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Hydrodynamic delivery of IL-28B (IFN-λ3) gene ameliorates lung inflammation induced by cigarette smoke exposure in mice

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ABSTRACT

Cigarette smoke (CS) is the principal cause of pulmonary inflammatory response. IL-28 (IFN-λ) is a novel group of class II cytokines targeting the epithelial cells and IL-28 responses prominent in lungs can exert important immunomodulatory effects. We tested the hypothesis that IL-28B may modulate the lung inflammation induced by CS. Groups of mice were exposed to CS two times per day for 11 consecutive days. CS exposure induced lymphocyte, neutrophil and macrophage infiltration and inflammatory cytokine (IL-1β, tumor necrosis factor-α (TNF)-α, IL-17, and IL-4) in the airways. More importantly, all these CS-induced pathogenic changes were significantly inhibited by hydrodynamic delivery of plasmid DNA encoding mouse IL-28B. Thus, our results suggest that IL-28 cytokines are beneficial for the suppression of CS-mediated airway inflammation and may be a therapeutic target in CS-related diseases.

1. Introduction

Cigarette smoke (CS) is a principal environmental risk factor closely associated with the development and exacerbation of a wide-range of inflammatory pulmonary diseases [1]. The mechanism by which CS contributes to the pathogenesis of lung inflammation is still poorly understood. Current studies suggest that CS has a great variety of biological and toxic effects on the structural and immune cells in the airways [2–4]. For instance, CS can lead to the generation of pathogenic T cells, and drive the recruitment and activation of macrophages and neutrophils, which induce the production of an array of inflammatory mediators including cytokines and chemokines in the airways [5,6].

Interleukin (IL)-28/29 family, which is a novel group of class II cytokines discovered independently by two research teams in 2003, consists of three members: IL-29, IL-28A, and IL-28B (also referred to as interferon (IFN)-λ1, IFN-λ2, and IFN-λ3, respectively) and collectively as type III IFNs [7–9]. IL-28A and IL-28B are virtually identical sharing 96% amino acid identity whereas IL-29 has 81% homology to IL-28 [7,8]. IL-28/29 signal through a heterodimeric receptor complex consisting of the IL-28Rα and IL-10Rβ chains, which mediates activation of both Janus kinases-signal transducers and activators of transcription (Jak–STAT) pathway and the mitogen-activated protein (MAP) kinases pathway [7,10,11]. Up to now, research has mainly focused on the role of IL-28/29 in their antiviral, anti-proliferative and antitumor activities. In fact, apart from these activities IL-28/29 can also exert important immunomodulatory effects, including increasing NK and T cell cytotoxicity, up-regulating MHC class I molecule expression on tumor cells to promote antigen presentation, activating monocytes to secrete a panel of cytokines, and modulating plasmacytoid dendritic cells (DCs) function and cytokine response as well as the Th1/Th2 response [7,8,12–16].

IL-28B, secreted primarily by DCs and macrophages, is known for its anti-inflammatory activity and considered to possess the highest specific activity of the IL-28/29 subtypes [17]. The solved crystal structure of it shows a close relationship to that of IL-19, which can promote Th2 immune deviation through a positive feedback system [18]. More recently, Morrow et al. [19] demonstrated that IL-28B is able to enhance adaptive immunity and reduce regulatory T-cell populations. Furthermore, it has been proved that IL-28/29 responses are prominent in the stomach, intestine and lungs, and the main IL-28/29 targets are the epithelial cells and specific subsets of immune cells, thus strongly suggesting the prominent role of IL-28/29 on mucosal surface [20–22]. Moreover, a recent study clearly demonstrated that IL-28A could effectively induce Th1 immunity and suppress Th2-mediated responses in the airways of a mouse model [23]. In humans, Miller and

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colleagues [24] found that IL-29 responses mediate exacerbations in asthma patients. Another study also showed that deficient IL-28/29 production correlates with the severity of asthma exacerbations and airway inflammation [25]. Together, we hypothesized that IL-28B may modulate the lung inflammation induced by CS.

We therefore investigated the therapeutic potential of IL-28B to block CS-induced airway inflammation in naive mice. Our data suggest that IL-28B gene delivery in vivo is able to alleviate airway inflammation in CS-exposed mice. Hence, IL-28 may be a new alternative therapy for CS-mediated inflammatory respiratory diseases.

