of allergic skin inflammation with features of AD.

Methods—Wild-type (WT), IL-4KO, IL-13KO and IL-4/13 double KO (DKO) mice were subjected to EC sensitization with ovalbumin (OVA) or saline and airway challenged with OVA. Systemic immune responses to OVA, skin and airway inflammation, and airway hyperresponsiveness (AHR) were examined.

Results—OVA sensitized DKO mice exhibited impaired Th2 driven responses with undetectable OVA specific IgE and severely diminished eosinophil infiltration at sensitized skin sites, but intact dermal infiltration with CD4⁺ cells. DKO mice mounted an exaggerated IL-17A, but normal IFN- γ and IL-5 systemic responses. Airway challenge of these mice with OVA caused marked upregulation of IL-17 mRNA expression in the lungs, increased neutrophilia in bronchoalveolar lavage fluid (BALF), airway inflammation characterized by mononuclear cell infiltration with no detectable eosinophils, and bronchial hyperresponsiveness to methacholine that were reversed by IL-17 blockade. IL-4, but not IL-13, was identified as the major Th2 cytokine that downregulates the IL-17 response in EC sensitized mice.

Conclusion—EC sensitization in the absence of IL-4/IL-13 induces an exaggerated Th17 response systemically, and in lungs following antigen challenge that results in airway inflammation and AHR.

Clinical implications—Blockade of IL-4 may promote IL-17-mediated airway inflammation in AD.

Keywords

IL-17; Th2 cytokines; atopic dermatitis; asthma

Introduction

Effector CD4⁺ T helper (Th) cells have been divided traditionally into two subsets: a Th1 subset, which produces IFN- γ and mediates cellular immunity, and a Th2 subset, which produces IL-4, IL-5 and IL-13 and mediates humoral immunity and allergic disease¹. Th2 cytokines are critical for the development of allergic disease and their expression is upregulated in lesional skin of patients with atopic dermatitis (AD) and in lungs of patients with active asthma. While IL-4 and IL-13 share a common signaling pathway, they make distinct contributions to allergic disease. IL-4 is crucial for the initiation of allergic disease by driving differentiation of uncommitted helper T cells towards a Th2 phenotype and inhibiting Th1 differentiation². IL-13 is crucial in sustaining allergic disease and is a cardinal mediator of airway hyperresponsiveness (AHR) and goblet cell hyperplasia in response to antigen inhalation³⁻⁵.

More recently, a third Th subset, Th17, has been identified. Th17 cells produce IL-17A, IL-17F and IL-22^{6, 7}. IL-17A and IL-17F share a high degree of homology and several biological functions such as induction of expression of neutrophil-attracting chemokines, that include CXCL2⁸, and recruitment of neutrophils⁹. IL-4 and IFN- γ negatively regulate the development of Th17 cells *in vivo* and *in vitro*¹⁰. IL-17 plays a central role in murine models of autoimmune diseases, such as experimental autoimmune encephalitis¹¹. IL-17 is present at a significant level in airways of patients with moderate to severe asthma^{12, 13}, however, its role in asthma is still controversial¹⁴⁻¹⁶. IL-17 is also present in acute AD skin lesions¹⁷. We recently showed that epicutaneous (EC) sensitization of mice with ovalbumin (OVA) elicits a systemic and local Th17 immune response¹⁸, and that IL-17 expression is upregulated in OVA challenged lungs of EC sensitized mice and contributes partially to airway inflammation in these mice.

AD is a common allergic skin inflammatory disease which often begins in infancy. Acute AD skin lesions are dominated by local expression of Th2 cytokines with some expression of IL-17, whereas chronic AD skin lesions are characterized by mixed expression of both Th2 and Th1 cytokines^{17,19}. The majority of infants with AD develop asthma and allergic rhinitis²⁰. In patients with AD, initial sensitization to antigen, including airborne allergens, may occur through an abnormal skin barrier^{21, 22}. Subsequent exposure to the same antigens by inhalation could precipitate airway inflammation and AHR. Because of the important role of IL-4 and IL-13 in AD and asthma, it has been proposed that blockade of these Th2 cytokines could be beneficial in these diseases. In the current study, we used IL-4/13 double knockout (DKO) mice to assess the role of IL-4 and IL-13 in antigen-induced skin and lung inflammatory responses in a mouse model of allergic skin inflammation elicited by EC sensitization with OVA²³.

