Dissertation

The Role of JAK/STAT Signaling in Neutrophilic Airway Inflammation

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Disclosures

Parts of this thesis have been published in Nayak BB et al. **"Brepocitinib, a selective TYK2/JAK1 inhibitor, mitigates neutrophilic inflammation and glucocorticoid receptor-β expression in COPD"** Am J Physiol Lung Cell Mol Physiol. 2026;330(2):L178-L197 and Nayak BB et al**. "Interleukin-23 in lung and airway diseases: from pathogenesis to precision-guided therapeutic targeting"** Front Pharmacol. 2026;17:1784434. doi:10.3389/fphar.2026.1784434. The candidate has also contributed to collaborative research, resulting in several co-authored publications (Vámos et al., 2023; Teppan et al., 2024; Vámos et al., 2025).

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Abbreviations

ACT1 – Activator of NF-κB 1

APC – Antigen-Presenting Cell

ARDS – Acute Respiratory Distress Syndrome

BAL – Bronchoalveolar Lavage

BCA – Bicinchoninic Acid Assay (protein estimation method)

C/EBP – CCAAT/Enhancer Binding Protein

C5a – Complement Component 5a (anaphylatoxin)

COPD – Chronic Obstructive Pulmonary Disease

CS – Cigarette Smoke

CXCL – C-X-C Motif Chemokine Ligand

ERK – Extracellular Signal-Regulated Kinase

FEV1 – Forced Expiratory Volume in 1 second

FVC – Forced Vital Capacity

GOLD – Global Initiative for Chronic Obstructive Lung Disease

GR – Glucocorticoid Receptor

γδ T – Gamma Delta T cell

HDAC2 – Histone Deacetylase 2

IL – Interleukin

ILC – Innate Lymphoid Cell

IPF – Idiopathic Pulmonary Fibrosis

JAK – Janus Kinase

LPS – Lipopolysaccharide

MAPK – Mitogen-Activated Protein Kinase

MyD88/TRIF – Myeloid Differentiation Primary Response 88 / TIR-domain-containing adapter-inducing interferon-β

NCR – Natural Cytotoxicity Receptor

NF-κB – Nuclear Factor kappa-light-chain-enhancer of Activated B cells

NR3C1- Nuclear Receptor Subfamily 3 Group C Member 1

PI3K – Phosphoinositide 3-Kinase

PLC–IP_3–Ca^{2+} – Phospholipase C-Inositol 1,4,5-trisphosphate-Calcium signaling pathway

PMNL – Polymorphonuclear Leukocytes

ROS – Reactive Oxygen Species

SOCS3 – Suppressor of Cytokine Signaling 3

STAT – Signal Transducer and Activator of Transcription

TGF-β – Transforming Growth Factor Beta

Th – T helper cell

TLR4 – Toll-Like Receptor 4

TNF-α – Tumor Necrosis Factor Alpha

TYK2 – Tyrosine Kinase 2

Zusammenfassung

Die chronisch obstruktive Lungenerkrankung (COPD) ist eine progrediente Atemwegserkrankung, die durch eine persistierende Entzündung der Atemwege gekennzeichnet ist. Diese Entzündung ist überwiegend neutrophil dominiert, häufig gegenüber Kortikosteroiden unempfindlich und eng mit der IL-23/IL-17A-Achse assoziiert, einem zentralen Treiber Th17-vermittelter Immunantworten. Diese Achse steht zudem in Verbindung mit dem Januskinase/Signal Transducer and Activator of Transcription (JAK/STAT)-Signalweg, insbesondere über die Kinasen TYK2 und JAK1, deren Rolle bei der neutrophilen Entzündung bei COPD bislang nicht vollständig geklärt ist.

Ziel der vorliegenden Arbeit war es, die Expression und Aktivierung von JAK-Kinasen, insbesondere TYK2 und JAK1, in Immunzellen aus Blutproben von COPD-Patienten, Rauchern ohne COPD sowie gesunden Kontrollpersonen zu untersuchen und deren Bedeutung für neutrophil vermittelte Entzündungsprozesse zu charakterisieren. Mittels Durchflusszytometrie konnten signifikant erhöhte Phosphorylierungsniveaus von TYK2 und JAK1 in Neutrophilen von COPD-Patienten und Rauchern im Vergleich zu gesunden Kontrollpersonen nachgewiesen werden.

Zusätzlich wurde der Einfluss der IL-23/IL-17A-Achse auf neutrophile Effektorfunktionen sowie das therapeutische Potenzial des TYK2/JAK1-Inhibitors Brepocitinib untersucht. IL-23 und IL-17A verstärkten sowohl die Aktivierung von Neutrophilen als auch die Freisetzung von IL-8 aus bronchialen Epithelzellen. Diese Effekte konnten durch Brepocitinib wirksam gehemmt werden. Zudem zeigten Neutrophile von COPD-Patienten und Rauchern eine erhöhte Expression des Glukokortikoidrezeptors β (GRβ), der mit Kortikosteroidresistenz assoziiert ist. Dieser Phänotyp ließ sich durch Stimulation mit IL-23 und IL-17A reproduzieren und ebenfalls durch Brepocitinib unterdrücken. In vivo-Experimente zeigten außerdem, dass Brepocitinib die durch IL-23 oder Lipopolysaccharid induzierte Rekrutierung von Neutrophilen in Modellen akuter Entzündung verhindern konnte.

Zusammenfassend zeigen die Ergebnisse, dass die IL-23/IL-17A-Achse eine wichtige Rolle bei der neutrophilen Entzündung und der Entwicklung von Kortikosteroidresistenz bei COPD spielt. Die pharmakologische Hemmung von TYK2/JAK1 durch Brepocitinib schwächte diese Prozesse wirksam ab. TYK2 und JAK1 stellen somit potenzielle therapeutische Zielstrukturen dar, die bestehende Behandlungsstrategien ergänzen könnten, um Atemwegsentzündung zu reduzieren und die Steroidsensitivität bei schwerer COPD zu verbessern.

Abstract

Chronic obstructive pulmonary disease (COPD) is a progressive respiratory disorder characterized by persistent airway inflammation. This inflammation is predominantly neutrophilic, often corticosteroid-insensitive, and associated with the IL-23/IL-17A axis, a key driver of the Th17-mediated immune responses. The IL-23/IL-17A axis is connected to the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway, especially through TYK2 and JAK1, whose roles in neutrophilic inflammation in COPD are still under investigation.

In this study, we examined the expression and activation of JAKs, particularly TYK2 and JAK1, in immune cells isolated from blood samples of patients with COPD, smokers without COPD and matched healthy controls to better understand the role of JAK kinases in neutrophil-related inflammation in COPD. Notably, flow cytometric analysis revealed significantly higher phosphorylation levels of TYK2 and JAK1 in neutrophils from COPD patients and smokers compared to healthy controls. We also investigated how the IL-23/IL-17A axis regulates neutrophil effector functions and evaluated the potential of a TYK2/JAK1 inhibitor, brepocitinib, as a therapeutic strategy for COPD. Our results showed that IL-23 and IL-17A enhanced neutrophil activation and IL-8 release from bronchial epithelial cells, effects that were effectively suppressed by brepocitinib. Additionally, neutrophils from COPD patients and smokers exhibited higher expression of glucocorticoid receptor β, a mechanism linked to corticosteroid resistance. This phenotype was recapitulated by treatment with IL-23 and IL-17A and was suppressed by brepocitinib. In vivo experiments further demonstrated that brepocitinib prevented neutrophil recruitment induced by IL-23 or LPS in models of acute inflammation.

Collectively, these findings suggest that the IL-23/IL-17A axis promotes neutrophil-driven inflammation and corticosteroid resistance in COPD, which can be mitigated by TYK2/JAK1 inhibition with brepocitinib. This highlights TYK2/JAK1 as a promising therapeutic target that may complement existing treatments to reduce airway inflammation and restore steroid sensitivity in severe COPD.

1. Introduction

1.1. General aspects of lung inflammation

The lungs are constantly exposed to environmental stimuli such as airborne pathogens, allergens, pollutants, and particulate matter. To ensure effective gas exchange while protecting the host, the respiratory system employs tightly controlled immune responses that balance defense and tissue preservation [1]. Under physiological conditions, the lung maintains immune homeostasis and tolerance to innocuous inhaled antigens, preventing unnecessary inflammation while remaining poised to mount rapid immune responses to pathogens. Disruption of this balance promotes pathological inflammation and disease. Lung inflammation is the immune response to these irritants, infections, or allergens, involving immune cells and mediators that facilitate rapid detection and removal of harmful stimuli through coordinated innate and adaptive immunity [2]. Key lung-resident immune cells, including alveolar macrophages, dendritic cells, and innate lymphoid cells, play central roles in sensing danger signals and shaping early inflammatory responses [3]. Airway structural cells, especially epithelial cells, serve as a physical barrier and also act as an active immune barrier by releasing cytokines, chemokines, and danger-associated molecular patterns to initiate inflammation [3]. Pulmonary endothelial cells further contribute to inflammatory responses by regulating leukocyte recruitment and vascular permeability, which can promote tissue edema and impair gas exchange during severe inflammation [4]. Generally, this inflammation can be classified as acute, such as pneumonia and acute respiratory distress syndrome (ARDS), or chronic, as seen in asthma and chronic obstructive pulmonary disease (COPD) [4]. Although acute inflammation helps in tissue protection and repair, chronic persistent inflammation can damage lung tissue, impair airway exchange, and lead to serious conditions like COPD, asthma, ARDS, and idiopathic pulmonary fibrosis. Sustained inflammatory signaling promotes airway remodeling, extracellular matrix deposition, and fibrotic changes that can result in irreversible structural alterations and progressive loss of lung function. The shift from acute to chronic lung inflammation involves a complex network of immune responses in the lungs [5]. The magnitude and persistence of lung inflammation are further influenced by host-related factors such as genetic susceptibility, aging, smoking status, occupational exposures, and the airway microbiome [6,7].

The airway epithelium acts as the first line of defense by trapping and neutralizing harmful substances through the secretion of mucins, defensins, lysozyme, lactoferrin, and nitric oxide [2,8]. During these inflammatory responses, epithelial cells further release proinflammatory cytokines and mediators, and reactive oxygen species, which lead to the recruitment, activation, and interactions of immune cells within the pulmonary microenvironment. This cascade causes excessive mucus production, airway sensitivity, and tissue damage, resulting in diverse inflammatory patterns with distinct cellular and molecular mechanisms [8]. Understanding this complex network of interactions has led to the concept of inflammatory endotypes, in which different immune mechanisms drive disease variation and influence clinical outcomes. Currently, chronic lung inflammation is simplified as Type 2-driven and non-Type 2 pathways, which include Th1- and IL17-mediated endotypes, each characterized by distinct cytokine profiles, cellular infiltrates, and therapeutic responses [9,10].

Chronic airway disease, therefore, emerges from maladaptive crosstalk between epithelial barrier cells and innate immune cells, creating self-sustaining inflammatory loops that perpetuate tissue injury.

1.2. Inflammatory endotypes of chronic airway disease

Type 2 Inflammation

One of the most well-characterized inflammatory endotypes in the lung is Th2 or Type 2 inflammation. This pathway emerges when epithelial injury or allergen exposure triggers epithelial-derived alarmins like IL-33, IL-25, and thymic stromal lymphopoietin [11]. These alarmins promote the differentiation and activation of $CD4^+$ T helper 2 cells and type 2 innate lymphoid cells. The cytokine environment, mainly IL-4, IL-5, and IL-13, drives eosinophil recruitment, immunoglobulin E production, mucus overproduction, and structural airway changes [11]. Type 2 inflammation is also associated with mast cell activation, basophil recruitment, and airway hyperresponsiveness, which contribute to bronchoconstriction and symptom exacerbation. This pathway's coordinated action explains common features across various chronic inflammatory lung diseases, such as asthma, and has facilitated the development of targeted treatments that specifically block Type 2 signaling [10]. Biomarkers such as blood

and sputum eosinophil counts, periostin, and fractional exhaled nitric oxide (FeNO) are commonly used to identify Type 2–high inflammatory profiles in patients [12].

Non-Type 2 Inflammation

While Type 2 pathways are prominent, a significant portion of chronic lung inflammation cannot be solely explained by Th2-driven mechanisms. This underscores the importance of non-Th2 inflammatory pathways, mainly involving Th1 and Th17 cells, along with innate immune activation [13]. Additional contributors include cytotoxic T lymphocytes, natural killer cells, and pattern recognition receptor–mediated signaling in response to microbial products and tissue damage [14]. These pathways produce cytokines such as interferon-γ, IL-17, IL-6, and tumor necrosis factor-α [15]. Such cytokines promote neutrophil recruitment and macrophage activation, thereby sustaining inflammation and causing further epithelial damage [15]. Non-Th2 inflammation is frequently linked to bacterial colonization, viral infections, and exposure to cigarette smoke or environmental pollutants, which further amplify inflammatory signaling [16]. This non-Th2 endotype is characterized by its lower responsiveness to corticosteroids and is associated with increased severity in conditions like COPD [9,17]. Despite the fundamental difference, Th2 and non-Th2 inflammatory endotypes often coexist or change over time due to environmental factors, infections, and host influences [18]. This plasticity contributes to clinical heterogeneity and poses challenges for patient stratification and targeted therapy.

IL-17-driven Inflammation

In the context of inflammatory airway diseases, particular attention has been given to the non–Type 2 inflammatory endotype, especially IL-17-driven inflammation. Chronic airway inflammation in COPD is increasingly associated with persistent IL-17-driven immune responses [9]. Continuous inflammatory signals in the lung environment sustain Th17 cells and develop a cytokine network that promotes their activity and survival. Th17 cells also interact with epithelial cells, neutrophils, and macrophages, amplifying local inflammatory loops and perpetuating tissue injury. In this context, the IL-23/IL-17A axis is a critical pathway that helps explain how Th17-related cytokines drive inflammatory processes in COPD [9,19]. Dysregulation of this axis has been linked to neutrophilic inflammation, mucus hypersecretion, airway remodeling, and steroid-resistant disease phenotypes. Given the growing evidence linking IL-17-driven immune responses and the IL-23/IL-17A axis to neutrophilic airway inflammation, it is essential to consider these mechanisms in the context of chronic obstructive

pulmonary disease (COPD), a condition in which persistent airway inflammation and immune dysregulation are central to disease development and progression.

1.3.Chronic Obstructive Pulmonary Disease (COPD)

COPD is a chronic inflammatory lung disease characterized by persistent airflow limitation that is usually progressive and associated with an abnormal inflammatory response of the lungs to harmful particles or gases, as well as chronic bronchitis and emphysema [20,21]. Pathologically, COPD is marked by small airway inflammation, mucus hypersecretion, airway wall thickening, and destruction of alveolar structures, leading to impaired gas exchange. The FEV_1/FVC ratio (ratio of the forced expiratory volume in the first second to the forced vital capacity of the lungs) in COPD is usually <70%, reflecting an obstructive spirometry pattern. According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), COPD is classified in four stages from 1-mild to 4-very severe based on the degree of airflow limitation [20,21]. Beyond airflow limitation, disease severity is also influenced by symptom burden and exacerbation frequency, reflecting the heterogeneity of COPD phenotypes.

1.3.1. Causes and epidemiology

The primary cause of COPD is tobacco smoke exposure. Other risk factors include prolonged exposure to air pollution, occupational dust and particulate matter, environmental toxins, and pathogens [21,22]. Genetic risk factors, such as α1-antitrypsin deficiency, account for only a small percentage of cases [21]. Host susceptibility factors, including sex, age, and genetic polymorphisms in inflammatory and antioxidant pathways, further modulate disease risk and progression. Recent studies also indicate that factors such as impaired lung development, recurrent childhood respiratory infections, socioeconomic challenges, and early environmental exposures may increase the risk of COPD in the later phases of life [23]. These early-life influences are increasingly recognized as contributors to reduced lung function trajectories that predispose individuals to COPD in adulthood.

Epidemiologically, COPD is the third leading cause of death worldwide, making it a serious global health problem [20]. Its prevalence is increasing, particularly in low- and middle-income countries, driven by continued exposure to environmental pollutants, smoking, and aging populations [24]. Underdiagnosis and delayed diagnosis further contribute to the high global

burden of disease. It has become a systemic disease due to comorbidities like cardiovascular disease, metabolic disorders, osteoporosis, and lung cancer, all of which contribute to a poorer overall prognosis [25]. Systemic inflammation is thought to be a central mechanism linking pulmonary pathology with extrapulmonary comorbidities in COPD.

1.3.2. Symptoms, endotypes, and pathology

COPD shows symptoms such as shortness of breath, cough, sputum production, and frequent exacerbations. Patients may also experience wheezing, chest tightness, exercise intolerance, fatigue, and weight loss, particularly in advanced disease stages. The disease is heterogeneous, encompassing a range of inflammatory endotypes. Neutrophilic inflammation is a major characteristic of COPD, reflected by increased neutrophil counts in blood and sputum. Notably, this neutrophilic inflammatory endotype has been associated with corticosteroid resistance [26]. This endotype is often linked to heightened activity of Th17-related cytokines, including IL-17A and IL-23, and to increased bacterial colonization of the airways. A subset of COPD patients also shows eosinophilic inflammation, marked by higher eosinophil counts in sputum and blood. Eosinophilia in COPD has been seen as a shared characteristic with asthma, linked to elevated IL-5 levels [27]. In fact, high blood eosinophil levels serve as a biomarker for predicting the response to inhaled corticosteroids in acute exacerbations of COPD [28]. These inflammatory endotypes are not fixed and may shift over time or during exacerbations, contributing to clinical heterogeneity and variable treatment responses.

Pathologically, COPD is characterized by 2 main features: chronic bronchitis and emphysema. Chronic bronchitis is characterized by thickened airway walls, increased goblet cells, excess mucus, and impaired mucociliary clearance, leading to airflow obstruction. Emphysema, on the other hand, leads to irreversible destruction of alveolar walls, loss of elasticity, and enlarged air spaces that affect gas exchange [29,30]. Additional pathological features include small airway fibrosis, smooth muscle hypertrophy, vascular remodeling, and infiltration of inflammatory cells in the airway wall and lung parenchyma. The progression of the disease is characterized by recurrent exacerbations. These exacerbations are often caused by viral or bacterial infections and environmental pollutants. Acute exacerbations are accompanied by transient surges in airway and systemic inflammation, often marked by increased neutrophil activation and cytokine release. Repeated episodes speed up lung function decline, raise the risk of death, and

add to the overall disease burden [31–33]. Frequent exacerbators represent a distinct clinical phenotype with worse prognosis and higher healthcare utilization.

