Research Article

IL-17 regulates DC migration to the peribronchial LNs and allergen presentation in experimental allergic asthma

Adan Chari Jirmo1,2, Mandy Busse3, Christine Happle1,2, Jelena Skuljec1,2, Kathleen Dalige1, Anika Habener1,2, Ruth Grychtol1,2, David S. DeLuca2, Oliver D. Breiholz4, Immo Prinz5 and Gesine Hansen1,2,6

1 Department of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany
2 Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Member of the German Center for Lung Research (DZL), Hannover, Germany
3 Experimental Obstetrics and Gynecology, Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany
4 Research Core Unit Genomics (RCUG), Hannover Medical School, Hannover, Germany
5 Institute of Immunology, Hannover Medical School, Hannover, Germany
6 Excellence Cluster RESIST (EXC 2155), Hannover Medical School, Hannover, Germany

IL-17 is associated with different phenotypes of asthma, however, it is not fully elucidated how it influences induction and maintenance of asthma and allergy. In order to determine the role of IL-17 in development of allergic asthma, we used IL-17A/F double KO (IL-17A/F KO) and WT mice with or without neutralization of IL-17 in an experimental allergic asthma model and analyzed airway hyperresponsiveness, lung inflammation, T helper cell polarization, and DCs influx and activation. We report that the absence of IL-17 reduced influx of DCs into lungs and lung draining LNs. Compared to WT mice, IL-17A/F KO mice or WT mice after neutralization of IL-17A showed reduced airway hyperresponsiveness, eosinophilia, mucus hypersecretion, and IgE levels. DCs from draining LNs of allergen-challenged IL-17A/F KO mice showed a reduction in expression of migratory and costimulatory molecules CCR7, CCR2, MHC-II, and CD40 compared to WT DCs. Moreover, in vivo stimulation of adoptively transferred antigen-specific cells was attenuated in lung-draining LNs in the absence of IL-17. Thus, we report that IL-17 enhances airway DC activation, migration, and function. Consequently, lack of IL-17 leads to reduced antigen-specific T cell priming and impaired development of experimental allergic asthma.

Keywords: airway hyperresponsiveness · bronchial LNs · DCs · IL-17A · experimental asthma · IL-17F · immunoglobulins · Th2 cells

Additional supporting information may be found online in the Supporting Information section at the end of the article.
Introduction

According to the statistics of both the World Health Organization and Global Asthma Reports, asthma affects more than 300 million people worldwide with an incidence of one in eight children and one in 12 adults [1, 2]. These statistics make it one of the most common chronic diseases [1, 2]. Asthma is clinically characterized by shortness of breath, wheeze, and cough due to bronchial obstruction caused by chronic inflammation of the airways with bronchial edema increased mucus secretion, and constriction of bronchial smooth muscle tissue. Airway inflammation can broadly be differentiated in type-2 high and type-2 low inflammation [3]. Both in humans and in experimental murine models, type-2 high asthma has been well defined and characterized based on presence of airway and blood eosinophilia, allergen-specific IgE, and other biomarkers that are driven by IL-13, IL-5, and IL-4, which are the signature cytokines associated with type 2 responses [3].

Type-2 low inflammation, on the other hand, is characterized by increased neutrophilic airway inflammation, sometimes in addition to eosinophilic inflammation. Th17 cells have been shown to play a determining role in this phenotype [3, 4]. Increased levels of IL-17 have been reported in lung specimens, BALF, sputum as well as in peripheral blood of patients with asthma [5-7]. High levels of IL-17 in severe asthma regulates the expression of inflammatory cytokines, chemokines, and cell adhesion molecules [8-11]. Through its influence on chemokines and growth factors such as G-CSF, GM-CSF, and TNF-α, IL-17 further promotes eosinophilia in airways in addition to causing asthma exacerbation and airway remodeling by triggering release of matrix metalloproteases (MMPs) or other inflammatory molecules such as IL-6, IL-8, IL-11, GM-CSF, and VEGF within the lung tissue [12, 13]. In genome-wide association studies, asthma development was linked to polymorphisms in IL-17 determining genes, and IL-17 secretion in cord blood correlated with the risk for later asthma development [14, 15].

