

Influence of an Allergen-Specific Th17 Response on Remodeling of the Airways

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Abstract

We showed previously that sensitization of mice with dendritic cells (DCs) via the airways depends on activation of these cells with LPS. Allergen-pulsed DCs that were stimulated with low doses of LPS induce a strong Th2 response *in vivo*. Our objective was to investigate whether airway sensitization of mice by the application of DCs with a phenotype that is able to induce Th17 cells results in increased remodeling of the airways. We generated DCs from the bone marrow of mice and pulsed them with LPS-free ovalbumin. Subsequently, cells were activated with LPS with or without ATP for inflammasome activation. The activated cells were used to sensitize mice via the airways. Intranasal instillation of DCs that were activated with 0.1 ng/ml LPS induced a Th2 response with airway eosinophilia. High doses of LPS, particularly when given in combination with ATP, led to induction of a mixed Th2/Th17 response. Interestingly, we found a correlation between IL-17A production and the remodeling of the airways. Stimulation of mouse fibroblasts with purified IL-17A protein *in vitro* resulted in transforming growth factor- β 1 secretion and collagen transcription. Interestingly, we found enhanced secretion of transforming growth factor- β 1 by fibroblasts

after costimulation with IL-17A and the profibrotic factor wingless-type MMTV integration site family, member 5A (Wnt5a). We showed that an allergen-specific Th17 response in the airway is accompanied by increased airway remodeling. Furthermore, we revealed that increased remodeling is not only based on neutrophilic inflammation, but also on the direct impact of IL-17A on airway structural cells.

Keywords: mouse model; asthma; IL-17; fibrosis

Clinical Relevance

We developed a mouse model for the induction of a mixed Th2/Th17 response by transfer of allergen-pulsed LPS plus ATP-activated dendritic cells via the airways. In this model, we provide evidence that a Th17 response is associated with increased remodeling of the airways. Furthermore, we show that IL-17A affects remodeling of the airways by mediating direct impact on structural cells, such as fibroblasts.

Atopic asthma is a Th2-dominated disease. The pathomechanism involves IgE-dependent mast cell activation and eosinophilic airway inflammation, often resulting in hyperreactivity of the airways and mucus deposition. Under circumstances that are not completely understood, asthma becomes persistent; a process which is associated with irreversible remodeling of the airways.

Newer studies suggest that a Th17 response may be involved in the process of remodeling, because, in the case of chronic asthma, neutrophilia is frequently found in the airways of patients with asthma (1–3). Neutrophilic inflammation, as part of an adaptive immune response, is based on IL-17A production of Th17 cells. These Th17 cells are antigen-specific Th cells

that are activated by the cytokines IL-1 β and IL-6 (4). Furthermore, IL-23 production seems to be important to maintaining the Th17 response. These cytokines are produced by dendritic cells (DCs) due to activation of these cells by ligands of the pattern recognition receptors, such as LPS. In the case of IL-1 β , a further inflammasome-stimulating

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mechanism seems to be important, resulting in the release of the mature bioactive cytokine. Damage-associated molecular pattern molecules (DAMPs), such as ATP or uric acid, can deliver these inflammasome-activating stimuli. Recently, however, it was shown that higher concentrations of LPS are also able to activate the inflammasome via a noncanonical pathway, particularly in DCs (5, 6). Once established, the antigen-specific Th17 response results in the release of IL-17A by Th cells, in turn activating structural cells, such as fibroblast and epithelial cells, to produce chemokines that attract neutrophils, resulting in an inflammatory immune response (7). This inflammation leads to tissue destruction that is repaired by profibrotic processes that can culminate in the irreversible remodeling of the airways (8).

There are some hints from the literature that *in vitro*-generated, antigen-specific Th cells induce a steroid-resistant airway inflammation in mice (9). Moreover, it was shown that *in vitro*-generated Th17 cells support the induction of a *de novo* immune response to allergens in the lung of mice (10). These results show that Th17 cells may be involved in the exacerbation of asthma in murine models (11).

We have previously shown in a model of sensitization of mice with allergen-loaded DCs via the airway that activation of cells with LPS is needed to arm cells for the induction of a Th2 response (12). We have chosen an LPS-driven allergy model, because more and more studies from the literature argue for a contribution of this pathogen-associated molecular pattern in the process of initial allergic sensitization (13, 14). In this study, we wanted to analyze the impact of the mode of DC activation in more detail. We used different concentrations of LPS to activate allergen-pulsed DCs in the presence or absence of ATP for inflammasome activation to test whether these conditions would lead to a shift from Th2- to Th17-inducing DCs. With the help of this model, we sought to study whether airway sensitization of mice by the application of DCs with a phenotype that is able to induce Th17 cells results in increased remodeling of the airways.

Materials and Methods

A complete description of all methods can be found in the online supplement.

Animals

Female BALB/c mice (Charles River, Sulzfeld, Germany), aged 5–6 weeks, were used and acclimated to the animal facility for 14 days before experiments. Animals had access to food and water *ad libitum*. All experimental procedures were approved by the animal ethics committee at Landesamt für Natur, Umwelt und Verbraucherschutz, Nordrhein-Westfalen, Germany.

Generation of Bone Marrow-Derived Cells *In Vitro*

Bone marrow-derived cells (BMDCs) were generated by cultivating bone marrow cells in the presence of granulocyte/macrophage colony-stimulating factor, as described elsewhere (15). On Day 8, cells were pulsed with 100 µg/ml ovalbumin (OVA) free of LPS (Hyglos GmbH, Bernried am Starnberger See, Germany). Chromatographically purified *Escherichia coli* LPS (16) was used at different concentrations, as indicated for activation of the BMDCs (12). An additional ATP stimulus was added in some experiments to activate the inflammasome. ATP was freshly solubilized and used at a concentration of 0.125 mM to stimulate BMDCs for 45 minutes after a 4-hour stimulation with 1 ng/ml LPS.

