IL-17A contributes to maintenance of pulmonary homeostasis in a murine model of cigarette smoke-induced emphysema

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Chronic obstructive pulmonary disease (COPD) is expected to become the fourth leading cause of death worldwide by 2030 (23). Major risk factors for the development of COPD are cigarette smoke (CS) and exposure to indoor air pollution (2). Pivotal in COPD is chronic airway inflammation leading to tissue destruction resulting in irreversible overinflation of the lung (33).

IL-17A is a member of the IL-17 cytokine family (IL-17 A to F), which is recognized by specific members of the IL-17 receptor family (10). IL-17A is of importance in the clearance of extracellular pathogens (41). IL-17A is expressed and released by a variety of immune cells such as activated CD4+ Th17 cells, γδ-T cells, and macrophages (41). Together with IL-17F, IL-17A regulates the recruitment of neutrophils to the site of infection via the induction of the release of inflammatory mediators by target cells. IL-17A and -F induce, for instance, the expression of antimicrobial peptides, mucins, and inflammatory cytokines in respiratory epithelial cells by binding to the IL-17 receptors IL-17RA and IL-17RC (11, 15, 19, 22, 39, 45–47).

Recent studies indicate that IL-17A and Th17 cells may contribute to the pathogenesis of COPD. Increased levels of IL-17A and enhanced numbers of IL-17A+ cells have been detected in the bronchial mucosa and in sputum of COPD patients (4, 8, 9). A study by Roos et al. (30) showed that the expression of IL-17A is increased (primarily in mast cells) in end-stage COPD. The function of IL-17A and the IL-17RA was also studied in animal models of CS- and oxidant stress-induced lung damage (5, 28, 35). By quantitative micrometric tomography (μCT), Shan et al. (35) demonstrated that IL-17A-deficient mice exposed to cigarette CS for 4 mo were protected from increases in lung volume and decreased lung density, whereas overexpression of IL-17A resulted in increases in lung volume and decreased lung density. In addition, IL-17RA-deficient mice exposed to CS for 6 mo did not show an increase in the mean linear intercept, as a measure of emphysema, compared with CS-exposed wild-type (WT) mice (5). In contrast, IL-17RA deficiency did not protect mice from an ozone-induced increase in mean linear intercept. Moreover, the emphysema score was increased in 17RA-deficient mice compared with WT mice after ozone exposure (28).

In this study, we aimed to investigate the contribution of IL-17A to CS-induced changes of pulmonary function and lung damage. Using invasive pulmonary function and stereology, we studied the physiological significance of IL-17A in the development of emphysematous lung disease.

Methods

CS exposure. IL-17A-deficient mice (complete knockout) were a gift from Dr. Yoichiro Iwakura (Institute of Medical Science, University of Tokyo) (25). All animal experiments were approved by the Landesamt für Soziales, Gesundheit und Verbraucherschutz of the State of Saarland following the national guidelines for animal treatment. Mice were maintained under pathogen-free conditions. Sevento nine-week-old female WT C57BL/6 and IL-17A-deficient C57BL/6 mice were exposed to CS (3R4F; College of Agriculture, Reference Cigarette Program, University of Kentucky, Lexington, KY) in a TE-10 smoking machine (Teague Enterprises, Woodland, CA). A smoking period was 1:21 h. Mice were exposed to CS for three smoking periods per day and exposed to air for 40 min between...
the smoking periods. Mice were exposed to CS at 5 days/wk. Pulmonary inflammation was determined in lungs of mice exposed to CS or air for 3 mo. Histology, morphometry, pulmonary function, and protease activity were determined in lungs of mice exposed to CS or air for 7 mo. The CS concentration was 120 mg/m³ total suspended particles. Carbon monoxide concentrations in the exposure chamber were monitored using the Standard Data Logger (Lascar Electronics). CO concentrations were between 400 and 550 ppm during the smoking period.