Methods

Mice and Sensitization

IL4/13 DKO, IL-4 KO and IL-13 KO mice were previously described^{24,25}. Wild type (WT) BALB/c mice were obtained from Charles River Laboratory. EC sensitization of six- to eight-week-old female mice with OVA (grade V, Sigma-Aldrich) was described previously²³, the content of endotoxin was < 100 pg/mg in OVA preparation. Experimental procedures were in accordance with the Animal Care and Use Committee at Children's Hospital Boston.

Cell preparation and culture

Single-cell suspensions of spleen, regional draining lymph nodes (DLN) and lungs were prepared and cultured with OVA (100 μ g/ml) for 4 days as previously described²⁶.

Intracellular cytokine staining and cytokine ELISA

Cells were stained with anti-CD4-PE, anti-IL-17A-Alexa Fluor 647 and anti-IFN- γ -FITC (eBioscience) as previously described¹⁸. Cytokines determined by ELISA following the manufacturer's instructions using a BD- Pharmingen kit for IL-4 and IFN- γ and an R&D system kit for IL-13 and IL-17A.

Quantitative PCR analysis of cytokines

Total RNA extraction, generation of cDNA and quantitative real-time PCR were done as previously described¹⁸.

Histological and Immunohistochemical analysis

Skin and lung specimens were prepared for histology and immunohistology as previously described²³. Lung sections were also stained by periodic acid-Schiff (PAS) and blindly scored as described previously²⁷. Eosinophils and CD4⁺ T cells were counted blinded in 10 high-power fields (HPFs) at 400 $\times$.

Airway challenge and analysis of BALF

Intranasal (i.n.) challenge with OVA was performed as described previously¹⁸ starting day 47 of sensitization by three daily intranasal application of OVA (50 μ g/mouse) followed by methacholine challenge on day 51. For some experiments, animals were treated intravenously with 100 μ g of neutralizing anti-IL-17A mAb, or rat IgG2a isotype control (both from R&D Systems), 7 days and 4 days before the first airway challenge. Enhanced pause (Penh) was measured using whole body plethysmography (BUXCO, Troy, New York, USA) as previously described²⁸. Lung resistance (R_L) and dynamic compliance (C_{dyn}) changes were measured in response to methacholine, administered by nebulization to anesthetized and ventilated mice as described previously^{16, 29}. Immediately after sacrifice, BALF was collected, total cells were counted, and the number of leukocytes was determined from cytopspins stained with Diff-Quik stain set (Baxter Healthcare Corp.).

Statistical analysis

The Student's t-test was used for unpaired data and two-way ANOVA for the results of Penh and invasive measurements.

Results

DKO mice exhibit an exaggerated systemic Th-17 response to EC sensitization

Spleens and DLN cells from DKO mice EC sensitized with OVA secreted significantly more IL-17A when cultured *in vitro* with OVA than those from WT controls (Fig. 1A). Splenocytes and DLN cells from DKO mice secreted comparable amounts of IFN- γ to those from WT controls, even though DKO mice had significantly increased OVA-specific serum IgG2a levels (Supplementary Fig. 1). DKO mice had no detectable OVA-specific serum IgE and significantly decreased OVA-specific serum IgG1 responses (Supplementary Fig. 1).

Increased production of IL-17A, but not IFN- γ , by OVA EC-sensitized DKO mice was confirmed by staining for intracellular cytokines in CD4⁺ cells from splenocytes stimulated with OVA. Very few CD4⁺ cells that express IL-17A or IFN- γ were detected in splenocytes from saline-sensitized WT and DKO mice (data not shown). As previously shown¹⁸, CD4⁺IL-17A⁺ cells were readily detectable in OVA stimulated splenocytes from WT mice (Fig.1B). There were significantly higher percentages of CD4⁺IL-17A⁺ cells in OVA stimulated splenocytes from DKO mice (mean of 16.5 $\pm$ 2.1% for DKO versus 3.7 $\pm$ 0.2% for WT controls, n=3, p<0.05). There was no significant difference in the percentages of

CD4⁺IFN- γ ⁺ cells between OVA stimulated splenocytes from the two groups ($4.6 \pm 0.6\%$ vs. $3.4 \pm 1.2\%$, $n=3$, $p < 0.05$).

IL-17A was not detectable in serum of unmanipulated mice (data not shown), but became detectable following EC sensitization, with significantly higher levels in OVA sensitized mice compared to saline sensitized controls (Fig. 1C). OVA sensitization resulted in significantly higher serum IL-17A levels in DKO mice compared to WT controls. Serum levels of IFN- γ were undetectable in both WT and DKO mice EC sensitized with OVA (data not shown). These results suggest that EC sensitization in the absence of Th2 cytokines results in an exaggerated Th17 response.

