doi: 10.4149/gpb_2025026

Tripterygium glycosides alleviate CSE-induced lung injury by inhibiting IL-33 in bronchial epithelial cells

Nan Zhang[1], Jian Fan[1] and Zhiping Deng[1]

[1] *Pneumology Department, Zigong First People's Hospital, Zigong, China*

Abstract. Chronic obstructive pulmonary disease (COPD) is characterized by airway remodeling and inflammation. Cigarette smoke extract (CSE) induces apoptosis, inflammation, and oxidative stress in COPD. *Tripterygium* glycosides (TG) are an active compound found in the root extracts of *Tripterygium wilfordii Hook F* (TWHF) that possesses anti-inflammatory and immunosuppressive effects. However, its role in COPD remains elusive. Herein, 2.5% CSE was used to treat human bronchial epithelial cells (BEAS-2B) to construct a cell injury model. Cell viability, apoptosis, and proliferation were assessed using MTT, flow cytometry, and EdU. Gene expression was analyzed using ELISA, Western blot, and RT-qPCR. TG treatment abolished 2.5% CSE-induced BEAS-2B cell viability and proliferation inhibition, apoptosis and inflammatory response promotion, and IL-33 level increase. Moreover, the repression of TG treatment on 2.5% CSE-triggered BEAS-2B cell damage was abrogated by IL-33 overexpression. Phosphorylation of JNK, ERK1/2, and p38 in 2.5% CSE-treated BEAS-2B cells was enhanced, manifesting that MAPK signaling pathways were activated. TG administration attenuated 2.5% of CSE-activated MAPK pathways through IL-33 upregulation. TG treatment repressed CSE-induced BEAS-2B cell damage partly by regulating the IL-33-mediated MAPK signaling pathway, providing a better understanding of the role of TG in the anti-inflammatory therapeutics for COPD treatment.

Key words: *Tripterygium* glycosides — Chronic obstructive pulmonary disease — Cigarette smoke extract — IL-33 — Inflammatory response

Abbreviations: COPD, chronic obstructive pulmonary disease; CS, cigarette smoke; CSE, CS extract; EdU, 5-ethynyl-2'-deoxyuridine; ELISA, Enzyme-linked immunosorbent assay; ERK1/2, extracellular-signal-regulated kinase 1/2; IL, interleukin; JNK, c-Jun N-terminal kinase; PI, propidium iodide; TG, *Tripterygium* glycosides; TCM, traditional Chinese medicine; TNF-α, tumor necrosis factor-α.

Introduction

As a form of chronic bronchitis and/or emphysema, chronic obstructive pulmonary disease (COPD) has been characterized by incomplete reversible airflow obstruction linked to pulmonary inflammation involving a wide range of inflammatory cells, leading to the development of heart disease and respiratory failure (Barnes 2016; Bernocchi et al. 2018; André et al. 2019). In recent years, COPD has continued to be a significant health problem and an increasingly heavy social and economic burden (Rabe and Watz 2017; Safiri et al. 2022). Currently, the etiology of COPD is recognized as associated with an aberrant inflammatory response of the lungs to noxious particles or gases, notably cigarette smoke (CS) and pollutants (Guan et al. 2020). Accumulating evi-

Electronic supplementary material. The online version of this article (Doi: 10.4149/gpb_2025026) contains Supplementary material.

Correspondence to: Zhiping Deng, Pneumology Department, Zigong First People's Hospital, No. 42, Shangyi Hao Yi Branch Road, Ziliujing District, Zigong 643000, China
E-mail: dengzp2121103@163.com

© The Authors 2025. This is an **open access** article under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

dence has indicated that repeated long-term environmental exposure to CS might disrupt the structure of the airway wall, and impair the septum of alveolar walls, resulting in interstitial fibrosis (Hou et al. 2019; Zong et al. 2019). Apart from that, CS also contributes to enhanced mucous gland secretions and obstructive bronchiolitis, thereby exacerbating the development of lung tissue lesions (Song and Chen 2021). In addition, CS-triggered airway epithelial barrier dysfunction has been reported to expose sub-epithelial layers to exogenous substances, which are responsible for a series of pathological processes such as airway inflammation and airway remodeling, thus increasing exacerbation rates of COPD (Aghapour et al. 2018). Although some treatments, such as dual phosphodiesterase 3/4 inhibitors, bronchodilators, and antibiotics, can relieve airway obstruction and enhance COPD patients' quality of life, the treatment outcomes are still frustrating due to side effects and antibiotic resistance (Martin and Burgel 2021; Vogelmeier et al. 2021). Hence, developing more effective therapeutic approaches is urgent and of great clinical importance for patients with COPD.

In recent years, researchers discovered that traditional Chinese medicine (TCM) has become increasingly popular for the clinical treatment of COPD, attributing benefits to protecting lung tissue from carcinogens such as cigarette smoke and optimizing the effects of chemotherapy drugs (Cao et al. 2023; Zhang et al. 2023). As a woody vine pertaining to the *Tripterygium* genus, *Tripterygium wilfordii Hook F* (TWHF) represents an important TCM possessing anti-inflammation, anti-rheumatism, and immunosuppressive impact (Xue et al. 2010). Notably, *Tripterygium* glycosides (TG) is the major active ingredient of TWHF, which has been widely used in China for the treatment of various inflammatory diseases (Li et al. 2021; Huang et al. 2023). Furthermore, some randomized controlled trials have reported that treatment combined with TG might significantly alleviate the inflammatory state of patients with chronic glomerulonephritis and oral lichen planus (Luo et al. 2020; Li et al. 2021). It has been reported that TG treatment might significantly reduce inflammatory cytokines. For example, a recent study suggested that TG exposure might hinder the inflammatory mediators in interleukin (IL)-1β-stimulated RSC-364 cells (Huang et al. 2023). Beyond that, TG treatment might induce the upregulation of IL-37 (anti-inflammatory cytokine) by regulating MAPK signal pathways (Wang et al. 2017). However, it remains unknown whether TG is involved in the regulation of COPD progression.

As a member of the IL-1 family, IL-33 has been reported as an alarmin cytokine with significant roles in tissue homeostasis and repair (Cayrol and Girard 2018). Furthermore, it is abundant in epithelial cells from barrier tissues and fibroblastic stromal cells from many tissues (Molofsky et al. 2015). Previous studies have suggested that lung IL-33 expression level was upregulated in animal models of COPD and patients with severe COPD (Byers et al. 2013; Kearley et al. 2015). It has been reported that the lack of IL-33 might protect mice from CS-triggered inflammatory responses to influenza virus infection (Kearley et al. 2015). In CS-induced COPD mice, IL-33 might elevate the production of IL-6 and IL-8 in systemic inflammation by the p38 MAPK pathway (Wu et al. 2014). These data implied that IL-33 might exert a vital function in COPD progression.

