Supporting materials

Methods

Fibroblast cultures

MRC-5 were cultured in Dulbecco's modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS, GIBCO, Grand Island, NY, USA), 100 U/ml of penicillin and 100 mg/ml of streptomycin (GIBCO) in a humidified incubator at 5% CO_2. Passages between 4 and 8 were used for this study. Fibroblasts were seeded in 6-well plates and grown to sub-confluence. Thereafter, cells were washed in sterile PBS for 3 times and serum-starved for 24 hours before challenge with the recombinant human IL-25, human transforming growth factor (TGF)-β1 or both (10 ng/ml of IL-25 and TGF-β1) as indicated in the main text.

Histologic analysis

To be briefly, samples were fixed in 4% formaldehyde, and were dehydrated by using a serial alcohol gradient in an automated Leica Tissue Processor. Thereafter, samples were embedded in paraffin wax blocks and sliced into 3 μm-thick lung tissue sections for Hematoxylin-Eosin (HE) and Masson-Trichrome staining as per the instructions of the Kits (Solarbio Science & Technology Co., Ltd. Beijing, China).

Immunohistochemistry of lung tissues

Immunohistochemistry (IHC) of lung tissues was performed on paraffin-embedded lung tissue sections. Briefly, immunostaining was performed by using the

avidin–biotinylated HRP enzyme complex method (ThermoFisher Scientific, Waltham, MA, USA) with antibodies against human IL-25, IL-17BR, and mouse Collagen I, Collagen III, Fibronectin, CTGF, FGFR4 (for fibroblast detecting) (all from Abcam, Cambridge, MA, USA), or polyclonal non-immune IgG controls. After incubation for overnight at 4°C, the slides were incubated with a biotin-conjugated secondary antibody for 30 minutes at room temperature, and subsequently with Vector ABC complex as per manufacturer's protocol. Color development was performed by using 3,3-diaminobenzidine (DAB, Solarbio) as a chromogen for 3-5 minutes. Slides were counterstained with haematoxylin for nucleus staining. The slides were observed by independent professors on a Leica microscope (Leica, Wetzlar, Germany).

BALF collection and harvest of lung homogenate

BALF was collected by using a tracheal cannula with 1.0 ml of cold sterile saline. The fluid recovery rate was approximately 80-90% in this study. Then the sample was centrifuged at 1000 g for 5 min at 4 °C. The cell-free supernatant was stored at −80 °C for the measurements of IL-5 and IL-13. Next, partial tissues from right lung were homogenized in 1 ml of PBS containing 1% Triton X-100 and a protease inhibitor cocktail tablet (Roche, Mannheim, Germany) by using hand-held homogenizer. Debris was removed by centrifugation at 12000 g for 15 minutes, and the supernatant were harvested for the detection of total collagen, and the expression levels of IL-5 and IL-13.

Protein extraction and Western blot

Lung tissues were washed in ice-cold phosphate buffered saline (PBS), and treated with RIPA lysis buffer (ThermoFisher) containing complete protease and phosphatase inhibitor cocktail (Roche) for 15 minutes at 4℃. Thereafter, cell debris was discarded by centrifugation at 12,000g for 15 minutes, and protein concentration was detected by using the BCA assay (ThermoFisher) before degeneration in loading buffer at 96℃ for 5 minutes. 10 μg of total proteins was isolated by 10% SDS-PAGE and electrotransferred to nitrocellulose (NC) membranes (Millipore, Billerica, MA, USA). Membranes were blocked with 5% nonfat dry milk in TBST (10 mmol/L Tris-HCl, pH 7.6; 150 mmol/L NaCl; 0.1% Tween-20) for at least 1 hours at room temperature, and incubated with the following primary antibodies overnight at 4℃: mouse monoclonal anti-IL-17BR, mouse monoclonal anti-IL-17RA, and mouse monoclonal anti-IL-25(all from Abcam, Cambridge, MA, USA). After 3 extensive washing for 30 minutes, membranes were treated with horseradish peroxidase–conjugated anti-mouse secondary antibody and assessed by an enhanced chemiluminescence detection buffer (KPL) with ChemiDoc XRS (Bio-Rad, Hercules, CA, USA). The band intensity was quantified using Image J image processing program (NIH Image, Bethesda, MD, USA).