1.3.3. Immune cell activity

Inflammation in COPD involves both innate and adaptive immune responses. Neutrophils are key innate immune cells present in the airways and blood of many COPD patients, contributing to tissue damage through the release of proteases, reactive oxygen species, and neutrophil extracellular traps. In addition to their tissue-destructive capacity, activated neutrophils contribute to mucus hypersecretion and impair host defense by altering epithelial barrier integrity. An important factor in lung damage is the imbalance of protease activity from neutrophils [34]. Enzymes like neutrophil elastase and matrix metalloproteinases, when released excessively, degrade the extracellular matrix and damage alveolar walls [34]. This proteolytic activity is closely linked to emphysema and lasting airflow obstruction. Furthermore, repeated injury and abnormal repair lead to airway remodeling, fibrosis, and small airway narrowing, worsening the disease [34]. Persistent neutrophil activation is also associated with increased bacterial colonization and recurrent exacerbations. Macrophages in COPD also display altered polarization states, contributing to impaired resolution of inflammation.

Alveolar macrophages increase in number and show altered functions, including reduced phagocytosis and increased production of inflammatory mediators. These dysfunctional macrophages are central to maintaining chronic inflammation and increasing infection risk [35]. Adaptive immune responses also play a significant role in COPD. Lung tissue exhibits higher levels of $CD8^+$ cytotoxic T lymphocytes, which are associated with severe forms of the disease and are implicated in alveolar cell death and tissue damage [36]. $CD4^+$ T helper cells, especially Th1 and Th17 subsets, contribute to ongoing inflammation by releasing interferon-γ and IL-17, respectively, which primarily lead to neutrophil recruitment and are linked to resistance to steroids [35]. These adaptive immune responses often persist even after smoking cessation, indicating long-lasting immune imprinting in the lung microenvironment. The detection of lymphoid follicles containing B cells and plasma cells in some airways of a subset of patients suggests that humoral immunity might also be connected to the development of COPD [37]. These tertiary lymphoid structures are associated with chronic antigen exposure and may contribute to sustained local immune activation.

Innate lymphoid cells (ILCs), particularly group 3 innate lymphoid cells (ILC3s), have also emerged as important contributors to COPD-associated inflammation. ILC3s respond to IL-23 stimulation by producing IL-17A and IL-22, thereby promoting neutrophil recruitment and amplification of airway inflammation. Increased IL-17-producing ILC populations have been associated with cigarette smoke-induced immune dysregulation, neutrophilic inflammation, and disease severity in COPD, highlighting the contribution of innate IL-17-producing cells alongside Th17 lymphocytes in COPD pathogenesis [38,39] .

Among the immune cells contributing to COPD pathogenesis, neutrophils represent a dominant inflammatory population and are strongly associated with disease severity, mucus hypersecretion, and corticosteroid resistance.

1.4. Neutrophils

Neutrophils are the most abundant circulating leukocytes and serve as a vital first line of defense in innate immunity [40,41]. They promptly respond to tissue damage or infection signals, carrying out vigorous effector actions to eliminate pathogens [40]. The key effector functions include phagocytosis, degranulation, and generation of reactive oxygen species (ROS) [42]. Neutrophils internalize pathogens and degrade them using hydrolytic enzymes and ROS [43]. Degranulation releases antimicrobial peptides, proteases, and enzymes such as the serine proteases, neutrophil elastase, cathepsin G, and proteinase 3, the cationic antimicrobial defensins, myeloperoxidase (MPO), lactoferrin, lysozyme, and matrix metalloproteinase-9 (MMP-9) from distinct granule subsets that contribute to microbial killing and tissue remodeling [44]. ROS generation through the NADPH oxidase complex provides an additional and crucial antimicrobial pathway, particularly effective against engulfed pathogens [45]. Neutrophils can also form neutrophil extracellular traps (NETs), extracellular web-like structures composed of DNA, histones, and antimicrobial proteins that immobilize and neutralize microbes [46].

In addition to frontline host defense, neutrophils maintain tissue and immune homeostasis under steady-state conditions. They continuously migrate through tissues, clearing debris and interacting with macrophages and dendritic cells to influence immune regulation [47]. Neutrophils also contribute to tissue repair, supporting angiogenesis and remodeling through

factors such as VEGF and matrix [48,49]. During inflammation, neutrophils are rapidly recruited to infected or damaged tissues in response to chemokines such as CXCL8, complement fragments, and formyl peptides [41]. At inflammatory sites, they deploy antimicrobial strategies including ROS production, degranulation, and NET release to eliminate pathogens [43]. Neutrophils also secrete cytokines and chemokines that amplify immune responses and recruit additional immune cells, thereby shaping the inflammatory environment [50]. Interactions between neutrophils, endothelial cells, platelets, and lymphocytes influence vascular permeability, thrombosis, and the transition from innate to adaptive immunity [51]. However, excessive or uncontrolled neutrophil activation contributes to pathology. For example, in acute lung injury where high ROS and protease production damage epithelial barriers [52].

1.5. IL-23 and IL-17A

1.5.1. History

In the late 1990s, it was found that using IL-12p40 neutralizing antibodies or disrupting the IL-12p40 gene to block IL-12 signaling provides protection in models of autoimmune diseases [53–55]. However, targeting the other IL-12 subunit, IL-12p35 worsened disease outcomes [56]. This was clarified through a computational sequence screening, which showed that the IL-12p40 subunit can form dimers not only with IL-12p35 but also with another subunit, p19. The resulting heterodimer composed of IL-12p40 and p19 was termed IL-23. Thus, a novel member of the IL-12 cytokine family, now known as IL-23, was discovered by Oppmann et al. in 2000 [57]. This discovery helped resolve discrepancies in earlier IL-12 functional studies and led to the recognition of IL-23 as a key driver of chronic inflammatory and autoimmune pathology rather than classical Th1-mediated immunity.

Interleukin-17A (IL-17A), originally known as cytotoxic T-lymphocyte-associated antigen 8 (CTLA-8), was first discovered in 1993 at the transcriptional level in a rodent T-cell hybridoma by Rouvier et al. [58]. IL-17A is the first IL-17 cytokine family member to be discovered. Human and mouse IL-17A were subsequently cloned [59,60]. The initial functional studies showed that IL-17A is a strong proinflammatory cytokine that can induce IL-6 and other inflammatory mediators owing to its role in immune regulation and host defense [60,61]. Eventually, IL-17A was identified as the key cytokine of the Th17 subset of $CD4^+$ T helper cells,

which are distinct from Th1 and Th2 lineages and play a key role in inflammation associated with various autoimmune and inflammatory diseases [62,63]. The subsequent identification of the IL-17A provided a mechanistic framework linking IL-23 signaling to IL-17A-driven chronic inflammation.

1.5.2. Cellular sources and synthesis

IL-23 is a cytokine that plays an important role in the immune system. It is classically secreted by antigen-presenting cells (APCs) such as dendritic cells, macrophages, and monocytes [64] in response to pathogen-associated molecular patterns and other immune signals, and contributes to the T helper (Th) 1 and Th17 immune response [57,64,65]. This process is essential for initiating and maintaining immune responses crucial in inflammation and autoimmunity [56]. IL-23 expression is tightly regulated by pattern recognition receptors, including Toll-like receptors and NOD-like receptors, which integrate microbial and danger signals in inflamed tissues. Recent research has expanded the understanding of IL-23 sources in chronic inflammatory diseases and infections. Notably, airway epithelial cells have been shown to produce IL-23, particularly in respiratory diseases such as COPD and severe asthma [66]. Upon chronic exposure to proinflammatory mediators or acute viral infections, these epithelial cells release IL-23, promoting the activation and expansion of Th17 cells [66]. Similarly, keratinocytes in skin diseases [67] and even neutrophils, under certain inflammatory conditions, have been observed to be sources of IL-23 [68,69]. This expands the understanding of IL-23's role beyond traditional sources, highlighting its involvement in pathological immune responses across different tissues. The production of IL-23 by non-APC sources including epithelial cells, highlights its importance in chronic inflammatory disease development, positioning it as a key target for treatments that aim to control inflammation in these conditions. Persistent IL-23 production contributes to the maintenance of pathogenic Th17 responses and the amplification of local inflammatory circuits within affected tissues.

For IL-17A, the primary source is Th17 cells. However, recent studies indicate that IL-17A can be synthesized by a heterogeneous network of immune cells beyond classical Th17 lymphocytes, like innate-like lymphocytes such as γδ T cells and type 3 innate lymphoid cells (ILC3). Under inflammatory conditions, neutrophils have also been reported to release IL-17A and IL-17RC, enabling autocrine functions like reactive oxygen species generation and antimicrobial responses [70]. Immunohistochemical analysis of COPD lung tissue shows that

IL-17A is produced not only by Th17 lymphocytes but also by neutrophils and macrophages in the small airway subepithelium [71]. Experimental emphysema murine models show that γδ T cells are a major source of IL-17A, which initiates early inflammatory responses and supports neutrophil-related pathways in the lung [72]. Altered ILC3 populations in inflammatory conditions can produce IL-17A and are associated with neutrophilia and increased disease severity in COPD [38]. These findings collectively show that, although Th17 cells are major contributors to IL-17A production, IL-17-driven inflammation such as COPD likely reflects coordinated contributions from both adaptive and innate immune cell populations. The multiplicity of IL-17A–producing cell types underscores the redundancy and robustness of IL-17-associated inflammatory circuits in chronic lung disease.

1.5.3. Structure, receptors, and signaling pathways

The IL-23 signaling cascade is a tightly regulated pathway that plays a central role in the differentiation and function of Th17 cells, which are critical in autoimmunity and inflammation. The cascade is initiated with the binding of IL-23, a heterodimeric cytokine composed of two subunits: p19, which is unique to IL-23, and p40, which is shared with IL-12 [57], to the IL-23 receptor complex, a heterodimer consisting of IL-23R and IL-12Rβ1 [57,73]. Structurally, IL-23 belongs to the IL-12 cytokine family, whose members share common receptor subunits and exhibit overlapping yet distinct biological functions. This receptor is expressed on various immune cells, including memory T cells, natural killer (NK) cells, dendritic cells, Th17 cells, and innate lymphoid cells (ILCs), especially ILC3. On release from antigen-presenting cells such as macrophages and dendritic cells, IL-23 binds to its receptor complex, initiating the intracellular Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway [74]. The interaction between receptor and ligand causes autophosphorylation and activates JAK2 and TYK2, which subsequently phosphorylate specific tyrosine residues on the cytoplasmic tails of the IL-23 receptor subunits [74]. These phosphorylated residues serve as docking sites for STAT proteins, which are crucial transcription factors involved in cytokine signaling [74]. Among the STAT proteins, STAT3 is recruited to the phosphorylated receptor complex [75]. Upon phosphorylation, STAT3 dimerizes and translocates into the nucleus. In the nucleus, it attaches to specific DNA sequences in the promoter regions of target genes, triggering the transcription of genes that play roles in inflammatory responses, cell survival, proliferation, and differentiation [75]. In addition to STAT3, STAT4 and STAT5 can also be

activated in certain cellular contexts, contributing to the functional heterogeneity of IL-23 responses. The IL-23 signaling pathway also interacts with other cytokine pathways to balance protective immunity and harmful autoimmunity. For example, IL-6 and TGF-β help initiate Th17 cell differentiation, while IL-23 is crucial for their expansion, promotion, and pathogenicity [76]. Additionally, the pathway is subject to regulatory feedback mechanisms, including the induction of Suppressor of Cytokine Signaling (SOCS) proteins. For instance, SOCS3 attaches to JAK2 or the IL-23 receptor complex, blocking STAT3 activation and inhibiting further signaling to prevent excessive inflammation [77,78]. Dysregulation of these feedback loops contributes to sustained IL-23/IL-17 signaling observed in chronic inflammatory diseases such as COPD. These regulatory mechanisms ensure that the immune response is controlled and help prevent potential tissue damage.

IL-17A interacts with a heterodimeric receptor made up of IL-17RA and IL-17RC, found on both immune and non-immune cells [79]. IL-17 receptor family members share a conserved SEFIR (similar expression to fibroblast growth factor genes and IL-17Rs) domain that is essential for downstream adaptor protein recruitment and signal transduction. Normally, IL-17A mainly targets epithelial cells and myeloid cells such as monocytes, macrophages, and dendritic cells, aiding in host defense and tissue surveillance [80]. During inflammatory states, increased IL-17A expands and intensifies its cellular targets to include neutrophils, fibroblasts, endothelial cells, and smooth muscle cells, thereby boosting inflammatory responses throughout tissues [81].

Activation of the IL-17 receptor complex triggers ACT1-dependent signaling pathways, which then activate NF-κB, MAPK, and C/EBP pathways [66]. ACT1 functions as a central adaptor molecule that couples IL-17 receptor engagement to downstream pro-inflammatory gene transcription and mRNA stabilization. This activation leads to the production of pro-inflammatory cytokines like CXCL1, CXCL8 (IL-8), and granulocyte colony-stimulating factor (G-CSF), which promote neutrophil recruitment and activation [82]. In addition to cytokine induction, IL-17A signaling enhances the stability of inflammatory mRNAs, thereby prolonging inflammatory mediator production at sites of tissue injury.

Although IL-17A classically signals via ACT1-dependent NF-κB and MAPK pathways, several studies demonstrate indirect and direct involvement of the JAK/STAT. IL-17A itself promotes the production of IL-6 and other cytokines that trigger JAK/STAT activation, thus indirectly

enhancing STAT-dependent inflammatory responses [83]. Another study demonstrated that IL-17A can trigger tyrosine phosphorylation of several JAKs (JAK1, JAK2, JAK3, TYK2) and STATs (STAT1, STAT2, STAT3, STAT4) in U937 human monocytic cells. This finding supports the idea that IL-17A directly activates JAK/STAT signaling, independently of IL-23/Th17 differentiation [84]. It has also been reported that IL-17A activates JAK/STAT signaling to affect drug-metabolizing enzymes and transporters in HepaRG cells [85]. This indicates that IL-17A signaling can interact with the JAK/STAT pathway in a cell-type- and context-dependent manner, extending beyond its canonical signaling pathway. This signaling plasticity may contribute to heterogeneous therapeutic responses observed in IL-17A–targeted and JAK-inhibitor–based interventions.

1.6. The IL-23/IL-17A axis in IL-17 driven inflammation

While Th17 cells contribute to protection and tissue homeostasis under physiological conditions [86], their activation by IL-23 leads to sustained inflammation and prolonged tissue damage. This dual role highlights the context-dependent nature of Th17 responses, which can be either protective or pathogenic depending on the inflammatory microenvironment. Alongside other cytokines like IL-6, TGF-β, IL-1β, and IL-21, IL-23 promotes and maintains the differentiation of naive CD4+ T cells into Th17 cells. IL-23 and T cell receptor engagement in response to external stimuli, along with cytokine signaling from TGF-β, IL-1, and IL-6, activate the master transcription regulator Retinoic acid receptor-related orphan receptor gamma t (RORγt). This initiates Th17 polarization and promotes Th17 family cytokine and IL-23R expression [65,87]. Additional transcription factors, including BATF and IRF4, cooperate with RORγt to stabilize Th17 lineage commitment. This is followed by IL-23 binding to IL-23R, activating the transcription factor STAT3, which enhances the transcription of IL-23R and RORγt. Consequently, IL-23 creates a positive feedback loop that promotes a more pathogenic phenotype in Th17 cells by supporting their polarization and survival [65], as well as the expression of pro-inflammatory cytokines, while suppressing anti-inflammatory cytokines such as IL-10 [88]. This self-amplifying IL-23/IL-17 axis contributes to the persistence of inflammatory responses and limits the resolution of inflammation.

Upon activation by IL-23, Th17 cells secrete a range of cytokines including IL-17A, IL-17F, IL-22, GM-CSF and TNF-α [75]. These cytokines act on epithelial, endothelial, and stromal

cells to amplify inflammatory signaling and reshape the local tissue microenvironment. In addition they mediate neutrophilic inflammation by promoting neutrophil recruitment to sites of infection and tissue damage [75,89]. IL-17A and IL-17F are potent inducers of chemokines like IL-8/CXCL8, which attract neutrophils and amplify the inflammatory response [89,90]. Besides chemokine induction, IL-17 family cytokines enhance granulopoiesis by promoting G-CSF production, further increasing circulating neutrophil numbers. Although the neutrophilic response plays a crucial role in defending against infections, excessive IL-23-driven Th17 polarization can cause chronic inflammation in autoimmune diseases like psoriasis, rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis. In these conditions, continuous recruitment and activation of neutrophils by Th17 cytokines lead to tissue damage and worsening of the disease [68,91,92]. These pathogenic processes are frequently associated with resistance to conventional anti-inflammatory therapies, highlighting the clinical relevance of targeting the IL-23/IL-17 axis. IL-23-driven Th17 polarization also contributes to chronic lung inflammation seen in conditions like COPD and severe asthma. In the lungs, cytokines produced by Th17 cells lead to ongoing neutrophil recruitment and activation, which causes persistent neutrophilic inflammation, airway remodeling, and continuous impairment of lung function [17,90]. Sustained activation of this axis contributes to steroid-resistant inflammatory phenotypes and disease progression in chronic airway diseases.

1.7. The IL-23/IL-17A axis in COPD

1.7.1. IL-23/IL-17A signalling and downstream mechanisms in COPD

In COPD, exposure to stimuli like cigarette smoke, oxidative stress, and microorganisms eventually leads to the activation of APCs, leading to the release of IL-23 [26,93]. Airway epithelial cells can also contribute to IL-23 production in response to chronic injury and infection, further amplifying local Th17-supportive environments. APC-derived IL-23 binds to its receptor on T helper cells, promoting Th17 polarization and stabilizing ILC3 cells [19]. Meanwhile, TGF-β increases IL-23R expression, which helps drive the differentiation process [94], wherein IL-23 primarily supports Th-17 cell survival, expansion, and maintenance [19]. This establishes a sustained inflammatory niche within the airway mucosa that favors persistent Th17 activity. Th17 cells produce IL-17A, leading to activation of epithelial cells. This results in the release of chemokines like CXCL1 and CXCL8/IL-8 from these cells, which help sustain

and attract neutrophils to the airways [19]. IL-17A also enhances G-CSF production, promoting neutrophil production in the bone marrow and contributing to systemic neutrophilia. Besides the NF-κB and MAPK pathways [5], JAK/STAT signaling plays a vital role in these processes, with IL-23 signaling through JAK2-TYK2, which then activates STAT3 and STAT4 to promote Th17 polarization [17]. Sustained activation of these pathways contributes to chronic neutrophilic inflammation and steroid-resistant disease phenotypes in COPD.