In the present work, our data for the first time found that TG treatment might relieve CS extract (CSE)-induced BEAS-2B cell apoptosis and inflammation. Furthermore, TG exposure might decrease IL-33 expression in CSE-treated BEAS-2B cells. Therefore, this project aimed to explore whether TG might attenuate CSE-triggered BEAS-2B cell injury *via* targeting IL-33.

Materials and Methods

Cell culture and treatment

In this research, human bronchial epithelial cells (BEAS-2B, CRL-3588, ATCC, Manassas, Virginia, USA) were cultured in Airway Epithelial Cell Basal Medium (PCS-300-030, ATCC) and Bronchial Epithelial Cell Growth Kit (PCS-300-040, ATCC) with 5% CO_2 at 37°C. In addition, cells were counted using an automated cell counter (Invitrogen, Paisley Scotland, UK).

For CSE preparation, after the five cigarettes (Da Qianmen, Shanghai, China, 2.5 mg nicotine and 12 mg tar *per* cigarette) were burned in turn, cigarette smoke (CS) was sucked into the syringe and bubbled into 10 ml of sterile PBS in bubble absorption bottle. After dissolving the CS, the CSE solution was sterilized by filtration through a 0.2 μm filter, regarded as 100% CSE solution. CSE was standardized by measuring the absorbance at wavelength 320 nm, freshly prepared for each experiment. These solutions were then diluted to the designated dose with a culture medium and used in the assay within 15 minutes of preparation.

For cell treatment, BEAS-2B cells were exposed to 2.5% CSE in culture medium for 24 h, followed by treatment with 1.25 μg/ml of TG (Fig. 1A) (dissolved in anhydrous alcohol) for 24 h.

MTT assay

In brief, 3×10^5 treated or un-treated BEAS-2B cells were incubated with MTT reaction solution (5 mg/ml, Sigma-Aldrich, St. Louis, MO, USA, an indicator of metabolic activity) for another 4 h later. After removing the medium, the formed precipitates were dissolved according to 150 μl DMSO. Finally, a microplate reader was employed for cell absorbance at 490 nm.

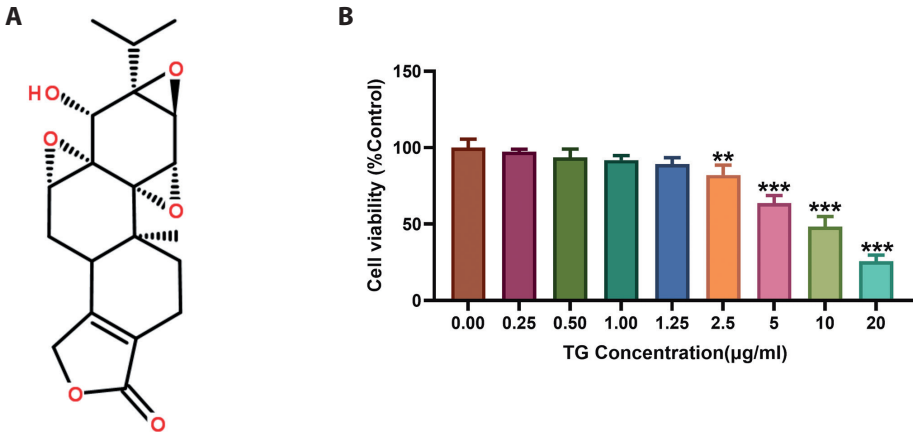

Figure 1. Effects of *Tripterygium* glycosides (TG) on BEAS-2B cell viability. **A.** Chemical structure of TG. **B.** BEAS-2B cells were exposed to different concentrations of TG for 24 h. Then, the viability of BEAS-2B cells was assessed using MTT assay. ** $p < 0.01$, *** $p < 0.001$; $n = 3$.

Flow cytometry for cell apoptosis

In short, 5×10^5 treated or un-treated BEAS-2B cells were resuspended in binding buffer with 5 μl Annexin incubation for 10 min, followed by staining with 5 μl PI (propidium iodide) avoiding light. At last, apoptotic rates were determined based on a FACSCalibur flow cytometer with CellQuest software (BD Biosciences, Heidelberg, Germany)

5-ethynyl-2'-deoxyuridine (EdU) assay

In brief, 5×10^3 BEAS-2B cells were added with EdU working solution (RiboBio, Guangzhou, China) at 37°C. After 2 h of incubation, cells were subjected to 4% formaldehyde fixture for 30 min. After being stained with Apollo Dye Solution and DAPI (identifying the nuclei), EdU-positive cells (red cells) were detected using a fluorescence microscope.

Enzyme-linked immunosorbent assay (ELISA)

After treatment or without, the BEAS-2B cell culture supernatant was taken and centrifuged at 13,000×*g* for 15 min at 4°C. After removing cellular debris, the secretion of IL-6, tumor necrosis factor-α (TNF-α), and IL-8 of the generated supernatants was assessed based on ELISA kits (eBioscience, San Diego, CA, USA).

Western blot assay

In general, BEAS-2B cell lysates were obtained with RIPA buffer (Keygen, Nanjing, China) with protease and phosphatase inhibitors. After quantification using a BCA protein kit, equivalent amount of cell lysates (20 μg/lane) were loaded onto 10% separating gel, followed by shifting to nitrocellulose membranes (Millipore, Molsheim, France). Following blocking with 5% non-fat milk for 1 h at room temperature, the membranes were probed with appropriate concentrations of primary antibodies: Bax (ab32503, 1:1000, Abcam, Cambridge, MA, USA), Bcl-2 (ab32124, 1:1000, Abcam), Cleaved caspase-3 (ab2302, 1:500, Abcam), IL-33 (ab207737, 1:1000, Abcam), c-Jun N-terminal kinase (JNK, #9252, 1:1000, CST, Danvers, MA, USA), phospho-JNK (p-JNK, #4668, 1:1000, CST), extracellular-signal-regulated kinase1/2 (ERK1/2, #9102, 1:1000, CST), p-ERK1/2 (#8544, 1:1000, CST), p38 MAPK (p38, #8690, 1:1000, CST), p-p38 (#4511, 1:1000, CST), and β-actin (ab213262, 1:1000, Abcam) at 4°C overnight. After incubation with secondary antibody (ab205718, 1:2000, Abcam) at 37°C for 2 h, the bands were visualized using ECL reagent (Millipore, Molsheim, France) and analyzed with Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

Cell transfection

In this experiment, GenePharma (Shanghai, China) provided oligonucleotides: si-IL-33 and its control si-NC, and plasmids: pcDNA-based IL-33 (XM_054364148) overexpression (OE-IL-33) and pcDNA, which were transfected into BEAS-2B cells using Lipofectamine 3000 (Invitrogen) for 48 h. After examining the transfection efficiency, these harvested cells were stimulated with or without 2.5% CSE and/or TG.