DKO mice EC sensitized with OVA develop a dermatitis characterized by infiltration with CD4⁺ cells, but lack of eosinophils

EC sensitization of DKO mice with OVA caused epidermal thickening and accumulation of inflammatory cells in the dermal layer comparable to those in WT controls (Fig. 2A). Immunohistochemical analysis revealed that OVA sensitization caused comparable infiltration by CD4⁺ cells in DKO mice and WT controls (Fig. 2B). However, in contrast to WT mice, DKO mice exhibited no detectable increase in dermal eosinophils following EC sensitization with OVA (Fig. 2C). As previously reported^{18, 23}, OVA sensitization caused significant upregulation of expression of IL-4, IL-13 and IL-17 mRNA in the skin of WT mice. As expected, no IL-4 or IL-13 mRNA was detected in sensitized skin sites of DKO mice (Fig. 2D). OVA sensitized skin sites of DKO mice exhibited higher IL-17A and IL-17F mRNA expression than WT controls, but the difference did not reach statistical significance. There was no significant increase in IFN- γ mRNA expression in OVA sensitized sites either in WT or DKO mice (Fig. 2D). These results suggest that EC sensitization in the absence of Th2 cytokines induces skin inflammation characterized by dermal infiltration of CD4⁺ cells with absence of eosinophils and increased IL-17 expression.

EC sensitized DKO mice exhibit a dramatically increased expression of IL-17 and CXCL2 in the lung following airway challenge

As previously reported¹⁸, mRNA expression of the Th2 cytokines IL-4, IL-13, and the Th17 cytokines IL-17A and IL-17F, but not IFN- γ , was upregulated in lungs of OVA sensitized WT mice following antigen challenge. There was drastic upregulation of IL-17A and IL-17F, but not IFN- γ , mRNA expression, in lungs of EC sensitized DKO mice following antigen challenge (Fig. 3A and B). As expected, mRNA for IL-4 and IL-13 was not detected (Fig. 3A). We confirmed the increase in IL-17A expression in lungs of DKO at the protein level. Significantly higher amounts of IL-17A were secreted by lung cells from airway challenged OVA sensitized DKO mice than similarly sensitized and challenged WT controls (Fig. 3C).

IL-4 and IL-13 induce the expression of CCL11 and CCL22, two chemokines associated with the recruitment of eosinophils and Th2 cells, respectively^{30, 31}, while IL-17 induces the expression of the CXCL2, a chemokine associated with recruitment of neutrophils⁸. Fig. 3D shows that airway antigen challenge of WT mice EC sensitized with OVA caused upregulation of CCL11, CCL22 and CXCL2 mRNA expression in lungs of WT mice. There was no detectable upregulation of CCL11 and CCL22 mRNA in challenged lungs of DKO mice. In contrast, mRNA expression of CXCL2 in lungs of these mice was significantly upregulated compared to WT controls. These results suggest that OVA airway challenge of EC sensitized mice that lack IL-4 and IL-13 induces an exaggerated Th17 response with increased expression of the neutrophil attracting chemokine CXCL2.

DKO mice EC sensitized with OVA develop airway inflammation and AHR following airway challenge

OVA airway challenge of EC sensitized WT BALB/c mice causes airway inflammation with BALF eosinophilia and AHR¹⁸. BALF from DKO mice EC sensitized then i.n. challenged with OVA contained very few eosinophils but significantly more neutrophils than BALF from the WT controls (Fig. 4A). The number of lymphocytes and macrophages in BALF was comparable in the two groups. Histological analysis of lung tissue revealed peribronchial infiltrates of mononuclear cells, eosinophils and some neutrophils in OVA sensitized/challenged WT mice. OVA sensitized/challenged DKO mice developed peribronchial mononuclear cells infiltrates comparable to those of WT controls (Fig. 4B). However, eosinophils and neutrophils were virtually undetectable. The number of mast cells in tracheas of DKO and controls was comparable (data not shown). OVA challenge of WT mice EC sensitized with OVA caused strong accumulation of PAS positive material in the airway (Fig. 4C), with a mean score of 2.2 ± 0.5 on a scale of 4. In contrast, there was significantly less accumulation of PAS positive material in challenged lungs from DKO mice EC sensitized with OVA (mean score 1.2 ± 0.2 , $n=6$, $p < 0.05$). There was no detectable PAS material in lungs of OVA challenged saline sensitized WT controls. Airway challenge of EC sensitized WT mice causes a significant increase in airway responsiveness to methacholine^{23, 28}. Whole body plethysmography revealed that EC-sensitized DKO mice displayed enhanced responsiveness to methacholine, which was comparable to WT controls (Fig. 4D). Similar results were obtained using invasive measurements of lung resistance (R_L) and dynamic compliance (C_{dyn}) (Fig. 4 E). These results suggest that IL-4 and IL-13 are not obligatory for the development of airway inflammation and AHR in airway challenged EC sensitized mice, but are important for mucus hypersecretion.