Professional antigen-presenting cells and specifically DCs are required for the induction of asthma-associated features in murine models in sensitization and challenge phases [16, 17]. In humans, adaptive immunity and especially antigen-specific immune response against allergens is a hallmark of allergic asthma [3]. Furthermore, genetic polymorphisms at the 6p21, 32 locus encoding HLA-DRB1 and HLA-DQA1 antigen-presenting MHCII molecules are linked with asthma in humans [18]. DCs are central for priming and inducing efficient T helper cell responses in asthma and allergy [19-21]. Optimal activation of naive T cells in the draining LNs requires the presentation of processed antigen taken up in the peripheral tissues by migratory DCs [22, 23]. Thus, DCs ability to migrate is a critical pre-requisite for the priming of an effective immune response in the LN [16]. On its part, IL-17 affects DCs by enhancing functional differentiation of DC progenitors and, in a murine model for allograft rejection, it seems to modulate DC function and their Th1 priming capacity [24, 25]. In the skin, IL-17 induces Langerhans cell migration [26].

In this study, we investigate IL-17A/F KO mice and monoclonal antibodies to block IL-17A in an experimental allergic asthma model. We show that in the absence of IL-17A and IL-17F, the allergic asthma phenotype is reduced and this attenuation correlates with reduced frequencies of infiltrating DCs, both in the lungs and draining LNs of allergic mice. Through in-depth proteome and transcriptome analyses, we furthermore demonstrate that the migration, antigen presentation, and activation of airway DCs are affected by IL-17. To our knowledge, this is the first study that links missing or low IL-17 in experimental asthma to reduced migration of DCs with a possible effect in attenuation of the severity of inflammation and AHR.

In summary, we propose that the presence of IL-17 in allergic asthma leads to enhanced activation, migration, and antigen presentation by DCs, which contributes to the development and course of the disease. Thus, a targeting approach aimed at attenuating DC migration and activation would be plausible in IL-17-dependent asthma/allergic conditions that are notoriously difficult to control.

Results

IL-17 expression enhances inflammation and airway hyperresponsiveness in experimental asthma

To explore how IL-17 influences the establishment and development of murine asthma, we subjected WT and IL-17A/F KO mice to an experimental allergic asthma model based on OVA immunization (Fig. 1A). Upon provocation with methacholine, allergic IL-17A/F KO mice showed reduced AHR as reflected by decreased resistance and increased compliance compared to WT mice (Fig. 1B). The inflammatory response in the airways was diminished in IL-17A/F KO compared to WT mice. Total cell numbers and numbers of eosinophils in the BALF of allergic WT mice were enhanced upon local administration of OVA. In contrast, in IL-17A/F KO mice, inflammation was lower as compared to allergic WT mice (Fig. 1C). In addition, H&E as well as Periodic-Acid Schiff (PAS) stained lung sections revealed reduced eosinophilic inflammation and mucus hypersecretion in OVA treated IL-17A/F KO compared to WT mice (Fig. 1D), which we further confirmed through analysis of pixel densities (Fig. 1E). In our setup, we did not observe neutrophilic influx in the allergic-asthma model both in WT as well as in IL-17A/F KO mice. In OVA-re-stimulated cell culture supernatants of BLN cells, the levels of Th2 cytokines IL-5, IL-10, and IL-13 (Fig. 1F) were also lower in allergic IL-17A/F KO mice compared to their WT counterparts. Also, serum antigen-specific IgE was significantly lower in IL-17A/F KO than in WT mice (Fig. 1G). In summary, these data demonstrate that KO of IL-17A/F significantly impacts development of experimental allergic asthma in our model.