1.7.2. Role of IL-23/IL-17A in COPD pathogenesis and progression

Lung inflammation and the development of emphysema in COPD are believed to be regulated by this IL-23/IL-17 pathway [95]. Higher serum levels of IL-23 have been observed in patients with COPD [96]. Serum IL-23 levels are positively correlated with GOLD grading, mMRC scores (Modified Medical Research Council), and a longer clinical medical history. Conversely, they are negatively correlated with FEV1/FVC and FEV1% [96]. These associations suggest that IL-23 may serve as a biomarker of disease severity and progression. IL-23 levels in sputum show a significant correlation with the percentage of neutrophils in both healthy individuals and COPD patients. Furthermore, IL-23 expression is elevated in patients with neutrophilic inflammation compared to those with eosinophilic airway inflammation [97]. This supports the link between IL-23 signaling and the neutrophilic endotype of COPD. IL-23 has also been associated with mucus production in COPD, especially evident in murine models wherein Mucin-5B (Muc5B) −/− mice, apoptotic macrophages accumulated in the lungs, and IL-23 production was decreased [98], whereas in Muc5b transgenic mice, IL-23 production was higher, along with improved macrophage activity [98]. These findings highlight the interplay between mucus biology, macrophage clearance, and IL-23-driven immune regulation.

Studies suggest that increased IL-23 expression in the bronchial mucosa of stable COPD patients might play a role in the development of the disease, as a higher number of IL-23-positive cells have been observed in the bronchial epithelium and submucosa of COPD patients compared to non-smokers [99]. This indicates that IL-23 signaling is active even in stable disease and may contribute to subclinical inflammation. Meanwhile, the study from Liang et al. highlights IL-23's key role as a mediator in cigarette smoke-induced lung inflammation, a hallmark of COPD. In a mouse model, IL-23, produced by activated dendritic cells after cigarette smoke exposure, promoted the differentiation of innate lymphoid cells into NCR− ILC3s. These NCR−ILC3s released IL-17A, which promotes neutrophilic inflammation and

contributes to chronic lung damage. The IL-23/ILC3 pathway may be a key mechanism behind immune imbalance and tissue injury in smoking-related COPD [100]. This provides evidence that IL-23 drives both adaptive and innate lymphoid inflammatory circuits in COPD. Recent studies have demonstrated that inhibiting IL-23 signaling using a monoclonal antibody in a mouse model of emphysema significantly reduced smoke-induced emphysema, as well as immune cell infiltration, oxidative stress, and apoptosis [101]. These preclinical findings support IL-23 as a disease-modifying target rather than solely a symptom-modifying mediator.

IL-17A has been reported to be increased in COPD patients and associated with disease progression in COPD. IL-17A expression is observed to be elevated in lung tissue and linked with severe airflow limitation and lymphoid neogenesis in advanced COPD patients [102]. Lymphoid neogenesis reflects persistent antigen-driven immune activation within the lung microenvironment. IL-17A is primarily known to promote neutrophil recruitment in COPD pathogenesis by inducing neutrophil-recruiting chemokines and cytokines such as IL-8, CXCL1, and GM-CSF from airway epithelial and structural cells, as discussed earlier. Experimental studies show IL-17A is also essential for smoke- and pathogen-related neutrophilia, and that neutralizing IL-17A significantly reduces neutrophil recruitment, underscoring its significant role in COPD [103]. These findings position IL-17A as a key amplifier of innate immune cell recruitment during both stable disease and exacerbations.

It has been reported that patients with COPD exhibit elevated IL-17A levels in sputum and serum, which correlate with disease severity and neutrophilic infiltration into the airways, suggesting that IL-17A contributes to persistent inflammation and exacerbation risk [104]. Elevated systemic IL-17A levels also indicate spillover of airway inflammation into the circulation, linking pulmonary and systemic disease manifestations. In murine models using COPD-relevant stimuli, it has been shown that IL-17A/IL-17RA axis-driven airway inflammation and fibrosis can be disrupted by genetic or antibody-mediated targeting of the pathway [105]. This supports a causal role of IL-17A signaling in airway remodeling and fibrotic progression.

Despite IL17A's primary role in neutrophil recruitment and worsening of COPD, some studies suggest a protective function of other Th17 cytokines, particularly IL-22. In a cigarette smoke (CS)-induced murine model with bacterial infection, defective IL-22 production was observed in conventional T cells [106], while exogenous administration of IL-22 protected CS-exposed

mice from bacterial infection. This was accompanied by reduced IL-23 production in alveolar macrophages and dendritic cells of CS-exposed mice. Likewise, in COPD patients, the Th17 cytokine responses (IL-22, IL-17A) to bacterial infections were also diminished, which correlated with lower IL-23 production from peripheral blood mononuclear cells (PBMCs) [106]. These findings suggest that impaired IL-23/IL-17 axis regulation may compromise antimicrobial defense in COPD. Therefore, a delicate balance exists between the different Th17 cytokines, with IL-23 likely serving as the primary polarizing cytokine. The study by Mardi et al. found that in patients with moderate and severe COPD, nanocurcumin supplementation significantly reduced IL-23 levels. This reduction downregulated Th17 cells and related pro-inflammatory cytokines such as IL-17. The modulation of the IL-23/IL-17A axis decreased systemic inflammation, further highlighting the importance of targeting this axis in COPD patients [107]. These findings support the concept that partial modulation, rather than complete blockade, of the IL-23/IL-17 axis may offer therapeutic benefit while preserving host defense.

It has been reported that an IL-17A airway epithelial gene expression signature defines a specific COPD patient subgroup characterized by higher neutrophil levels, more airway obstruction, and reduced response to corticosteroids, regardless of eosinophilic or type 2 inflammation. This indicates IL-17A's role in steroid resistance [108]. This molecular stratification highlights the potential of IL-17A–related biomarkers for patient endotyping and treatment selection. Studies in airway cells and disease models show that IL-17A can work together with glucocorticoids to activate neutrophil-promoting genes like CSF3. This interaction may weaken the effectiveness of glucocorticoids and promote ongoing neutrophilic inflammation [109]. This synergistic pro-neutrophilic effect provides a mechanistic explanation for glucocorticoid resistance in IL-17A–high COPD.

These findings support the broader idea of an IL-23/IL-17A axis, where IL-23 maintains pathogenic IL-17 responses that lead to ongoing neutrophilic inflammation and tissue injury. In COPD, the IL-23/IL-17-axis driven neutrophilic response is especially significant because neutrophils are key players in mucus hypersecretion, protease release, and oxidative tissue damage. These processes contribute to airway obstruction and resistance to steroids. IL-17A's role in promoting the production of chemokines and growth factors that attract neutrophils creates a mechanistic link between Th17 cell polarization, IL-23 regulation, and the neutrophilic endotype seen in many COPD patients. Collectively, these mechanisms place the IL-23/IL-17A axis at the center of COPD immunopathogenesis and treatment resistance.

1.8. The IL-23/IL-17A axis as a major driver of steroid resistance

The glucocorticoid receptor (GR) has several isoforms, primarily generated via alternate splicing and translation initiation. The main isoform, GRα, binds glucocorticoids and moves to the nucleus, where it promotes anti-inflammatory gene expression and inhibits pro-inflammatory factors like NF-κB [110,111]. In addition to transrepression of pro-inflammatory transcription factors, GRα can induce the expression of anti-inflammatory mediators such as MKP-1 and GILZ, which further dampen inflammatory signaling cascades. In contrast, GRβ results from alternative splicing at exon 9β and acts as a dominant-negative inhibitor: it can bind to DNA response elements but lacks the ligand-binding domain, which prevents glucocorticoid binding [112]. GRβ can also sequester transcriptional coactivators, thereby further limiting GRα–mediated gene regulation. Other isoforms, such as GRγ, GRA, and GRP, show reduced transcriptional activity. Nonetheless, their relative abundance can regulate an individual's sensitivity to glucocorticoids. Cell type–specific expression patterns of GR isoforms further contribute to heterogeneous steroid responses across different immune and structural cell populations in the lung. Epigenetic regulation and microRNA-mediated control of GR isoform expression have also been implicated in shaping cell-specific steroid responsiveness.

A lower GRα/GRβ ratio characterizes corticosteroid resistance and can arise from increased GRβ levels, decreased GRα levels, or a combination of both [113]. Reduced corticosteroid sensitivity—particularly in immune and epithelial cells—is frequently caused by inflammatory signals or oxidative stress. These factors can shift isoform balance toward GRβ or hinder GRα signaling via mechanisms like phosphorylation, disrupted coregulator interactions, or decreased HDAC2 activity, leading to resistance [111,113]. Post-translational modifications of GRα, including phosphorylation and nitrosylation, further modulate receptor stability and transcriptional competence under inflammatory conditions. Additional mechanisms, including altered nuclear translocation of GRα and impaired glucocorticoid-induced transrepression of inflammatory genes, further contribute to reduced steroid efficacy in chronic inflammatory lung disease. Defective resolution pathways, including impaired induction of anti-inflammatory macrophage phenotypes, also contribute to persistent steroid insensitivity in chronic airway inflammation. Recent evidence suggests that IL-23 is crucial in developing corticosteroid resistance in lung inflammation by affecting the functions of both immune and structural cells. In peripheral blood mononuclear cells from asthma patients, IL-23 combined with IL-17A

increases GRβ expression and lowers the GRα/GRβ ratio, which reduces the effectiveness of dexamethasone and weakens the suppression of inflammatory genes [114]. This mechanism provides a molecular link between IL-17-driven inflammation and steroid-refractory disease phenotypes. Besides affecting immune cells, IL-23 and IL-17A make airway structural cells like fibroblasts and endothelial cells resistant to steroids by blocking glucocorticoid-triggered cell death. This resistance leads to ongoing inflammation and plays a role in airway remodeling, a characteristic feature of severe, treatment-resistant asthma [115]. In addition, IL-17A–mediated activation of MAPK pathways can directly antagonize GRα transcriptional activity, further weakening steroid responsiveness in inflamed lung tissue.

The significance of IL-23 signaling in corticosteroid resistance has also been shown in vivo. In mouse asthma models, IL-23 expression in the epithelium rises during allergen exposure. Blocking IL-23 pharmacologically results in notable decreases in airway inflammation, mucus secretion, and levels of Th2/Th17 cytokines—characteristics linked to corticosteroid-resistant disease types [66]. Furthermore, in chronic and fibrotic lung diseases, heightened activity of the IL-17/IL-23 pathway has been linked to higher GRβ levels and reduced responsiveness to corticosteroids [116]. Mechanistically, this resistance is enhanced via downstream signaling pathways such as STAT3 and MAPK, along with decreased HDAC2 activity, all of which hinder GRα function and interfere with normal glucocorticoid signaling [11,116]. These pathways converge to create a feed-forward loop in which persistent inflammation further suppresses steroid sensitivity, thereby perpetuating disease severity. These findings collectively emphasize IL-23 as a central driver of corticosteroid resistance in various cellular compartments and disease settings, reinforcing its promise as a therapeutic target in severe, treatment-resistant airway inflammation.

Any therapeutic strategy targeting this axis must therefore balance suppression of pathogenic inflammation with preservation of antimicrobial host defense, a particularly critical consideration in infection-prone COPD patients.

1.9. Therapeutic management of COPD and limitations

Currently, there is no cure for COPD. Available treatments include bronchodilators, corticosteroids, and antibiotics [117]. Small-molecule anti-inflammatory drugs, such as phosphodiesterase 4 (PDE4) inhibitors, are also used to reduce exacerbations [118]. Long-

acting bronchodilators, such as β_2-agonists and muscarinic antagonists, are essential in managing COPD because they relax airway smooth muscle and enhance airflow. Systemic and inhaled corticosteroids (ICS) are primarily used during acute exacerbations to reduce inflammation and improve lung function. Inhaled corticosteroids (ICS) are administered in combination with long-acting bronchodilators in stable COPD with eosinophilic inflammation to reduce exacerbations [117]. However, COPD inflammation is mainly neutrophilic and resistant to steroids, which reduces the effectiveness of corticosteroids [119]. Long-term ICS increases pneumonia risk, and repeated use of systemic steroids can cause systemic toxicity [120]. Since corticosteroids don't prevent disease progression or reverse lung damage, alternative therapies for steroid-resistant inflammation are needed. Antibiotics are also used in COPD to manage acute exacerbations associated with bacterial infections. Common agents used include amoxicillin–clavulanate, macrolides (like azithromycin), doxycycline, and respiratory fluoroquinolones [121]. Macrolides are also prescribed long-term in selected patients for their immunomodulatory properties, which help reduce exacerbation frequency by suppressing neutrophilic inflammation, but prolonged use leads to the development of antibiotic resistance [122]. Besides antibiotic resistance, there are other limitations due to overuse of antibiotics, such as limited efficacy against non-bacterial infections, and risks of toxicity and microbiome disruption [123,124]. Therefore, none of the current treatments can reverse lung structural damage or improve the long-term decline in lung function associated with COPD. These limitations highlight the need for mechanism-based therapies that address disease-driving inflammatory pathways rather than only symptom control.

1.10. Emerging therapeutic targeting IL-23/IL-17A axis

IL-23

Given the established role of the IL-23/IL-17A axis in the pathogenesis and corticosteroid resistance in COPD, it represents a potential therapeutic target for the development of precision medicines, particularly for inflammation-driven phenotypes. While IL-23 Monoclonal antibodies that selectively inhibit the IL-23 p19 subunit, like guselkumab [125], risankizumab [126], tildrakizumab [127], mirikizumab [128] as well as the IL-12/IL-23 p40 inhibitor ustekinumab [129] have been quite effective in psoriasis and autoimmunity, there are limited studies in the context of lung inflammation. CS- and elastase-induced emphysema models in

mice demonstrated that IL-23-neutralizing antibodies can reduce the severity of the disease [101]. However, so far, there is no clinical evidence that IL-23 antibodies are effective in treating COPD, and they are limited by the risks of increased infections resulting from reduced Th17-mediated antimicrobial defense [130]. This underscores the challenge of balancing inflammation suppression with preservation of host defense in the lung.

IL-17A

Similarly, IL-17-neutralizing antibody treatment, even after lung damage had been established, showed effectiveness in a CS-induced in vivo model [131]. However, clinical trials in asthma using IL-17A pathway blockade (e.g., anti-IL-17RA brodalumab) failed to improve lung function or symptom control, indicating that IL-17A is not a dominant disease driver in heterogeneous airway diseases [132]. Secukinumab, a human IgG1κ monoclonal antibody targeting IL-17A, is currently approved for treating plaque psoriasis and psoriatic arthritis. However, in a Phase II study evaluating its safety, tolerability, and efficacy in patients with severe asthma, no improvement in the "Asthma Control Questionnaire" was observed, and the study was discontinued by the manufacturer [133]. This is the only known clinical study of secukinumab in severe asthma.

Another major limitation is that IL-17A inhibition is consistently associated with increased respiratory infections in COPD patients [130]. In a study by Christeson et al., COPD patients were primarily identified as having an IL-17A-high molecular phenotype characterized by neutrophilia and reduced corticosteroid responsiveness, but in vivo IL-17A blockade did not restore steroid resistance [134]. Consequently, despite a few promising preclinical data, clinical evidence supporting IL-17A antibodies in COPD remains weak. These findings suggest that IL-17A may act more as an amplifier of disease rather than a single dominant driver in COPD pathogenesis.

JAK inhibitors

Recent preclinical studies have demonstrated that JAK inhibitors can reduce lung inflammation and tissue damage in COPD models [135–137]. Furthermore, inhaled frevecitinib (KN-002; NCT05006521) was well tolerated by COPD patients and outcomes support further evaluation to determine its clinical efficacy [138]. Inhaled delivery may reduce systemic immunosuppression and infection risk compared to systemic JAK inhibition. Beyond COPD, JAK inhibitors such as tofacitinib, baricitinib, and upadacitinib have been approved for

autoimmune diseases, including rheumatoid arthritis, psoriatic arthritis, ulcerative colitis, and atopic dermatitis, due to their high efficacy in targeting neutrophilic inflammation driven by the IL-23/IL-17A axis [139]. Taken together, these observations underscore the need for more detailed exploration of JAK inhibitors as potential therapies for neutrophilic inflammation in COPD driven by the IL-23/IL-17A axis. Targeting shared downstream signaling nodes may offer broader anti-inflammatory effects than cytokine-specific blockade.

To summarize, in the current scenario, COPD remains a heterogeneous disease, with current therapies often failing to comprehensively target the various inflammatory endotypes and molecular mechanisms that drive its progression. Many patients continue to experience steroid-resistant inflammation, persistent symptoms, and frequent exacerbations despite optimal treatment. Existing therapies mainly focus on symptom relief, exercise, and preventing exacerbations, but don't target underlying disease mechanisms. Therefore, there's increasing interest in developing precision medicine and treatments that address these core mechanisms. Stratifying patients based on molecular endotypes, such as IL-23/IL-17A–high neutrophilic inflammation, may improve therapeutic efficacy and reduce unnecessary immunosuppression.

1.11. Research gaps and rationale of the thesis

Despite extensive evidence linking the IL-23/IL-17A axis to neutrophilic airway inflammation in COPD, several key mechanistic aspects remain unresolved. In particular, it remains unclear whether IL-23 and IL-17A directly reprogram human neutrophil effector functions under COPD-relevant conditions or primarily act indirectly via epithelial and stromal intermediates. Furthermore, although IL-23 classically signals via JAK2 and TYK2 in lymphoid cells, the extent to which TYK2 and JAK1 signaling pathways are engaged in neutrophils in smokers and COPD patients has not been systematically investigated. Importantly, the contribution of TYK2/JAK1 activation to the development of steroid-resistant neutrophilic inflammation in COPD remains poorly defined. These gaps limit the translation of IL-23/IL-17A biology into effective, mechanism-based therapies for neutrophilic COPD endotypes.

Investigating whether IL-23 and IL-17A directly affect neutrophil functions, along with the roles of TYK2 and JAK1 signaling pathways and glucocorticoid receptor expressions in neutrophils from smokers and COPD patients, could provide valuable mechanistic insights into the factors driving steroid-resistant inflammation in COPD. Additionally, pharmacological

inhibition of TYK2 and JAK1 could help determine the functional importance of these signaling pathways in regulating neutrophilic inflammation in COPD. Gaining a better understanding of these pathways will assist in identifying new therapeutic targets within the IL-23/IL-17A axis and support the development of more effective treatment strategies for neutrophilic COPD endotypes.