Sensitization of Mice with OVA-Pulsed BMDCs and Airway Challenge

To sensitize the mice with DCs via the airways, animals were anesthetized with a mixture of ketamine and xylazine. An amount of 10⁶ OVA-pulsed BMDCs was administered intranasally in 50 µl PBS. Mice were challenged on Days 12, 19, and 26 with 1% OVA aerosol for 30 minutes using a PARI-Boy aerosol generator (PARI, Starnberg, Germany) for induction of airway inflammation and IgE production. Controls were sensitized with BMDCs that were not pulsed with OVA and, subsequently, treated with OVA aerosol.

Histology

Lungs were fixed by inflation with 1 ml of 4% buffered paraformaldehyde (PFA) via a tracheal tube. Further fixation was performed by incubation of the lungs in the same fixation medium for 24 hours. Thereafter, lungs were embedded in paraffin and sliced into 5-µm sections with a microtome. After deparaffinization, slices were stained for goblet cells, fibrotic tissue,

and α-smooth muscle actin (SMA), essentially as described in Wegmann and colleagues (17). Stained slices were analyzed using a light microscope (Olympus BX40; Olympus; Hamburg, Germany). Digital image analysis was performed using ImageJ (National Institutes of Health, Bethesda, MD). The thickness of the basement membrane was measured to analyze fibrosis. Mucus-producing cells in periodic acid Schiff-stained tissue were counted, the length of the airway epithelium was measured and the number of positive cells per micrometer was calculated. Phase analysis was performed to quantify α-SMA-positive cells.

Isolation of Lung Fibroblasts and Stimulation *In Vitro*

We used a modified protocol of the mice fibroblast isolation method described previously (18).

Real-Time PCR

The following primers were used for detection (19):

proCol1a2_for 5'-TGCTGCTTGCGAGTA
ACGTCG-3'
proCol1a2_rev 5'-TCAACACCATCTCTG
CCTCG-3'
HPRT_for 5'-GATTCAACTTGCGCTCAT
CTTA-3'
HPRT_rev 5'-GTTGGATACAGGCCAGA
CTTTGTTG-3'

Statistical Analysis

Data were analyzed by the Kruskal-Wallis test. If a significant difference was found, treated groups were compared with the control group by using Dunn's post test. The nonparametric Spearman's test was applied for the analysis of correlation. GraphPad Prism Software (Version 3.03; GraphPad Software Inc., La Jolla, CA) was used for the analysis. Values of *P* less than 0.05 were considered statistically significant.

Results

Cytokine Production of DCs after Stimulation with LPS and ATP

We tested different doses and time points for the stimulation of DCs with LPS in the absence or presence of additional DAMPs, namely, ATP, to investigate which stimuli are best suited to achieve Th17-inducing conditions.

As has already been described for macrophages, we found abundant release of IL-1 β when DCs were prestimulated with LPS for 4 hours, followed by stimulation with ATP for 45 minutes (Figure 1A), whereas ATP alone did not induce IL-1 β production (data not shown). As expected, we also found high concentrations of IL-6 due to LPS stimulation (Figure 1B). However, the production of this cytokine is not enhanced by costimulation with ATP; rather, there seems to be a decrease in production of this cytokine in response to ATP costimulation.

DCs were pulsed with OVA and, subsequently, cocultivated with OVA-specific DO11.10 Th cells to test whether the differentially stimulated DCs differ in their ability to induce a distinct T cell response. T cells that were cocultivated with LPS and ATP-stimulated DCs show a stronger proliferation (Figure 2B) than T cells that were cultivated in the presence of DCs stimulated with LPS only (Figure 2A). The BMDCs stimulated with LPS or LPS in combination with ATP did not differ in the production of IL-13 (Figure 2C). By contrast, LPS plus ATP-stimulated cells induced a stronger Th17 response than cells that were stimulated only with LPS (Figure 2D), whereas their Th1 response was slightly lower than that of cells that were stimulated with LPS only (Figure 2E).

Characterization of the Immune Response after Intranasal Application of Allergen-Pulsed LPS/ATP-Activated DCs

DCs were pulsed with OVA and, subsequently, activated with different doses of LPS with or without the addition of ATP to test whether these LPS with or without ATP-activated DCs differ in their ability to polarize T cells *in vivo*. These cells were then used to sensitize mice via intranasal transfer. At 10 days after intranasal transfer, mice were challenged with OVA aerosol a total of three times. Subsequently, mice were killed, a bronchoalveolar lavage (BAL) was prepared, and lung cells were cultivated to analyze cytokine production.

Production of IL-17A of lung lymphocytes increased with rising doses of LPS used to activate the DCs used for the sensitization of mice. There is a

significant increase in IL-17A production after sensitization with DCs activated with LPS plus ATP (Figure 3A). The infiltration of neutrophilic granulocytes was observed in the airway lumen correlating with the increasing IL-17A production (Figure 3B).

By contrast, the Th2 response, as detected by IL-5 (Figure 3C), infiltration of eosinophilic granulocytes (Figure 3D) into the airways, and IgE production in serum (Figures 3E and 3F), is already induced by a lower LPS dose (i.e., 0.1 ng LPS), showing that DCs activated with a

low dose of LPS are already able to induce a Th2 response.