Neutralization of IL-17A impairs development of allergic asthma and ameliorates AHR

To assess the role of IL-17 in vivo and thereby determine its impact in our model, we neutralized IL-17A before we subjected mice to...
Figure 1. Attenuated airway hyperresponsiveness and eosinophilia in absence of IL-17A/F. Female WT C57BL6/J and IL-17A/F KO mice were subjected to the model of experimental allergic asthma (A). IL-17A/F KO mice show reduced airway hyperresponsiveness on challenge with increasing doses of methacholine, total cell counts in BALF determined using automated counter, eosinophilia (Eos), and macrophages (Mac) in cytospins as compared to WT animals (B and C). Lung inflammation and mucus production (H&E/ PAS) staining of paraffin-embedded lung-slices at magnification ×200, scale bar = 100 μm and an objective quantification based on pixel densities (D and E). Th2 cytokines concentration in re-stimulated bronchial LN cells (F) and serum titres of allergen-specific Ig (G) were measured by ELISA. The error bars represent mean ± SEM and *<0.05, **<0.01, and ***<0.001 and one-way ANOVA (C, E, F, and G) or two-way ANOVA (B) was used to compare differences in a representative experiment from —two to three independent experiments with n = 4–5 animals/group in each experiment.

the asthma protocol (Fig. 2A). Neutralization of IL-17A led to reduced symptoms in the murine asthma model compared to the control group. Lung function parameters were improved (Fig. 2B) and BALF total cell counts as well as airway eosinophilia were lower after neutralization of IL-17A (Fig. 2C). Furthermore, less lung inflammation was observed (Fig. 2D–E) and Th2 associated cytokines after re-stimulation of LN cells were reduced while IFN-γ was elevated (Fig. 2F). We furthered this observation through establishment of a model we called ’therapeutic’ (Fig. 3A) in which we assessed whether neutralization of IL-17 in already sensitized mice would ameliorate experimental asthma through blocking IL-17A before mice were re-exposed to the sensitizing
Figure 2. Blockage of IL-17A impairs development of experimental allergic asthma: IL-17A was blocked in WT mice before starting and throughout asthma protocol (A). Airway hyper responsiveness after methacholine challenge (B), BALF total cell count determined using automated counter, eosinophils (Eos), and macrophages (Mac) in cytospins (C). Lung inflammation and mucus production (H&E/PAS) staining of paraffin-embedded lung-slices at magnification $\times 200$, scale bar $= 100 \mu m$, and pixel quantifications (D and E). Th2 and Th1 cytokine concentrations in re-stimulated bronchial LN cells using ELISAs (F) and serum titres of allergen-specific immunoglobulin measured using ELISA (G) comparing influence of blocking IL-17A on experimental asthma. The error bars represent mean $\pm$ SEM and * $< 0.05$, ** $< 0.01$, and *** $< 0.001$. One-way ANOVA (C, E, F, and G) or two-way ANOVA (B) was used to compare differences in a representative experiment from —two to three independent experiments with $n = 4–5$ animals/group in each experiment. The red filled arrow heads show the days on which α-IL-17A was administered.
antigen. Blocking IL-17A before challenge led to reduced AHR, eosinophilic inflammation, and mucus hypersecretion in allergic mice (Fig. 3B–E). However, this later neutralization of IL-17A did not lead to attenuation of Th2 helper cell-associated cytokines or reduction in IgE but correlated with elevated levels of IFN-γ (Fig. 3F). Interestingly, we observed a significant and very strong increase of IFN-γ in supernatants of OVA-restimulated LN cells of OVA-immunized WT mice that were treated with a monoclonal anti-IL17 antibody. This strong increase of IFN-γ production might be due to a compensatory upregulation of IFN-γ producing Th1 cells after inhibition of IL-17 via a monoclonal antibody. It has been observed previously that IL-17 and IFN-γ have compensatory
effects on each other. For instance, in tuberculosis infection model increase of IL-17 diminished IFN-γ production [27]. In contrast to our observation in OVA-immunized WT mice that were treated with anti-IL-17A antibody, we did not detect IFN-γ in IL-17A/F KO mice after OVA immunization (see Fig. 1F). This observation is in line with previous reports in which constitutive deficiency of IL-17 in mice is associated with an altered microbiota as well as an impaired Th1 response due to reduced IFN-γ positive CD4+ tissue-resident memory T cells [28]. Taken together, neutralization of IL-17 before sensitization or re-exposure during challenge attenuates allergic airway inflammation and Th2 polarization.