2. Hypothesis

I hypothesize that the IL-23/IL-17A axis directly primes neutrophil effector functions in COPD and that this process is mediated through dysregulated JAK/STAT signaling. Furthermore, pharmacological inhibition of JAK signaling may attenuate pathogenic neutrophilic inflammation while preserving essential host defense mechanisms. Therefore, this thesis is based on the following hypotheses:

1. The IL-23/IL-17A axis primes neutrophil effector functions in airway inflammation
2. The JAK/STAT pathway is dysregulated in patients with neutrophilic respiratory inflammation
3. Pharmacological inhibition of JAK signaling may complement existing therapeutic strategies in COPD by attenuating pathogenic inflammatory signaling with the potential to preserve essential host defense mechanisms.

3. Aim and objectives

The aim of this thesis is to investigate the role of JAK inhibition in IL-23/IL-17A-driven neutrophilic inflammation in COPD, with a focus on identifying mechanistic links between cytokine signaling, neutrophil function, and steroid resistance. To achieve this aim, the following objectives were addressed:

1. Evaluate JAK and phosphor-JAK protein abundance in COPD patients and smokers.
2. Investigate the contribution of the IL-23/IL-17A axis to neutrophil effector function including migration, activation, and inflammatory mediator release.
3. Assess the impact of JAK inhibition on neutrophil-mediated inflammatory responses under IL-23/IL-17A-driven inflammatory conditions.

To achieve these objectives, this thesis integrates complementary experimental approaches across human patient samples, in vitro neutrophil and epithelial cell systems, and in vivo airway inflammation models. This integrated strategy enables systematic dissection of IL-23/IL-17A-driven signaling in human neutrophils, including the characterization of TYK2/JAK1 activation in smokers and COPD patients, the functional reprogramming of neutrophil effector responses

by IL-17A cytokine, and the modulation of glucocorticoid receptor isoform balance under inflammatory conditions. By interrogating shared downstream signaling nodes rather than single cytokines, this work provides mechanistic insight into how IL-23/IL-17A-driven inflammatory circuits can be targeted across epithelial, immune, and tissue compartments in COPD.

In this study, we employed a TYK2/JAK1 inhibitor, Brepocitinib, a dual TYK2/JAK1 inhibitor that has demonstrated efficacy in phase II clinical trials for psoriatic arthritis, a disease similarly characterized by IL-23-driven neutrophilic inflammation [140]**.** The use of brepocitinib allows interrogation of shared downstream signaling nodes within the IL-23/IL-17A axis that are not directly targeted by cytokine-specific biologics.

4. Materials

All reagents, antibodies, and kits used in this study are listed below; detailed buffer compositions are provided in Appendix.

Table 1: List of reagents

Reagents	**Catalog number**	**Company**
FIX&PERM	Szabo-Scandic	NORGAS-002-1
dihydrorhodamine-123	D1054	Sigma
Recombinant mouse IL-23	589002	Biolegend
LPS	L-2880	Sigma
Ketamidor 100 mg/mL (ketamine)	_	Vetviva
Rompun 20 mg/mL (xylaxine)	_	Elanco
Human IL-8/CXCL8 DuoSet ELISA kit	DY208-05	R&D Systems
LEGENDplex Human Inflammation Panel 1	740809	Biolegend
Recombinant Human IL-23	11340233	ImmunoTools
Recombinant Human IL-17A	11340174	ImmunoTools
Brepocitinib	S8804	Selleck Chemicals
Deucravacitinib	HY-117287	Medchemexpress MCE
IL-8	11349084	Immunotools
C5a	2037-C5-025	R&D
Polycarbonate filters (3 µm pore size, PVP-free)	PCTF30258050	Sterlitech
Purecol	5005-B	Advanced BioMatrix
Human fibronectin	C-43060	PromoCell
BSA	A7906	Sigma-Aldrich
BD lysing solution	349202	BD
PBMC spin medium	60-00092-12	Pluriselect
PET membrane inserts	3460	Corning

12-well plates	3506	Corning
BEpiCM-b	SCC3211-b	ScienCell Research Laboratories
DMEM	36250	STEMCELL Technologies
BEpiCGS	3262	ScienCell Research Laboratories
HEPES	15630056	Thermo Fisher Scientific
GlutaMAX	35050038	Thermo Fisher Scientific
Penicillin/ streptomycin (Pen/Strep)	SCC0513	ScienCell Research Laboratories
EC-23	4011	Tocris
3R4F reference cigarettes	_	University of Kentucky, Lexington, KY, USA
Triton X-100	T8787	Merck
TrueVIEW® Autofluorescence Quenching Kit	SP-8400	Vector Laboratories
Vectashield Vibrance mounting medium with DAPI	H-1800	Vector Laboratories

Table 2: List of antibodies

Antibody	**Catalog number**	**Company**	**Dilution factor**
Fc receptor blocking solution	422302	Biolegend	1:50
TYK2	NBP2-76968	Novus Biologicals	1:50
pTYK2 (Tyr1054/Tyr1055)	bs-3437R-TR	Bioss	1:50

JAK2	bs-0908R-TR	Bioss	1:50
pJAK2 (Tyr1007/Tyr1008)	bs-2485R-TR	Bioss	1:50
JAK1	bsm-54138R	Bioss	1:50
pJAK1 (Tyr1022/Tyr1023)	bs-3238R-TR	Bioss	1:50
Glucocorticoid receptor α	PA1-516	Invitrogen	1:50
Glucocorticoid receptor β	BS-13385R-TR	Bioss	1:50
AF647-conjugated secondary antibody	A78957	Invitrogen	1:500
CD14 BV421	301830	Biolegend	1:100
CD3 APC-Cy7	300426	Biolegend	1:150
CD8 BV510	344732	Biolegend	1:100
CD4 AF488	317420	Biolegend	1:100
CD16-PerCP-Cy5.5	302009	Biolegend	1:200
CD11b-PE	301305	Biolegend	1:50
True stain fcX	101320	Biolegend	1:100
CD11b (PE-Cy7)	552850	BD Pharmingen	1:200
CD11c (BV421)	562782	BD Pharmingen	1:200
Ly6G (APC)	127614	Biolegend	1:500
CD3 (FITC)	100203	Biolegend	1:100
Siglec-F (PE)	552128	BD Pharmingen	1:100

5. Methods

Some parts of this section have been adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

5.1. Ethical approval

All experiments involving human material were conducted in accordance with the Declaration of Helsinki and were approved by the appropriate Institutional Review Boards and carried out with written consent from all donors prior to sample collection. All samples were processed and analyzed in a coded, pseudonymized manner to protect donor identity. The use of human samples was approved by the Institutional Review Board of the Medical University of Graz (Healthy Blood donors: EK 17-291 ex 05/06; COPD blood donors: 30-537 ex 17/18; lung tissue conditioned media: EK Nr: 1170/2024). Animal experiments were approved by the Austrian Federal Ministry of Science and Research's Animal Ethics Committee, adhering to the European Community's Council Directive (2022-0.137.219). All animal procedures were performed in accordance with national and institutional guidelines for animal welfare and complied with the principles of the 3Rs (Replacement, Reduction, and Refinement).

Primary bronchial epithelial cells were isolated from macroscopically normal lung tissue obtained from patients undergoing resection surgery for lung cancer at the Leiden University Medical Center, the Netherlands. Patients from which this lung tissue was derived were enrolled in the biobank via a no-objection system for coded anonymous further use of such tissue (www.coreon.org). Samples from this Biobank were approved for research use by the institutional medical ethical committee (BB22.006/AB/ab). Since 01-09-2022, patients are enrolled in the biobank using written informed consent in accordance with local regulations from the LUMC biobank with approval by the institutional medical ethical committee (B20.042/KB/kb).

5.2. Blood donors

Blood was collected every day from healthy volunteers for functional assays. All donors were adults and provided written informed consent prior to participation. The volunteers were

classified into allergic and non-allergic donors based on symptoms reported in response to aeroallergens like house dust mite, grass, or tree pollen. The presence of allergen-specific IgE antibodies confirmed allergic sensitization. Total (≥100 kU/l) and specific IgE (class 1: 0,35-0,69 kU/l; class 2: 0,7-3,49 kU/l; class 3: 3,5-17,4 kU/l; class 4: 17,5-49,9 kU/l: class 5: 50-100; class 6 >100) was measured at the Department of Dermatology and Venerology of the Medical University of Graz. Donors were excluded if they reported acute infections, chronic inflammatory diseases, or immunomodulatory medication use at the time of blood donation.

For whole blood staining, blood was collected from individuals with COPD, smokers without COPD, allergic individuals, and their respective controls, such as non-COPD individuals, non-smokers, and non-allergic individuals, respectively. All participants were clinically stable at the time of sampling and had not experienced acute infections within the preceding weeks. The blood donors among these groups are age- and sex-matched to avoid disparity between the groups. Medication use, smoking status, and recent respiratory infections were recorded to account for potential confounders. The diagnosis of COPD and the severity of airflow limitation were established according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) recommendations [21] by respiratory physicians at the Division of Respiratory Medicine at the Medical University of Graz. Clinical data, including lung function parameters and smoking history, were obtained from medical records where available.

Table 1: Demographic table of COPD patients and healthy controls

Parameter	**COPD Patients (n=17)**	**Controls (n=7)**	**p-value**
Age in years (mean ± SD)	64.1 ± 9.3	60.3 ± 7.7	0.29
Sex (Male: Female)	9: 8	4:3	-
Vital Capacity [% predicted]	82.9 ± 20.4	-	-
FVC [% predicted]	47.4 ± 16.8	-	-
FEV1 [% predicted]	45.3 ± 12.3	-	-
Stage / Clinical notes	COPD II–IV (GOLD II–IVE; COPD III–E; mixed COPD/asthma)	-	-

Table 2: Demographic table of smokers and non-smokers

Parameter	Non-smoker (n=10)	Smoker (n=8)	p-value
Age in years (mean ± SD)	35.5 ± 10.9	30.4 ± 6.7	0.24
Sex (Male: Female)	6:4	4:4	—
Cigarettes Smoked (mean ± SD/day)	None	9.3 ± 5.9	—

Table 3: Demographic table of allergic and non-allergic blood donors

Parameter	Non-allergic (n = 6)	Allergic (n = 6)	p-value
Age in years (mean ± SD)	33.5 ± 9.2	27.33 ± 1.36	0.16
Sex (Male: Female)	1:5	3:3	—
IgE (IU/mL)	55.0 ± 54.6	276.2 ± 190.4	0.01

5.3. Preparation of human peripheral blood leukocytes

Peripheral blood leukocytes were isolated from citrated whole blood. Plasma was separated from the blood by centrifugation at 400 × g for 20 minutes at room temperature using a low brake setting. After plasma removal, erythrocyte sedimentation was performed using dextran for 30 minutes at room temperature. The upper leukocyte-rich dextran phase was carefully layered onto 15 mL of PBMC spin medium (Pluriselect) without disturbing the gradient. This was followed by density gradient centrifugation at 400 × g for 20 minutes at room temperature. After centrifugation, the peripheral blood mononuclear cell (PBMC) layer and PBMC spin medium were gently aspirated and discarded. The cell pellet, containing polymorphonuclear leukocytes (PMNL), was resuspended in washing buffer and centrifuged at 400 × g for 7 minutes with maximum brake to remove residual plasma proteins and platelets. To eliminate remaining erythrocytes, the PMNL pellet was subjected to hypotonic lysis by resuspension in 0.2% saline, followed by gentle mixing. The suspension was then made isotonic by adding 1.6% saline. An additional washing buffer was added, and the suspension was centrifuged again at 400 × g for 7 minutes with maximum brake. The final PMNL pellet was collected and used for

functional assays. Neutrophils were identified within PMNL by flow cytometric analysis either by excluding eosinophils based on their characteristic autofluorescence or by using an antibody against the neutrophil surface marker CD16 [141].

5.4. Flow cytometric staining and phospho-flow

Whole blood collected from blood donors was lysed using BD FACS lysing solution. This was followed by cell fixation and permeabilization using FIX&PERM (Mubio). For fixation, Mubio FIX&PERM solution A was added and incubated for 15 minutes in the dark at room temperature. The sample was then centrifuged at 400 × g for 7 minutes, and the supernatant discarded. Next, the cells were permeabilized using Mubio FIX&PERM solution B, added to the cells and incubated for 15 minutes in the dark at room temperature. The cells were centrifuged again at 400 × g for 7 minutes, and the supernatant discarded. The cells were blocked using Fc receptor blocking solution (Biolegend) for 20 minutes at room temperature. Isotype and fluorescence minus one (FMO) controls were included to define background staining and gating thresholds. Intracellular staining was performed using primary antibodies targeting TYK2 (Novus Biologicals), pTYK2(Tyr1054/Tyr1055), JAK2, pJAK2(Tyr1007/Tyr1008), JAK1 and pJAK1(Tyr1022/Tyr1023) (Bioss), and glucocorticoid receptor α/β (Invitrogen/Bioss) for 30 minutes at 4°C. Following this, the samples were centrifuged again at 400 × g for 7 minutes, and the supernatant discarded. The samples were then incubated with the AF647-conjugated secondary antibody (Invitrogen) for 30 minutes at 4°C. The samples were centrifuged again at 400 × g for 7 minutes, and the supernatant discarded. Surface markers were stained using antibodies against CD14 BV421, CD3 APC-Cy7, CD8 BV510, and CD4 AF488 (Biolegend) for 15 minutes at 4°C. The samples were centrifuged once more at 400 × g for 7 minutes, and the supernatant was discarded; the pellet was resuspended in fixation buffer. Compensation controls were prepared using single-stained samples. Finally, the samples were acquired using a BD FACSCanto II flow cytometer and analyzed with FlowJo v10.7.1. Results were reported as ΔGeoMean = GeoMean_stained sample – GeoMean_FMO (mean ± SD) [142]. All analyses were performed using identical gating strategies across donor groups to ensure comparability.

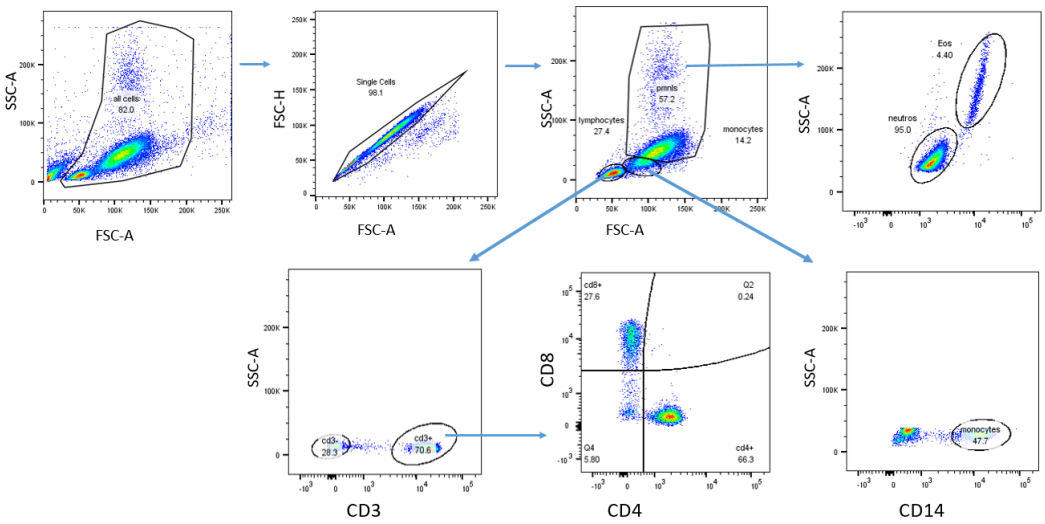
For the flow cytometric staining of the glucocorticoid receptors α/β, PMNLs were first treated with IL-17A and IL-23 (10 ng/mL) in the presence or absence of brepocitinib (300 nM) for 3

hours at 37°C. Vehicle-treated controls were included for all conditions. In another set of experiments, PMNLs were stimulated with other cytokines or chemokines like IL-8 (10 nM) for 1 hour at 37°C or C5a (30 nM) for 20 minutes at 37°C.

This was followed by cell fixation and permeabilization using FIX&PERM (Mubio). For fixation, Mubio FIX&PERM solution A was added and incubated for 15 minutes in the dark at room temperature. The sample was then centrifuged at 400 × g for 7 minutes, and the supernatant was discarded. Next, the cells were permeabilized using Mubio FIX&PERM solution B, added to the cells and incubated for 15 minutes in the dark at room temperature. The cells were centrifuged again at 400 × g for 7 minutes, and the supernatant was discarded. The cells were blocked using Fc receptor blocking solution (Biolegend) for 20 minutes at room temperature. Isotype and fluorescence minus one (FMO) controls were included to define background staining. Intracellular staining was performed using primary antibodies targeting glucocorticoid receptor α/β (Invitrogen/Bioss) for 30 minutes at 4°C. Following this, the samples were centrifuged again at 400 × g for 7 minutes, and the supernatant was discarded. The samples were then incubated with the AF647-conjugated secondary antibody (Invitrogen) for 30 minutes at 4°C. The samples were centrifuged again at 400 × g for 7 minutes, and the supernatant discarded. The samples were centrifuged once more at 400 × g for 7 minutes, and the supernatant discarded and resuspended in fixation buffer. Then the samples were acquired using a BD FACSCanto II flow cytometer and analyzed with FlowJo v10.7.1. Neutrophils were gated out of PMNL by excluding eosinophils based on their autofluorescence. Gating strategies were applied identically across all experimental conditions. Results were reported as fold change in the geometric mean fluorescence intensity (MFI) in response to the secondary antibody [141].

5.5. Cytokine treatments

Human PMNL were stimulated with 10 ng/mL recombinant IL-17A and IL-23, a concentration widely used in neutrophil and airway-inflammation studies [143,144]. This dose is more likely to approximate local cytokine levels within inflamed tissue than systemic concentrations, as serum levels in COPD patients are typically in the pg/mL range [145]. Consistent with this rationale, reported EC_{50} values for IL-17A- and IL-23-receptor activation also fall within the ng/mL range [146–148].