RT-qPCR

For mRNA studies, Trizol reagent (Invitrogen) was employed to extract total RNAs from BEAS-2B cells, which were qualified using NanoDrop 2000 system. Subsequently, complementary DNA (cDNA) synthesis from 1 μg of total RNA was conducted using Prime Script RT Master Mix (Takara, Tokyo, Japan). After that, qPCR reaction was carried out based on Thermal Cycler CFX6 System (Bio-Rad with SYBR Green SuperMix (Roche, Basel, Switzerland) to determine IL-33 mRNA transcript level. After being normalized

Table 1. Primers sequences used for PCR

Name		Primers for PCR (5'-3')
IL-33	Forward	GTGACGGTGTTGATGGTAAGAT
	Reverse	AGCTCCACAGAGTGTTCCTTG
β-actin	Forward	GATTCCTATOTGGOCGACGA
	Reverse	GCGTACAGGATAGCACAGC

to β-actin, relative expression of IL-33 was analyzed using the $2^{-\Delta\Delta Ct}$ method. Primers used are exhibited in Table 1.

Statistical analysis

All data were displayed as the mean ± standard deviation (SD) from at least three independent experiments. The screening criteria for all data were p value < 0.05. Based on GraphPad Prism7 software, differences between two groups or among multiple groups were analyzed by Student's t-test or one-way ANOVA with Tukey's tests.

Results

Cytotoxicity of TG on BEAS-2B cells

Firstly, to avoid cytotoxicity, the influence of TG on the BEAS-2B cells was checked using MTT assay. As shown in Figure 1B, the concentration of TG up to 1.25 μg/ml did not obviously affect the viability of BEAS-2B cells in comparison with the un-treated group, implying that TG had no cytotoxic impacts on BEAS-2B cells at a dose of 1.25 μg/ml. Meanwhile,

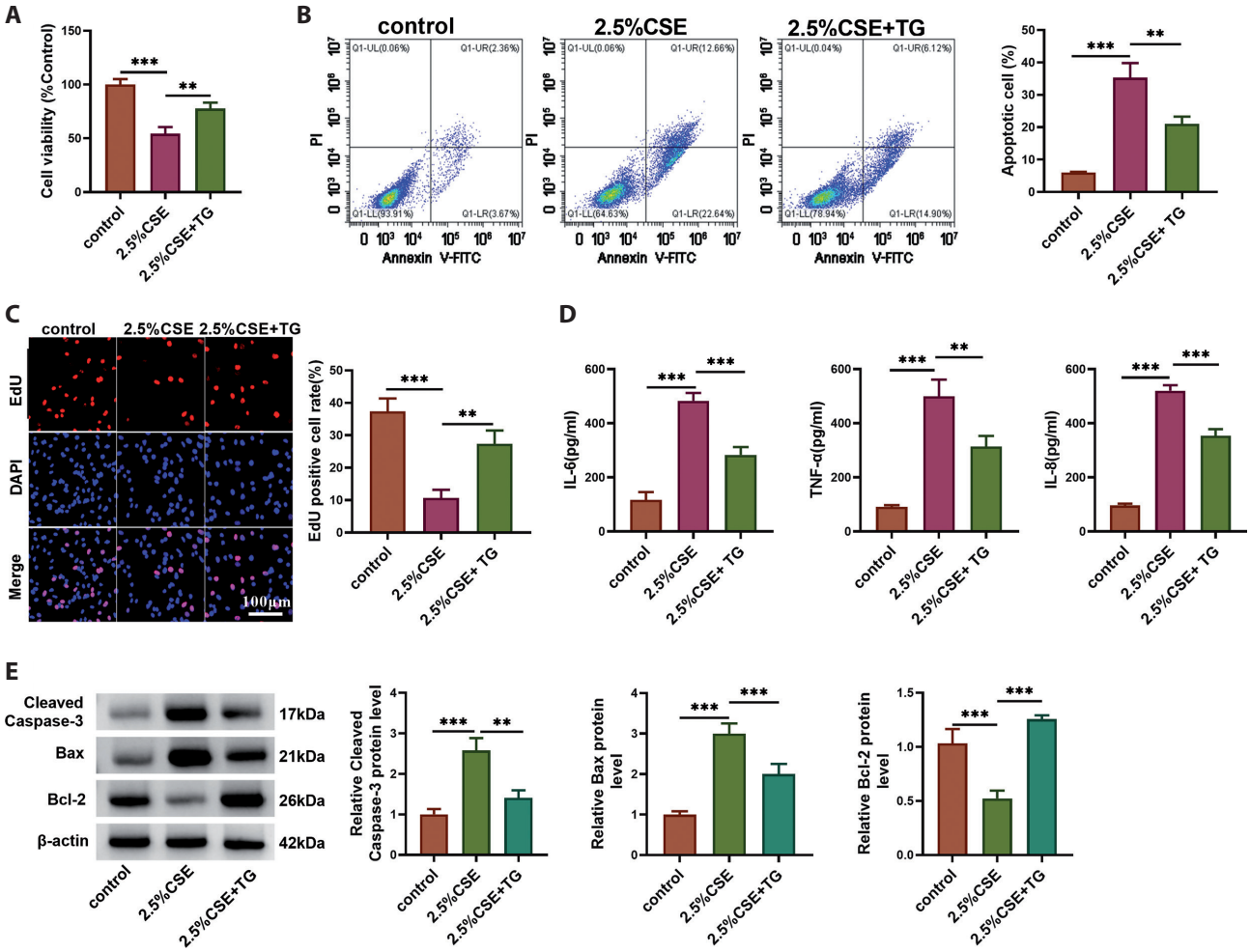

Figure 2. Effects of CSE and TG on BEAS-2B cell proliferation, apoptosis, and inflammation. BEAS-2B cells were treated with 2.5% CSE, 2.5% CSE+TG, or an equivalent volume of ethanol (control). **A.** Cell viability was detected using MTT assay. **B.** Cell apoptosis was tested using flow cytometry assay. **C.** Cell proliferation was measured using EdU assay. **D.** IL-6, TNF-α, and IL-8 levels were determined using ELISA. **E.** Cleaved caspase-3, Bcl-2, and Bax protein levels were detected using Western blot assay. ** $p < 0.01$, *** $p < 0.001$; $n = 3$.