Absence of IL-17 attenuates DC frequencies and antigen responses in draining LNs

Antigen uptake and processing by professional antigen-presenting cells such as DCs are required for an effective priming of naive T cells. To elucidate the role of IL-17 in airway inflammation, we analyzed immune responses in lung draining LNs. DCs sample antigen in the periphery and migrate to local LNs where they present to T cells and prime immune response [16, 29, 30]. We hypothesized that reduced airway inflammation in IL-17A/F KO mice was due to attenuated DC-T cell interaction and Th2 priming in LNs. To address this hypothesis, we adoptively transferred OT-II cells intravenously via the tail vein followed by intranasal application of OVA (Fig. 4A). In the absence of IL-17, we observed a reduction in vivo proliferation of OT-II cells in lung draining LNs (Fig. 4B and C and Supporting information Fig. 1). In addition, we also observed significantly fewer cells in lung draining LNs of IL-17A/F KO mice as compared to their WT counterpart after treatment (Fig. 4D). More so, in the lungs and draining LNs of IL-17A/F KO mice, frequencies of DCs after intranasal challenge were much lower than in WT animals (Fig. 4E and F and Supporting Information S1). However, frequencies of myeloid progenitor cells in NM were not affected in IL-17A/F KO mice (Fig. 4G-H).

Impaired DC activation and migration in IL-17A/F KO mice

Our finding that T cell responses are attenuated in mediastinal LNs of IL-17A/F KO mice could partly be explained by reduced frequencies of DCs in the LNs as compared to WT mice. Furthermore, IL-17 could also affect DC functions such as their ability to take up antigen. To address this, we analyzed in vivo antigen uptake by pulmonary DCs through intranasal administration of APC-conjugated OVA to WT and IL-17A/F KO mice (Fig. 5A and Supporting Information Fig. S2). Subsequent ex vivo analysis revealed a significant reduction in frequencies of antigen-positive DCs in IL-17A/F KO mice as compared to their WT counterparts (Fig. 5B and C). In order to further understand impact of IL-17 on activation and migration potential of DCs, we applied chip cytometry for identification of pulmonary DCs (Fig. 5D), a novel technique that is suitable for in-depth single-cell characterization [31]. We have shown in our previous analysis that chip cytometry measurements correlate very well with flow cytometry observations [32, 33]. In addition, chip cytometry allows comprehensive phenotyping of single cells and small cell populations thereby increasing the analytical power of samples with few cells such as DCs in LNs. Based on CD11c and MHC-II expression, we thus analyzed phenotypic characteristics of DCs in lung draining LNs from both WT and IL-17A/F KO mice after asthma protocol (Fig. 1A) using chip cytometry. As shown in Fig. 5E, unbiased hierarchical clustering of single cells based on surface expression of CCR7, MHC-II, CD40, CCR2, and CD62L on DCs derived from lung draining LNs shows that whereas WT cells have an enhanced expression of these molecules and can be segregated based on whether they are from antigen exposed animals or not, this unbiased separation does not occur in cells derived from IL-17A/F KO mice (Fig. 5E). We confirmed this lack of hierarchical clustering of DCs isolated from IL-17A/F KO mice through further unbiased analytical approaches involving linear discriminant analysis (LDA), which revealed that no significant changes occur on DCs from IL-17A/F KO mice after OVA challenge (Supporting Information Fig. S3). Further quantitative analysis revealed substantial reduction in expression of MHC-II, CD40, CCR7, and CCR2 in DCs isolated from IL-17A/F KO mice when compared to the DCs from WT mice (Fig. 5F). To test differences in activation or functional capacities of DCs derived from IL-17AF KO compared to WT mice, we co-cultured splenic and lung derived DCs with T cells and compared IL-2 levels in the supernatants 2 days after co-culture with similar results (Supporting Information Fig. E2). This finding supports a comparable stimulatory capacity of WT and IL-17AF KO DCs in vitro. However, when we performed a migration assay toward Ccl19, the number of DCs migrating toward Ccl19 in absence of IL-17 was significantly reduced (see Supporting Information Fig. E2). Our data further support our finding that IL-17 attenuates the migratory potential of DCs. Thus, deficiency of IL-17A/F in our model correlated with lower expression levels of activation and migration associated molecules on DCs derived from lung draining LNs.