Methods Figure 1. The flow cytometric gating strategy used for the whole blood staining identifying various immune populations like CD3+, CD4+, CD8+, CD14+, neutrophils and

5.6. Migration assay

PMNL were resuspended in assay buffer to attain a concentration of 2 million cells per mL. The assay buffer consists of 90 mg Glucose, 50 mg BSA, and 500 µL HEPES, then the total volume is brought to 50 mL by adding PBS ($+Ca^{2+}/+Mg^{2+}$). Then the PMNL were pretreated with cytokines (IL-17A (10 ng/mL) or/and IL-23 (10 ng/mL)) for 30 minutes in the presence or absence of brepocitinib for 1 hour at 37°C. Vehicle-treated controls were included for all conditions. Cells were added into the upper wells of a 48-well Boyden chamber fitted along with polycarbonate filters (5µm pore size, PVP-free) and migrated towards the stimulants like IL-8 (10 nM), IL-17A (10 ng/mL), IL-23 (10 ng/mL), conditioned media obtained from lung tissue, or PBEC-ALI cultures placed in the lower wells in the Boyden chamber. All conditions were tested in parallel using cells from the same donor to minimize inter-donor variability. The Boyden chamber containing the cells and stimulant was incubated for 60 minutes at 37°C. The cells migrating towards the stimulant into the lower wells were collected and counted using flow cytometry. Neutrophils were gated out of PMNL by excluding eosinophils based on their autofluorescence. Identical gating strategies were applied across all experimental conditions. Results were reported as response normalised to the vehicle response [149]. All migration experiments were performed in technical replicates and repeated with cells from multiple

independent donors. This assay does not distinguish chemotaxis from chemokinesis; therefore, we refer to the response as increased migration.

5.7. CD11b activation assay

PMNLs were freshly isolated and co-cultured for 2 hours with cytokine- and/or brepocitinib-treated PBECs and stained with CD16-PerCP-Cy5.5 for 5 minutes at room temperature, followed by staining with CD11b-PE for 20 minutes at 37°C. Vehicle-treated controls and unstimulated controls were included in each experiment. Cells were fixed with 4% formaldehyde and analyzed using flow cytometry. Results were reported as response normalised to the vehicle control [150]. All conditions were performed in parallel using cells from the same donor to minimize inter-donor variability.

Besides the co-culture setup, PMNL were first treated with cytokines IL-17A (10 ng/mL) and IL-23 (10 ng/mL) for 30 minutes at 37°C, stained with CD16-PerCP-Cy5.5 (to divide them into CD16+ neutrophils and CD16- eosinophils) and CD11b-PE for 5 minutes at room temperature. Then the PMNL are stimulated with serial dilutions of C5a for 20 minutes at 37°C and analyzed by flow cytometry. Results were reported as response normalised to the vehicle control.

5.8. ROS production

To study the priming effects of cytokines on PMNL, cells were pretreated with cytokines such as IL-17A (10 ng/mL) or IL-23 (10 ng/mL) for 30 minutes at 37°C, followed by addition of 1 µM dihydrorhodamine-123 (Fisher Scientific) for 5 minutes at 37°C and then stimulated with serial dilutions of C5a for 20 min at 37°C. Unstimulated and vehicle-treated controls were included to define baseline ROS production.

To further understand the effects of brepocitinib along with the cytokines, PMNL were pretreated with brepocitinib for 1 hour at 37°C and with cytokines like IL-17A (10 ng/mL) or IL-23 (10 ng/mL) in the last 30 minutes of the one hour and incubated with 1 µM dihydrorhodamine-123 (Fisher Scientific) for 5 min at 37°C followed by C5a stimulation for 20 min at 37°C. All treatments were performed in parallel using cells from the same donor to minimize inter-donor variability. Production of reactive oxygen species (ROS) was quantified as the increase in fluorescence in the B530/30 channel due to oxidation of nonfluorescent dihydrorhodamine-123 into fluorescent rhodamine-123 by flow cytometry (BD FACSCanto II)

and analyzed by FlowJo 10.7.1 software. ROS production was expressed as fold change in the geometric mean fluorescence intensity (MFI) in response to the vehicle group. Neutrophils were gated out of PMNL by excluding eosinophils based on their autofluorescence [151]. Identical gating strategies were applied across all conditions, and experiments were repeated with cells from multiple independent donors.

5.9. Calcium flux assay

PMNLs were isolated from peripheral blood samples and subsequently resuspended in assay buffer to achieve a final concentration of 1 × 10^6 cells/mL The assay buffer consists of 90 mg Glucose, 50 mg BSA, and 500 µL HEPES, then the total volume is brought to 50 mL by adding PBS ($+Ca^{2+}/+Mg^{2+}$). For each experimental setup, 500 µL of the cell suspension was mixed thoroughly with 0.5 µL of Fluo-3 AM (at a concentration of 2 mM, obtained from Thermo Fisher Scientific) and an equal volume of Pluronic F-127 (20% in DMSO, sourced from Sigma-Aldrich) and was then incubated at room temperature, in the dark, for 1 hour. PE-Cy5-conjugated anti-CD16 antibody (BioLegend) was added after 50 minutes to gate out neutrophils from PMNL. PMNLs were pretreated for 30 minutes at 37°C with cytokines, either IL-17A (10 ng/mL), IL-23 (10 ng/mL), or a vehicle control. All conditions were performed in parallel using cells from the same donor to minimize inter-donor variability. Following pretreatment, the cells were stimulated with IL-8 at a final concentration of 10 nM to induce calcium flux. As controls, IL-8 acted as a positive stimulant while unstimulated cells served as a negative baseline reference. Fluorescence was recorded continuously to capture peak calcium responses and response kinetics. Calcium mobilization was immediately monitored after stimulation using an Accuri C6 flow cytometer (BD Biosciences), with fluorescence signals detected in the FITC channel [152]. Identical acquisition settings were used for all samples within an experiment.

5.10. Primary Human Bronchial Epithelial Cell (PBEC) culture and co-culture

5.10.1. PBEC culture

PBECs (passage 1) were cultured in T75 flasks coated with 30 µg/mL Purecol, 5 µg/mL human fibronectin, and 10 µg/mL BSA for 5 days. Cells from five non-COPD donors were pooled and seeded at high density (160,000 cells) on 0.4 µm PET membrane inserts (cellQART) in 12-well

plates. Cells were maintained in a 1:1 mixture of BEpiCM-b (ScienCell) and DMEM (STEMCELL Technologies), supplemented with BEpiCGS, 12.5 mM HEPES, two mM GlutaMAX, 100 U/mL penicillin, and 100 µg/mL streptomycin—referred to as complete BD medium (cBD). 1 nM EC-23 (a light-stable retinoic acid analogue) was added during submerged culture. Once confluent, ALI (air–liquid interface) differentiation was initiated by removing the apical medium and increasing EC-23 to 50 nM. Medium was changed three times per week, and the apical surface was washed with 200 µL warm PBS for 10 minutes at 37°C. Cultures were used after 14 days of ALI differentiation when ciliary activity and all major airway epithelial cell types were observed [153,154].

5.10.2. PBEC treatment and neutrophil co-culture

ALI-differentiated PBECs were treated for 7 days starting with day 0, with a positive control (TNFα + IL-1β at 20 ng/mL), either alone or in combination with cytokines (IL-17A or IL-23 at 10 ng/mL), with or without JAK1/TYK2 inhibitor (brepocitinib or deucravacitinib) (300 nM) added to the 1 mL basal medium. On day 7, the medium was replaced with fresh medium and incubated for 24 hours. On day 8, 200µL of the basal medium was collected as conditioned medium, and replaced with 200 µL fresh medium. Freshly isolated PMNLs were then added to the basal side of the inserts and co-cultured for 2 hours. Conditioned medium collected prior to PMNL co-culture was used for downstream analyses.

5.10.3. Chronic cigarette smoke exposure

In a separate experiment, ALI-differentiated PBECs were exposed to freshly generated whole cigarette smoke using 3R4F reference cigarettes (University of Kentucky, Lexington, KY, USA) for 7 days. During this smoke exposure, cells were placed in modified hypoxic chambers for 4–5 minutes and exposed to either cigarette smoke from one cigarette or to room air. After exposure, smoke was removed by ventilation with air for over 10 minutes, and cells were then returned to the incubator overnight. About 18–20 hours later, ALI-PBECs were washed apically with PBS and, four hours afterward, exposed again to cigarette smoke. This cycle was repeated daily from day 0 to day 7, with or without brepocitinib (300 nM) added to the basal medium

[155,156]. On day 7, the medium was replaced, and the cells were incubated for 24 hours. On day 8, conditioned media were collected and used for ELISA.

5.11. Cytokine measurement

IL-8 levels in conditioned media were determined using the DuoSet ELISA kit (R&D Systems) following the manufacturer's protocol. Absorbance was measured at 450 nm, with background correction at 540 nm, using a microplate reader as recommended in the instructions.

Serum levels of IL-23, IL-17A, and IL-8—from COPD patients and healthy donors were quantified using the LEGENDplex Human Inflammation Panel 1 (BioLegend, Cat. #740809), according to the manufacturer's instructions. In brief, serum samples or standards were incubated with fluorescence-labeled capture beads in a V-bottom 96-well plate, then sequentially with detection antibodies and streptavidin-PE. Following the final wash, bead populations were analyzed by flow cytometry. Cytokine concentrations were expressed as median fluorescence intensity (MFI) for each analyte and analyzed with LEGENDplex software.

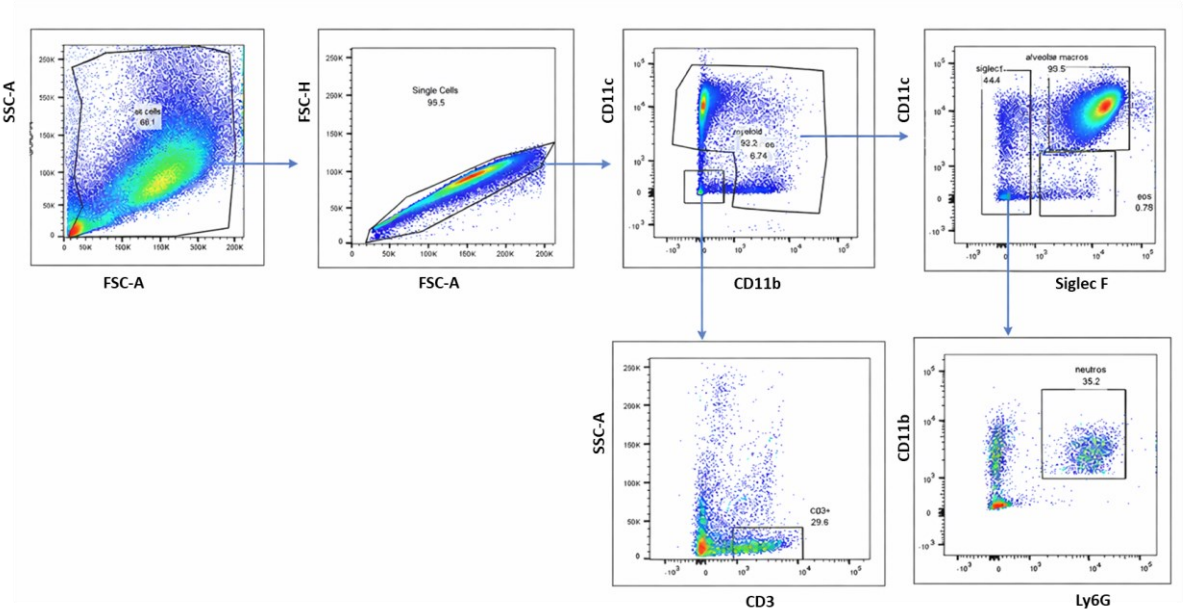
5.12. In vivo models

Eight- to 12-week-old C57BL/6 female mice were used in two experimental models. In the first model, the mice were treated intranasally with IL-23 (2 µg) or saline (vehicle control) in combination with brepocitinib (3 mg/kg) or saline, given via oral gavage, once daily for three consecutive days.

In the second setup, mice were treated intranasally with lipopolysaccharide (LPS, 5 µg) or saline (vehicle control) to induce acute lung inflammation, along with oral administration of brepocitinib (10 mg/kg) or saline, also once daily for three days. On day 4, animals were sacrificed and bronchoalveolar lavage fluid (BALF) samples were collected. The immune cell populations in the BALF were analyzed by flow cytometric staining. All procedures were performed at the same time of day to minimize circadian variability.

After the collection, BALF is immediately placed on ice and kept on it throughout the experiment. Then the collected BALF was washed with BAL buffer (centrifuged for 7 minutes

at 400 x g). Next, the pellet was hemolysed with a NH4Cl lysis solution for 5 minutes under constant shaking. The samples were then washed in BAL buffer and resuspended in FC block solution (1:100 prepared in BAL buffer). This is followed by marker staining with antibodies against CD11b PE-Cy7 (1:200), CD11c BV421 (1:200), Ly6G APC (1:500), CD3 FITC (1:200), and Siglec-F PE (1:100) in staining buffer, incubated for 30 minutes on ice. The cells are then washed, fixed for 30 minutes, and resuspended in staining buffer. Fluorescence minus one (FMO) and single-stain compensation controls were included for accurate gating. The immune cell populations in the BALF were measured using flow cytometry, and the results are expressed as cells per mL of BALF [157]. Identical gating strategies were applied across all treatment groups, and analyses were performed blinded to treatment conditions.



Methods Figure 2. The flow cytometric gating strategy used for BALF staining identifies various immune populations, including CD3+ cells, alveolar macrophages, neutrophils, and

5.13. Immunofluorescence (IF)

Paraffin-embedded mice lung tissue sections were deparaffinized by incubating in xylol for 40 minutes, followed by a 50% xylol/100% ethanol mixture (1:2) for 2 minutes. The tissue sections were then rehydrated by passing the slides through graded ethanol dilutions (100%, 96%, 80%, 70%, and 50%) for 15 seconds each, followed by a 5-minute wash in distilled water. The slides were washed three times in PBS for 10 minutes each on a shaker. Antigen retrieval was performed in 10 mM sodium citrate buffer (pH 6.0) by heating in a microwave at 850 W for 10

minutes. The slides were cooled at room temperature for 30–60 minutes, then washed three times in PBS for 10 minutes each. Non-specific binding sites were blocked with 10% normal goat serum in PBS containing 0.2% Triton X-100 for 1 hour at room temperature. The sections were then incubated overnight at room temperature with the primary antibody (pTYK2, 1:100 dilution in 1% goat serum with 0.2% Triton X-100) in a humidified chamber. After three PBS washes, the slides were incubated with the AF647-labeled secondary antibody (Invitrogen, 1:500 in PBS with 0.2% Triton X-100) for 2 hours at room temperature in a humidified chamber. Slides were washed again with PBS three times. Autofluorescence was quenched using the TrueVIEW® Autofluorescence Quenching Kit (Vector Lab SP8400 kit) for 5 minutes, then the slides were washed once more in PBS for 5 minutes with gentle shaking. Finally, sections were mounted using VECTASHIELD Vibrance antifade mounting medium containing DAPI (Vector Lab H1800) and prepared for imaging [158]. Photomicrographs of stained slides were captured using the Olympus IX73 fluorescence microscope. All images from each treatment group across experiments were captured simultaneously and with identical settings in a blinded manner. The images were then analyzed using Image J software. Regions of interest were defined using consistent criteria across samples, and background fluorescence was subtracted prior to quantification.

5.14. Western blot

Twenty million PMNL treated with vehicle, IL-17A, or IL-23 for 15 minutes were resuspended in 65 µL of RIPA lysis buffer (Thermo Fisher Scientific) supplemented with 3× Halt Protease and Phosphatase Inhibitor (Thermo Fisher Scientific). Cells were incubated on ice for 30 minutes to allow lysis and subsequently centrifuged at 14,000 × g for 15 minutes. The supernatants (protein lysates) were collected, and the pellets discarded. Total protein concentrations were determined using the Pierce BCA assay kit (Thermo Fisher Scientific). Equal amounts of protein (50 µg per sample) were separated by SDS–PAGE on 4%–20% Tris-glycine gradient gels (Thermo Fisher Scientific) and transferred to polyvinylidene fluoride membranes using the iBlot dry transfer system (Thermo Fisher Scientific), following the manufacturer's instructions. Membranes were blocked in 3% BSA for 1 hour at room temperature and subsequently incubated with primary antibodies (rabbit anti-pTYK2, 1:1,000; rabbit anti-TYK2, 1:1,000; or mouse anti-β-actin, 1:5,000) overnight at 4 °C. After washing

with Tris-buffered saline containing 0.1% Tween-20, membranes were incubated with HRP-conjugated secondary antibodies (goat anti-rabbit, 1:5,000 for pTYK2 and TYK2; goat anti-mouse, 1:5,000 for β-actin). Protein bands were visualized using HRP detection reagent (Bio-Rad) and imaged with the iBright Imaging System (Thermo Fisher Scientific). Densitometric analysis of blots was performed using the iBright analysis software, with β-actin serving as the loading control.

5.15. Microarray dataset analysis

mRNA abundance data obtained from the publicly available microarray dataset GSE22148 [159], including samples from patients with moderate (stages II) and severe COPD (stages III and IV) were analyzed for the levels of *TYK2, JAK1, JAK2, IL23A, IL23R, IL17A, IL17RA* and *IL8* using RStudio (v4.1.3). Statistical significance was assessed using the Mann–Whitney U test.

5.16. Statistical analysis

All data are presented as means ± SD for n observations. n refers to the sample size, meaning the number of biological replicates included in the analysis (e.g., number of patients, donors, or animals). Sample size is determined based on feasibility and sample availability, while ensuring sufficient biological replicates to allow meaningful statistical comparisons and consistency with similar published studies. Statistical analyses were conducted using GraphPad Prism software 10.0 (La Jolla, CA, USA) and RStudio (v4.1.3). Statistical outliers were identified by the Rout or Grubbs test. The Shapiro–Wilk test was used to confirm the normal distribution. Comparisons between two groups were performed using either a t-test (paired or unpaired) or the Mann-Whitney U test. More than two groups were compared using one-way or 2-way ANOVA, followed by a post hoc test as indicated. Probability values of $p < 0.05$ were considered statistically significant.

6. Results

This section summarizes the experimental findings of our study and addresses the research objectives outlined in the introduction earlier. The findings are supported by the relevant figures and tables, with statistical significance is reported to highlight relevant differences between experimental groups. Some parts of this section have been adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.1. mRNA abundance of the IL-23/IL-17 axis is associated with disease severity

Previous studies show that the IL-23/IL-17A axis is closely associated with neutrophilic inflammation in COPD. Primarily because the IL-23/IL-17A axis stimulates the secretion and release of neutrophil-recruiting chemokines such as IL-8 [160,161]. Given the crucial role of neutrophils in COPD pathogenesis due to their persistent recruitment and activation, there is a need to understand the clear connection of this axis with neutrophil activation, recruitment, and inflammation. In particular, linking transcriptional regulation of this pathway to clinical disease severity may provide mechanistic insight and biomarker potential. Thus, to further explore the role of the IL-23/IL-17A axis in detail, we first examined the publicly available microarray data set GSE22148 from Singh et al. [159], which provided mRNA abundance profiles of immune cells derived from the sputum of COPD patients. The cells derived from the sputum of COPD patients consisted primarily of neutrophils and macrophages, which are two prominent cell populations to be found in the inflammatory milieu of COPD lungs. This dataset therefore provides a relevant window into inflammatory signaling within the airway microenvironment.

