

Article

STAT1 Antisense Oligonucleotides Attenuate the Proinflammatory Cytokine Release of Alveolar Macrophages in Bleomycin-Induced Fibrosis

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To investigate the effect of signal transducers and activators of transcription 1 (STAT1) antisense oligonucleotides (ASON) on concentrations of TNF- α , IL-8, NO secreted by alveolar macrophages (AMs) in bleomycin-induced rat pulmonary fibrosis, five adult female Wistar rats were intratracheally instilled with bleomycin. After 7 days, the rats were killed by right ventricle of heart exsanguinations under ketamine anaesthesia and bronchoalveolar lavage (BAL) was performed to obtain AMs. AMs were divided into four groups, treated with STAT1 ASON, STAT1 sense oligonucleotides (SON), dexamethasone (DEX) and medium alone (control), respectively. AMs and media were collected after culture for 36 h. The mRNA and protein expressions of STAT1 and ICAM-1 in AMs were detected by RT-PCR and ELISA, respectively. The concentrations of TNF- α , IL-8, NO in cultured medium were detected. The STAT1 mRNA expression by AMs in the STAT1 ASON group was lower than those of AMs in the STAT1 SON group, the DEX group and the control group ($p < 0.05$). Moreover, the STAT1 mRNA expression by AMs in the DEX group was also lower than those of AMs in the STAT1 SON group and the control group ($p < 0.05$), but the STAT1 mRNA expression by AMs in the STAT1 SON group was not different from that of the control group ($p > 0.05$). The protein expressions of STAT1 and ICAM-1 and the mRNA expression of ICAM-1 showed similar changes to the STAT1 mRNA expression by AMs. The concentrations of TNF- α , IL-8, NO in cultured medium from STAT1 ASON group were lower than those from STAT1 SON, DEX and the control groups ($p < 0.05$). Moreover, the concentrations of TNF- α , IL-8, NO in cultured medium from DEX group were also lower than those from the control and STAT1 SON group ($p < 0.05$), but no difference between STAT1 SON group and the control ($p > 0.05$). The results suggest that STAT1 ASON could inhibit the secretion of TNF- α , IL-8, NO in AMs, and STAT1 could become a target of treating pulmonary fibrosis. *Cellular & Molecular Immunology*. 2005;2(3):211-217.

Key Words: STAT1, pulmonary fibrosis, alveolar macrophage, ASON

Introduction

Idiopathic pulmonary fibrosis (IPF) is a lung disease that is characterized by epithelial cell damage and areas of denuded basement membrane resulting in inflammation, fibroblast proliferation, excessive extracellular matrix deposition, and

remodeling of alveolar gas exchange units. The progressive loss of lung gas exchange units in patients with IPF leads to respiratory failure and eventually to death (1). The pathogenesis of this disease remains incompletely understood. Standard treatment of this disorder with corticosteroids and/or cytotoxic agents is generally disappointing, and accordingly, it tends to progress to respiratory failure within several years of diagnosis. In view of the poor outcomes and therapeutic options available in IPF, there is an urgent need for new insights into their pathobiology that can be translated into therapeutic alternatives (2). Because of its pathophysiological similarity to IPF, bleomycin-induced pneumopathy has been frequently used as an animal model of IPF (3).

Bleomycin is a well-known toxic substance which produces lung injury and pulmonary fibrosis in both humans and other animals. The intratracheal instillation of bleomycin into rodents elicits a substantial inflammatory lung reaction followed by the development of fibrosis. The cellular constituents in the bronchoalveolar lavage fluid, the histologic characteristics, the changes in lung function, and a

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number of aspects related to collagen metabolism have been studied in this experimental model, and it is widely accepted that the phenomena described in this system resemble those occurring in human IPF. So bleomycin-induced lung injury has been extensively used as a model of IPF in the world (4).