Transcriptional signature of lung draining LN DCs in the absence of IL-17A/F

The above observations led us to investigate if the differences between WT and IL-17A/F KO mice exist already at transcriptional level. Thus, we carried out transcriptomic analysis of DCs sorted from lung-draining LNs of either naive or mice subjected to intranasal allergen challenge (Fig. 6A). Using a well-established protocol [34,35] for preparation of single cells from LNs without having to digest using collagenase and DNase, we isolated lung draining LNs from both WT and IL-17A/F KO mice after exposure to OVA via intranasal challenge (Fig. 5A). We then analyzed transcriptomic patterns of a set of genes involved in migration and function of non-lymphoid-tissue DCs into the draining LNs in response to tissue injury or immunization that were previously described [36]. Analysis of the 321 genes that were described as determinants of inflammatory DCs (Supporting Information...
Figure 4. Impaired in vivo proliferation of antigen-specific T cells in lung draining LNs and reduced influx of DCs in absence of IL-17: CFSE-labeled OT-II cells were adoptively transferred into either WT or IL-17A/F KO mice followed by challenge with OVA and subsequent analysis of in vivo proliferation (A). Identification of transferred cells and comparative analysis of proliferation and total cell counts in mediastinal lung draining LNs was determined by flow cytometry (B–D). Influx of DC into the lungs and lung draining LNs was analyzed after intranasal application of OVA (E and F). Quantification of various progenitor cell populations in BM of either WT or IL-17A/F KO mice (G and H). All bars display mean ± SEM with * < 0.05, and ** < 0.01. One-way ANOVA (C–F) and unpaired Student’s t-test (H) was used to compare differences in data pulled from two to three independent experiments. MP (myeloid Progenitors), LP (Lymphoid Progenitors), LSK (Linage−, Sca-1+, cKit+).

Table 2) as reported by Miller and colleagues [36] revealed that they are significantly upregulated in a challenged WT animals compared to their unchallenged WT counterparts, which is not seen in IL-17A/F KO mice after challenge (Fig. 6B and C). A closer look into the gene sets revealed that these changes occurred in genes that are involved in antigen presentation, co-stimulation, and cytokine production by DCs (Fig. 6D). These patterns were confirmed when looking at individual genes involved in the processes of antigen presentation, co-stimulation, and cytokine production (Fig. 7A and B). Importantly, these transcriptomic changes were not significant between the WT and IL-17A/F KO mice in those gene sets that are reported to be associated with the expression of chemokine receptors or involved in DC lineage commitment [36] (Fig. 7C and D). Due to a reduction in several genes that have been shown to be associated with inflammatory DCs ability to present antigen, provide co-stimulation and also migration to peripheral LNs in IL-17A/F KO mice, we think IL-17A/F is necessary for an efficient DC stimulation in an inflammatory environment.

Discussion

In our study, we aimed to characterize the role of IL-17 in initiation and establishment of airway inflammation and AHR using a well-established experimental mouse model of allergic asthma. We found that absence of IL-17A/F or neutralization of IL-17A alone led to attenuation of severity of experimental allergic asthma, either before sensitization or challenge. These findings are in
Figure 5. Lack of IL-17 leads to reduced frequencies and activation of conventional DCs: WT or IL-17A/F KO mice were intranasally inoculated with fluorescence conjugated OVA (A). Flow cytometry analysis of antigen positive DCs (see supplementary information regarding gating strategy for lung DC) in the lung after 12 h (B and C). Chip cytometry based single cell identification of DC and alveolar macrophages (AM) in the lung (D) followed by single cell analysis of various co-stimulatory and migratory molecules on the surface of lung draining LN DCs using chip cytometry in WT and IL-17A/F KO mice (E and F). The error bars represent mean ± SEM and *<0.05, **<0.01, and ***<0.001 and unpaired two-tailed Student's t-test (C) or one-way ANOVA (F) was used to compare differences in data pulled from—two to three independent experiments.
IL-17 impacts on genes associated with inflammatory DCs: Both WT and IL-17A/F KO mice were intranasally challenged with OVA followed by DC sorting from lung draining LNs (A). Transcriptomic patterns of 321 genes associated with inflammatory DCs in LNs showing fold changes after exposure to antigen in both WT and IL-17A/F KO mice (B) and a 2D scatter plot showing distribution of various transcripts on a X-Y axis (C). A heatmap showing log2 fold change for gene-sets involved in antigen presentation and co-stimulation, chemokine receptors, cytokines, and chemokines and DC lineage within a selected set of genes that have been reported to be expressed by inflammatory DC after exposure to allergen both in WT and IL-17A/F KO mice (D; and Supporting Information Table 2). RNA used in this analysis was extracted from sorted DCs from two different intranasal challenge experiments with \( n = 10 \) animals/group in total. Wilcox Ranked test was used to calculate significance between fold changes in WT and IL-17A/F KO mice.