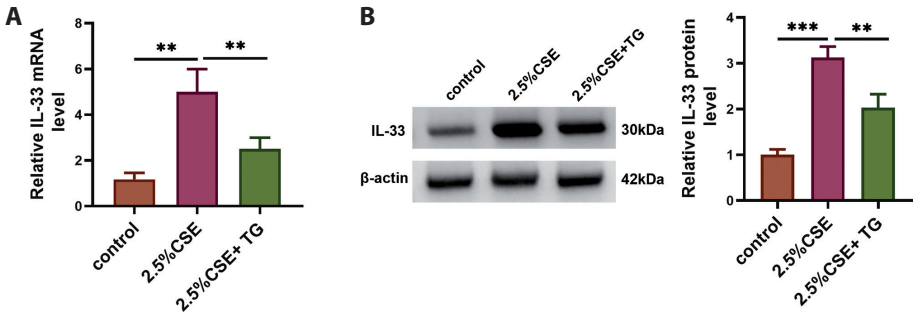

Figure 3. TG treatment affected IL-33 expression in CSE-treated BEAS-2B cells. **A, B.** IL-33 mRNA level and protein level were determined in BEAS-2B cells treated with 2.5% CSE, 2.5% CSE+TG, or control using RT-qPCR and Western blot assays. ** $p < 0.01$, *** $p < 0.001$; $n = 3$.

our data also displayed that 2.5–20 μg/ml could gradually reduce BEAS-2B cell viability. Therefore, the concentration of 1.25 μg/ml was selected for the following experiments.

TG treatment might relieve CSE-induced BEAS-2B cell apoptosis and inflammation

Previous studies have indicated that smoking is a main risk factor for COPD (Christenson et al. 2022). To mimic *in vitro* COPD pathological conditions, 2.5% CSE was used to treat BEAS-2B cells. After that, the biological role of TG in 2.5% CSE-triggered BEAS-2B cell damage was further explored. According to the data displayed in Figure 2A, 2.5% CSE exposure might apparently block BEAS-2B cell viability, while these effects were partly abolished by TG treatment. On the contrary, flow cytometry assay presented that applying TG might effectively overturn 2.5% CSE-induced BEAS-2B cell apoptosis (Fig. 2B). Besides, EdU results exhibited that 2.5% CSE might obviously reduce the EdU-positive cell rate in BEAS-2B cells, which was partially abrogated by TG treatment (Fig. 2C). In terms of inflammatory response, secretions of pro-inflammatory cytokines IL-6, TNF-α, and IL-8 were highly induced in response to 2.5% CSE in BEAS-2B cells, which was attenuated after TG exposure (Fig. 2D). Similar to the flow cytometry results, Western blot analysis presented that 2.5% CSE-mediated Cleaved caspase-3 and Bax (pro-apoptosis markers) enhancement and Bcl-2 (anti-apoptosis marker) decrease in BEAS-2B cells were remarkably reversed through TG treatment (Fig. 2E). Collectively, these results indicated that 2.5% CSE-induced BEAS-2B cell injury was partly ameliorated by TG exposure.

TG treatment repressed IL-33 expression in CSE-treated BEAS-2B cells

It has been reported that cigarette smoke might promote the accumulation of IL-33 in bronchial epithelial cells, thereby exacerbating inflammation (Pace et al. 2014). Thus, we further identified the effects of TG on IL-33 expression in 2.5% CSE-induced BEAS-2B cells. As displayed in Figure 3A and 3B, the mRNA level and protein level of IL-33 were markedly improved in the presence of 2.5% CSE exposure in BEAS-2B cells, whereas these effects were partly weakened by TG treatment. Overall, these results indicated that applying TG might be involved in regulating 2.5% CSE-induced IL-33 expression in BEAS-2B cells.

IL-33 knockdown might attenuate CSE-induced BEAS-2B cell injury

Furthermore, *in vitro* loss-of-function analyses were conducted to check the influence of IL-33 on 2.5% CSE-evoked BEAS-2B cell damage. At first, the knockdown efficiency of IL-33 was detected and presented in Figure S1A in Supplementary material. Then, Western blot analysis displayed that IL-33 protein level was significantly reduced in si-IL-33-transfected BEAS-2B cells under 2.5% CSE treatment compared with the si-NC group (Fig. 4A), implying that the knockdown efficiency was available. Functionally, 2.5% CSE-mediated cell viability inhibition and apoptosis promotion in BEAS-2B cells were clearly abolished after si-IL-33 transfection (Fig. 4B,C). Meanwhile, the lack of IL-33 might evidently weaken the suppressive role of 2.5% CSE on BEAS-2B cell proliferation (Fig. 4D). Beyond that, 2.5% CSE-stimulated inflammatory response in BEAS-2B cells was distinctly relieved by IL-33 silencing, as evidenced by decreased IL-6, TNF-α, and IL-8 (Fig. 4E). In addition, the downregulation of IL-33 might strikingly reverse the 2.5% CSE-induced increase in Cleaved caspase-3 and Bcl-2 protein levels and decrease in Bax level in BEAS-2B cells (Fig. 4F). Overall, these data suggested that CSE-induced BEAS-2B cell injury was partly overturned by IL-33 knockdown.

TG treatment might mitigate CSE-induced BEAS-2B cell injury by targeting IL-33

Additionally, rescue experiments were performed to clarify whether TG administration might mediate the functional role of IL-33 in 2.5% CSE-treated BEAS-2B cells. First of all, the overexpression efficiency of IL-33 was measured and displayed in Figure S1B in Supplementary material. Subsequently, the transfection of OE-IL-33 might significantly

overturn the promoting role of TG treatment on cell viability in 2.5% CSE-treated BEAS-2B cells (Fig. 5A). Meanwhile, reduced apoptosis and enhanced proliferation due to TG administration in 2.5% CSE-stimulated BEAS-2B cells were partly abolished by IL-33 overexpression (Fig. 5B,C). Beyond that, the inhibitory role of TG administration on inflammatory response in 2.5% CSE-treated BEAS-2B cells was effectively ameliorated through the forced expression of IL-33, as described by increased IL-6, TNF-α, and IL-8 secretions (Fig. 5D). Besides, in 2.5% CSE-stimulated BEAS-2B cells, apoptosis-related markers Bax and Cleaved caspase-3 protein levels were reduced and Bcl-2 level was increased with TG administration, which was significantly reversed by IL-33 upregulation (Fig. 5E). All these results indicated that elevated IL-33 might partly reverse the repression of TG treatment on CSE-stimulated BEAS-2B cell injury.

TG treatment relieved IL-33-activated MAPK signaling pathway in CSE-treated BEAS-2B cells

MAPK signaling pathway has been reported to elicit an appropriate physiological response, containing cellular