Whatever the initial factor is, the pathologic process of IPF is divided into both the early alveolitis stage and the late over-repaired pulmonary fibrosis stage after the damage of inflammation. Moreover, in bleomycin-induced rat pulmonary fibrosis, after intratracheal instillation of bleomycin, the alveolitis is the severest on day 7. With the development of cell biology and molecular biology, it has been demonstrated that alveolar macrophages (AMs) play a key role in the pathogenesis of IPF by virtue of their ability to release a variety of cytokines and inflammatory mediators (5). Abnormal expression of cytokine and imbalance of cytokine network induced by abnormal activation of AMs may occupy an important place in the initiation and progression of IPF. Among these cytokines and inflammatory mediator, TNF- α , IL-8, NO, and PDGF are thought to be potential participants in the inflammatory and fibrotic process of IPF.

However, cytokine effects on immunity and inflammation depend on the transcription factors termed signal transducers and activators of transcription (STAT) (6). In the last decade, the STAT protein has been identified as critical transcription factors in mediating virtually all cytokine-driven signaling. These proteins are latent in the cytoplasm and become activated through tyrosine phosphorylation which typically occurs through cytokine receptor associated Janus kinases (JAKs). Activated STATs form homo- or hetero-dimers, enter the nucleus and work coordinately with other transcriptional co-activators or transcription factors leading to increase in gene transcription. In normal mammalian cells, ligand-dependent activation of the STATs is a transient process, lasting for only several minutes to several hours. In contrast, in many diseases, the STAT proteins are persistently activated. Quite a few studies support that abnormal STAT activation was correlated with many diseases such as tumors, inflammatory diseases and so on (6-8). The first STAT protein discovered was STAT1, which correlated with immunity and inflammation. Several studies showed that STAT1 was correlated with the occurrence and development of inflammatory and immune diseases (6-9). On the basis of nuclear localization, Sampath et al. found that STAT1 in airway epithelial cells was constitutively activated in asthmatic patients compared with healthy control subjects (6). In previous study, using the animal model of bleomycin-induced IPF, we also found that there was abnormal STAT1 activation in AMs of rats with bleomycin-induced IPF based on nuclear translocation of STAT1, and that the abnormal STAT1 activation led to upregulation of STAT1-dependent immune-response gene ICAM-1 expression. The increased ICAM-1 expression gave rise to the accumulation and activation of several types of inflammatory cells in lung tissue. These inflammatory cells released some cytokines which in turn activated STAT1 and increased further releasing of the cytokines. Such positive feedback effect may lead to alveolitis and pulmonary fibrosis. It was hypothesized that

abnormal STAT1 activation in AMs may play a pivotal role in this process. Therefore, STAT1 might be a promising molecular target for the treatment of IPF (9). In the present study, we applied STAT1 antisense oligonucleotides (ASON) aiming at AMs to stop the expression of STAT1 mRNA and protein of AMs in bleomycin-induced rat pulmonary fibrosis to observe the change of level of TNF- α , IL-8, NO secreted by AMs, and to evaluate the treatment effect of STAT1 ASON on IPF.

Materials and Methods

Animals and reagents

Wistar rats were obtained from the Laboratory Animal Department of Sichuan University (Chengdu, China). Bleomycin was purchased from Tianjin Taihe Pharmaceutical Co., Ltd. (Tianjin, China). Trizol, RPMI 1640 and other cell culture reagents were from Gibco BRL (USA). The TNF- α ELISA kit and IL-8 ELISA kit were purchased from Jingmei (Shenzhen, China); NO kit was purchased from Jiancheng Co, Ltd (Nanjing, China). M-MLV kit was purchased from Sangon Biotechnology Engineering Company of Shanghai (Shanghai, China).

Bleomycin-administration

Induction of bleomycin-induced rat pulmonary fibrosis was performed using methods as previously described (9). Briefly, five adult female wistar rats were used in this study. Rats were acclimatized to their housing condition for one week before starting the experiment. The rats were anaesthetized (Ketamine anaesthesia) and fixed. Tracheostomies were performed so as to facilitate the intratracheal instillation of bleomycin A5 (0.5 mg/100 g body weight) in 0.2-0.3 ml of 0.9% NaCl solution. After the instillation, the rats were immediately rotated uprightly to make the drug distributed in the lungs thoroughly and evenly. After 7 days, five bleomycin-treated rats were killed by right ventricle of heart exsanguinations under Ketamine anaesthesia.