Figure 6. IL-17 impacts on genes associated with inflammatory DCs: Both WT and IL-17A/F KO mice were intranasally challenged with OVA followed by DC sorting from lung draining LNs (A). Transcriptomic patterns of 321 genes associated with inflammatory DCs in LNs showing fold changes after exposure to antigen in both WT and IL-17A/F KO mice (B) and a 2D scatter plot showing distribution of various transcripts on a X-Y axis (C). A heatmap showing log2 fold change for gene-sets involved in antigen presentation and co-stimulation, chemokine receptors, cytokines, and chemokines and DC lineage within a selected set of genes that have been reported to be expressed by inflammatory DC after exposure to allergen both in WT and IL-17A/F KO mice (D; and Supporting Information Table 2). RNA used in this analysis was extracted from sorted DCs from two different intranasal challenge experiments with \( n = 10 \) animals/group in total. Wilcox Ranked test was used to calculate significance between fold changes in WT and IL-17A/F KO mice.

line with the results of other studies using related mouse models [37, 38]. Moreover, we report that the observed reduction in pulmonary eosinophilic inflammation, AHR and goblet cell hyperplasia correlated with reduced conventional DC frequencies both in the lung and lung draining mediastinal LNs of IL-17A/F KO allergic animals. In concomitance with reduced DC frequencies, we observed diminished T cell responses to cognate antigen in lung draining LNs in absence of IL-17; probably an effect that resulted from the dearth of DCs and thereby reduced antigen presentation in LNs [21, 39, 40]. To our knowledge, this is the first evidence that links availability of IL-17 in an experimental asthma model to reduced migration of DCs with a possible effect in attenuation of severity of inflammation and AHR. Given that IL-17 and Th17 cells are involved in asthma development and shown to be
associated with severity and course of disease [7-9, 37, 38], we believe this is an important mechanism by which this cytokine enhances inflammation and associated pathologies such as those in asthma and allergy.

Induction of immunologically protective as well as pathogenic responses are a product of a cascade of events involving antigen sampling by professional antigen presenting cells, their activation, migration and eventually presentation to naive or memory effector cells such as T cells. Being the most important APC when it comes to activating naive T cells, DCs are indispensable during induction of immune responses to foreign antigens or in maintaining tolerance to self [41]. For immature DCs to effectively traffic from the peripheral to the LNs, where antigen presentation and priming takes place, specific adhesion molecules and maturation-dependent chemo-attractant cues such as CCR2-CCL2, CCR5-CCL20, or CCR5-CCL5 chemokine receptor–ligand pathways have been conclusively shown to be necessary [42,43]. In addition, to migrate to draining LNs, mature DCs upregulate CCR7 [44-46]. Thus, paucity of antigen-specific OT-II cells in lung draining LNs in our study is likely a result of impaired functional and migratory potential of DCs. Indeed, our single-cell analysis of surface molecules expressed on DCs showed that, levels of MHC-II, CD40, CCR7, and CCR2 were reduced in IL-17A/F KO mice as compared to their WT counterparts after exposure to antigen. However, to track the fate of DCs, we used a cell transfer model (see Figs. 4 and 5) while we used a sensitization model with systemic antigen application to test the effect of IL-17 deficiency on the asthma phenotype (see Figs. 1–3). This is a limitation of our study since we cannot directly translate the information on DCs gained in the cell transfer experiments to the immunization model. Nevertheless, the aim of our cell transfer experiments was to compare the impact of IL-17 on the antigen-presenting potency of DCs. By applying the same number of antigen-specific OT-II cells in vivo, we standardized the effector cell population to selectively focus on differences in DC function between WT and IL-17 KO mice in response to OVA stimulation. By this means, we avoided different T cell frequencies and differences in T cell activation as possible confounders during the sensitization phase, which depends on the frequency and functional potential of antigen-presenting DCs. In addition, the DCs used in our analysis were not isolated from animals that were sensitized via peritoneal cavity but were from intranasally challenged animals using a second model, which we believe is more suited to clarify the impact of IL-17 on DCs.