Neutralizing antibody to IL-17A inhibits airway inflammation and AHR in DKO mice

To determine the role of IL-17A in lung inflammation of EC sensitized DKO mice, we used neutralizing antibody for IL-17A for cytokine blockade. Anti-IL-17A mAb, but not isotype control, significantly diminished neutrophil accumulation in BALF, with no significant effect on the accumulation of macrophages, eosinophils and lymphocytes (Fig. 5A), significantly reduced the level of CXCL2 mRNA expression in lungs (Fig. 5B), and strongly reduced peribronchial cellular infiltrates (Fig. 5C). Anti-IL-17A mAb, but not control mAb, reduced the Penh response to inhaled methacholine of OVA sensitized DKO mice to that of saline sensitized controls (Fig. 5D). Similar results were obtained with i.n. administration of 30 μ g of anti-IL-17A mAb 30 min before each of the three OVA airway challenges (data not shown). These data suggest that IL-17A is critical for the development of airway inflammation and AHR in EC-sensitized DKO mice.

The IL-17 response is only weakly increased in *i.p.* immunized IL4/13 DKO mice

Airway inflammation is abolished in *i.p.* immunized DKO mice airway challenged with OVA²⁵. Fig. 6A shows that OVA stimulated splenocytes from *i.p.* immunized DKO mice secreted significantly lower amounts of IL-17A than splenocytes from EC sensitized DKO mice. More importantly, upregulation of IL-17A mRNA in OVA challenged lungs was significantly lower in *i.p.* immunized DKO mice than in those from EC sensitized DKO mice (Fig. 6B). These results suggest that the modest IL-17 response of *i.p.* immunized DKO mice may have not been sufficient to drive airway inflammation in response to airway challenge with antigen.

IL-4 is the major Th2 cytokine that downregulates the IL-17 response to EC sensitization

To determine the relative role of IL-4 and IL-13 in downregulating the Th17 response to EC sensitization, we examined the systemic IL-17A response of single IL-4 KO and IL-13 KO

mice. Production of IL-17A by OVA stimulated splenocytes from mice EC sensitized with OVA was significantly higher in IL-4 KO mice, but not in IL-13 KO mice, compared to WT controls (Fig. 7A). There was significantly decreased IL-13 secretion in IL-4 KO mice, but no decrease in IL-4 secretion in IL-13 KO mice, while IFN- γ secretion was intact in both single KO mice.

IL-17A and CXCL2 mRNA expression were significantly higher following OVA airway challenge in IL-4 KO mice, but not IL-13 KO mice. As expected, IL-13 mRNA was not upregulated in IL-4 KO mice, IL-4 mRNA upregulation was intact in IL-13 KO mice, and there was no upregulation of CCL11 mRNA in either KO mice. These results suggest that IL-4, but not IL-13, is the major Th2 cytokine that downregulates the Th17 response to EC sensitization.

Discussion

We demonstrate that the Th17 response to EC sensitization is exaggerated in DKO mice and drives airway inflammation and AHR in response to airway challenge with antigen independently of Th2 cytokines.

EC sensitization with OVA elicits a systemic and local Th17 immune response that is partially decreased in MyD88^{-/-} mice¹⁸, suggesting that it is partially dependent on endotoxin. In the absence of IL-4 and IL-13, we observed an exaggerated systemic Th17 response and more importantly, an exaggerated IL-17 response in antigen challenged airways that drove airway inflammation and AHR. IL-4 was identified as the major Th2 cytokines that downregulates the Th17 response to EC sensitization, because IL-4 KO mice, but not IL-13 KO mice, had an increased systemic Th17 response and increased IL-17 expression in antigen challenged lungs (Fig. 7). This is consistent with the fact that T cells express IL-4R, but not IL-13R³². Absence of IL-4 and IL-13 had no demonstrable effect on the infiltration of sensitized skin by CD4⁺ cells (Fig. 2), but was associated with lack of infiltration by eosinophils and with higher, albeit not significant, expression of IL-17A and IL-17F mRNA (Fig. 2). DKO mice had markedly exaggerated expression of IL-17A and IL-17F mRNA in lungs and increased secretion of IL-17A by OVA stimulated lung cells following OVA airway challenge (Fig. 3). The observation that the local Th17 response in lungs was markedly more exaggerated than in the skin, suggests that tissue specific factors regulate IL-17 expression by locally infiltrating T cells or that IL-17-secreting cells may home less efficiently to skin than to lung.