Bronchoalveolar lavage (BAL) and isolation and purification of AMs

The lavage was performed under Ketamine anaesthesia. The left lung was ligated at the hilus and a small plastic tube was inserted into the trachea and placed in the right main bronchus. The tube attached to a 10 ml syringe, and 40 ml (in 5 ml, aliquots) of sterile phosphate-buffered saline (PBS) at 37°C was instilled. The fluid was retrieved by gentle aspiration after each infusion, filtered through a double layer of sterile gauze on crushed ice, and then centrifuged at 4°C at 400 g for 15 min. The cell pellet was resuspended in RPMI 1640 medium supplemented with 10% fetal bovine serum and incubated for two hours at 37°C in 5% CO₂. The medium was then removed and the AMs were collected by washing with PBS to remove non-adherent cells.

Design and synthesis of STAT1 ASON

According to the theory of designing ASON and the analysis

of STAT1 functional domain (10), we designed phosphorothioate-modified STAT1 ASON and STAT1 sense oligonucleotides (SON), synthesized by Sangon Biotechnology Engineering Company of Shanghai (Shanghai, China). The sequences for STAT1 ASON and STAT1 SON are 5'-GAT GTA TCC AGT TCT CTT AG-3' and 5'-CTA AGA GAA CTG GAT ACA TC-3', respectively. The synthesized oligodeoxynucleotides were purified by HPLC, stored at -20°C.

The generation of ASON-LipofectamineTM 2000 complexes

Preparing ASON-LipofectamineTM 2000 complexes was as follows: 1) Diluting ASON in DMEM without serum and antibiotics and mixing gently; 2) Mixing LipofectamineTM 2000 gently before use, then diluting the appropriate amount in DMEM without serum and antibiotics. Mixing gently and incubating for 5 minutes at room temperature; 3) After the 5 minutes incubation, combining the diluted ASON with the diluted LipofectamineTM 2000. Mixing gently and incubating for 20 minutes at room temperature to allow the ASON-LipofectamineTM 2000 complexes to form. At the same time, sense oligonucleotides (SON)-LipofectamineTM 2000 complex was prepared.

Transfection of oligodeoxynucleotides

AMs were subjected to different treatments in poly-plicate in 24-well culture plates with 1 ml/well. Experiments were conducted as follows: control group, AMs cultured in DMEM medium with 10% FCS; ASON group, AMs cultured in complete DMEM medium with ASON; SON group, AMs cultured in complete DMEM medium with SON; DEX group, AMs cultured in complete DMEM medium with dexamethasone (DEX, final concentration 2.5 µg/ml). The total number of AMs in each well was 2×10^5 . Cell transfection was performed according to the manufacturer's instructions. AMs and AM-conditioned supernatant were collected after cultured for 36 h.

RNA extraction and reverse transcriptase polymerase chain reaction analysis of STAT1 and ICAM-1 mRNA levels

Total cellular RNA was extracted from AMs subjected to various treatments using the TRIzol reagent. The purity of the RNA samples was assessed by OD₂₆₀/OD₂₈₀ spectrophotometric measurements. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed using M-MLV reagent as described by manufacturer's instructions. Primers used for the reactions were as follows: STAT1 sense primer was 5'-AGA ACT CAT TAA GAA GCA CCT G-3', antisense primer was 5'-GTA TCC AGT TCT CTT AGG GTC A-3' (Product length, 418 bp); ICAM-1 sense primer was 5'-AGA CAC AAG CAA GAG AAG AAA AGG-3', antisense primer was 5'-TTG GGA ACA AAG GTA GGA ATG TAT-3' (Product length, 425 bp); Glyceraldehydes-3-phosphate dehydrogenase (GAPDH) served as the control of reaction efficacy. Its sense primer was 5'-GTG CTG AGT ATG TCG TGG A-3', antisense primer was 5'-CAC AGT CTT CTG AGT GGC A-3' (Product length, 298 bp). The PCR reactions were as follows: cDNA synthesis, 20 min at 40°C; PCR, 94°C for 2 min predenaturation; 94°C for 15 s, 56°C for 30 s and