2. Materials and methods

2.1. Mice

Male C57BL/6 mice were obtained from the Animal Centre of Shantou University Medical College. Mice were housed in sterilized cages with filter tops in specific-pathogen-free conditions at Shantou University Medical College, China in accordance with animal experimentation guidelines.

2.2. Cigarette smoke exposure

Mice (n = 10 per group) were whole-bodily exposed passively to CS in the atmosphere of a perspex chamber using a modified method [26,27]. Briefly, groups of mice were exposed to the smoke of eight cigarettes (Reference Cigarette 1R5F; University of Kentucky, Lexington, USA) for two separate 1 h periods per day (4 h smoke-free intervals) for 11 consecutive days. Control groups of mice were exposed to ambient room air for the same time period. The mice were sacrificed on day 15. Exposure details are further depicted in Fig. 1A.

2.3. Hydrodynamic injection of plasmid DNA

The hydrodynamic injection (referred to intravenous injection with a large volume of plasmid DNA solution) took no longer than 10 s, and the apnea period in mice ranged from 4 to 6 s with previously reported methods [28,29]. On days 1 and 8, 100 μg purified plasmid pcDNA3.1-IL-28B or empty plasmid pcDNA3.1 were diluted in ~2 ml of PBS and injected into the tail vein of C57BL/6 mice (Fig. 1A). Specifically, the total volume of plasmid DNA solution injected into one mouse was calculated according to 0.1 ml per 1 g body weight.

For study of the kinetics of IL-28B gene delivery, mice received an intravenous injection of plasmid DNA (100 μg; pcDNA3.1-IL-28B or control pcDNA3.1) on day 0, mice were sacrificed on days 1, 4, 7, 10, 13, 20, and 23 after the plasmid injection. Concentrations of IL-28B in serum and BALF were measured using an ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

2.4. Bronchoalveolar lavage (BAL)

BAL was performed in some mice using a tracheal cannula as previously described [30,31]. Briefly, mice were anaesthetized lightly by intraperitoneal injection of ~50 μl 2% sodium pentobarbitone, then the trachea was exposed and the cannula was inserted via a small transverse cut, and lungs were lavaged in situ with three aliquots (500 μl) of PBS. Total cells and cell differentials were counted using a hemocytometer and Diff-Quik stain (Fisher