Using this data set we studied the mRNA abundance of *IL23A*, *IL17A* and *IL8* in the sputum-derived immune cells and compared them between the patients with moderate (stage II) and severe (stage III, IV) COPD in order to assess the disease severity correlation with transcriptional regulation of these cytokines. The analysis revealed that there is a significant increase in *IL23A* and *IL8* mRNA levels in the severe COPD group when compared to the moderate group, whereas no statistically significant change was observed in *IL17A* levels despite a noticeable upward trend in the severe COPD group (Fig. 1A). This pattern suggests

that IL-23 and downstream neutrophil-recruiting signals may be more tightly linked to disease progression than IL-17A transcription alone.

Since IL-23 signals through the JAK/STAT pathway, particularly TYK2 and JAK2 via IL-23 receptor (IL23R) engagement, and IL-17A may associate with several JAKs, including JAK1, JAK2, TYK2, we further analysed the mRNA abundance of *IL23R, IL17RA* and these kinases (TYK2, JAK1 and JAK2) in relation to COPD severity. Our analysis showed that patients with severe COPD had significantly higher *TYK2* and *JAK1* mRNA levels compared to those with moderate COPD (Fig. 1C). This suggests the possibility of enhanced cytokine signaling through TYK2-dependent pathways, which may be associated with increasing disease severity and worsening. This finding provides a molecular rationale for targeting TYK2/JAK1 signaling in severe neutrophilic COPD. In contrast, there was no significant difference observed for *JAK2* levels between the two groups (Fig. 1C). Interestingly, *IL23R* mRNA level was significantly reduced in patients with severe COPD compared to the moderate ones (Fig. 1B). This reduction may reflect a compensatory feedback mechanism due to receptor desensitization, internalization and downregulation in response to chronic overstimulation by elevated IL-23 levels [162]. Such receptor downregulation is consistent with sustained pathway activation in chronic inflammatory settings. Similar to *IL17*, there was no difference observed in the *IL17RA* levels between severe and moderate COPD groups (Fig. 1C).

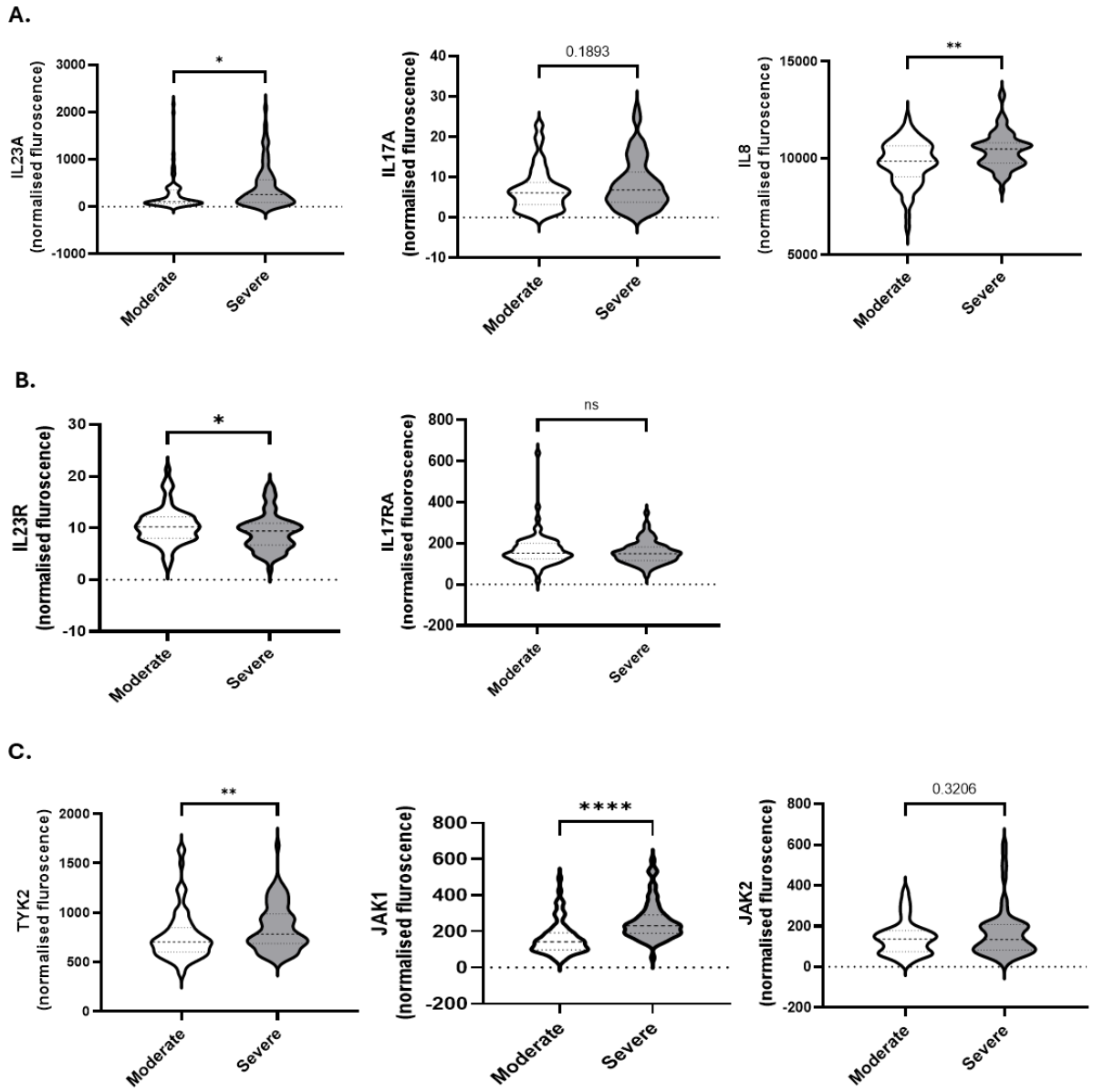


Figure 1. mRNA abundance of the *IL23A, IL17A, IL8, IL23R, IL17RA, TYK2, JAK1, JAK2* in sputum cells from moderate and severe COPD patients. A: *IL23A, IL17A,* and *IL8* mRNA abundance in sputum-derived immune cells from patients with moderate and severe COPD. B: *IL23R, IL-17RA* mRNA abundance in sputum-derived immune cells from patients with moderate and severe COPD. C. *TYK2, JAK1* and *JAK2* mRNA levels in sputum-derived immune cells from patients with moderate and severe COPD. For statistical analyses, Normal distribution was confirmed using the Shapiro–Wilk test, followed by either t-test or Mann-Whitney U test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p< 0.0001, ns = not significant. Some parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.2. IL-23, IL-17A, and IL-8 protein levels in serum from COPD

To compare the levels of IL-23, IL-17A, and IL-8 proteins between healthy individuals and COPD patients, we analyzed serum samples from our available COPD cohort and matched controls. This approach allowed validation of transcriptional trends at the protein level in a clinically accessible compartment. Consistent with microarray results, we observed a trend toward higher IL-23 levels (Fig. 2A), particularly in patients with severe COPD compared to healthy controls and those with moderate disease. This trend supports a link between IL-23 abundance and disease severity. Notably, serum IL-17A levels (Fig. 2B) were significantly elevated in patients with severe COPD, whereas IL-8 levels (Fig. 2C) were increased irrespective of disease severity. The widespread elevation of IL-8 is consistent with its role as a general neutrophil chemoattractant in chronic airway inflammation.

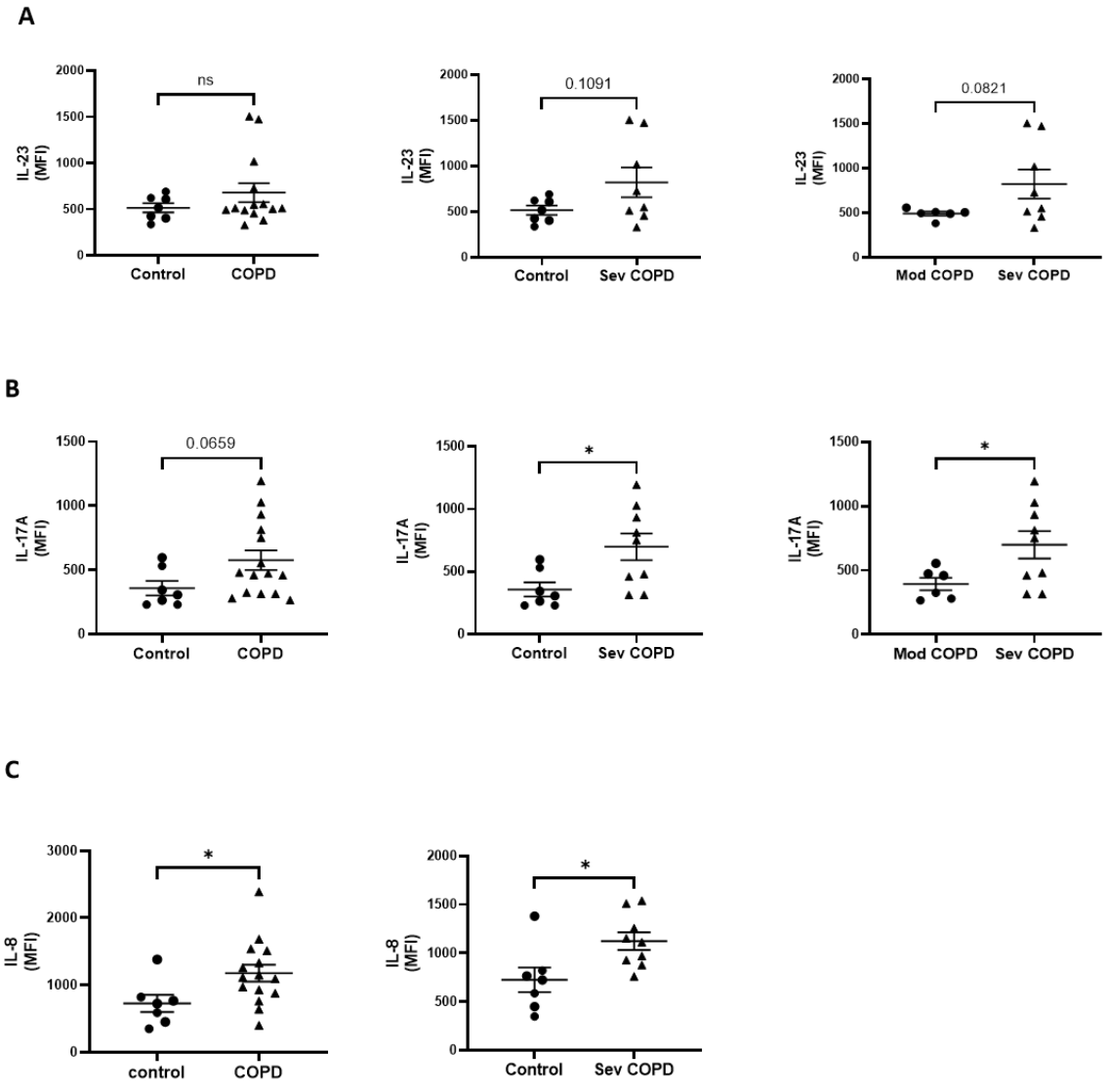


Figure 2. IL-23, IL-17A, and IL-8 serum levels in healthy donors compared to COPD patients. A: IL-23 cytokine levels in serum from healthy donors compared to COPD patients; healthy donors compared to severe COPD; Moderate COPD compared to severe COPD. B: IL-17A cytokine levels in serum from healthy donors compared to COPD patients; healthy donors compared to severe COPD; Moderate COPD compared to severe COPD. C: IL-8 cytokine levels in serum from healthy donors compared to COPD patients; healthy donors compared to severe COPD; Moderate COPD compared to severe COPD. Outliers were assessed using the Grubbs' test. For statistical analyses, Normal distribution was assessed using the Shapiro–Wilk test, followed by either t-test or Mann-Whitney U test. GeoMean-Geometric Mean; MFI-Mean Fluorescence intensity. *$p < 0.05$, ns = not significant. Some parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.3. TYK2 protein abundance and phosphorylation in immune cells from COPD and healthy controls

To further validate the initial observations regarding mRNA abundance levels at the protein level, we optimized a comprehensive whole-blood staining protocol to study both total and phosphorylated forms of TYK2 (pTYK2 (Tyr1054/Tyr1055)) by flow cytometry. Blood samples were collected under the same conditions and at the same time of the day from two groups: patients diagnosed COPD and age- and sex-matched healthy control subjects, with demographic details outlined in Methods Table 1.

In our analysis, we focused on the following immune cell populations present in whole blood: neutrophils, T lymphocytes identified by CD3, CD4+ helper T cells, CD8+ cytotoxic T cells, monocytes identified by CD14, and eosinophils. Our quantitative assessment revealed that the levels of total TYK2 protein and its phosphorylated form (pTYK2 (Tyr1054/Tyr1055)) did not show significant differences in the examined immune cell populations of CD3- (Fig. 3A), CD4- (Fig. 3B), CD8- (Fig. 3C), and CD14-positive cells (Fig. 3D) between COPD patients and healthy controls. These findings suggest that TYK2 phosphorylation is not globally altered across all circulating immune subsets in COPD. However, neutrophils from COPD patients showed a significant increase in pTYK2 (Tyr1054/Tyr1055) levels compared to controls (Fig. 3E). This selective increase in neutrophil pTYK2 (Tyr1054/Tyr1055) aligns with the proposed role of TYK2 in neutrophilic inflammation. In addition, there was also a trend toward higher total TYK2 levels in neutrophils ($p=0.0521$) (Fig. 3E). Interestingly, eosinophils from COPD patients showed a significant increase in both TYK2 (p-values of 0.0406) and pTYK2

(Tyr1054/Tyr1055) (p= 0.009) levels when compared to eosinophils from healthy controls (Fig. 3F). This suggests that TYK2 signaling may also be enhanced in eosinophils in COPD, reflecting overlapping or mixed inflammatory endotypes in subsets of patients.

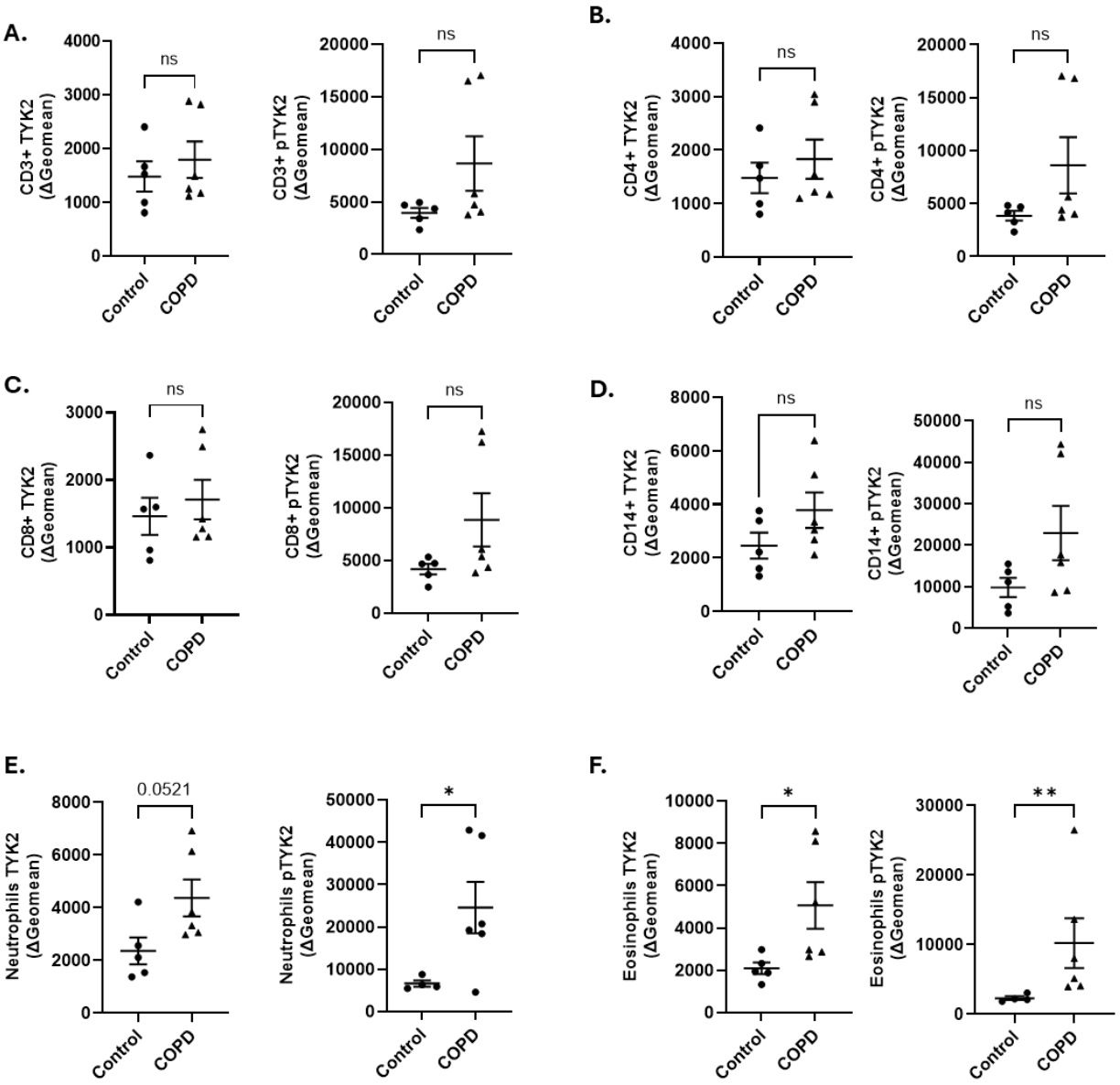


Figure 3. Differential TYK2 protein abundance and phosphorylation in the immune cells from COPD patients compared to healthy individuals. A: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in CD3+ cells from healthy donors compared to COPD patients. B: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in CD4+ cells from healthy donors compared to COPD patients. C: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in CD8+ cells from healthy donors compared to

COPD patients. D: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in CD14+ cells from healthy donors compared to COPD patients. E: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in neutrophils from healthy donors compared to COPD patients. F: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in eosinophils from healthy donors compared to COPD patients. For statistical analyses, Normal distribution was confirmed using the Shapiro–Wilk test, followed by either t-test or Mann-Whitney U test. ΔGeoMean= $GeoMean_{stained\ sample}$-$GeoMean_{FMO}$. GeoMean-Geometric Mean. *p < 0.05, **p < 0.01, ns = not significant. Some parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.4. JAK1 protein abundance and phosphorylation in immune cells from COPD patients and healthy controls

To study JAK1 abundance, blood was collected from COPD patients and from age- and sex-matched healthy non-smokers under the same conditions and at the same time of the day. Using flow cytometry, we performed whole-blood staining to evaluate total JAK1 and its phosphorylated form (pJAK1 (Tyr1022/Tyr1023)) across multiple immune cell populations, including neutrophils, $CD3^+$ T lymphocytes (Fig. 4A), $CD4^+$ helper T cells (Fig. 4B), $CD8^+$ cytotoxic T cells (Fig. 4C), $CD14^+$ monocytes (Fig. 4D), and eosinophils (Fig. 4F). This analysis allowed cell-type–specific assessment of JAK1 pathway phosphorylation in circulating immune compartments. Across all examined immune subsets, the differences in total JAK1 abundance between COPD patients and healthy controls were not statistically significant. However, there was a significant increase in pJAK1 (Tyr1022/Tyr1023) in CD8+ cytotoxic T cells and neutrophils in the COPD group. This indicates selective phosphorylation of JAK1-dependent signaling rather than global changes in JAK1 abundance. Although not statistically significant, the CD3+, CD4+, and CD14+ populations showed a trend toward increased pJAK1 (Tyr1022/Tyr1023) levels in COPD patients compared to healthy controls. These trends suggest broader low-grade phosphorylation of JAK1 signaling across other immune subsets in COPD.