Indeed, the involvement of IL-17 in the migration of various immune cells has been shown in several other studies albeit using different models. Notably, IL-17 directly enhances B cell migration both in viral infection and asthmatic conditions through its action on structural lung cells that led to the production of migration influencing chemokines such as Cxcl12 and Cxcl13 [8, 47]. Here, we provide evidence that IL-17 impacts on the functional and migratory potential of DCs and thereby directly influences severity of experimental allergic asthma. A direct link of how DCs affect Th17 cells and thereby maintain inflammation in the lung was recently shown by Shalaby and colleagues using the house dust extract augmented OVA model [48]. According to Shalaby and colleagues, activation of DCs via TLR4 after exposure to common house dust extracts in a murine model of allergic asthma applying OVA/HDE led to revival and maintenance of effector/memory
mice are sensitized intraperitoneally (i.p.) with Polymyxin-treated
We have established an experimental asthma protocol in which
Induction of allergic asthma
This study. All mice used in this study had C57BL/6 background.
the Committee on Animal Welfare in the State of Lower Sax-
Animals
This observation augments previous work
by Lajoie and colleagues showing that presence of IL-17 leads to
the production of IL-23 by DCs and thereby to the sustenance of
Th17 cells in the lung. This observation augments previous work
by Lajoie and colleagues showing that presence of IL-17 leads to
the production of IL-23 by DCs and thereby to the sustenance of
Th17 cells in a model of severe asthma [49].
An important observation in our current study is the attenuation
of DC frequencies both in the lungs and lung draining LNs in
the absence of IL-17. Although we show that CCR7 expression at
transcript and protein level is reduced in absence of IL-17, never-
theless, we are still searching for more molecular partners that
are involved in this impaired influx into the lung and lung drain-
ing LNs. Our current tenet is that reduced DC frequencies coupled
with impaired antigen presentation potential and co-stimulation
in absence of IL-17 explain observed ameliorative effects in our
asthma model. Since it is well established that DCs are the main
leukocyte sub-population that primes Th cell subset differentia-
tion was further analyzed objectively using computer-based pixel
periodic acid–Schiff reagent (PAS, Sigma, Taufkirchen, Germany)
comparable to the left lung. Sections were stained with H&E
and scanned using Keyence BZ-9000 microscope (Keyence, Osaka,
Switzerland).

Analysis of BALF
In vivo antibody treatments

C57BL/6J WT mice were injected i.p. with 100 μg anti-IL-17A
mAb (clone eBioMM17F3, eBioscience Inc., San Diego, USA) or
with isotype control mouse IgG1 κ mAb (eBioscience, Inc., San
Diego, USA) as outlined in Figs. 2A and 3A.

Measurement of airway responsiveness
Invasive lung function was assessed as described previously
[35, 60, 61]. Briefly, 24 h following the last allergen challenge
anesthetized tracheotomized mice were connected to the flex-
vEnt system (SCIREQ, Montreal, Canada) and exposed to dif-
ferent concentrations of aerosolized methacholine (0, 10, 20, and
30 mg/mL) in 0.9% NaCl.

Analysis of BALF
BALF was performed by flushing the right lung with PBS/2 mM
EDTA. Total number of BALF cells was measured by automated
cell counting (CedexHiRes, Innovatis, Bielefeld, Germany). BALF
differential cell counts were performed by staining cytospin
preparations with Diff-Quick (Medion Diagnostics, Duedingen,
Switzerland).