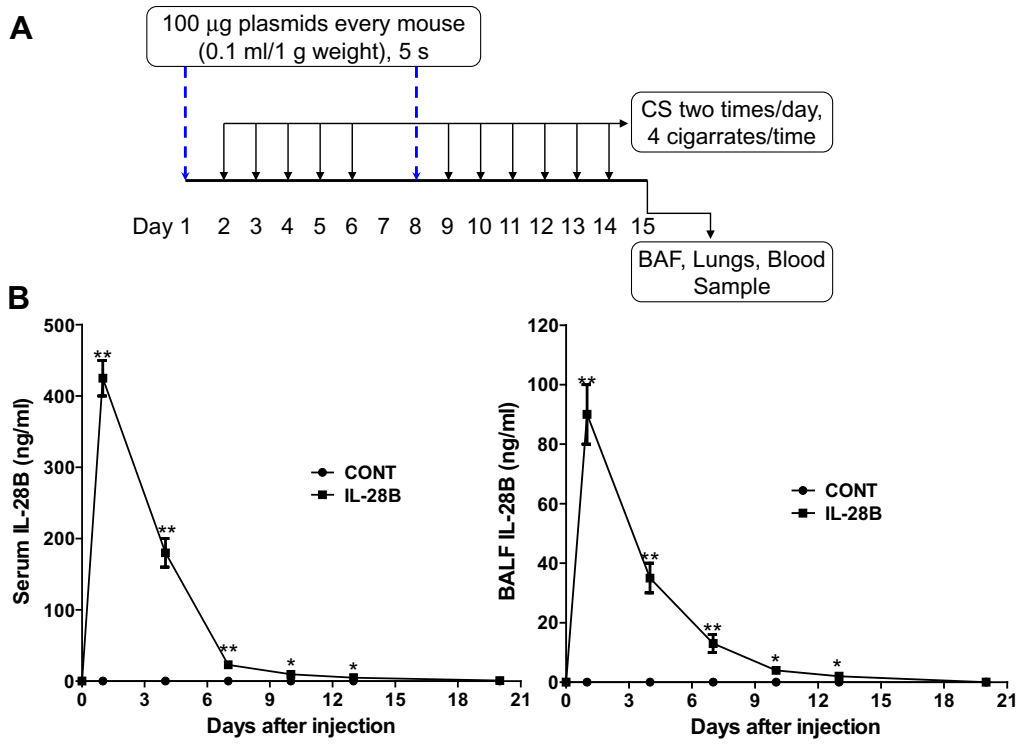

Fig. 1. Summary of prophylactic and therapeutic study protocols for IL-28B in a murine model of cigarette smoke and IL-28B concentrations in serum and in BALF after hydrodynamic delivery of plasmid DNA. (A) Mice were treated with 100 μg purified plasmid pcDNA3.1-IL-28B or plasmid pcDNA3.1 on days 1 and 8 by hydrodynamic injection. On days 2–6 and 9–14, mice were nose-only exposed to the smoke of eight 1R5F reference cigarettes per day. Lung tissues, serum and BAL were performed on day 15. (B) Mice received an intravenous injection of pcDNA3.1-IL-28B or control pcDNA3.1 plasmid on day 0. Concentrations of IL-28B in serum and in BALF were measured at the indicated times after the injection using ELISA. Values are presented as means ± SEM for 10 mice per group. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the value of control plasmid group.

Scientific, Pittsburgh, PA), respectively. The supernatant was stored at −80 °C for cytokine analysis.

2.5. Histological analysis

Lung tissues (which had not been lavaged) were fixed in 10% buffered formalin solution and embedded in paraffin using standard methods. Sections (5 μm) were stained with hematoxylin and eosin (H&E) to detect cellular infiltration. For each animal, 10 fields at a magnification of ×200 were captured in a blinded fashion using an image analyzer platform (Olympus Corporation, Tokyo, Japan). Peribronchial and perivascular inflammation was scored using a semi-quantitative scoring system as described [30].

2.6. ELISA assay

The protein level of cytokines and chemokines in BAL fluid (BALF) and serum were quantified by ELISA analysis. Commercially available ELISA kits for mouse IL-1β, IL-4, tumor necrosis factor (TNF)-α (R&D Systems) and IL-17 (ExCellBiology, Shanghai, China) were used and assayed according to their recommend protocols.

2.7. RT-PCR

Total RNA in mouse lung tissue was isolated using Trizol (Invitrogen) and reverse transcription was performed using a PrimeScript II 1st strand cDNA Synthesis Kit (Takara, Dalian, China) according to the manufacturer's protocol. The cDNA was amplified with paired primers using Premix Ex Taq™ Hot Start Version (Takara, Dalian, China) according to the manufacturer's protocol. The endogenous β-actin gene was used as an RT-PCR control. PCR products were electrophoresed in 1.2% agarose gels and stained with SYBR safe dye, then visualized digitally with a UV illuminator. The band intensities were semi-quantified using Gel-Pro Analyzer 3.2 software (Media Cybernetics).

2.8. Statistical analysis

Data are presented as mean ± SEM. Differences between groups were determined by one-way analysis of variance (ANOVA) followed by the Bonferroni *post hoc* test for multiple comparisons or the two-tailed Student's *t*-test as appropriate.

3. Results

3.1. IL-28B expression in serum and in BALF after hydrodynamic delivery of plasmid DNA

First, we examined the expression of IL-28B gene delivery after intravenous injection of the plasmid DNA. Samples were collected at the indicated times after the injection of plasmid (100 μg; pcDNA3.1-IL-28B or control pcDNA3.1). Once over-expressed, by intravenous injection of IL-28B plasmid, IL-28B levels in blood and BALF, as expected, are significantly elevated and lasted for more that 11 days (until the end of 11 day CS exposure) with peak expression at day 2 (Fig. 1B). In contrast, there was no detectable IL-28B protein in the control plasmid-treated group.