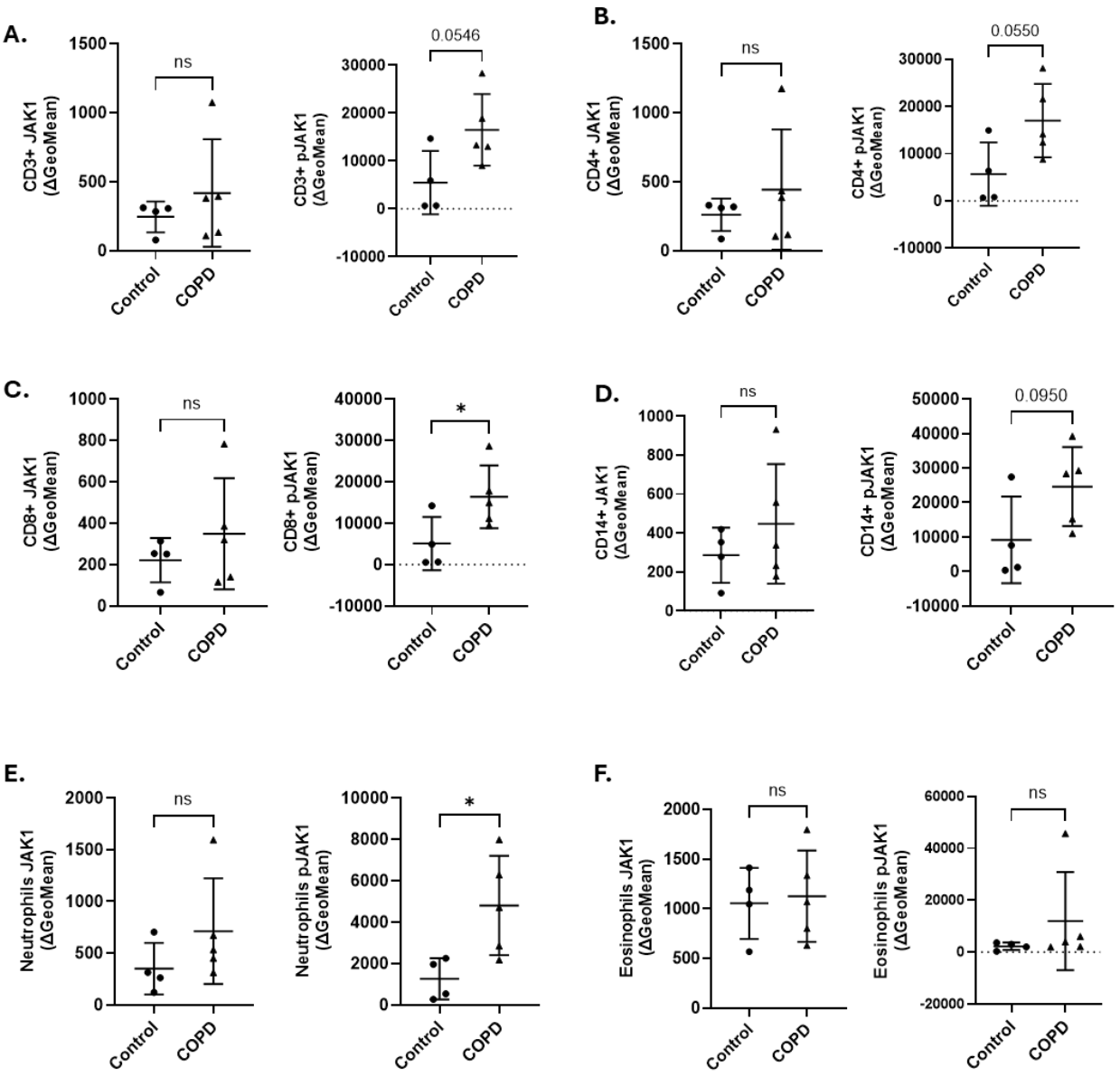


Figure 4. Differential JAK1 abundance and phosphorylation in the immune cells from COPD patients compared to healthy individuals. A: JAK1 abundance and phosphorylation (pJAK1 (Tyr1022/Tyr1023)) in CD3+ cells from healthy donors compared to COPD patients. B: JAK1 abundance and phosphorylation (pJAK1 (Tyr1022/Tyr1023)) in CD4+ cells from healthy donors compared to COPD patients. C: JAK1 abundance and phosphorylation (pJAK1 (Tyr1022/Tyr1023)) in CD8+ cells from healthy donors compared to COPD patients. D: JAK1 abundance and phosphorylation (pJAK1 (Tyr1022/Tyr1023)) in CD14+ cells from healthy donors compared to COPD patients. E: JAK1 abundance and phosphorylation (pJAK1 (Tyr1022/Tyr1023)) in neutrophils from healthy donors compared to COPD patients. F: JAK1 abundance and phosphorylation (pJAK1 (Tyr1022/Tyr1023)) in eosinophils from healthy donors compared to COPD patients. For statistical analyses, Normal distribution was confirmed using the Shapiro–Wilk test, followed by either t-test or Mann-Whitney U test. ΔGeoMean= $GeoMean_{stained\ sample}$-$GeoMean_{FMO}$. GeoMean-Geometric Mean. *$p < 0.05$, ns = not significant.

Some parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.5. JAK2 protein abundance and phosphorylation in immune cells from COPD patients and healthy controls

Using the same staining protocol and blood samples, we also evaluated total and phosphorylated JAK2 (pJAK2 (Tyr1007/Tyr1008)), since IL-23 signals through JAK2 as well as TYK2. Again, whole blood staining was performed in T lymphocytes identified by CD3 (Fig. 5A), CD4+ helper T cells (Fig. 5B), CD8+ cytotoxic T cells (Fig. 5C), monocytes identified by CD14 (Fig. 5D), neutrophils (Fig. 5E), and eosinophils (Fig. 5F). This enabled direct comparison of JAK2 phosphorylation patterns with those observed for TYK2 and JAK1. Our analysis showed no significant difference in the levels of total JAK2 protein and its phosphorylated form in all immune cells except eosinophils. Eosinophils from COPD patients showed a significant increase in pJAK2 (Tyr1007/Tyr1008) ($p=0.0308$) levels compared to controls. Again, this selective phosphorylation of JAK2 in eosinophils suggests endotype-specific JAK pathway engagement in subsets of COPD patients, potentially reflecting overlapping neutrophilic and eosinophilic inflammatory phenotypes.

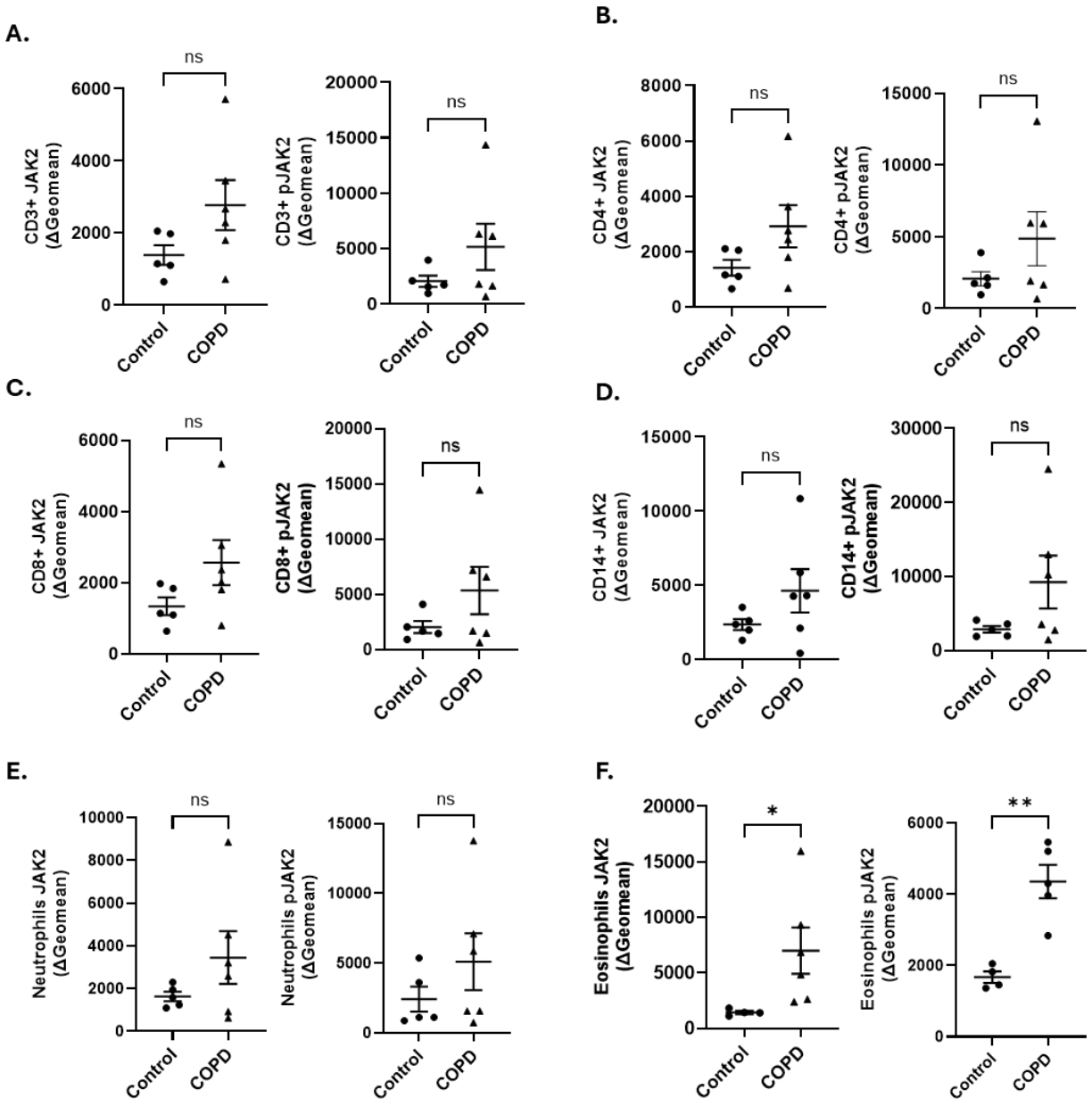


Figure 5. Differential JAK2 abundance and phosphorylation in the immune cells from COPD patients compared to healthy individuals. A: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in CD3+ cells from healthy donors compared to COPD patients. B: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in CD4+ cells from healthy donors compared to COPD patients. C: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in CD8+ cells from healthy donors compared to COPD patients. D: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in CD14+ cells from healthy donors compared to COPD patients. E: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in neutrophils from healthy donors compared to COPD patients. F: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in eosinophils from healthy donors compared to COPD patients. For statistical analyses, Normal distribution was confirmed

using the Shapiro–Wilk test, followed by either t-test or Mann-Whitney U test. ΔGeoMean= $GeoMean_{stained\ sample}$-$GeoMean_{FMO}$. GeoMean-Geometric Mean. *p < 0.05, **p < 0.01, ns = not significant. Parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.6. TYK2 protein abundance and phosphorylation in immune cells from smokers and healthy controls

In addition to blood from COPD patients, we also collected blood from younger smokers without COPD and age and sex-matched healthy non-smokers as the control group (Material and method table 2) under the same conditions and at the same time of the day. Including these groups was particularly valuable, as smoking is a major risk factor for the development of COPD. This comparison allowed assessment of smoking-related inflammatory signaling independent of established disease.

Using flow cytometry, we performed whole-blood staining to study total TYK2 and its phosphorylated form (pTYK2 (Tyr1054/Tyr1055)) in various immune populations, including T lymphocytes identified by CD3 (Fig. 6A), CD4+ helper T cells (Fig. 6B), CD8+ cytotoxic T cells (Fig. 6C), monocytes identified by CD14 (Fig. 6D), neutrophils (Fig. 6E) and eosinophils (Fig. 6F). Notably, all immune cell populations from smokers also depicted a significant increase in pTYK2 (Tyr1054/Tyr1055) levels compared to healthy controls. However, there was no significant change in the total TYK2 levels. This pattern indicates enhanced pathway downstream phosphorylation rather than altered protein abundance in response to cigarette smoke exposure. Given that smoking is a major risk factor in COPD, these findings suggest that TYK2 may contribute to early inflammatory responses associated with disease initiation. These results further support TYK2 as an early signaling node that may be targetable before overt disease develops.

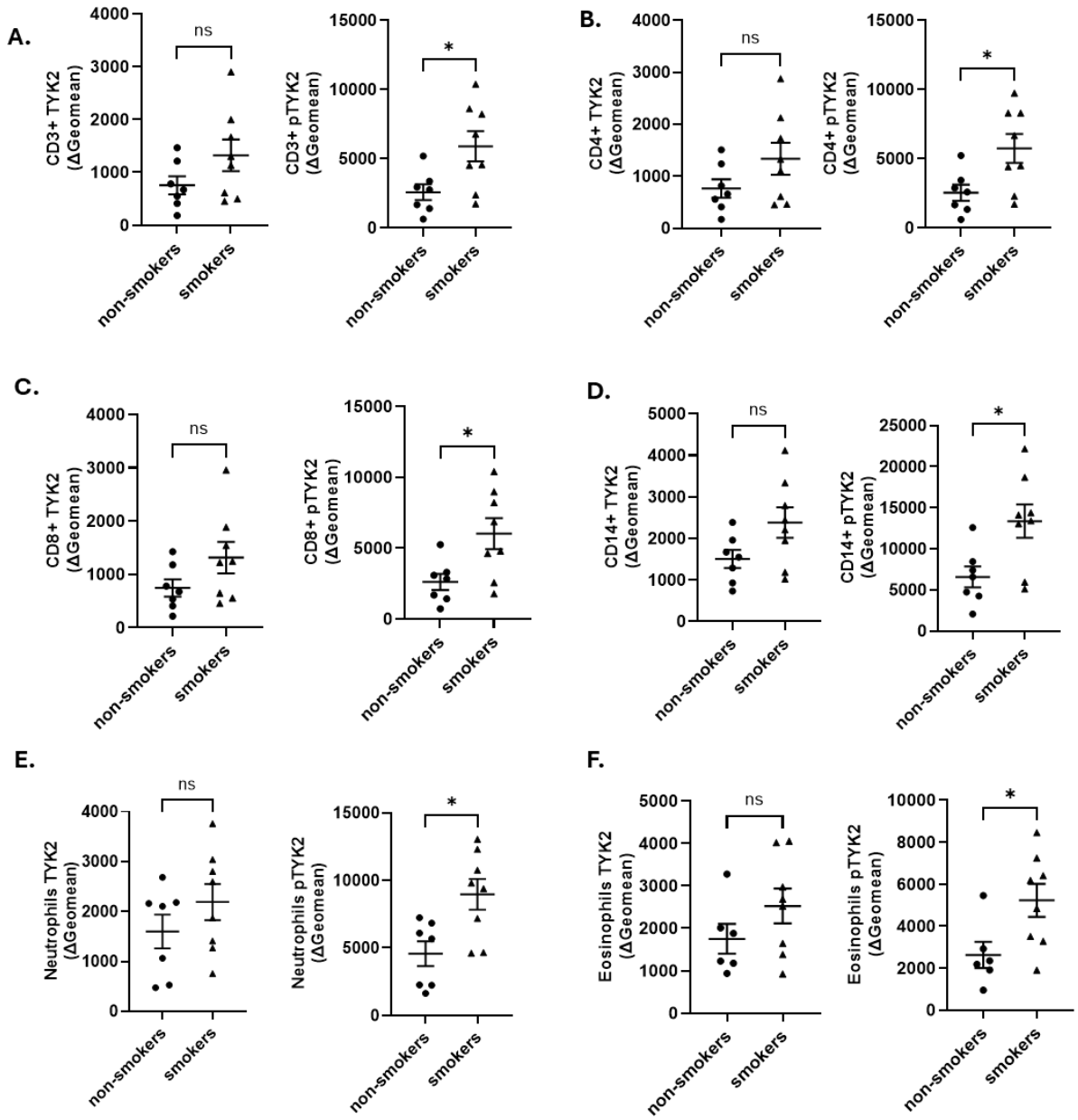


Figure 6. Differential TYK2 protein abundance and phosphorylation in the immune cells from smokers compared to healthy non-smokers. A: TYK2 protein abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in CD3+ cells from healthy non-smokers compared to smokers. B: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in CD4+ cells from healthy non-smokers compared to smokers. C: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in CD8+ cells from healthy non-smokers compared to smokers. D: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in CD14+ cells from healthy non-smokers compared to smokers. E: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in neutrophils from healthy non-smokers compared to smokers. F: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in eosinophils from healthy non-smokers compared to smokers. For statistical analyses, Normal

distribution was confirmed using the Shapiro–Wilk test, followed by either t-test or Mann-Whitney U test. ΔGeoMean= $GeoMean_{stained\ sample}$-$GeoMean_{FMO}$. GeoMean-Geometric Mean. *p < 0.05, ns = not significant. Parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.7. JAK1 protein abundance and phosphorylation in immune cells from smokers and non-smokers

Blood was collected from younger smokers without COPD and from age- and sex-matched healthy non-smokers as the control group (Materials and methods table 2). This comparison enables assessment of smoking-associated immune activation prior to clinical COPD development. Using flow cytometry, we performed whole-blood staining to examine total JAK1 and its phosphorylated form (pJAK1 (Tyr1022/Tyr1023)) in various immune cell populations, including T lymphocytes identified by CD3 (Fig. 7A), CD4+ helper T cells (Fig. 7B), CD8+ cytotoxic T cells (Fig. 7C), monocytes identified by CD14 (Fig. 7D), neutrophils (Fig. 7E), and eosinophils (Fig. 7F). Immune populations such as neutrophils, CD4+ cells, and eosinophils from smokers showed a significant increase in pJAK1 (Tyr1022/Tyr1023) levels compared to healthy controls. This indicates enhanced JAK1 pathway phosphorylation in key innate and adaptive immune subsets in response to cigarette smoke exposure. Although not statistically significant, CD3+, CD8+, and CD14+ populations showed a trend toward increased pJAK1 (Tyr1022/Tyr1023) levels in smokers compared to non-smokers. These trends suggest broader low-grade phosphorylation of JAK1 signaling across immune compartments. In addition, there was no significant change in total JAK1 levels in CD3+, CD4+, and CD8+ T cells. However, CD14+ monocytes, neutrophils, and eosinophils showed a notable increase in total JAK1 levels in smokers compared with non-smokers. This combination of increased abundance and phosphorylation in myeloid cells may reflect heightened sensitivity to cytokine signaling in smoke-exposed individuals. Since smoking is a significant risk factor for COPD, these findings suggest that JAK1 may also play a role in early inflammatory responses. Together with the TYK2 data, these results support the concept that smoking primes JAK-dependent inflammatory signaling prior to overt disease.