Lung histology
The left lungs were inflated with PBS and fixed in 4% forma-
lin before embedding in paraffin as previously described [35]. 4
μm sections were stained with H&E (Merck, Germany) or with
periodic acid–Schiff reagent (PAS, Sigma, Taufkirchen, Germany)
and scanned using Keyence BZ-9000 microscope (Keyence, Osaka,
Japan) at 200× magnification. Inflammation and mucus produc-
tion was further analyzed objectively using computer-based pixel
densities determination as previously done in our laboratory [34,
59, 60].

Cytokine production
Splenic and lung draining bronchial LNs cells (BLN) were re-
stimulated in vitro (5 × 10^6 cells/mL) with OVA (200 μg/mL)
in RPMI (supplemented with 10% FCS, 100 U/mL penicillin, and
100 μg/mL streptomycin). Cytokines were measured in super-
natants using DuoSet ELISA kits (R&D Systems, Minneapolis,
USA) according to the manufacturer’s instructions.
Serum levels of OVA-specific IgE were measured by ELISA according to a standard protocol as described previously [59].

Ovalbumin specific T helper (OT-II) cells were purified from LNs and spleens of OT-II mice using CD4\(^+\) T cells enrichment kit (Miltenyi Biotec, Bergisch Gladbach, Germany) followed by labeling with 1 \(\mu\)M CFSE (Life Technologies, ThermoFisher Scientific, Waltham, MA, USA). A total of \(3 \times 10^6\) CFSE-labeled OT-II cells were intravenously injected into recipient mice. Forty-eight hours later, mice were sacrificed and lung draining LNs cells were analyzed by flow cytometry using anti-CD3, anti-V\(\alpha\)2, anti-CD4, and CFSE on a FACS Canto flow cytometer (BD Biosciences, Mountain View, CA, USA). FlowJo software (Treestar Inc., Ashland, OR, USA) was used to analyze data.

Lung and lung-draining LN DCs and alveolar macrophages (AM) were sorted as previously described [62-64] following flowcytometry guidelines [65]. Briefly, single cells from lung tissue after digestion of the lungs using a mixture of collagenase and DNase (Milteny) were stained with CD11c, MHC-II, and siglec-F and MHC-II\(^hi\) CD11c\(^+\) Siglec-F\(^-\) cells in the lung were sorted as lung DC. For isolation of DC from lung draining LN, single cells were prepared through mashing LNs and separation using 70 \(\mu\)M nylon mesh followed by sorting of CD11c\(^-\) MHC-II\(^-\) cells as LN DCs. Antibodies used for identification and sorting of DCs and AM; CD11c, MHC-II, CD11b, CD103, Siglec-F while CD197, CD40, CCR2, and CD62L were used to analyze DCs and AM using chip cytometry (see Supporting Information Table 1 for clones, fluorophore, and manufacturer information).

Chip cytometry

Chip cytometry was performed as described previously [66, 67]. Briefly, cells were fixed on cell-adhesive microfluidic-chips according to company instructions (ZellSafe Chips, Zellkraftwerk GmbH, Leipzig, Germany). ZellSafe chips were then subsequently subjected to an iterative staining/bleaching cycles using ZellScannerONE (Zellkraftwerk GmbH, Leipzig, Germany). Data acquisition and analysis of fluorescence intensities was accomplished using the ZellExplorer App (Zellkraftwerk GmbH, Leipzig, Germany). SAS software (Treestar Inc., Ashland, OR, USA) was used to create heatmaps based on fluorescence intensities on individual cells.
Statistical analysis

GraphPad Prism software version 6 was used for statistical analysis (GraphPad Software, La Jolla, CA, USA). Unless otherwise indicated, one-way or two-way ANOVA was used to compare differences between more than two groups and Wilcoxon Ranked or Student’s t-test was used to determine the statistical significance of differences between two groups. p-Values less than 0.05 (*), p < 0.01 (**), and p < 0.001 (***)) were considered as significant.

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Abbreviations: AHR: airway hyperresponsiveness · AM: alveolar macrophages · BLN: bronchial LN · Th: T helper

Full correspondence: Dr. Gesine Hansen, Department of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Carl-Neuberg-Str.1, D-30625 Hannover, Germany.
e-mail: hansen.gesine@mh-hannover.de

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