70°C for 1 min for 35 cycles, followed by 10 min at 72°C for final extension. The PCR products were separated by electrophoresis using 1.5% agarose gels stained with ethidium bromide to visualize cDNA products. Bands of each target transcript were visualized by ultraviolet transillumination and captured using a digital camera. ODs for each band were quantified by image analysis software. The level of gene expression of each transcript was normalized to that of the housekeeping gene GAPDH.

Detection of ICAM-1 and STAT1 protein expressions in AMs by enzyme linked immunosorbent assay (ELISA)

The ICAM-1 and STAT1 protein expressions in AMs was quantified by ELISA similarly as described previously with minor modification (11). Briefly, after the medium was removed, AMs subjected to various treatments in 24-well culture plates were washed with PBS and fixed with 0.25% glutaraldehyde-PBS for 10 min. The glutaraldehyde solution in the wells was removed and the wells were washed with PBS. After being blocked the non-specific binding sites with horse serum for 30 min, these cells were incubated with 100 µl per well of monoclonal ICAM-1 antibody solution (dilution 1:500) or monoclonal STAT1 antibody solution (dilution 1:1,000) overnight at 4°C. After washing with 0.05% Tween 20-PBS, HRP-conjugated horse anti-mouse IgG was added to the wells, and they were allowed to stand at room temperature for 1 h. Next, the wells were washed four times with 0.05% Tween 20-PBS, and then 100 µl per well of tetramethylbenzidine solution was added as a substrate. Subsequently, 100 µl per well of 1 mol/L HCl was added to stop the enzyme reaction after incubation for 10 min at room temperature. Absorbance at 450 nm (A₄₅₀) was measured with a microtiter plate reader. After further washing, 0.08% crystal violet was added to the wells to color the nuclei for 30 min at room temperature, and then, the wells were washed and 200 µl per well of 33% glacial acetic acid was added to split the cells for 30 min at room temperature. Absorbance at 550 nm (A₅₅₀) was measured with a microtiter plate reader. The ratio of A₄₅₀ to A₅₅₀ represents the relative value of ICAM-1 or STAT1 protein expression in AMs.

Assay for TNF-α, IL-8 and NO

For the assay, the frozen AM-conditioned supernatants prepared from treated AMs were thawed at room temperature and added to wells of rigid flat bottom microtiter plates coated with murine mAbs against rat TNF-α or IL-8. After incubation of the samples and thorough washing of the wells, HRP-conjugated anti-TNF-α antibody or anti-IL-8 antibody, respectively, was added to the test wells. After a second incubation, the excess HRP-conjugated antibody was removed by washing. The HRP substrate was then added, and the color intensity measured with a microtiter plate reader. At the same time, assay for NO was performed according to the manufacturer's instructions.

Statistical analysis

Data were showed as mean ± SD. Statistical analysis was

Table 1. The effect of STAT1 ASON on STAT1 mRNA and ICAM-1 mRNA expression of AMs in bleomycin-induced rat pulmonary fibrosis

Groups	STAT1 mRNA (%)	ICAM-1 mRNA (%)
Control	65.49 ± 4.62	62.63 ± 4.08
ASON	31.76 ± 3.48*	32.86 ± 3.41*
SON	64.16 ± 4.27 [#]	59.04 ± 5.34 [#]
DEX	44.09 ± 4.60* [#] △	43.32 ± 2.83* [#] △