RNA purification and real time RT PCR

Total cellular RNA from fibroblasts or lung tissues was isolated by using RNeasy

mini kit extraction columns (Tiangen, Beijing, China) and 2 μg of RNA was reverse-transcribed at 37℃ for 1 hours with the Omniscript RT kit (Tiangen) by using oligo dT primers (1 mmol/L). Real-time quantification of mRNA levels was performed by ABI PRISM 7500 instrument (Applied Biosystems, Foster City, CA, USA) using SYBRGreen PCR reagents (Tiangen). Reaction mixtures were incubated for 2 min at 94℃, followed by another 40 cycles (94℃ for 15 second, 55℃ for 20 second, and 68℃ for 35 second). β-actin was performed as a housekeeping gene to correct for variations in cDNA content between different samples. The relative expression level of target genes was calculated by using the $2^{-\Delta Ct}$ or $2^{-\Delta\Delta Ct}$ method. The mRNA expression levels derived from fibroblasts were expressed as the fold changes in comparison to vehicle-treated controls. Primer sequences were shown in Table S1.

Cell proliferation assay

For CCK-8 assay, cells were placed in 96-well plates at an initial density of 5×10^3/well and incubated for 24 hours before serum-starved for 24 hours. Thereafter, cells were challenged with hIL-25 and TGF-β1 as indicated before. After culture for another 24, 48 and 72 hours, the supernatant was discarded, and 10 μl of CCK-8 solution was added to each well. The absorbance was detected at 450 nm via enzyme-linked immunometric meter (ThermoFisher). Furthermore, DNA synthesis was determined by using an EdU incorporation assay. Briefly, cells were cultured in 24-well plates and treated with hIL-25 and TGF-β1 for 72 hours after sufficient serum

starvation. Thereafter, the cells were incubated with EdU solution for 3 hours, and were fixed and incubated with 1× Apollo buffer for 30 minutes at room temperature. The DNA contents were stained with 1× DAPI buffer. Finally, the numbers of positive cells were observed and recorded with a fluorescent microscope (Olympus, Tokyo, Japan).

Figure legends

Fig.S1 Lung expression levels of IL-25, IL-17RA, IL-17BR determined by Real time PCR and WB

The mRNA levels of IL-25 (A), IL-17RA (B) and IL-17BR (C) in normal and IPF lung tissues, as quantified by real-time RT-PCR are shown by $2^{-\Delta ct}$ method. Also, Example immuno-blots and densitometric ratios of IL-25/IL-17RA/IL-17BR to β-actin are depicted as bar graphs (D). Data are shown as Means±SEMs of 3 independent experiments. *N*=9 of IPF patients and *N*=6 of normal healthy controls. Student *t* test was used for the comparison. **P*<.05, ***P*<.01, ****P*<.001 compared with normal controls.

Fig.S2 IgG-isotype control for human IHC assay

Representative photomicrograph of lung tissue slide from IPF patients incubated with mouse IgG isotype control. We showed that no positive staining in lung tissue sections. Original magnification: bar = 100 μm.

Fig.S3 Lung histological features induced by mIL-25 intra-nasal instillation

Mice were intranasal injected with mIL-25 or saline and were sacrificed at day 3, 7, 19, 31 for lung tissue process. Representative HE staining with low-power histology images (*N*=5 per group) depicting infiltration of inflammatory cells (termed as lymphoid follicles or aggregates) and collagen deposition within the lung are shown. Squares in F and H indicate infiltrated lymphoid follicles.

Fig.S4 IgG-isotype control for mice IHC assay

Representative photomicrograph of lung tissue slide from IL-25 or saline treated mice incubated with IgG isotype control. We showed that no positive staining in lung tissue sections. Original magnification: bar = 100 μm.