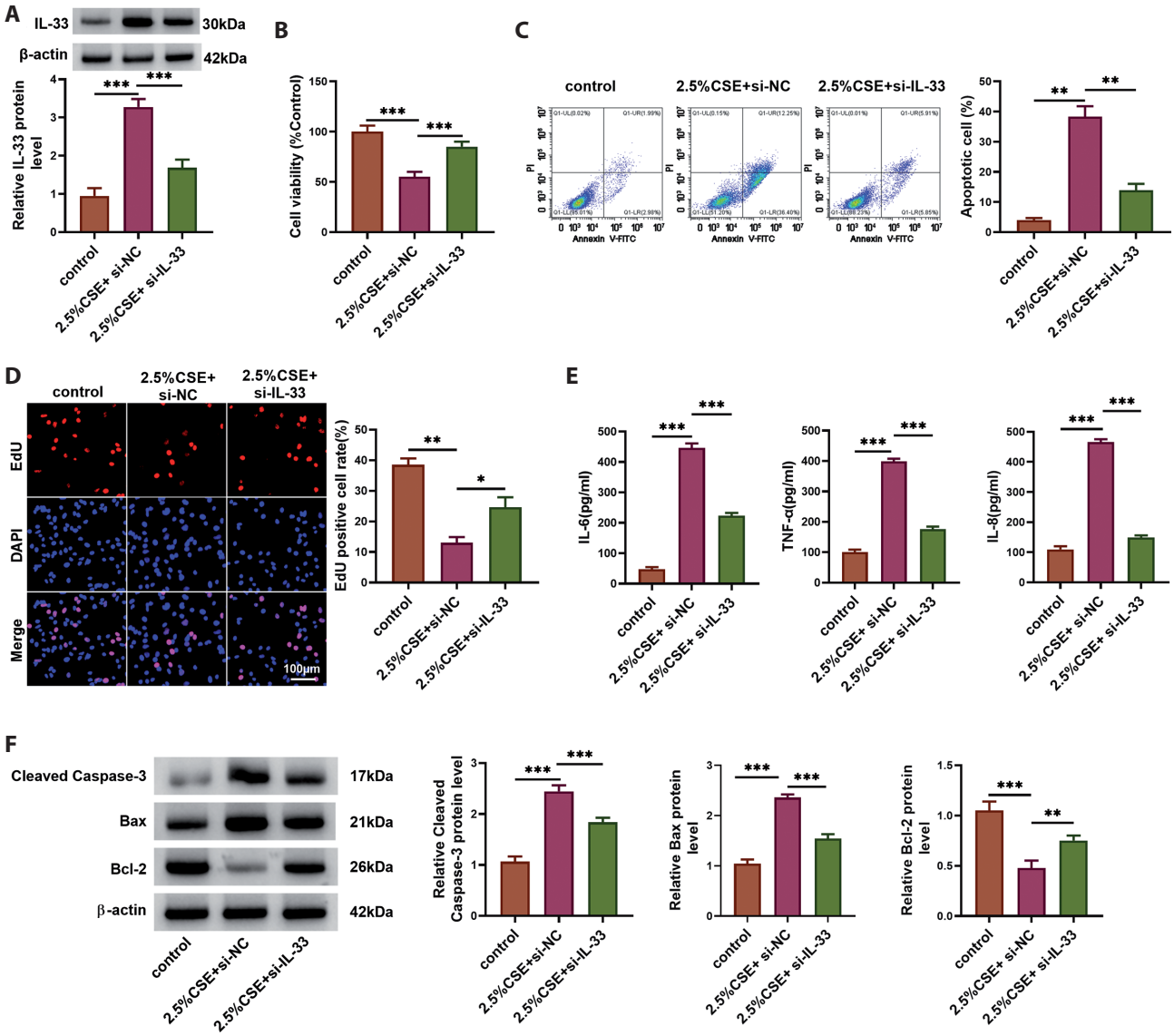

Figure 4. IL-33 knockdown repressed CSE-triggered BEAS-2B cell apoptosis and inflammation. BEAS-2B cells were treated with 2.5% CSE+si-NC, 2.5% CSE+si-IL-33, or control. **A.** IL-33 protein level was detected using Western blot assay. **B, C.** Cell viability and apoptosis were analyzed using MTT and flow cytometry assays. **D.** EdU assay was used to examine cell proliferation. **E.** ELISA was applied to measure IL-6, TNF-α, and IL-8 levels. **F.** Western blot analysis of Cleaved caspase-3, Bcl-2, and Bax protein levels. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $n = 3$.