**p* < 0.05 vs. control group; [#]*p* < 0.05 vs. ASON group; △*p* < 0.05 vs. SON group, *n* = 5.

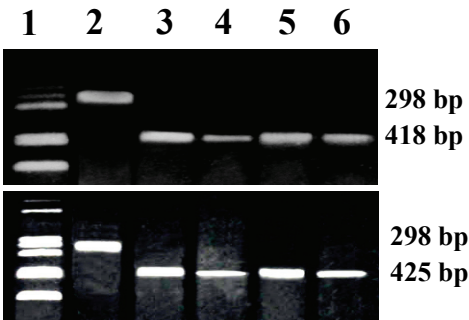


Figure 1. Representative RT-PCR results of STAT1 and ICAM-1 of AMs. Lane 1, marker; Lane 2, GAPDH; Lane 3, control group; Lane 4, ASON group; Lane 5, SON group; Lane 6, DEX group.

performed using one-way ANOVA with SPSS 10.0 software. Student’s *t* test was also used for the parametric data analysis between two groups. Probability value *p* < 0.05 was considered statistically significant.

Results

The effect of STAT1 ASON on STAT1 mRNA and ICAM-1 mRNA expressions of AMs in bleomycin-induced rat pulmonary fibrosis

The STAT1 mRNA and the ICAM-1 mRNA expressions of AMs in ASON group were obviously lower than those in SON group, DEX group, and control group (*p* < 0.05). The STAT1 mRNA and the ICAM-1 mRNA expressions of AMs in DEX group were obviously lower than those in SON group and control group (*p* < 0.05), but there was no statistical difference between control group and SON group (*p* > 0.05) (Table 1 and Figure 1).

The effect of STAT1 ASON on STAT1 protein and ICAM-1 protein expression of AMs in bleomycin-induced rat pulmonary fibrosis

The STAT1 protein and the ICAM-1 protein expression of AMs in ASON group were obviously lower than those in SON group, DEX group, and control group (*p* < 0.05). The

Table 2. The effect of STAT1 ASON on STAT1 protein and ICAM-1 protein expression of AMs in bleomycin-induced rat pulmonary fibrosis

Groups	STAT1	ICAM-1
Control	7.6 ± 0.6	16.3 ± 1.9
ASON	4.4 ± 0.6*	5.3 ± 0.3*
SON	7.7 ± 0.7 [#]	15.6 ± 1.3 [#]
DEX	5.9 ± 0.4* [#] △	8.9 ± 1.1* [#] △

**p* < 0.05 vs. control group; [#]*p* < 0.05 vs. ASON group; △*p* < 0.05 vs. SON group, *n* = 5.

Table 3. The effect of STAT1 ASON on TNF-α, IL-8, NO secreted by AMs in bleomycin-induced rat pulmonary fibrosis

Groups	TNF-α (pg/ml)	IL-8 (pg/ml)	NO (μmol/L)
Control	175.94 ± 30.29	326.47 ± 46.13	75.78 ± 14.59
ASON	62.23 ± 8.49*	112.97 ± 32.12*	16.89 ± 3.59*
SON	182.82 ± 30.52 [#]	332.71 ± 43.65 [#]	69.54 ± 19.22 [#]
DEX	95.3 ± 20.86* [#] △	232.13 ± 21.95* [#] △	35.50 ± 5.25* [#] △

**p* < 0.05 vs. control group; [#]*p* < 0.05 vs. ASON group; △*p* < 0.05 vs. SON group, *n* = 5.

STAT1 protein and the ICAM-1 protein expression of AMs in DEX group were obviously lower than those in SON group and control group (*p* < 0.05), but there was no statistical difference between control group and SON group (*p* > 0.05) (Table 2).

The effect of STAT1 ASON on TNF-α, IL-8, NO secreted by AMs in bleomycin-induced rat pulmonary fibrosis

The results indicated that the concentrations of TNF-α, IL-8, NO in AM-conditioned supernatants from STAT1 ASON group were lower than those from STAT1 SON group, DEX group, and control group (*p* < 0.05). Moreover, the concentrations of TNF-α, IL-8, NO in AM-conditioned supernatants from DEX group were also lower than those from control group (*p* < 0.05). But compared with those from control group, the concentrations of TNF-α, IL-8, NO in AM-conditioned supernatants from SON group were no different (*p* > 0.05) (Table 3).