3.2. IL-28B treatment inhibits CS-induced airway inflammation

We next investigated whether the IL-28B could block the CS-induced airway inflammation in mice by treating the mice with intravenous delivery of plasmid-encoded IL-28B. Morphometric assessment was performed to explore the effects of IL-28B

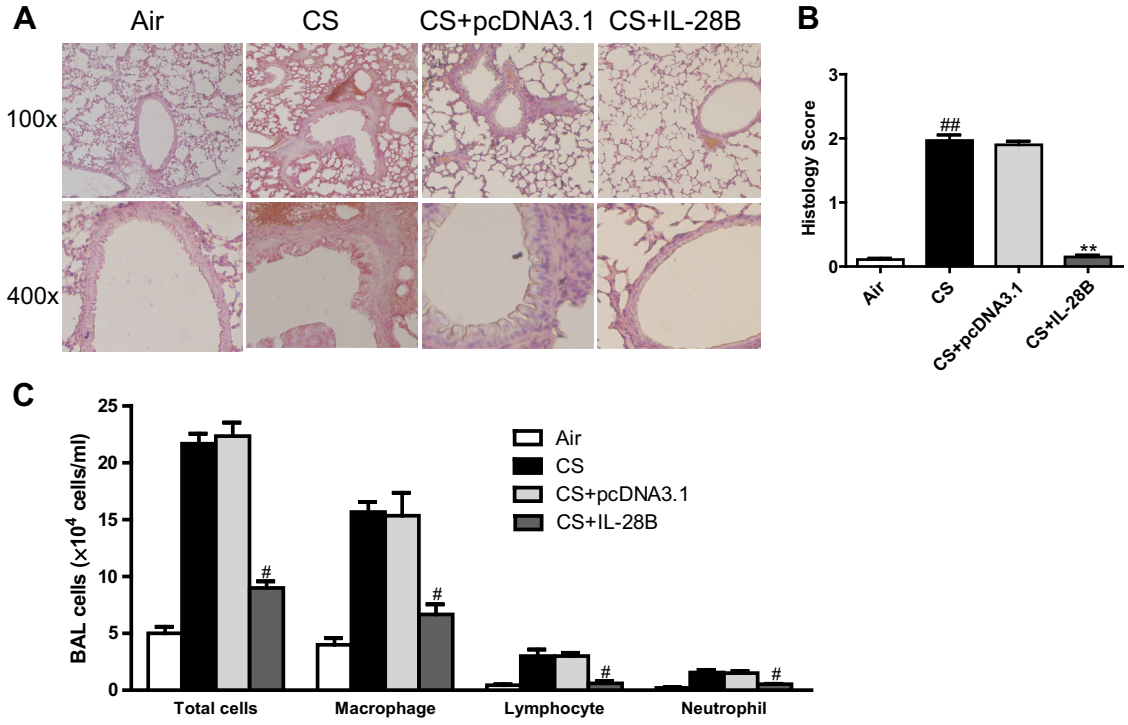

Fig. 2. IL-28B treatment inhibits CS-induced airway inflammation. Groups of mice were exposed to CS or room air as Fig. 1A and treated with pcDNA3.1-IL-28B plasmid or pcDNA3.1 plasmid as control. (A) Mice in room air, cigarette smoke exposure or received pcDNA3.1 plasmid developed inflammation in the lung but treated with IL-28B impaired lung inflammation. Upper sections are shown at 100× magnification and lower at 400× magnification. (B) Histological score of peribronchial and perivascular inflammation. Data are mean ± SEM ($n = 10$ mice/group). $^{\#\#}P < 0.01$ versus the Air group, $^{**}P < 0.01$ versus the CS + pcDNA3.1 group. (C) Effects of IL-28B on lung BAL inflammation in CS-exposured mice. The number of inflammatory cells in BALF was determined 24 h after the last CS exposure, as described in Section 2. Data represent means ± SEM of 6 mice per group. *Significant differences ($P < 0.05$) between CS-exposured control groups and CS-exposured IL-28B treatment groups.

treatment by histological examination of lung sections. The lungs of mice exposed to room air showed a normal parenchyma with normal airways (Fig. 2A). However, CS-exposure increased the number of inflammatory cells in the lung parenchyma and interstitial space of the airways as well as foci of inflammation and goblet cell metaplasia and hyperplasia, and administration of pcDNA3.1 had no significant effect on the airways histology or cytology (Fig. 2A). In contrast, treatment with IL-28B significantly reduced inflammatory cell infiltration in the peribronchiolar and alveolar regions as well as goblet cell metaplasia and hyperplasia (Fig. 2A, B).