We confirmed the importance of IL-4 and IL-13 in driving Th2 dependent responses in our model. DKO mice had undetectable serum levels of OVA-specific IgE, and severely diminished eosinophil infiltration in OVA sensitized skin and in BALF and parenchyma of OVA challenged lungs. In addition, they exhibited decreased mucus production evidenced by decreased PAS staining, consistent with IL-13 being a key mediator of mucus production in the airway⁵. EC sensitized DKO mice had a comparable increase in circulating eosinophils as WT controls and their splenocytes secreted normal amounts of IL-5 in response to OVA stimulation (Supplementary Fig 1B and C). The latter finding is in agreement with the previously reported normal IL-5 production in DKO mice infected with *Schistosoma mansoni*²⁴. A likely explanation of the absent tissue eosinophilia at sites of antigen challenge in DKO mice is the failure to upregulate the expression of the eosinophil attracting chemokine CCL11, which is induced by IL-4 and IL-13, and is essential for eosinophil recruitment to skin and lungs in our model²⁸.

IL-4 downregulates the expression of IFN- γ . DKO mice infected with *Schistosoma mansoni* have increased production of IFN- γ and IgG2a antibodies²⁴ and *i.p.* immunized, airway challenged DKO mice have increased expression of IFN- γ in bronchial DLN²⁵. We were unable to detect a change in the amount of IFN- γ secreted by OVA stimulated splenocytes or

in the expression of mRNA in sensitized skin or challenged lungs in DKO mice. The increased IgG2a response of these mice may be due to absence of the inhibitory effect of IL-4 on IgG2a producing B cells³³.

Airway challenge of EC sensitized DKO mice resulted in a dramatic influx of neutrophils in BALF with increased expression of CXCL2, dense peribronchial mononuclear cell infiltrate and increased response to methacholine. All these responses were inhibited by neutralizing anti-IL-17A mAb. Since this rat mAb is specific to IL-17A and does not cross-react with IL-17F, which was elevated in the lungs of DKO mice. This result suggests that exaggerated IL-17A expression was responsible for the airway inflammation and AHR in EC sensitized DKO mice. In contrast, in a previous study with EC sensitized WT mice, IL-17 contributed only partially to airway inflammation and AHR¹⁸. A role for IL-17 in human asthma is suggested by the observation that severe asthma is associated with accumulation of IL-17 and neutrophils in the airways^{12, 13, 34, 35}, and that IL-17 sputum level correlates with airway hyper-reactivity to methacholine³⁶.

Although BALF neutrophilia was present in EC sensitized DKO mice, their peribronchial infiltrate contained little, if any, neutrophils. This was unexpected given the fact that IL-17 mRNA expression was >20 fold higher in the challenged lungs of EC sensitized DKO mice than WT mice (Fig. 3). It was previously reported that transgenic mice that selectively overexpress IL-17A in the lung also develop a mononuclear peribronchial infiltrate with virtually no neutrophils³⁷. These observations suggest that high expression levels of IL-17A in the lungs may interfere with peribronchial accumulation of neutrophils. The reason for this is at present unknown. Although modest, the accumulation of PAS positive material in the airway of challenged DKO mice is consistent with the observation that PAS positive material accumulates in the airway of mice that selectively overexpress IL-17A in the lung³⁷.

The development of airway inflammation and AHR in EC sensitized DKO mice was rather unexpected because it has been reported that airway inflammation and AHR are absent in *i.p.* immunized DKO mice²⁵. We demonstrated that *i.p.* immunization, which does not induce a detectable Th17 response in WT mice¹⁸, induced only a modest Th17 response in DKO mice (Fig. 6). The weak Th17 response of *i.p.* immunized DKO mice may explain their failure to develop airway inflammation and AHR following airway challenge.

