Supplementary materials and methods

1. Lung histopathology

Lungs were fixed in 10% (volume fraction) neutral buffered formalin and embedded in paraffin. Sections of 4–5 μm thickness were prepared and stained with hematoxylin and eosin (H&E). Furthermore, Masson's trichrome (MTC) staining was conducted and inspected blindly under a light microscope (BX43, Olympus, Tokyo, Japan) to visualize the collagen-rich fibrotic regions in the lungs (Goss, 2009). Five non-overlapping fields from each lung section were evaluated and scored for inflammation and/or injury (0, no inflammation; 1, focal interstitial infiltrates; 2, diffuse interstitial infiltrates; 3, focal alveolar infiltrates; and 4, confluent alveolar infiltrates/consolidation) (Manali et al., 2011). Fibrosis was also scored using a 0–4-point scale, where 0 indicated normal lung; 1, thickening of $<50\%$ of interalveolar septa; 2, thickening of $\geq 50\%$ of interalveolar septa; 3, interalveolar septal thickening with a formation of isolated fibrotic foci; and 4, multiple fibrotic foci with a distortion of parenchymal architecture (Manali et al., 2011). Besides, fibrotic lung injury was histologically assessed and quantitatively graded in accordance with the scale defined by Ashcroft et al., (1988) and modified by Hübner et al., (2008). Five microscopic fields (×100) from each lung sample were stained with MTC and scored on a scale point of 0–8, where the final score represented the mean of individual scores recorded across all microscopic fields. To quantify the distribution of PF, the modified Ashcroft's scores were graded to three classes of increasing values: 0–3 (mild), 4 (moderate), and ≥ 5 (severe) (Ruscitti et al., 2017). The area percentage of fibrosis was blindly visualized and quantified using cellSens dimensions (Olympus Software).

2. Determination of IL-1β, TGF-β1, NF-κB p65, and caspase-3 in the lung tissues of bleomycin-injected rats

The immunohistochemical evaluation of IL-1β, TGF-β1, NF-κB p65, and caspase-3 was performed in the lung tissues. In brief, sections were incubated with rat monoclonal anti-IL-1β, anti-TGF-β1, anti-NF-κB p65, and anti-caspase-3 antibody (1:100 dilutions, volume ratio) (Santa Cruz Biotechnology, Dallas, TX, USA). The immune reaction was visualized using diaminobenzidine tetrachloride (Sigma Chemical Co., St. Louis, MO, USA). The positive immune reactive cells showed brown-stained cytoplasm and/or nuclei. The staining intensity and distribution were graded as negative (no staining), weak, moderate, or strong intensity. The positive expression was quantified using cellSens Dimensions (Olympus Software) as area percentage from five randomly selected fields in each section. The control negative slides were obtained by skipping the primary antibody incubation step.

3. Determination of gene expression of inflammasome-associated proteins in the lung tissues

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was conducted using an ABI 7500 Real-Time PCR System (Applied Biosystems, California, USA) and Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Waltham, USA). The sequences of qRT-PCR primer pairs used are shown in Table S1. The cycle threshold (Ct) values were normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as invariant endogenous control (reference gene). The fold change in the expression of studied genes was calculated by the comparative threshold cycle method ($2^{-\Delta\Delta C_T}$) (Livak and Schmittgen, 2001).

Table S1 Primers used for quantitative reverse transcriptase-polymerase chain reaction

Gene	Forward	Reverse
NLRP3	5′-GCAGCAAACTGGAAAGGAAG-3′	5′-CTTCTCTGATGAGGCCCAAG-3′
ASC	5′-GCACTTTATAGACCAGCACCG-3′	5′-GGCTGGTGTGAAACTGAAGA-3′
GAPDH	5′-ACGAAGCTGAAGCAGGAGAAG-3′	5′-GGATGAAACCCAGACACATAGC-3′

ASC: The apoptosis-associated speck-like protein containing a caspase recruitment domain, NLRP3: NOD-like receptor family, pyrin domain-containing protein-3, GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

4. Determination of NLRP3, fibronectin, and HIF-1α levels in the lung tissues

The level of NLRP3 protein in the lung tissue homogenates (20%) of rats were determined using commercial sandwich-enzyme-linked immunosorbent assay (ELISA) kits supplied from MyBioSource (San Diego, USA). The levels of fibronectin and HIF-1α in the lung homogenates (20%) of rats were determined using commercial sandwich-ELISA kits supplied from CUSABIO (Houston, USA) and MyBioSource, respectively.

5. Determination of oxidative stress markers, reduced glutathione (GSH) and malonialdehyde (MDA), in the lung tissue

The levels of glutathione (GSH) and malondialdehyde (MDA) were detected in the lung homogenates using colorimetric methods. For GSH, the method is based on the reduction of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) with GSH, as previously described (Beutler et al., 1963). For MDA, thiobarbituric acid (TBA) reacts with MDA in an acidic medium to form a TBA-reactive product that can be measured at 535 nm (Kei, 1978).

6. Statistical analysis

Statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software, California , USA). Testing for normality was done using the Shapiro-Wilk test. The data were presented as mean±standard deviation (SD) and n=6 in all biochemical assays, and n=3 in histological and immunohistological analyses. All data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. For non-parametric data, comparisons between each two groups were done using Mann-Whitney test for score. The level of significance was set as P<0.05.

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