We measured the expression of retinoic acid-related orphan receptor gamma t (ROR γ t) in Th cells to identify Th17 cells infiltrating the lung tissue (Figure 4) to prove that there is an increase in Th17 cells in the lung due to sensitization with LPS/ATP-activated cells. Indeed, the percentage (Figure 4A) of ROR γ t-expressing Th cells doubled in the lung of mice that were sensitized with DCs that were pulsed with OVA and

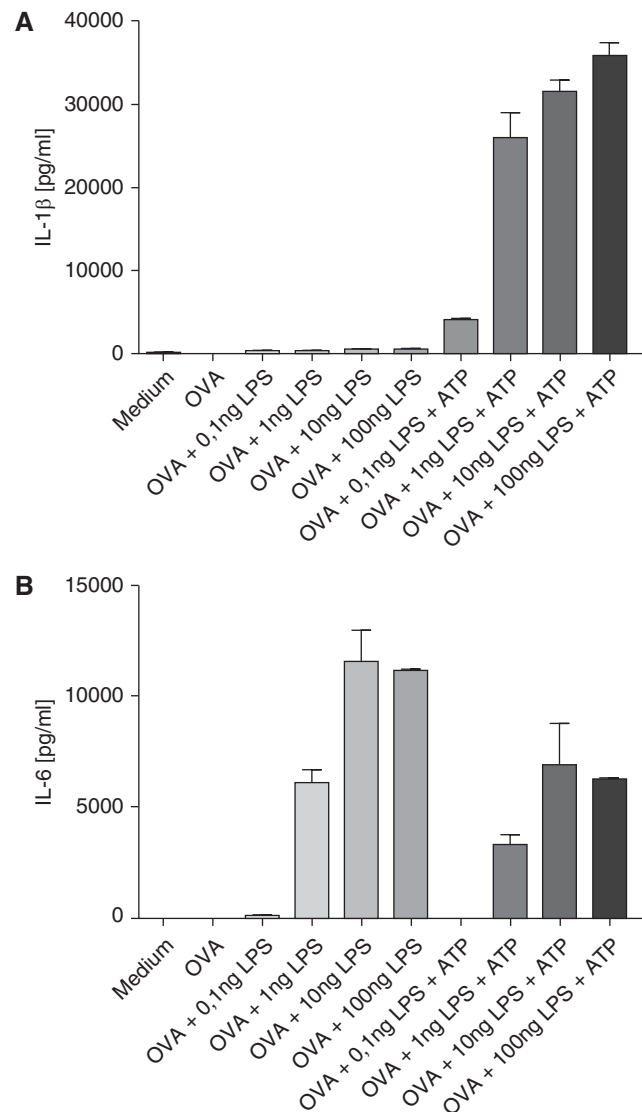


Figure 1. Cytokine production of bone marrow-derived cell (BMDCs) *in vitro*. Dendritic cells (DCs) were pulsed with ovalbumin (OVA). Subsequently, cells were stimulated with the designated LPS concentrations for 4 hours. Where indicated, Toll-like receptor stimulation was followed by stimulation with ATP for 45 minutes. Subsequently, IL-1 β (A) and IL-6 (B) were measured in the supernatant by means of ELISA. Mean $\pm$ SEM is shown ($n = 3$). The data are representative of three independent experiments.