Pulmonary function. Invasive pulmonary function was determined in mice with the forced oscillation technique using the FlexiVent system (Scireq, Montreal, Canada) as described earlier (42). The following maneuvers were used for specific measurements: the snapshot perturbation maneuver for respiratory system compliance, elastance, and resistance; the pressure-volume (PV) loops maneuver for total lung capacity, inspiratory capacity from zero pressure (parameter B of the Salazar-Knowles equation), estimate of the difference between total lung capacity and the predicted volume at zero pressure), deflating PV loop (parameter K of the Salazar-Knowles equation, reflects the curvature of the upper portion of the deflation PV curve), hysteresis, and quasi-static compliance; and the constant-phase model for tissue elasticity, Newtonian resistance, inerterance, hysteresivity, and tissue damping.

Determination of cytokine concentrations, FACS analyses, and real-time RT-PCR. Bronchoalveolar lavage (BAL) was performed using 1 ml of 1× PBS and flushing the lung three times. BAL was centrifuged for 10 min at 300 g and 4°C, and supernatants were frozen at −80°C. Total leukocyte cell counts were performed using a Neubauer cell counter. Differential cell counts were performed on cytospin preparations of BAL fluids cells stained with Diff Quik Staining kit (Medion Diagnostics). For FACS analysis blood free cytospin preparations of BAL fluids cells stained with Diff Quik were used as primary antibody (20, 31).

Statistical analysis. Values are displayed as means ± SE. Comparisons between groups were analyzed by unpaired t-test (Mann-Whitney). Kinetics of elastase and MMP-12 activity were analyzed by two-way ANOVA. Results were considered statistically significant for P < 0.05. All statistical tests were performed using the software Prism (GraphPad Software, San Diego, CA).

RESULTS

IL-17A deficiency affects CS-induced lung inflammation. To address whether deficiency for IL-17A affects pulmonary inflammation, age-matched WT and IL-17A-deficient (IL-17A−/−) mice were chronically exposed to CS for 3 mo. Concentrations of inflammatory mediators were determined in lung tissue. Exposure to CS resulted in significantly increased concentrations of IL-1β (Fig. 1A) and G-CSF (Fig. 1B) at a low level in lungs of WT mice but not in lungs of IL-17A−/− mice. Concentrations of IL-1x were significantly enhanced in both WT and IL-17A−/− mice (Fig. 1C). Concentrations of IL-17A in lungs of air- or CS-exposed WT mice were below the detection limit of 15 pg/ml. Numbers of macrophages in BAL fluids were decreased in air-exposed IL-17A−/− mice and increased in CS-exposed IL-17A−/− mice compared with air-exposed WT mice (Fig. 1D) whereas chronic CS exposure did not result in increased levels of neutrophils in BAL fluids of WT and IL-17A−/− mice (Fig. 1E).

Histology of lungs of air-exposed WT and IL-17A−/− mice did not result in enhanced levels of γδ T cells in lung tissue (Fig. 1G). IL-17A deficiency and CS exposure resulted in a reduced expression of elastin. The mRNA expression of elastin was decreased in lungs of air- and CS-exposed IL-17A−/− mice and in lungs of CS-exposed WT mice (Fig. 2A). The reduced expression of elastin in lungs of IL-17A-deficient mice and CS-exposed mice was also evident in the elastin protein content in lung tissue (Fig. 2B).

Loss of lung architecture in IL-17A-deficient mice. To address whether deficiency for IL-17A affects lung structure and development of emphysema, age-matched WT and IL-17A−/− mice were exposed to air or CS for 7 mo. Lungs were analyzed by histology and morphometry. Figure 3A shows representative histology of lungs of air- and CS-exposed WT and IL-17A−/− mice. IL-17A-deficiency per se resulted in a loss of alveolar structure. The mean chord length (Fig. 3B) and alveolar air space (Fig. 3C) were significantly increased whereas the parenchyma occupied by alveolar wall tissue (Fig. 3D) was decreased in air-exposed IL-17A−/− mice compared with air-exposed WT mice. Histology also showed that CS exposure resulted in loss of alveolar structures in WT and IL-17A−/− mice (Fig. 3A). The mean chord length (Fig. 3B) and alveolar air space (Fig. 3C) were significantly increased and the parenchyma occupied by alveolar wall tissue (Fig. 3D) was decreased in WT and IL-17A−/− mice exposed to CS compared with air-exposed WT mice. Table 1 shows the morphometric
data of CS-exposed WT and IL-17A−/− mice as relative changes (%) from air-exposed mice. There was no significant difference in the changes from baseline in the mean chord length, alveolar air space, and parenchyma occupied by alveolar wall tissue between CS-exposed WT and CS-exposed IL-17A−/− mice. There was no difference in the mean chord length between 8-wk-old WT and IL-17A−/− mice (Fig. 3E).