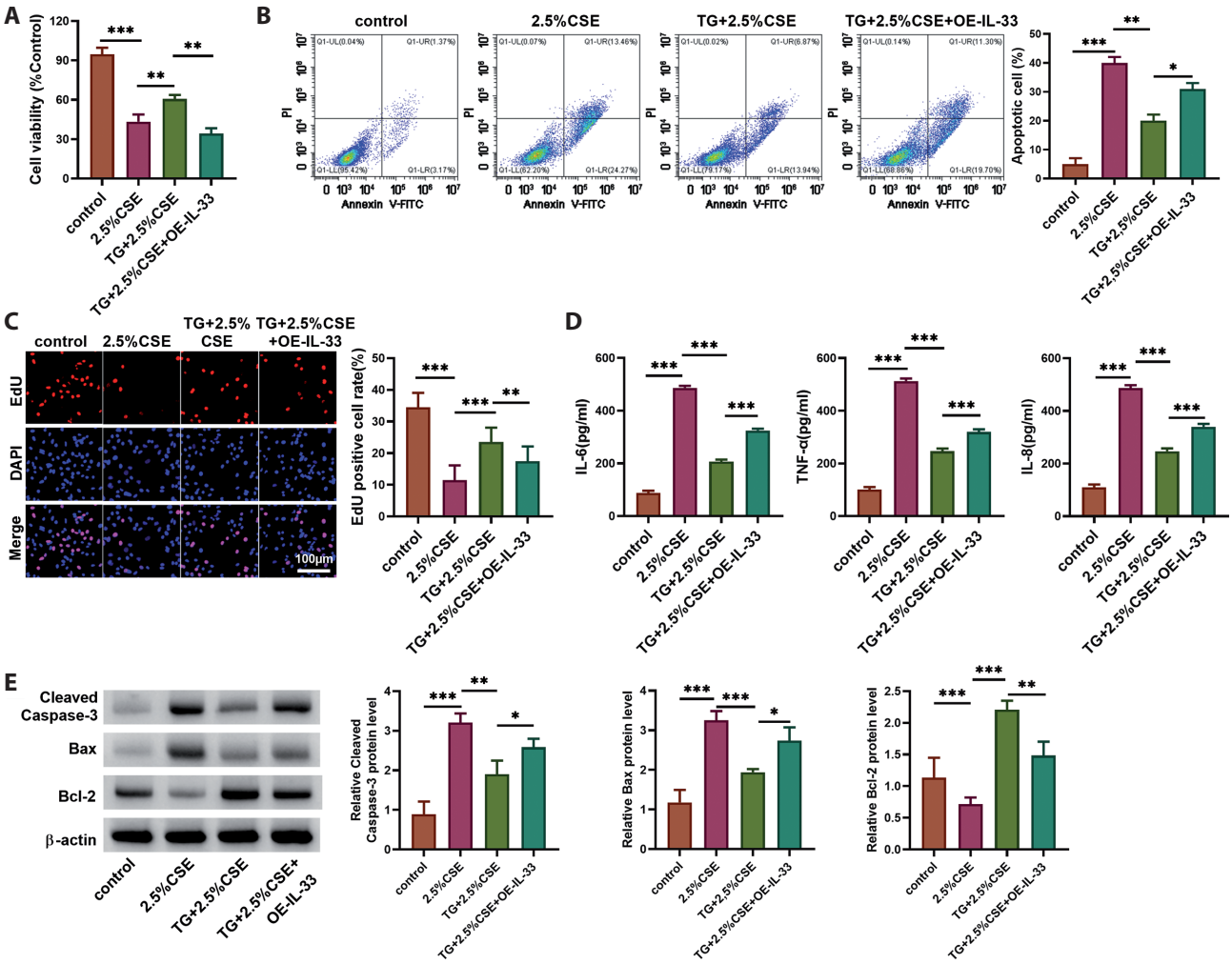

Figure 5. Effects of TG and IL-33 on CSE-induced BEAS-2B cell damage. BEAS-2B cells were treated with 2.5% CSE, TG+2.5% CSE, TG+2.5% CSE+OE- IL-33, or control. **A, B.** Measurement of cell viability and apoptosis was performed using MTT and flow cytometry assays. **C.** Assessment of cell proliferation was conducted using EdU assay. **D.** ELISA kits analyzed IL-6, TNF-α, and IL-8 levels. **E.** Western blot analysis of Cleaved caspase-3, Bcl-2, and Bax protein levels. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $n = 3$.

proliferation, apoptosis, differentiation, and inflammatory responses in mammalian cells (Zhang and Liu 2002). Meanwhile, this classical pathway is mainly composed of JNK, ERK1/2, and p38. In this study, we further explored the influences of TG and IL-33 on the MAPK signaling pathway. As displayed in Figure 6, the phosphorylation of JNK, ERK1/2, and p38 was respectively enhanced after 2.5% CSE exposure in BEAS-2B cells, suggesting that the MAPK signaling pathway was activated by CSE treatment. Meanwhile, TG administration significantly blocked 2.5% CSE-induced MAPK signaling pathway in BEAS-2B cells, whereas these effects were partly abrogated by IL-33 overexpression. Collectively, the above data illuminated that TG treatment might recede the MAPK signaling pathway by regulating IL-33.

Discussion

As a chronic, progressive, and incurable lung disease, COPD is typically recognized by persistent restriction airflow, which is linked to a boosted inflammatory constant reaction to pernicious substances or gases in the lungs and airways (Christenson et al. 2022). It has been confirmed that CS and infection are the main drivers of COPD (Vij et al. 2018). Typically, this inflammatory reaction leads to parenchymal impairment, fibrosis, and minor airway (King 2015). In recent years, numerous TCMs have been demonstrated to possess unique advantages and efficacy in reducing acute exacerbations of COPD, improving quality of life, and alleviating side effects of western medicine (Rahman et al. 2022; Cao et al. 2023). TG is extracted from a TCM herb,

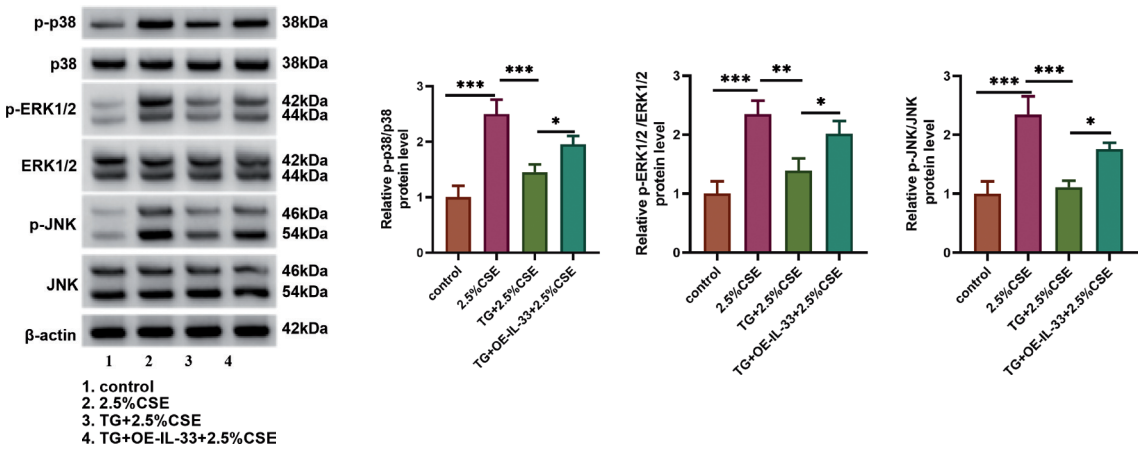

Figure 6. MAPK signaling pathway was regulated by the TG/IL-33 axis in CSE-treated BEAS-2B cells. The protein levels of p-p38, p-ERK1/2, and p-JNK were determined in BEAS-2B cells treated with 2.5% CSE, TG+2.5% CSE, TG+2.5% CSE+OE-IL-33, or control using Western blot assay. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $n = 3$.

TWHF, which has long been used in China to treat a variety of inflammatory diseases (Zhu et al. 2022). It has been reported that TG has multiple pharmacological activities, containing anti-inflammatory, anti-fertility, anti-tumor, and immune modulation (Cao et al. 2015). Nevertheless, whether TG influences COPD progression is still unknown. Herein, our data suggested that TG treatment has relatively lower toxicity to BEAS-2B cells, implying 1.25 μg/ml TG might be used to study human bronchial epithelial cells. More importantly, functional experiments displayed that CSE-induced BEAS-2B cell viability and proliferation repression and apoptosis and inflammation promotion were partly abolished after TG exposure. In total, these above observations provide first-hand information that treatment with TG might protect human bronchial epithelial cells from CSE-induced apoptosis and inflammation.