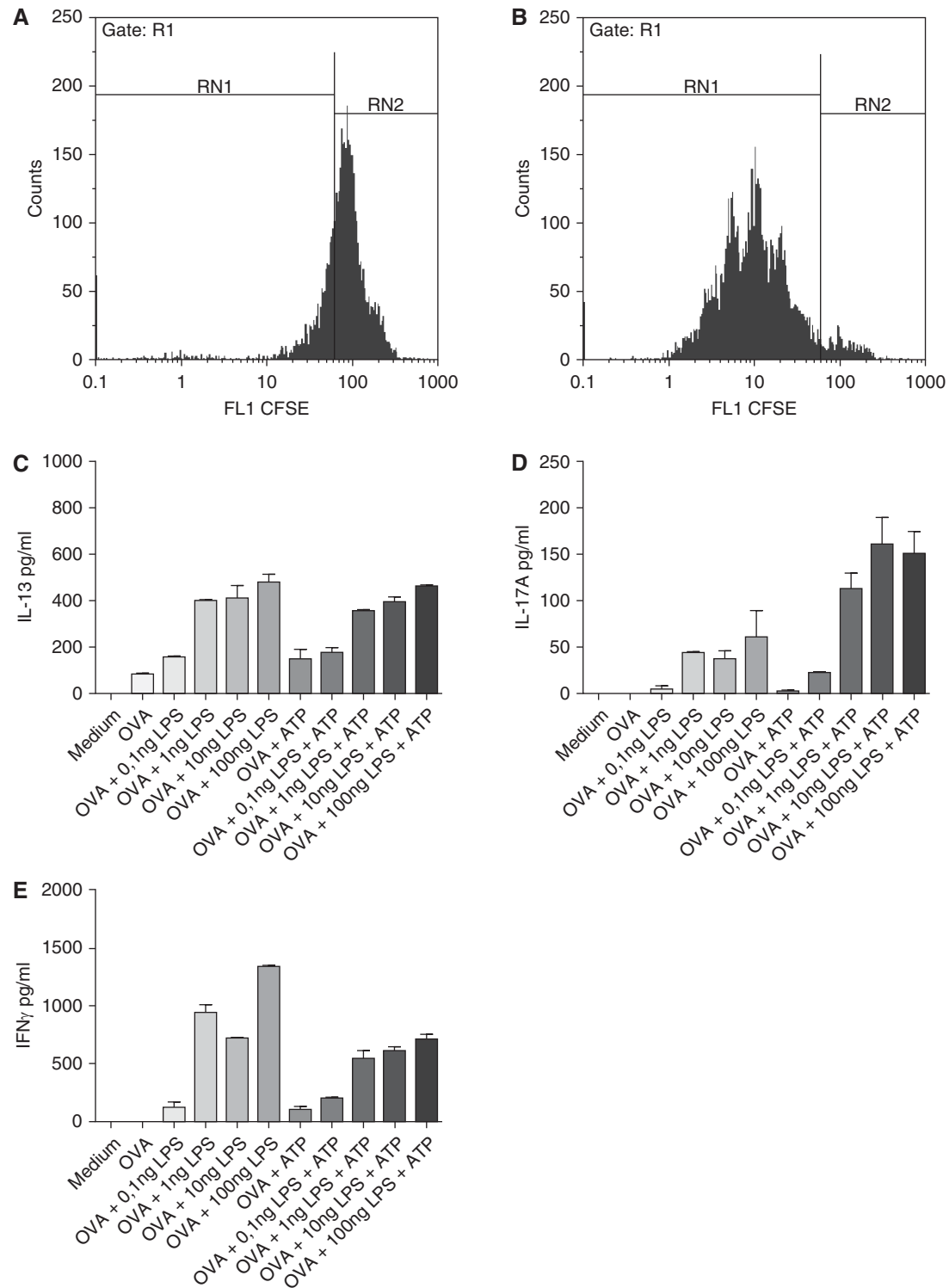


Figure 2. DCs were pulsed with OVA, followed by stimulation with LPS with and without ATP. After washing, cells were cocultivated with Th cells from DO11.10 mice. Cytokines IL-13 (C), IL-17A (D), and IFN- γ (E) were measured in the supernatant of lymphocytes after 24 hours of stimulation. DO11.10 T cells were labeled with carboxyfluorescein succinimidyl ester and cocultivated with DCs for 6 days for the evaluation of T cell proliferation. Representative results are shown for T cell proliferation induced by either DCs activated with 1 ng LPS (A) or 1 ng LPS plus ATP (B). Mean $\pm$ SEM is shown ($n = 2$). The data are representative of two independent experiments.