Total cell numbers in BALF were increased 24 h after the end of 11 day CS exposure compared with ambient room air exposure. The increase of total cell numbers was associated with macrophage, lymphocytes, and neutrophils (Fig. 2C). As compared with control pcDNA3.1, treatment of CS-exposured mice with pcDNA3.1-IL-28B significantly inhibited the increase in total cell numbers in BALF (Fig. 2C). Macrophages, lymphocytes, and neutrophils decreased after pcDNA3.1-IL-28B treatment ($P < 0.05$). Therefore, these data demonstrate that CS-induced airway inflammation can be effectively inhibited by administration of IL-28B in mice.

3.3. IL-28B treatment decreases protein expression of CS-induced inflammatory mediators

The expression of relative inflammatory mediators in the BALF and serum was quantified by ELISA assay. We found that IL-28B treatment suppressed significantly CS-induced expression of IL-1β, IL-4, IL-17 and TNF-α in the BALF (Fig. 3A). IL-28B treatment also decreased CS-induced expression of IL-1β and IL-4 and partly suppressed CS-induced expression of IL-17 and TNF-α in the serum (Fig. 3B). These data show that IL-28B can decrease protein expression of relative inflammatory mediators stimulated by CS exposure.

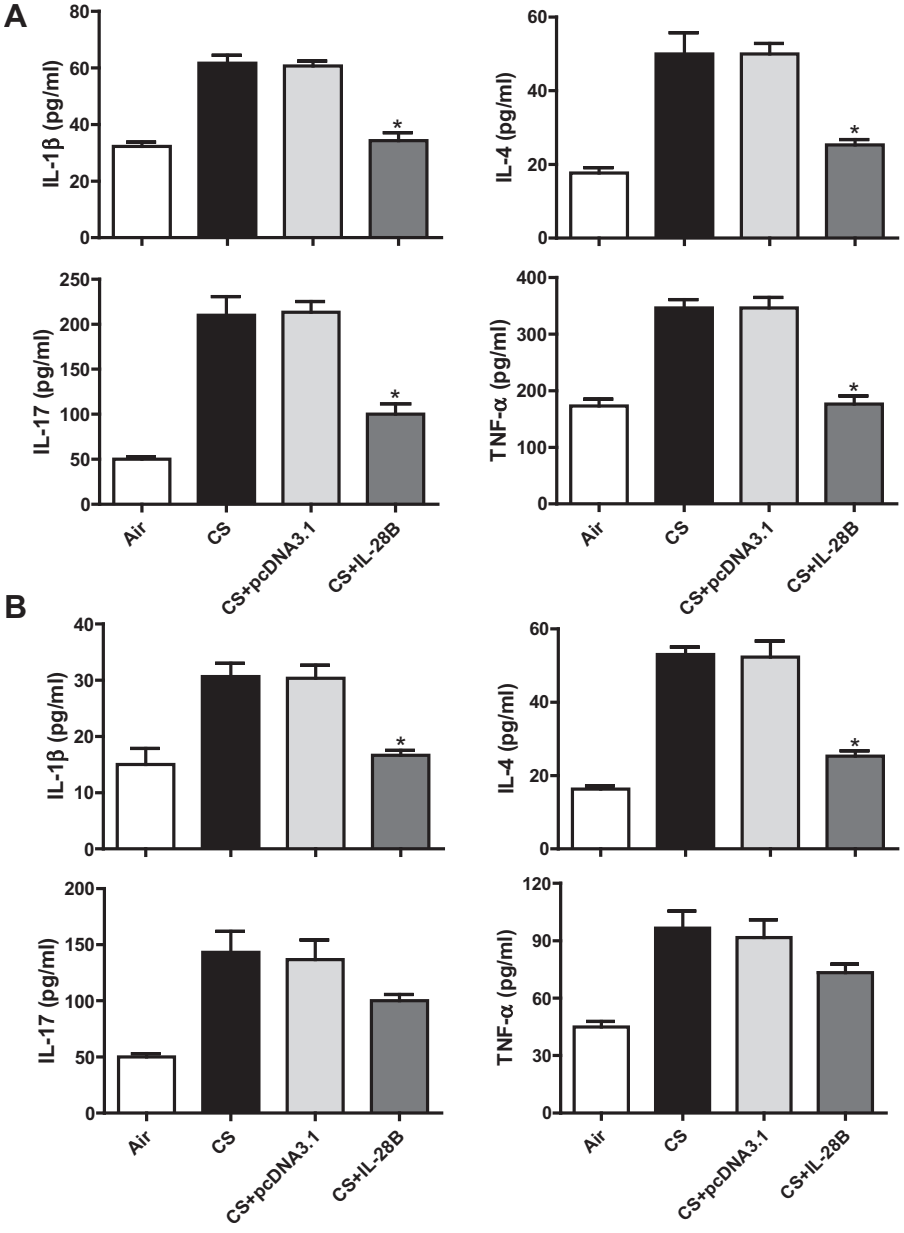

Fig. 3. IL-28B treatment decreases protein expression of CS-induced inflammatory mediators. The protein expression levels of IL-1β, IL-4, IL-17 and TNF-α in the BALF (A) and serum (B) of different groups were analyzed by ELISA respectively. Data are representative of 3 experiments, means ± SEM. *$P < 0.05$ versus the CS + pcDNA3.1 group.

3.4. IL-28B treatment reduces CS-induced inflammatory mediators gene expression