Recently, increasing evidence has indicated that TG treatment might effectively decrease the expression of pro-inflammatory cytokines, IL-6, IL-1β, and TNF-α, in some human diseases (Zhang and Chen 2017; Huang et al. 2023). For instance, the application of TG might increase IL-37 expression (an anti-inflammatory cytokine) by regulating the p38 MAPK signaling pathway, thereby relieving the development of inflammatory diseases (Wang et al. 2017). Moreover, TG treatment might significantly block inflammatory mediators in rat chondrocytes induced with IL-1β (Huang et al. 2023). Interestingly, some researchers displayed that IL-33, a cytokine of the IL-1 family, performed a critical role in allergic inflammation (Liew et al. 2016; Cayrol and Girard 2018). Widely expressed in the epithelium and endothelium of humans and mice, IL-33 is released when these cells are triggered by inflammation or necrosis (Wu et al. 2014). A related study indicated the association of lack of IL-33 with decreased COPD risk (Rabe et al. 2021). In agreement with previous studies (Byers et al. 2013; Kearley et al. 2015), the present work exhibited the upregulation of IL-33 in CSE-induced BEAS-2B cells. Therefore, we inferred TG might be involved in the regulation of IL-33 expression in BEAS-2B cells treated with CSE. Herein, our data firstly confirmed that CSE-induced IL-33 expression increase was partly relieved after TG exposure. Beyond that, we found that IL-33 silencing might significantly mitigate CSE-evoked BEAS-2B cell injury, consistent with former work (Kearley et al. 2015). As expected, our data validated that IL-33 overexpression might partially reverse the repression of TG administration on CSE-induced BEAS-2B cell damage. In addition, some studies have suggested that TG and IL-33 might participate in the modulation of the MAPK signaling pathway (Wu et al. 2014; Wang et al. 2017). Herein, our data verified that TG administration might suppress the MAPK signaling pathway by regulating IL-33 in CSE-treated BEAS-2B cells, supporting the TG/IL-33/MAPK pathway. The current work was limited in vitro studies, and more animal experiments and clinical tests about the novel mechanism in COPD will be performed in further work. In addition, clinical trials have displayed promising efficacy of monoclonal antibodies targeting IL-33 and its suppression of tumorigenicity 2 (ST2) receptor in COPD (Pelaia 2024). Therefore, we will further explore whether TG can also regulate the IL-33/ST2 pathway during COPD in future studies. Besides, previous studies have indicated that TG may lead to side effects, including hepatotoxicity, reproductive toxicity, nematotoxicity, and intestinal toxicity (Almuzaini et al. 2013). Therefore, current strategies to mitigate its toxicity contain the combined application with Chinese material medicine, TCM preparation, and chemical drugs (Niu et al. 2025). Meanwhile, during clinical application, patients should be closely monitored and potential adverse reactions should

be detected and treated in a timely manner. This suggests that individualized treatment plans are needed in clinical applications to accommodate the specific conditions of different patients (Shu et al. 2025).

Conclusion

Taken together, our data discovered that TG administration might repress CSE-induced BEAS-2B cell damage by regulating IL-33, providing a novel mechanism for TG in COPD and shedding insight into the development of a new therapeutic for COPD patients with clinical application.

Conflict of interest. The authors declare that they have no conflicts of interest.

References

Aghapour M, Raee P, Moghaddam SJ, Hiemstra PS, Heijink IH (2018): Airway epithelial barrier dysfunction in chronic obstructive pulmonary disease: Role of cigarette smoke exposure. Am. J. Respir. Cell. Mol. Biol. **58,** 157-169
https://doi.org/10.1165/rcmb.2017-0200TR

Almuzaini T, Sammons H, Choonara I (2013): Substandard and falsified medicines in the UK: a retrospective review of drug alerts (2001-2011). BMJ Open **3,** e002924
https://doi.org/10.1136/bmjopen-2013-002924

André S, Conde B, Fragoso E, Boléo-Tomé JP, Areias V, Cardoso J (2019): COPD and cardiovascular disease. Pulmonology **25,** 168-176
https://doi.org/10.1016/j.pulmoe.2018.09.006

Barnes PJ (2016): Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J. Allergy Clin. Immunol. **138,** 16-27
https://doi.org/10.1016/j.jaci.2016.05.011

Bernocchi P, Vitacca M, La Rovere MT, Volterrani M, Galli T, Baratti D, Paneroni M, Campolongo G, Sposato B, Scalvini S (2018): Home-based telerehabilitation in older patients with chronic obstructive pulmonary disease and heart failure: a randomised controlled trial. Age Ageing **47,** 82-88
https://doi.org/10.1093/ageing/afx146

Byers DE, Alexander-Brett J, Patel AC, Agapov E, Dang-Vu G, Jin X, Wu K, You Y, Alevy Y, Girard JP et al. (2013): Long-term IL-33-producing epithelial progenitor cells in chronic obstructive lung disease. J. Clin. Invest. **123,** 3967-3982
https://doi.org/10.1172/JCI65570

Cao LJ, Yan M, Li HD, Zhang BK, Fang PF (2015): Progress on mechanism of Tripterygium wilfordii-induced liver injury and detoxification mechanism of licorice. Zhongguo Zhong Yao Za Zhi **40,** 2537-2541 (in Chinese)

Cao X, Wang Y, Chen Y, Zhao M, Liang L, Yang M, Li J, Peng M, Li W, Yue Y, et al. (2023): Advances in traditional Chinese medicine for the treatment of chronic obstructive pulmonary disease. J. Ethnopharmacol. **307,** 116229
https://doi.org/10.1016/j.jep.2023.116229

Cayrol C, Girard JP (2018): Interleukin-33 (IL-33): A nuclear cytokine from the IL-1 family. Immunol. Rev. **281,** 154-168
https://doi.org/10.1111/imr.12619

Christenson SA, Smith BM, Bafadhel M, Putcha N (2022): Chronic obstructive pulmonary disease. Lancet **399,** 2227-2242
https://doi.org/10.1016/S0140-6736(22)00470-6

Guan R, Wang J, Cai Z, Li Z, Wang L, Li Y, Xu J, Li D, Yao H, Liu W, et al. (2020): Hydrogen sulfide attenuates cigarette smoke-induced airway remodeling by upregulating SIRT1 signaling pathway. Redox Biol. **28,** 101356
https://doi.org/10.1016/j.redox.2019.101356

Huang Z, Song S, Zhang D, Bian Z, Han J (2023): Protective effects of Tripterygium glycoside on IL-1β-induced inflammation and apoptosis of rat chondrocytes via microRNA-216a-5p/TLR4/NF-κB axis. Immunopharmacol. Immunotoxicol. **45,** 61-72
https://doi.org/10.1080/08923973.2022.2115924

Hou W, Hu S, Li C, Ma H, Wang Q, Meng G, Guo T, Zhang J (2019): Cigarette smoke induced lung barrier dysfunction, EMT, and tissue remodeling: A possible link between COPD and lung cancer. Biomed. Res. Int. **2019,** 2025636
https://doi.org/10.1155/2019/2025636

Kearley J, Silver JS, Sanden C, Liu Z, Berlin AA, White N, Mori M, Pham TH, Ward CK, Criner GJ, et al. (2015): Cigarette smoke silences innate lymphoid cell function and facilitates an exacerbated type I interleukin-33-dependent response to infection. Immunity **42,** 566-579
https://doi.org/10.1016/j.immuni.2015.02.011

King PT (2015): Inflammation in chronic obstructive pulmonary disease and its role in cardiovascular disease and lung cancer. Clin. Transl. Med. **4,** 68
https://doi.org/10.1186/s40169-015-0068-z

Li Q, Huang Y, Liu P, Yuan H, Zhao J (2021): Effect of Tripterygium wilfordii polyglycoside tablets on serum inflammatory factors and T cells in patients with chronic nephritis. Am. J. Transl. Res. **13,** 8385-8390

Liew FY, Girard JP, Turnquist HR (2016): Interleukin-33 in health and disease. Nat. Rev. Immunol. **16,** 676-689
https://doi.org/10.1038/nri.2016.95