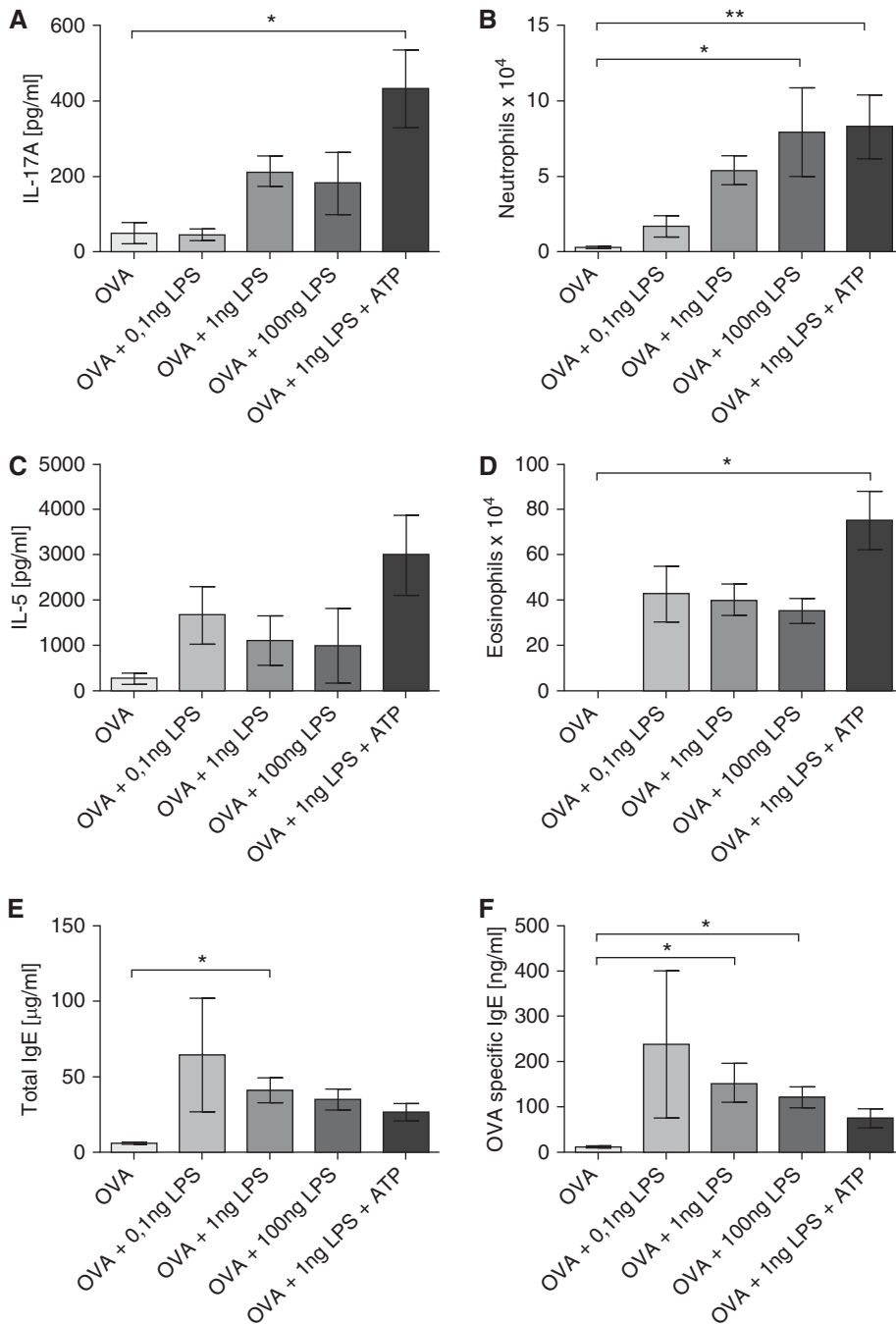


Figure 3. OVA and LPS $\pm$ ATP-pulsed BMDCs were used to sensitize mice via the airways. Subsequently, mice were challenged by the application of OVA aerosol. Bronchoalveolar lavages (BALs) were evaluated for eosinophilic (D) and neutrophilic (B) infiltration, and lung cells were restimulated with OVA. The cytokines IL-5 (C) and IL-17A (A) were measured in the supernatant by means of ELISA. Total (E) and OVA-specific IgE (F) was measured in serum of mice. Mean $\pm$ SEM is shown ($n = 4$). One-way ANOVA was performed. Subsequently, Dunn's post test was used to detect differences between the OVA group and all other groups. * $P < 0.05$, ** $P < 0.01$. Data are representative of two independent experiments.

activated with LPS plus ATP compared with mice that were sensitized with DCs that were pulsed with OVA alone. Likewise, the total number of ROR γ t-

expressing T helper cells significantly increased in lung tissue of mice sensitized with LPS/ATP-activated cells (Figure 4B).

Investigation of Remodeling in the Lung of Mice Sensitized with Th17-Inducing DCs

We studied goblet cell metaplasia by detection of mucus-producing cells in periodic acid Schiff-stained tissue and detection of fibrosis by Sirius red staining to test whether these effects on Th17 induction had consequences for remodeling in the lung. Both analyses were performed on thin slices of paraffin-embedded tissues. Airway smooth muscle thickness was measured by immunohistochemistry on frozen sections. We measured the profibrotic factor transforming growth factor (TGF)- β 1 in BAL fluid (BALF).

Interestingly, all signs of remodeling increased when mice were sensitized with DCs that were either activated with a high dose of LPS or the combination of LPS and ATP (see Figure E1 in the online supplement). Remodeling in the airway is accompanied by increased concentrations of TGF- β 1 in the airway (Figure 5). Absolute numbers of eosinophilic granulocytes ($r = 0.56$; $P < 0.01$) and lymphocytes ($r = 0.72$; $P < 0.001$) are both positively correlated with the concentration of TGF- β 1 in BALF.

Both TGF- β 1, as measured in the BALF, and IL-17A, as detected in the supernatant of lymphocytes that were restimulated with OVA *in vitro*, show a significant correlation with the number of goblet cells (Figures 6A–6C) and the degree of fibrosis (Figures 6D–6F) in the airways. There is increased deposition of α -SMA in the airways of mice that were sensitized with LPS-activated BMDCs. However, this increased deposition is not significant, nor is the correlation to IL-17A and TGF- β 1 production.

Effects of Recombinant Murine IL-17A on Mouse Lung Fibroblasts *In Vitro*

We aimed to study the response of airway structural cells upon stimulation with IL-17A *in vitro* and *in vivo* to shed further light on the influence of IL-17A production on remodeling of the airways. First, we studied the response of fibroblasts *in vitro*.

Therefore, we stimulated freshly isolated lung fibroblasts with recombinant murine IL-17A and studied the expression of the profibrotic factor TGF- β 1 and the fibrotic matrix component proCollagen1a2 (proCol1a2). Stimulation with rising levels of IL-17A significantly increased the

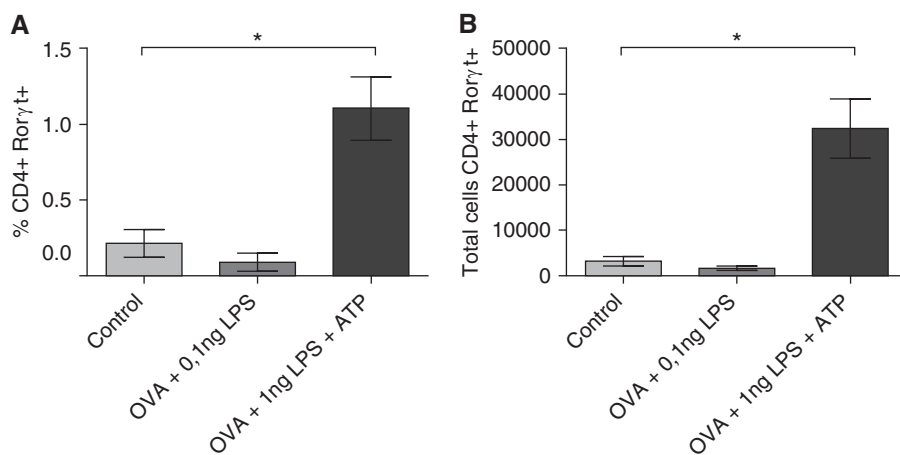


Figure 4. Percentage (A) and total number (B) of retinoic acid-related orphan receptor gamma t (RORγt)-expressing Th cells infiltrating the lung tissue of mice that were sensitized with OVA and LPS ± ATP-pulsed BMDCs. Mean ± SEM is shown ($n = 5$). One-way ANOVA was performed. Subsequently, Dunn's post test was used to detect differences between the control and all other groups. * $P < 0.05$. Data are representative of two independent experiments.

transcription of proCol1a2 after 72 hours of stimulation (Figure 7C).