**IL-17A deficiency affects pulmonary activity of proteases.** Studies showed that neutrophil elastase and MMP-12, but not MMP-9, are required for the development of CS-induced emphysema in mice (1, 12, 36). Moreover, intranasal stimulation of mice with IL-17A resulted in increased concentrations of MMP-9 in lungs of mice (29). Thus we determined the activity of elastase and MMP-12 in lung tissue of mice exposed...
to air or CS for 7 mo. Exposure to CS resulted in an increased activity of MMP-12 in WT mice compared with air-exposed WT mice, but not in IL-17A−/− mice (Fig. 4A). Baseline elastase activity was increased in lungs of air-exposed IL-17A−/− mice (Fig. 4B). CS exposure was associated with lower elastase activity. IL-17A deficiency and CS exposure resulted in reduced protein levels of MMP-9. Protein concentrations of MMP-9 were decreased in lungs of air- and CS-exposed IL-17A−/− mice and in lungs of CS-exposed WT mice compared with air-exposed WT mice (Fig. 4C).

CS exposure impairs pulmonary function in IL-17A-deficient mice. In addition to the histological analysis, pulmonary function was determined in mice exposed to air or CS for 7 mo. Invasive pulmonary function was determined with the forced oscillation technique applying the snapshot perturbation maneuver (Fig. 5), PV loops maneuver (Fig. 6), and constant-phase model (Fig. 7).

Chronic CS exposure of WT mice resulted in a loss of pulmonary function characteristic for CS-induced lung damage and emphysema. The respiratory system elastance was decreased (Fig. 5A) and the total lung capacity (Fig. 6A), inspiratory capacity (Fig. 6B), hysteresis (Fig. 6D), and quasi-static compliance (Fig. 6E) were increased in WT mice after CS exposure. The deflating PV loop (Fig. 6C) and tissue elasticity (Fig. 7A) were significantly decreased in WT mice after chronic CS exposure. Invasive pulmonary function analysis further revealed that IL-17A−/− mice were not protected against CS-induced lung damage. The respiratory system elastance was significantly decreased in CS-exposed IL-17A−/− mice compared with air- and CS-exposed WT mice (Fig. 5A).

In addition, the respiratory system (Fig. 5B) and the quasistatic...
Fig. 6. IL-17A deficiency affects pulmonary elastase and matrix metalloproteinase (MMP)-12 and -9. MMP-12 and neutrophil elastase activity and MMP-9 concentrations were measured in total lung tissue of mice exposed to air or CS for 7 mo. A: MMP-12 activity was measured using the Sensolyte 520 MMP-12 assay kinetic read. B: neutrophil elastase was measured using the Molecular Probes’ EnzChek Elastase Assay. *P < 0.05, as compared with air-exposed WT mice; n ≥ 4 per group. C: MMP-9 concentrations were determined by ELISA. Data are shown as means ± SE. Bars indicate significant differences of *P < 0.05; n ≥ 4 per group.

Fig. 7. A: IL-17A deficiency was significantly increased and the tissue elasticity (Fig. 7A) was significantly decreased in CS exposed IL-17A−/− mice compared with air-exposed mice. However, even though CS exposure resulted in a significant increase of the total lung capacity (Fig. 6A) in IL-17A−/− mice compared with air-exposed WT and IL-17A−/− mice, this parameter was significantly reduced in CS-exposed IL-17A−/− mice compared with CS-exposed WT mice. IL-17A−/− mice were also partially protected from CS induced changes in the deflating PV loop (Fig. 6C) and the CS-induced increase in the hysteresis (Fig. 6D). Chronic CS exposure did not cause any changes in respiratory resistance (Fig. 7C), as can be expected from the model. The Newtonian resistance (Fig. 7B), inertia, hysteresivity, and tissue damping were unaltered in WT and IL-17A−/− mice by chronic CS exposure, as well (data not shown).