Discussion

Antisense technology provides outstanding promise for treatment of human disease, having broad applicability and high specificity (12). Antisense oligonucleotides that hybridize to a specific mRNA or pre-mRNA have the potential to inhibit the expression of the targeted molecule. STAT1 belongs to a transcription factor and ICAM-1 is one

of the STAT1-dependent immune-response genes. The mechanism for the upregulation of ICAM-1 expression has been clarified (6, 10, 13). In previous study, we found that STAT1 activation was significantly correlated with ICAM-1 expression in AMs of bleomycin-induced IPF (9). In the present study, the STAT1 ASON targeting nucleotides sequences 2093-2111 (including Tyr-701) down-regulated both STAT1 mRNA and ICAM-1 mRNA expression in AMs of bleomycin-induced IPF, suggesting that design of STAT1 ASON is reasonable and effective. However, sometimes the change of protein expression is out of step with mRNA expression (14), therefore we also detected the STAT1 and ICAM-1 protein expressions in AMs using ELISA similarly as described previously with minor modification (11). In this method, the value of A_{450} represents total STAT1 or ICAM-1 protein expression in each well. Crystal violet can color nuclei, and 33% glacial acetic acid split the cells, so the value of A_{550} represents the total number of cells in each well. Accordingly, the ratio of A_{450} to A_{550} can reflect the protein level in an equivalent number of cells. In the present study, STAT1 ASON also inhibited both STAT1 protein and ICAM-1 protein expressions in AMs of bleomycin-induced IPF, further indicating that design of STAT1 ASON is reasonable and effective.

Abnormal expression of cytokine, imbalance of cytokine network plays an important role in the form of IPF. It is well known that AMs, neutrophils and other inflammatory cells were accumulated and activated in the lung, which secrete plenty of inflammatory and fibrinogenic factors such as IL-1, TNF- α , IL-8, NO, TGF- β , PDGF and so on, furthermore induce inflammatory cells to infiltrate and enlarge inflammatory reaction, thus directly or indirectly lead to lung injury and pulmonary fibrosis.

We have previously reported that there was abnormal activation of STAT1 in AMs of rats with bleomycin-induced IPF, which induced inflammatory cells to accumulate and to be activated in the lung by upregulating the expression of ICAM-1, then produced alveolitis and followed up formation of pulmonary fibrosis, and presumed that the abnormal STAT1 activation might play a pivotal role in the pathogenesis of acute alveolitis and pulmonary fibrosis (9). In this study, we applied STAT1 ASON to handle AM of BLM-induced rat pulmonary fibrosis, to observe the change of concentrations of TNF- α , IL-8, NO secreted by AMs, further investigate the effect of STAT1 ASON on preventing and treating pulmonary fibrosis.

The action of TNF- α and STAT1 ASON on pulmonary fibrosis

TNF- α is a proinflammatory cytokine with many biologic properties (15) and is thought to be critical in the development of pulmonary fibrosis (16). The expression of TGF- β is upregulated by TNF- α in primary mouse lung fibroblasts which is a key cell population in fibrogenesis (16). Expression of a TNF- α transgene in murine lung causes fibrosing alveolitis (17), and anti-TNF- α antibody attenuates both bleomycin-induced pulmonary fibrosis and silica-induced pulmonary fibrosis in mice (18, 19). In addition, a soluble

receptor for TNF- α has also been shown to lessen bleomycin induced pulmonary fibrosis (20). Importantly, TNF- α -deficient or receptor-deficient animals do not develop fibrotic lung disease (21, 22). TNF- α receptor knockout mice are also protected against pulmonary fibrosis due to silica, bleomycin, and asbestos (23-25).

At present, the role of TNF- α in pulmonary fibrosis is thought to be as follows: 1) TNF- α has the action of accumulating inflammatory cells and can make them infiltrate into injured lung. 2) TNF- α can cooperate with IL-1 to activate neutrophils and to induce alveolitis. 3) TNF- α is responsible for fibroblast proliferation and production of matrix including collagen and fibronectin. 4) Because of toxic effect of TNF- α , alveolar epithelium is frequently necrosis, desquamation and regeneration, which leads to the formation of pulmonary fibrosis (26).