Exposure to CS triggers the expression of an array of inflammatory cytokines, chemokines and other mediators in the airways [1,2,6,32]. We assessed the impact of IL-28B treatment on the expression of the key inflammatory genes, including IL-1β, IL-4, IL-17, and TNF-α, in the lung tissue in response to CS. Critically, the treatment with IL-28B significantly abolished CS-induced expression of IL-1β, IL-4, IL-17, and TNF-α (Fig. 4). Our results suggest that IL-28B can mediate varying degree of key pro-inflammatory gene expression in CS-induced airway inflammation.

4. Discussion

IL-28/29 cytokines exhibit potent antiviral and antitumor function, but their full spectrum of activities, especially immunomodulatory effects, remains poorly characterized. In the present study, we have uncovered a new role of IL-28B as an important regulator of adaptive immunity and CS-induced airway inflammation. Our results demonstrated for the first time that IL-28B can modulate the development of airway inflammation, including impairing pathological changes, suppressing the migration and activation of inflammatory cells including lymphocytes, neutrophils and macrophages into the lung tissue, and attenuating inflammatory mediators increase caused by CS exposure. Therefore, IL-28 may be a new immunotherapeutic agent for the treatment of CS-mediated airway inflammation or other CS-related diseases.

There is abundant evidence supporting that the neutrophil is as the primary effector cell in inflammatory pulmonary diseases and it can promote the development of chronic obstructive pulmonary disease (COPD) [33]. Our results show that CS exposure can significantly induce accumulation of neutrophils in the lung tissue. In consistent with this, the number of neutrophils is augmented in induced sputum and BALF of COPD patients and this correlates with disease severity [33,34]. Moreover, this increase persists even after smoking cessation [35]. However, with the mediation of IL-28B, the activation and accumulation of neutrophils are inhibited effectively, so it provides the possibility of hindering further development of airway inflammation. But how IL-28B has effect