We analyzed the supernatant with a TGF-β1 ELISA to test whether the transcription of proCol1a2 induced by the stimulation of fibroblasts with IL-17A is a result of TGF-β1 secretion. Stimulation with

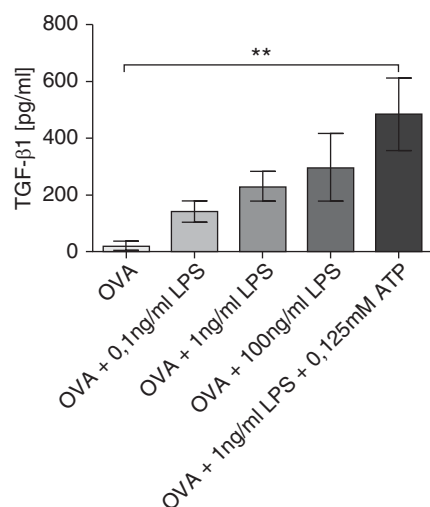


Figure 5. Transforming growth factor (TGF)-β1 concentration in the airways (bronchoalveolar lavage fluid [BALF]) of mice sensitized with OVA-pulsed and LPS ± ATP-stimulated BMDCs, followed by challenge with OVA aerosol. Mean ± SEM is shown ($n = 4$). One-way ANOVA Test was performed. Subsequently, Dunn's post test was used to detect differences between the "OVA + medium" only and all other groups. Mean ± SEM is shown. ** $P < 0.01$. Data are representative of two independent experiments.

IL-17A alone led to a dose-dependent increase of TGF-β1 secretion, which reaches significance at 50 ng/ml IL-17A with no or minor effect at lowest concentration of IL-17A (Figure 7B). Similarly, the lowest dose of IL-17A does not affect transcription of TGF-β1 (Figure 7A). Interestingly, when cells were stimulated with a combination of IL-17A and the profibrotic factor, wingless-type MMTV integration site family, member 5A (Wnt5a), TGF-β1 expression and secretion were enhanced significantly, even at the lowest concentrations of IL-17A (Figures 7A and 7B). Treatment of fibroblasts with Wnt5a alone had no or minor impact on transcription and production of TGF-β1 of lung fibroblasts at 24 and 48 hours, respectively (Figures 7A and 7B).

Discussion

In this study, we investigated whether airway sensitization of mice by the application of DCs with a phenotype that is able to induce Th17 cells results in increased remodeling of the large airways.

We started our experiments by achieving the best conditions for the induction of a Th17 response to the model allergen OVA by differential stimulation of DCs. IL-1β is an important differentiation factor for the induction of Th17 cells, and it is well known that its secretion depends on the activity of the inflammasome (20). Inflammasome-derived IL-1β production may even

substitute other cytokines, such as IL-6, in Th17 induction (21). Several studies were published investigating the activation of the inflammasome in macrophages (22). Efficient IL-1β production in these cells seems to rely on at least two signals. One signal must be received via a Toll-like receptor and the other by a DAMP-like receptor (e.g., ATP). By contrast, it was recently shown that IL-1β production in DCs is already observable after stimulation with LPS alone (5). Consequently, we tested different concentrations of LPS, sometimes in combination with ATP. Subsequently, we examined the cytokine pattern produced by DCs after exposure to these stimuli. As expected, we found increased IL-1β production of DCs that were stimulated with ATP in addition to LPS stimulation. IL-1β is an important factor for the induction of a Th17 response in mice (21). DCs that were treated with LPS without additional ATP were also able to produce moderate levels of IL-1β. However, this production is only observable after longer stimulation periods, particularly with high LPS concentrations (data not shown). LPS plus ATP stimulation synergistically activates DCs that, subsequently, induce strong proliferation of OVA-specific DO11.10 T cells *in vitro*. These T cells produced IL-17A, whereas production of the Th2 cytokine IL-13 seemed to be unchanged. Although LPS plus ATP-stimulated DCs activated higher IL-17A production in DO11.10 cells, production of IL-17A was already observable in T cells activated with DCs pulsed with LPS only. These Th17 cells may result from IL-6 production that is already observable after stimulation of DCs with LPS alone.

After administration of DCs *in vivo*, we showed that enhanced neutrophilic airway inflammation is present in mice that were sensitized with LPS plus ATP-activated DCs. Because we found a correlation between IL-17A production and neutrophilic infiltration, this inflammation was presumably caused by the production of IL-17A acting on structural cells, subsequently producing chemokines attracting neutrophils.

IL-17A is known to be produced from different cell types. Aside from Th17 cells that belong to the adaptive immune system, IL-17A can also be produced by innate immune cells, such as innate lymphoid cells or γδ-T cells (23). It is likely that the IL-17A production is derived from Th17