TNF- α is the only cytokine that fulfils all three criteria for key fibrogenic cytokines proposed by Coker and others (27): 1) a candidate cytokine should stimulate fibroblast replication or procollagen production; 2) mediator gene expression and protein production should increase in the lungs of patients with pulmonary fibrosis; and 3) inhibitors of mediators function should attenuate fibrosis in animal models of this disease. In this study, we found that using STAT1 ASON to handle AM of bleomycin-induced rat pulmonary fibrosis, the ability of AMs secreting TNF- α declined obviously. Therefore, STAT1 ASON might be benefit to the treatment of pulmonary fibrosis.

The effect of IL-8 and STAT1 ASON on pulmonary fibrosis

Besides AMs, neutrophils may play an important role in the pathogenesis of IPF. BAL from patients with IPF shows an increased number of neutrophils (28). IL-8, a cytokine that exerts its potent chemotactic effect mainly on neutrophils, may have an important role in the attraction of neutrophils to the lung in IPF (29). Several former studies have demonstrated that both the expression of IL-8 in BAL cells and IL-8 levels in BAL supernatants are increased in patients with IPF (30-32). The degree of neutrophilic alveolitis in IPF is associated with increased serum levels of IL-8. Furthermore, serum levels of IL-8 correlated significantly with impairment of lung function parameters and Pao₂. The elevated IL-8 levels in BAL fluid and serum from patients with IPF may be a useful marker of disease severity and activity (33, 34). However, AMs were thought to be the major source of IL-8 in the lower respiratory tract (35).

Our research found that using STAT1 ASON to handle AMs of bleomycin-induced rat pulmonary fibrosis, the ability of AMs secreting IL-8 declined obviously. So STAT1 ASON might be helpful to treat pulmonary fibrosis. But this conclusion still needs further study.

The effect of NO and STAT1 ASON on pulmonary fibrosis

In 1987, two laboratories in America and England meanwhile reported that NO was endothelium-derived relaxing factor. From then on, the experimental and clinical researches on the effect of NO have been performed. At present, it is thought that NO is a kind of endogenous vasodilator, inflammatory

mediator and nerve transmitter. Taking L-arginine and oxygen as substrate, NO can be synthesized by its synthase in many kinds of cells such as macrophages, neutrophils and so on. Superoxide anion and NO can react to form the highly oxidizing species peroxynitrite (OONO^-) and ONOOH , the former is a strong oxidant, the latter has cytotoxic, and both of them take part in lung injury. Recently, there is increasing evidence that NO is involved in the pathogenesis of pulmonary fibrosis (36-38).

In this study, we found that the ability of AMs secreting NO declined obviously after using STAT1 ASON to handle AMs of bleomycin-induced rat pulmonary fibrosis. So STAT1 ASON might be useful to treat pulmonary fibrosis. But this conclusion still needs further study.

As glucocorticoid, dexamethasone has been for treating IPF for several years in clinic. It can inhibit inflammatory and immune reaction, and slow the activation of inflammatory cells, the synthesis and secretion of inflammatory cytokines. In this study, we found that the ability of AMs secreting $\text{TNF-}\alpha$, IL-8, NO declined obviously after using DEX to handle AMs of bleomycin-induced rat pulmonary fibrosis, which had obvious difference compared with control group, which further proved that dexamethasone played a role in controlling inflammation in bleomycin-induced rat pulmonary fibrosis. However, in bleomycin-induced rat pulmonary fibrosis, the ability of AMs secreting $\text{TNF-}\alpha$, IL-8, NO in STAT1 ASON group was obviously lower than DEX group, which implied that STAT1 ASON had stronger anti-inflammation than that of DEX. As DEX has serious side-effect, STAT1 ASON has a broaden perspective in treating IPF, but it needs further study.

In summary, in our present study, we found that STAT1, which took part in pulmonary fibrosis, could become an interfering target, and STAT1 ASON might be a new means by which pulmonary fibrosis could be treated, but it needed further study.

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