Skin is an important portal for sensitization to environmental allergens in AD³⁸ and it is believed that asthma can be precipitated in patients with AD upon inhalation of allergens that have been first introduced via the skin³⁹. IL-17 is expressed in acute AD skin lesions¹⁷ and we recently found that serum IL-17 levels are elevated in 6 of 20 patients with AD, but undetectable in 20 healthy controls ($p < 0.01$). These observations suggest that EC sensitization in humans with AD may elicit a Th17 response. Although mouse models have limitations as to whether they replicate the pathogenesis of human disease, should a similar mechanism apply in humans, blockade of IL-4 and IL-13 in patients with AD could result in a heightened IL-17 response and IL-17 driven airway inflammation and AHR. This may explain the failure of therapies targeting IL-4R α in several clinical trials for asthma⁴⁰.

Acknowledgments

Source of funding: This work was supported by USPHS grants AR-047417 and the Atopic dermatitis and Vaccinia Immunization Network (ADVNI) N01 AI 40030.

Abbreviations used

AD atopic dermatitis

DKO	IL-4/IL-13 double knockout
KO	knockout
WT	Wild-type
OVA	ovalbumin
AHR	Airway hyperresponsiveness
BALF	bronchoalveolar lavage fluid
DLN	draining lymph node
EC	epicutaneous
IL-4	interleukin-4
IL-13	interleukin-13
IL-17	interleukin-17
IFN- γ	interferon-gamma
i.n.	intranasal
i.p.	intraperitoneal
HPFs	high-power fields

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Figure 1. Exaggerated systemic Th17 responses in DKO mice EC sensitized with OVA
A. IL-17A and IFN- γ secretion by OVA stimulated splenocytes and cells from skin DLN. **B.** Intracellular IL-17A and IFN- γ staining of CD4⁺ cells in cultures of OVA stimulated splenocytes. Data are representative of three experiments. **C.** Serum levels of IL-17A. Columns and error bars represent mean and SEM (n=5-7 per group). *p $\leq$ 0.05 **p $\leq$ 0.01 *** p $\leq$ 0.0001,

Figure 2. Skin inflammation in EC sensitized DKO mice lacks eosinophils

A. Representative photomicrographs of H&E-stained skin sections from sensitized sites at 200 $\times$. **B and C.** Numbers of CD4⁺ cells (**B**) and eosinophil (**C**) were counted in at 400 $\times$ HPF. **D.** Expression of cytokine mRNA in sensitized skin. Columns and error bars represent mean and SEM. (n=5-7 per group). **p $\leq$ 0.01 *** p $\leq$ 0.0001.

Figure 3. DKO mice EC sensitized with OVA exhibit markedly increased expression of IL-17 and CXCL2 in the lungs following airway challenge with allergen

A and B. mRNA expression of cytokines. **C.** IL-17A protein secretion by OVA stimulated lung cells. **D.** mRNA expression of chemokines. Columns and error bars represent mean and SEM. (n=5-7 per group). *p<0.05 **p<0.01 *** p<0.0001

Figure 4. DKO mice EC sensitized with OVA develop airway inflammation and AHR following airway challenge with allergen

A. Total and differential counts in BALF. **B and C.** Representative photomicrographs of lung sections stained with H&E (B) and PAS (C) at 200 $\times$. The inset in B shows the presence of eosinophils in WT but not IL-4/13 DKO lungs. **D and E.** Penh response (D) and R_L and C_{dyn} measurements (E). Columns or points and error bars represent mean and SEM (n=5-7 per group). **p<0.01 *** p<0.0001.

Figure 5. Blockade of IL-17A *in vivo* inhibits airway inflammation and methacholine hyperresponsiveness in EC sensitized DKO mice challenged with OVA

A. Total and differential counts in BALF. **B.** mRNA expression of CXCL2. **C.** Representative photomicrographs of lung sections stained with H&E and examined at 200 $\times$. **D.** Penh response to methacholine. Columns or points and error bars represent mean and SEM (n=5-7 per group). *-p $\leq$ 0.05 **-p $\leq$ 0.01 *** p $\leq$ 0.0001.

Figure 6. DKO mice mount a weaker IL-17 response to *i.p* immunization than to EC sensitization
A. IL-17A secretion by OVA stimulated splenocytes. **B.** IL-17A mRNA expression in OVA challenged lungs. **.p≤0.01 *** p≤0.0001.

Figure 7. IL-4, but not IL-13, downregulates the systemic IL-17 response and IL-17 expression in lungs of EC sensitized mice

A. Splenocyte secretion of cytokines in response to OVA stimulation. **B.** Expression of cytokines in lungs of EC sensitized mice airway challenged with OVA. *- $p \leq 0.05$ **- $p \leq 0.01$ *** $p \leq 0.0001$.