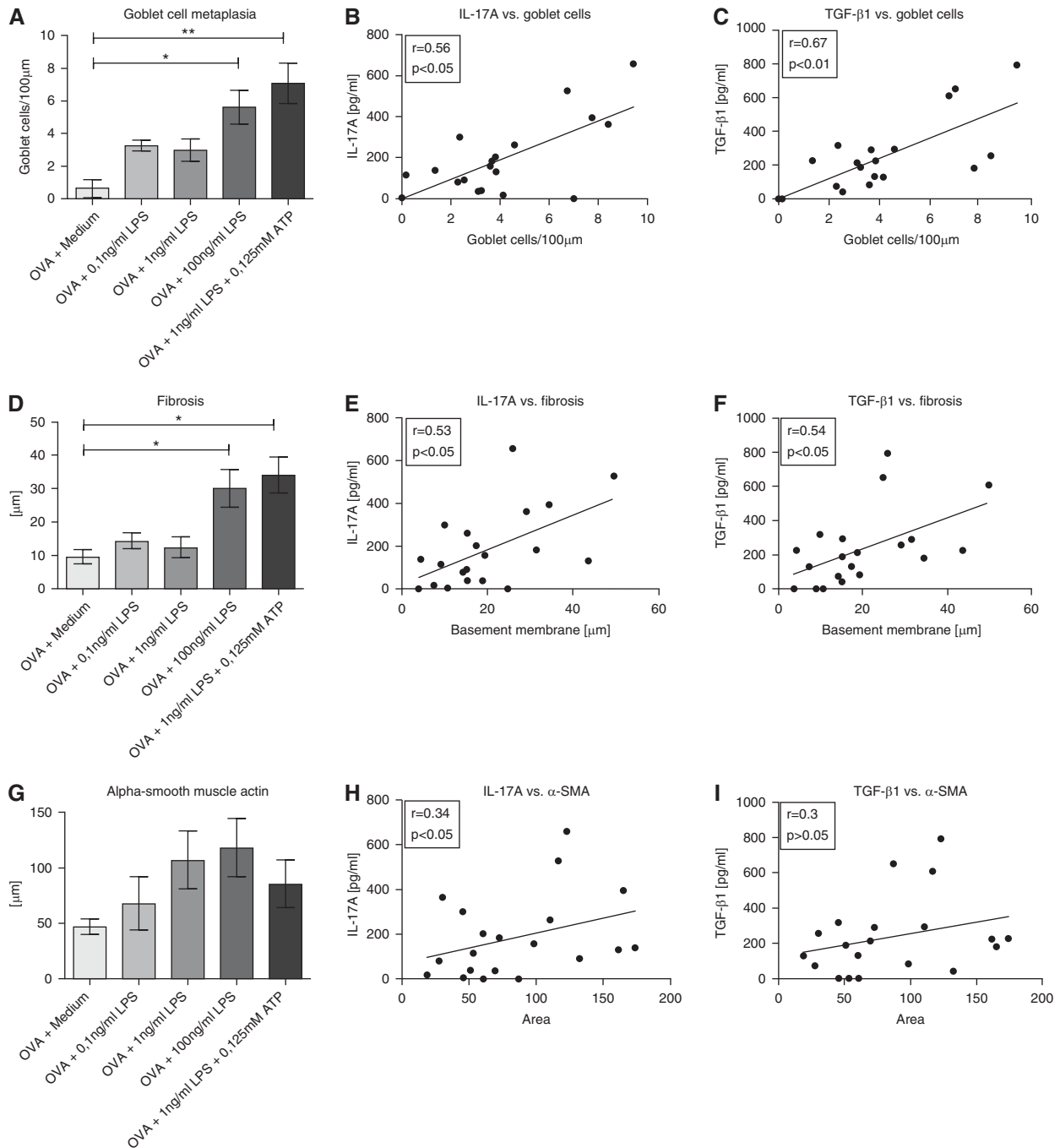


Figure 6. OVA-pulsed and LPS ± ATP-stimulated BMDCs were used to sensitize mice via the airways. Subsequently, mice were challenged by the application of OVA aerosol. Remodeling of the airways was studied by histology. Periodic acid Schiff staining was performed to count goblet cells (A), Sirius red staining was performed to measure the thickness of the basement membrane to evaluate the degree of fibrosis (D), and airway smooth muscle cells and myofibroblasts were stained with an antibody against α-smooth muscle actin (α-SMA) (G). All parameters were correlated against either IL-17A production of lung lymphocytes (B, E, and H) or TGF-β1 concentration of BALF supernatant (C, F, and I). Mean ± SEM is shown ($n=4$). One-way ANOVA test was performed. Subsequently, Dunn's post test was used to detect differences between the "OVA + medium" and all other groups. $*P < 0.05$, $**P < 0.01$. The correlations shown were analyzed with Spearman's test for nonparametric data. The r values and P values are given in each figure. Data are representative of two independent experiments.

cells and not from innate immune cells. First, the IL-17A production is antigen specific and only observable after the restimulation of lymphocytes with OVA. Second, we found a

significant increase of Th17 cells in the lung of mice by detecting RORγt expression in Th cells.

In this study, we aimed to investigate whether there is a correlation of IL-17A

production and remodeling of the large airways. Indeed, we were able to show this relationship. Although we are aware of the importance of remodeling of the small distal