DISCUSSION

In this study, we sought to determine whether the cytokine IL-17A mediates CS-induced lung damage. Our main findings are that IL-17A contributes to the maintenance of basal tissue homeostasis and that IL-17A-deficient mice are not protected from CS-induced emphysematous disease. IL-17A deficiency resulted in spontaneous loss of alveolar structure under basal conditions. While the expression of inflammatory mediators was partially reduced in lungs of IL-17A-deficient mice compared with WT mice, exposure to CS still resulted in destruction of lung tissue. Invasive pulmonary function showed that the respiratory system elastance declined and compliance increased in lungs of CS-exposed IL-17A-deficient mice.

Recent studies link the expression of the proinflammatory cytokine IL-17A in immune cells to the development of COPD. Enhanced numbers of IL-17A+ cells have been detected in bronchial mucosa of COPD patients and increased numbers of IL-17A-expressing mast cells were found in end-stage COPD (4, 8, 9, 30). As inflammation is central in the development of COPD (33), we initially hypothesized that IL-17A mediates CS-induced destruction of alveolar tissue and loss of pulmonary function. To study the contribution of IL-17A to CS-induced lung damage we used a murine model of CS-induced inflammation that leads to the development of emphysema-like lung damage. Our model resembles published emphysema models in which exposure to CS for 4–8 mo is required to detect emphysema in C57BL/6 mice (6, 14, 32, 44). Surprisingly, we found that exposure of IL-17A-deficient mice to CS for 7 mo resulted in a loss of alveolar structure comparable to WT mice. The respiratory system elastance was even decreased in CS-exposed IL-17A-deficient mice compared with WT mice. Thus, in our disease model, IL-17A undoubtably is not a central mediator of CS-induced emphysema, even though
IL-17A partially regulated the expression of inflammatory mediators and proteases, such as IL-1\beta and MMP-12.

We found evidence that IL-17A contributes to the maintenance of basal tissue homeostasis. Histology and morphometry of 8 mo old air-exposed mice showed that deficiency for IL-17A spontaneously results in a loss of lung structure at a low level. These differences in the lung structure were not present in young (8 wk old) mice and thus seem to develop during adulthood of IL-17A-deficient mice rather than being a developmental effect. Interestingly, the structural changes in lungs of air-exposed IL-17A-deficient mice were associated with a reduced expression of pulmonary elastin and increased elastase activity. Elastin is a structural component of the lung, and impaired expression of elastin has been linked to CS damage in mice before (3, 32, 38). Shifren et al. (38) showed that quantitative deficiencies in elastin predispose mice to CS-induced emphysema. Mice heterozygous for elastin developed a 1.8 times greater air space enlargement after CS exposure than WT mice (38). Thus loss of basal elastin expression may negatively affect pulmonary homeostasis in air- and CS-exposed IL-17A-deficient mice. Mechanistically, it is unclear how IL-17A deficiency affected the expression of elastin. Studies showed that inflammatory mediators regulate transcriptional expression of elastin (40). IL-1\beta reduces the expression of elastin in neonatal lung fibroblasts in a CCAAT/enhancer-binding protein (C/EBP)\beta-dependent manner but increases the expression of elastin in adult dermal fibroblasts (17, 18, 24). Further studies are required to reveal whether IL-17A and its receptor IL-17RA, which negatively regulates C/EBP\beta (37), directly regulate the expression of elastin in target cells or indirectly via the induction of additional inflammatory mediators, such as IL-1\beta. We also found that IL-17A deficiency resulted in reduced expression of MMP-9 in lung tissue, which is in line with a study showing that intranasal stimulation of mice with IL-17A increases lung concentrations of MMP-9 (29). As concentrations of MMP-9 were also reduced in CS-exposed WT and IL-17A-deficient mice, pulmonary expression of MMP-9 did not correlate with loss of alveolar structure. This
finding is in agreement with a study by Akkinson et al. (1) showing that MMP-9 is not required for the development of CS-induced emphysema in mice. Deficiency for MMP-9 does not prevent CS-induced pulmonary inflammation and air space enlargement in mice (1). Together, our data suggest that dysregulated activity of proteases that likely do not only have a role during pathologic conditions but also contribute to tissue homeostasis and wound healing in the lung (21) and aberrant expression of structural components of the lung may account for the basal loss of alveolar structure in IL-17A-deficient mice. Impaired basal pulmonary homeostasis may also predispose IL-17A-deficient mice to the CS-induced decline of pulmonary function, as seen for the respiratory system elastance.