Luo Y, Kuai L, Chen J, Sun X, Liu L, Luo Y, Ru Y, Xing M, Ding X, Zhou M, et al. (2020): Efficacy and safety of Tripterygium wilfordii Hook. F. for oral lichen planus: Evidence from 18 randomized controlled trials. Phytother. Res. **34,** 2180-2191
https://doi.org/10.1002/ptr.6672

Martin C, Burgel PR (2021): Inhaled dual phosphodiesterase 3/4 inhibitors for the treatment of patients with COPD: A short raaeview. Int. J. Chron. Obstruct. Pulmon. Dis. **16,** 2363-2373
https://doi.org/10.2147/COPD.S226688

Molofsky AB, Savage AK, Locksley RM (2015): Interleukin-33 in tissue homeostasis, injury, and inflammation. Immunity **42,** 1005-1019
https://doi.org/10.1016/j.immuni.2015.06.006

Niu Z, Zhang H, Cai C, Yang T, Ma T, Xu D, Cui D, Tang Y (2025): The mechanisms of tripterygium glycosides-induced reproductive toxicity and detoxification strategies. Reprod. Toxicol. **132,** 108830
https://doi.org/10.1016/j.reprotox.2025.108830

Pace E, Di Sano C, Sciarrino S, Scafidi V, Ferraro M, Chiappara G, Siena L, Gangemi S, Vitulo P, Giarratano A, et al. (2014):

Cigarette smoke alters IL-33 expression and release in airway epithelial cells. Biochim. Biophys. Acta **1842,** 1630-1637
https://doi.org/10.1016/j.bbadis.2014.06.009

Pelaia C (2024): Interleukin 33: a suitable target for biological therapies of COPD? ERJ Open Res. **10,** 00433-2024
https://doi.org/10.1183/23120541.00433-2024

Rabe KF, Celli BR, Wechsler ME, Abdulai RM, Luo X, Boomsma MM, Staudinger H, Horowitz JE, Baras A, Ferreira MA, et al. (2021): Safety and efficacy of itepekimab in patients with moderate-to-severe COPD: a genetic association study and randomised, double-blind, phase 2a trial. Lancet Respir. Med. **9,** 1288-1298
https://doi.org/10.1016/S2213-2600(21)00167-3

Rabe KF, Watz H (2017): Chronic obstructive pulmonary disease. Lancet **389,** 1931-1940
https://doi.org/10.1016/S0140-6736(17)31222-9

Rahman MM, Bibi S, Rahaman MS, Rahman F, Islam F, Khan MS, Hasan MM, Parvez A, Hossain MA, Maeesa SK, et al. (2022): Natural therapeutics and nutraceuticals for lung diseases: Traditional significance, phytochemistry, and pharmacology. Biomed. Pharmacother. **150,** 113041
https://doi.org/10.1016/j.biopha.2022.113041

Safiri S, Carson-Chahhoud K, Noori M, Nejadghaderi SA, Sullman MJM, Ahmadian Heris J, Ansarin K, Mansournia MA, Collins GS, Kolahi AA (2022): Burden of chronic obstructive pulmonary disease and its attributable risk factors in 204 countries and territories, 1990-2019: Results from the Global Burden of Disease Study 2019. BMJ **378,** e069679
https://doi.org/10.1136/bmj-2021-069679

Shu H, Chen XY, Zhao J, Li P, Sun Z (2025): Efficacy and safety of Tripterygium wilfordii glycosides tablets combined with western medicine for patients with rheumatic immune diseases. World J. Clin. Cases **13,** 95513
https://doi.org/10.12998/wjcc.v13.i6.95513

Song Q, Chen P (2021): The role of cigarette smoke-induced pulmonary vascular endothelial cell apoptosis in COPD. Respir. Res. **22,** 39
https://doi.org/10.1186/s12931-021-01630-1

Vij N, Chandramani-Shivalingappa P, Van Westphal C, Hole R, Bodas M (2018): Cigarette smoke-induced autophagy impairment accelerates lung aging, COPD-emphysema exacerbations and pathogenesis. Am. J. Physiol. Cell. Physiol. **314,** C73-C87
https://doi.org/10.1152/ajpcell.00110.2016

Vogelmeier CF, Naya IP, Maltais F (2021): Treatment of COPD with long-acting bronchodilators: Association between early and longer-term clinically important improvement. Int. J. Chron. Obstruct. Pulmon. Dis. **16,** 1215-1226
https://doi.org/10.2147/COPD.S295835

Wang S, Li R, He S, He L, Zhao H, Deng X, Chen Z (2017): Tripterygium wilfordii glycosides upregulate the new anti-inflammatory cytokine IL-37 through ERK1/2 and p38 MAPK signal pathways. Evid. Based Complement. Alternat. Med. **2017,** 9148523
https://doi.org/10.1155/2017/9148523

Wu H, Yang S, Wu X, Zhao J, Zhao J, Ning Q, Xu Y, Xie J (2014): Interleukin-33/ST2 signaling promotes production of interleukin-6 and interleukin-8 in systemic inflammation in cigarette smoke-induced chronic obstructive pulmonary disease mice. Biochem. Biophys. Res. Commun. **450,** 110-116
https://doi.org/10.1016/j.bbrc.2014.05.073

Xue M, Jiang ZZ, Liu JP, Zhang LY, Wang T, Wang H, Liu L, Zhou ZX (2010): Comparative study on the anti-inflammatory and immune suppressive effect of Wilforlide A. Fitoterapia **81,** 1109-1112
https://doi.org/10.1016/j.fitote.2010.07.007

Zhang H, Chen W (2017): Interleukin 6 inhibition by triptolide prevents inflammation in a mouse model of ulcerative colitis. Exp. Ther. Med. **14,** 2271-2276
https://doi.org/10.3892/etm.2017.4778

Zhang W, Liu HT (2002): MAPK signal pathways in the regulation of cell proliferation in mammalian cells. Cell Res. **12,** 9-18
https://doi.org/10.1038/sj.cr.7290105

Zhang Y, Zhao L, Yue Q, Su L, Li K, Zhao C, Li B, Zhang S (2023): Using traditional chinese medicine to alleviate chronic obstructive pulmonary disease. Stud. Health Technol. Inform. **308,** 696-704
https://doi.org/10.3233/SHTI230902

Zhu Y, Zhang L, Zhang X, Wu D, Chen L, Hu C, Wen C, Zhou J (2022): Tripterygium wilfordii glycosides ameliorates collagen-induced arthritis and aberrant lipid metabolism in rats. Front. Pharmacol. **13,** 938849
https://doi.org/10.3389/fphar.2022.938849

Zong D, Liu X, Li J, Ouyang R, Chen P (2019): The role of cigarette smoke-induced epigenetic alterations in inflammation. Epigenetics Chromatin **12,** 65
https://doi.org/10.1186/s13072-019-0311-8

Received: November 1, 2024
Final version accepted: June 13, 2025