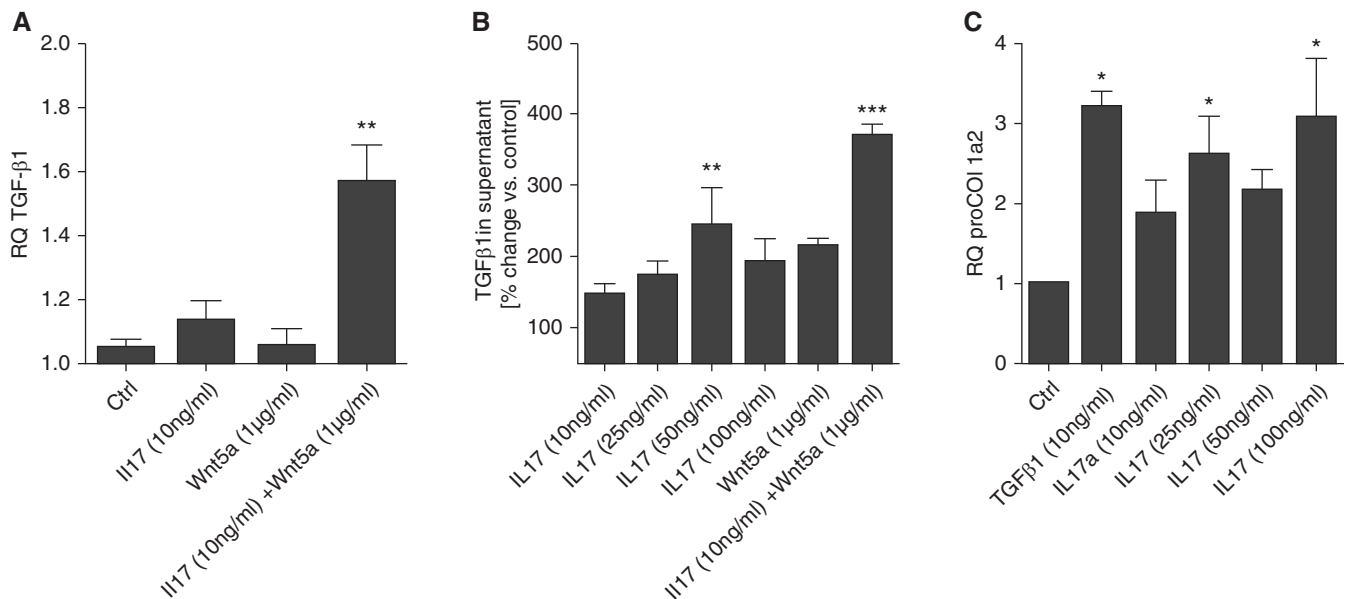


Figure 7. Detection of TGF-β1 mRNA in lung fibroblasts after 24 hours (A) and TGF-β1-protein in supernatant 48 hours (B) after stimulation of lung fibroblasts with recombinant murine IL-17A alone or in combination with wingless-type MMTV integration site family, member 5A (Wnt5a) *in vitro*. The expression of proCollagen 1a2 mRNA was measured in lung fibroblasts upon stimulation with recombinant murine IL-17A alone for 72 hours (C). Mean $\pm$ SEM is shown for $n \geq 3$ for all experiments. One-way ANOVA test (Kruskal-Wallis) was performed. Subsequently, Dunn's post test was used to detect differences between the control and all other groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Data are representative of two independent experiments.

airways in human asthma, we have concentrated on the analysis of the large airways here, as our data derived from analysis of the small airways was prone to a high variance. Therefore, this analysis was excluded here. There are hints from the literature that remodeling of the distal small airways is observable after extended challenge periods for several months (17). Further studies must show whether the elongation of the challenge period will lead to a more consistent remodeling of the distal airways.

Interestingly, IL-17A production induced by sensitization with DCs correlated with production of TGF-β1 in BALF. Moreover, there is a direct correlation of the production of these two cytokines with remodeling found by histological analysis of the large airways. The question is whether this results from destruction of lung tissue by neutrophilic inflammation or whether IL-17A has a direct impact on remodeling of the airways. One point that may argue for such a direct influence is our observation that stimulation of lung fibroblasts with IL-17A can induce an increase in collagen expression typical for the switch of fibroblasts to a profibrotic phenotype, namely, the differentiation of fibroblasts to myofibroblasts, one important feature of remodeling processes involved in asthma.

Furthermore, stimulation of fibroblasts with IL-17A leads to the secretion of TGF-β1 of these cells, a key factor involved in the initiation and maintenance of airway remodeling. IL-17A may act directly via IL-17R on the activation of lung fibroblasts, and/or partially by activating TGF-β1 signaling, as was shown in the context of pulmonary fibrosis for the epithelial-mesenchymal transition of alveolar epithelial cells *in vitro* (24).

Here, we revealed that the activating potency of even low levels of IL-17A is notably enhanced by Wnt5a. This factor was found to act as a translator/multiplier/enhancer of TGF-β1 signaling in airway smooth muscle cells (25), and is produced by, for example, airway epithelium and macrophages, suggesting an IL-17A/TGF-β1/Wnt5a signaling axis in (asthmatic) activated pulmonary fibroblasts. Thus, we hypothesize that IL-17A may have a direct impact on airway remodeling in asthma without the need of granulocytic inflammation. One possibility could be the microenvironmental coactivation of lung fibroblasts by IL-17A produced by allergen-specific Th17 cells, resulting in a local increase of TGF-β1 and Wnt5a produced by adjacent damaged airway epithelium. Further studies have to show whether this observation that is derived

from *in vitro* experiments by using quite high concentrations of recombinant cytokines also have implications *in vivo*. Moreover, it will be of great interest to find out whether cytokines produced by Th17 cells besides IL-17A—namely, IL-17F and IL-22—will also affect tissue remodeling.

Taken together, our data suggest that an allergen-specific mixed Th2/Th17 response results in increased remodeling of the airways in a mouse model of allergic airway inflammation. Further studies must show whether this results in an exacerbation of the allergic disease, for instance, by studying lung function. Because neutrophilic inflammation that is based on a Th17 response is poorly controllable by using corticosteroids (26), these results argue for advanced studies of controlling the remodeling in neutrophilic asthma (e.g., by treatment with inhibitors of the Th17 pathway). ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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