In a recent study, Shan et al. (35) reported that mice deficient for IL-17A were protected from CS-induced increases in lung volume and decreases in lung density, whereas overexpression of IL-17A resulted in increases in lung volume and decreased lung density. In that study, structural changes were only analyzed by μCT and no data obtained by pulmonary function or morphometry were provided (35). In the present study, we applied a different methodology, invasive pulmonary function analysis, and morphometric methods. Even though we found that IL-17A-deficient mice were partially protected against CS-induced increases in total lung capacity, which is in line with the data for the lung volume obtained by μCT (35), our additional data obtained by histology, morphometry, and pulmonary function do not confirm the conclusion by Shan et al. (35) that deficiency for IL-17A results in attenuation of CS-induced emphysema in mice. Methodological differences between our study and the study by Shan et al. could account for the different findings. Shan et al. used a CS exposure regime that resulted in detectable structural changes in lungs of mice exposed to CS for 4 mo, whereas in our protocol it takes 6 mo of CS exposure to detect emphysema. Moreover, in the study by Shan et al., pulmonary inflammation appears to be increased under chronic conditions of CS exposure compared with our study. In contrast to our study, Shan et al. found increased numbers of γδ T cells and neutrophils in lungs of mice chronically exposed to CS. However, a recent study by Pinart et al. (28) also showed that the deficiency for the IL-17A receptor IL-17RA does not result in an improved outcome in mice exposed to oxidative stress. In line with our study, mice deficient for the IL-17A receptor IL-17RA were not protected against lung damage induced by chronic ozone exposure. Moreover, exposure to ozone resulted in significantly increased emphysema scores in IL-17RA-deficient mice (28). The different outcomes in mouse studies underline the importance of data from clinical investigation to obtain more insight in a possible role of inflammatory cytokines, such as IL-17A, in the development of chronic respiratory tract diseases.

COPD is a complex disease and its pathogenesis involves multiple mechanisms. Microbial pathogens establish infections of the lung and evoke harmful inflammatory responses that contribute to clinical deterioration of the patient during exacerbations (33, 34, 43). As the expression of IL-17A by diverse immune cells as well as Th-17-dependent immunity is mainly induced by microbial agents (10, 41), it is conceivable that IL-17A plays a major role during exacerbations of COPD. Therefore, our finding that deficiency for IL-17A did not protect mice from CS-induced loss of pulmonary function and lung damage does not give insights in a possible role of microbial-driven expression of IL-17A during exacerbations of COPD. However, in our disease model, we found the pulmonary expression of IL-17A in mouse lungs solely dependent on exposure to nontypeable *Haemophilus influenzae* (NTHi), whereas chronic exposure to CS alone did not induce IL-17A expression (13 and unpublished data). NTHi are among the most frequent pathogens isolated from patients suffering from exacerbation (33). Thus our disease models suggest that microbial pathogens, but not CS are mainly responsible for the increased numbers of IL-17A-expressing immune cells seen in COPD patients (4, 8, 9, 30). Additional studies are required to examine whether IL-17A-dependent immune mechanisms evoked by microbial pathogens contribute to the development of COPD, especially during acute exacerbations.

In summary, our study provides additional insight into the contribution of IL-17A to the development of emphysema. Our data suggest that basal IL-17A expression contributes to tissue homeostasis, which may predispose mice to CS-induced emphysema. Thus IL-17A likely has a complex role in the CS-induced loss of pulmonary function and development of emphysema and cannot be characterized as a central mediator of CS-induced lung damage. Future studies are needed to examine the role of IL-17A during exacerbations of COPD induced by microbial pathogens.

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DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