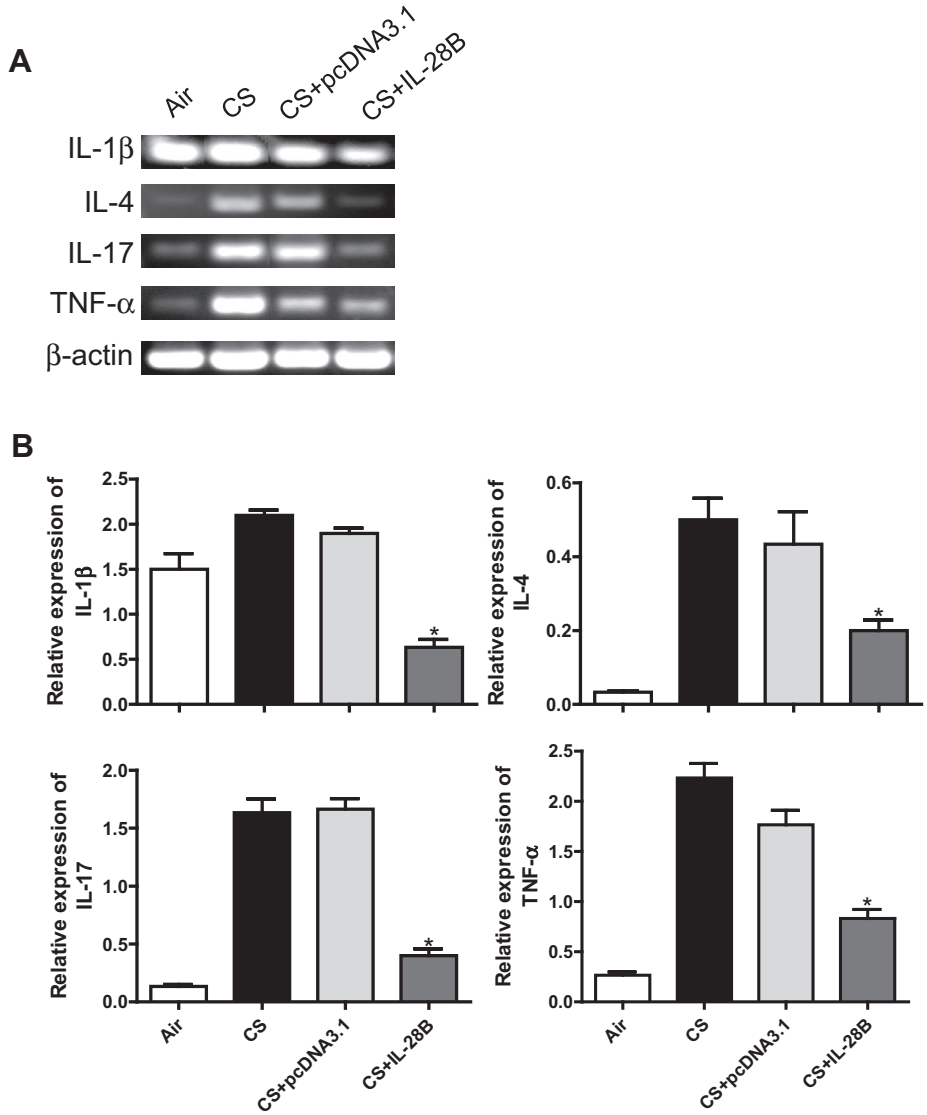

Fig. 4. IL-28B treatment reduces CS-induced pro-inflammatory gene expression. The gene expression levels of IL-1β, IL-4, IL-17, and TNF-α in the lungs of CS-exposed or control mice were analyzed by RT-PCR. (A) Gel images of PCR bands separated by electrophoresis and (B) result of RT-PCR score. Data are representative of 3 experiments, means ± SEM. $^{*}P < 0.05$ versus the CS + pcDNA3.1 group.

on neutrophils and decrease its number are still unclear in our study, it may be related with some chemokines such as IL-8 and Leukotriene B4, and further researches are needed.

Our data also demonstrate that IL-28B can significantly suppress CS-induced recruitment and activation of lymphocytes. The total number of T cells is increased in the lung parenchyma and peripheral and central airways of patients with COPD [36]. They can cause tissue injuries either by direct cytolytic activity or through the secretion of pro-inflammatory mediators. For instance, Th17 cells, a subset of $CD4^+$ T cells, can secrete pro-inflammatory cytokines IL-17 which is expressed highly in the lung after CS, thereby promoting the pulmonary inflammation [37]. In present study, we confirm that the mRNA level of IL-17 is high because of CS. However, their expression is impaired with the treatment of IL-28B. This may be due to the suppression of CS-induced T cells' activation and accumulation after treatment with IL-28B. In turn, the production of these pro-inflammatory cytokines is reduced so as to attenuate the pulmonary inflammation responses and decrease the number of T cells in lung tissues.

In addition, our study proves that the number of macrophages is increased in the CS-induced lung parenchyma but IL-28B can impair their increase. Previous researches have showed that macrophages are very important effectors cells which play a critical role in the initiation of airway inflammation in COPD [38]. Typically, their number is augmented in the airways, lung parenchyma, BALF, and sputum of smokers and in patients with COPD [39]. Macrophages can be activated by IL-1β and the count of them correlates positively with the concentration of IL-1β in sputum [40]. This is consistent with the expression of IL-1β in our experiments. When IL-1β as a potent stimulator of macrophages is mediated by IL-28B, its expression is significantly inhibited. Thus it illustrates, at least in part, why IL-28B can inhibit the CS-induced activation and accumulation of macrophages.

In conclusion, we have demonstrated that IL-28B gene delivery achieved a high concentration of protein in the lung and suppressed CS-induced subchronic pulmonary inflammation in mice by reducing the inflammatory cells infiltration and pro-inflammatory cytokines production in the lung. Although further investigation is needed to elucidate the detailed mechanisms of action, IL-28B gene delivery may represent a new effective strategy for treatment of CS-mediated inflammatory lung diseases.

Acknowledgments

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