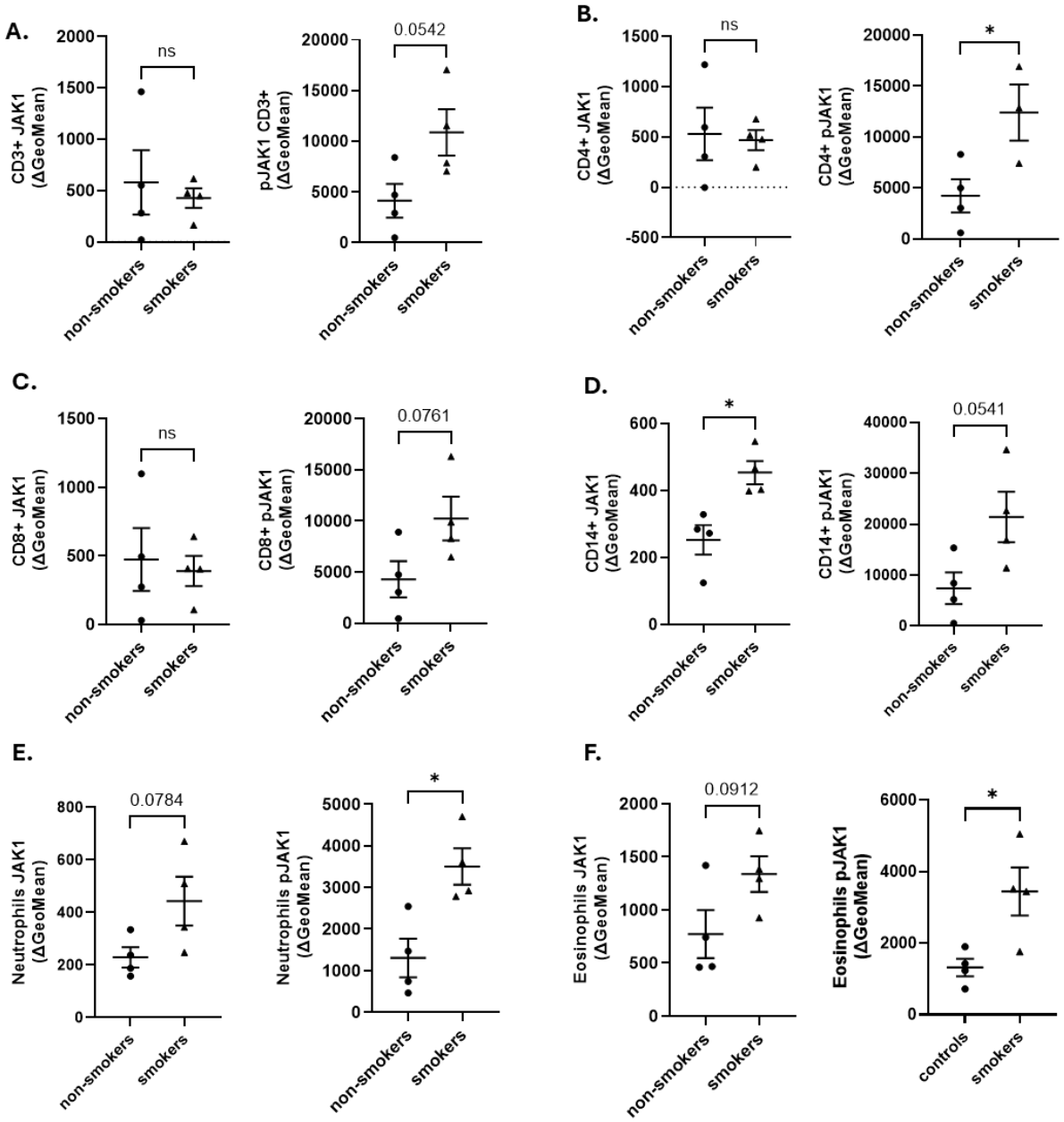


Figure 7. Differential JAK1 abundance and phosphorylation in the immune cells from smokers compared to healthy non-smokers. A: JAK1 abundance and phosphorylation (pJAK1 (Tyr1022/Tyr1023)) in CD3+ cells from healthy non-smokers compared to smokers. B: JAK1 abundance and phosphorylation (pJAK1 (Tyr1022/Tyr1023)) in CD4+ cells from healthy non-smokers compared to smokers. C: JAK1 abundance and phosphorylation (pJAK1 (Tyr1022/Tyr1023)) in CD8+ cells from healthy non-smokers compared to smokers. D: JAK1 abundance and phosphorylation (pJAK1 (Tyr1022/Tyr1023)) in CD14+ cells from healthy non-smokers compared to smokers. E: JAK1 abundance and phosphorylation (pJAK1 (Tyr1022/Tyr1023)) in neutrophils from healthy non-smokers compared to smokers. F: JAK1

abundance and phosphorylation (pJAK1 (Tyr1022/Tyr1023)) in eosinophils from healthy non-smokers compared to smokers. For statistical analyses, Normal distribution was confirmed using the Shapiro–Wilk test, followed by either t-test or Mann-Whitney U test. ΔGeoMean= $GeoMean_{stained\ sample}$-$GeoMean_{FMO}$. GeoMean-Geometric Mean. *p < 0.05, **p < 0.01, ns = not significant. Parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.8. JAK2 protein abundance and phosphorylation in the immune cells of blood collected from smokers and healthy controls

The blood collected from younger smokers without COPD and the age and sex-matched healthy non-smokers as the control group (Material and method table 2) were also studied for total JAK2 and its phosphorylated form (pJAK2 (Tyr1007/Tyr1008)) using the same flow cytometric whole-blood staining protocol for T lymphocytes identified by CD3 (Fig. 8A), CD4+ helper T cells (Fig. 8B), CD8+ cytotoxic T cells (Fig. 8C), monocytes identified by CD14 (Fig. 8D), neutrophils (Fig. 8E), and eosinophils (Fig. 8F). This allowed direct comparison of smoking-related JAK2 phosphorylation with the patterns observed for TYK2 and JAK1.

Across all examined immune populations, including neutrophils, CD3+, CD4+, CD8+, CD14+ cells, and eosinophils, there were no significant differences in total JAK2 levels between smokers and healthy controls. However, a significant increase in pJAK2 (Tyr1007/Tyr1008) was observed in the CD3+, CD4+, and CD8+ T cells in smokers compared to healthy controls. Interestingly, we also observed an increased trend of pJAK2 (Tyr1007/Tyr1008) in CD14+ cells and eosinophils in smokers compared to healthy controls. Together, these findings suggest that smoking primes JAK2 signaling across multiple immune subsets, potentially contributing to early immune dysregulation prior to overt COPD development.

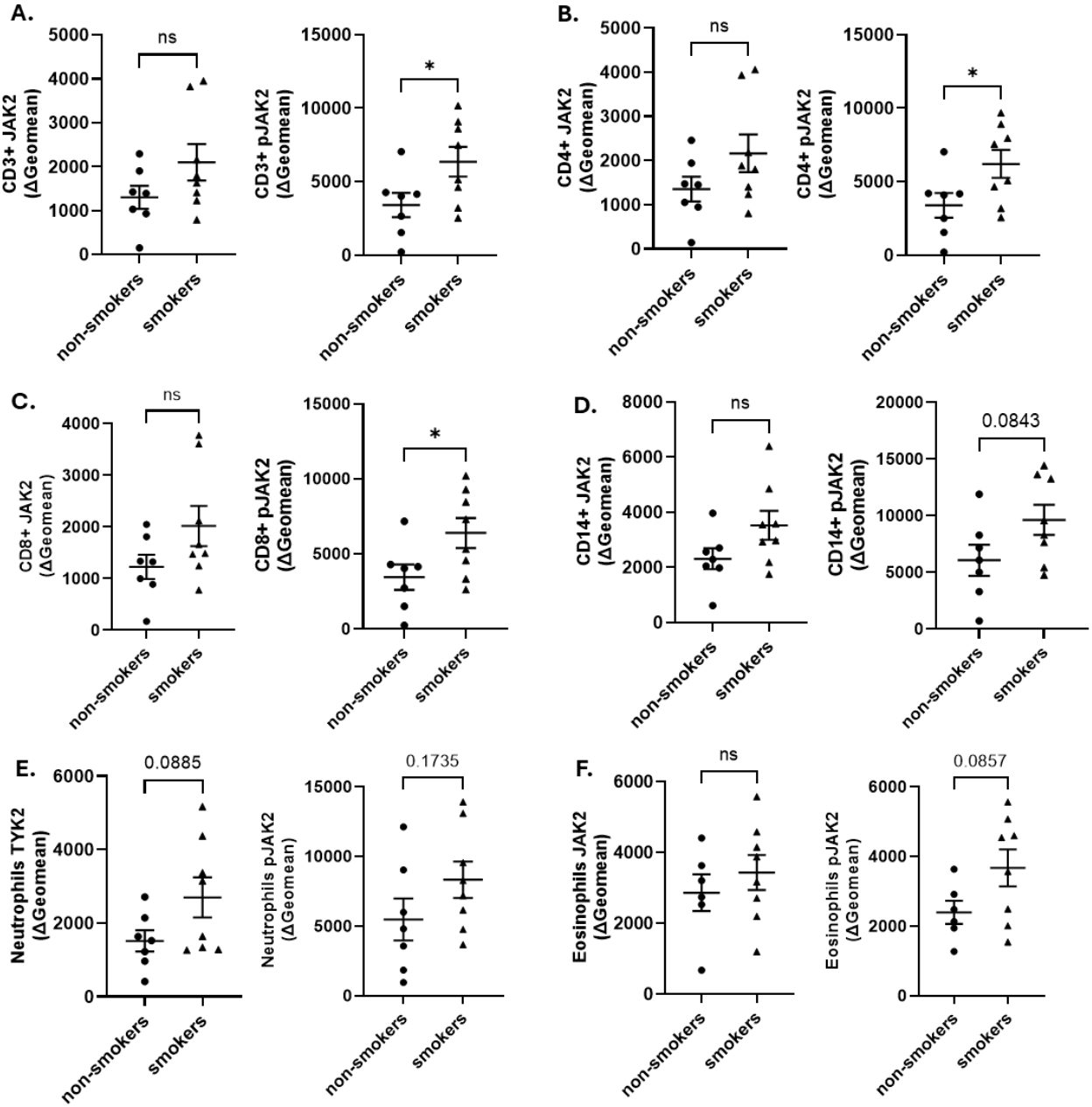


Figure 8. Differential JAK2 abundance and phosphorylation in the immune cells from smokers compared to healthy non-smokers. A: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in CD3+ cells from healthy non-smokers compared to smokers. B: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in CD4+ cells from healthy non-smokers compared to smokers. C: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in CD8+ cells from healthy non-smokers compared to smokers. D: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in CD14+ cells from healthy non-smokers compared to smokers. E: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in neutrophils from healthy non-smokers compared to smokers. F: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in eosinophils from healthy non-smokers compared to smokers. For statistical analyses, Normal distribution was confirmed

using the Shapiro–Wilk test, followed by either t-test or Mann-Whitney U test. ΔGeoMean= $GeoMean_{stained\ sample}$-$GeoMean_{FMO}$. GeoMean-Geometric Mean. *p < 0.05, ns = not significant. Parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.9. TYK2 protein abundance and phosphorylation in immune cells from allergic and non-allergic individuals

In addition to COPD patients and smokers, we collected samples from allergic and non-allergic donors (Material and method table 3) under the same conditions and at the same time of the day and performed the same whole-blood staining to compare their TYK2 abundance and phosphorylation (Figure 9). The allergic cohort consisted of volunteers reporting symptoms in response to aeroallergens (house dust mite, grass, or tree pollen) with confirmed allergen-specific IgE positivity, whereas non-allergic donors had no reported allergic symptoms or sensitization. Including allergic donors allowed assessment of whether TYK2 phosphorylation is a general feature of airway inflammation or specific to neutrophilic COPD-associated inflammation. Neither neutrophils, CD3+, CD4+, CD8+ cells, nor eosinophils, showed significant differences in either total TYK2 levels or pTYK2 (Tyr1054/Tyr1055) between the non-allergic and allergic group. However, a considerable decrease in both total TYK2 and pTYK2 (Tyr1054/Tyr1055) was observed in the CD14+ monocyte population from allergic donors compared to non-allergic healthy controls (Figure 9D). This reduction may reflect differential regulation of TYK2 signaling in monocytes under type 2–skewed inflammatory conditions and further supports disease-specific modulation of TYK2 pathways.

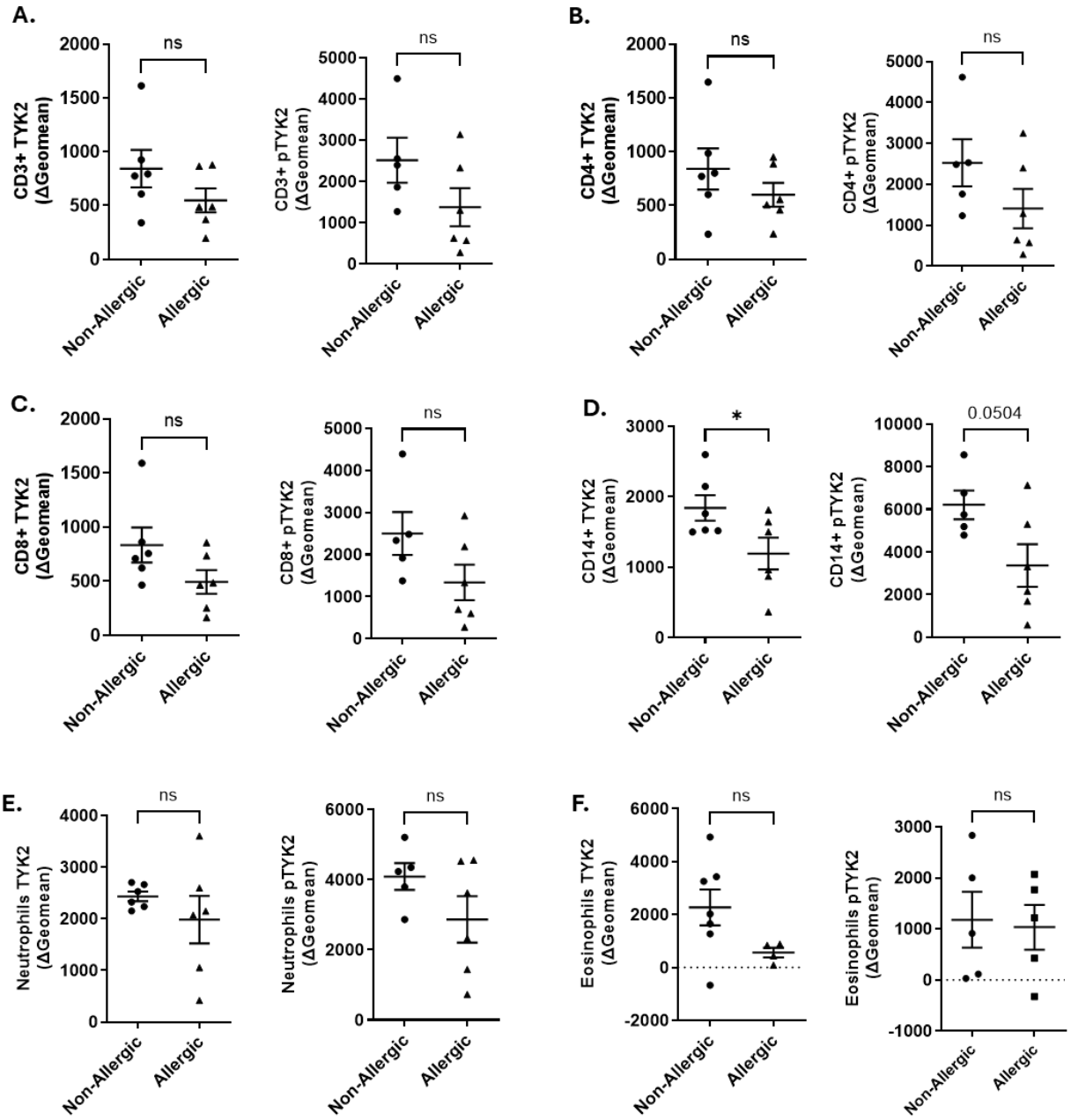


Figure 9. Differential TYK2 abundance and phosphorylation in the immune cells from non-allergic donors compared to allergic donors. A: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in CD3+ cells from non-allergic donors compared to allergic donors. B: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in CD4+ cells from non-allergic donors compared to allergic donors. C: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in CD8+ cells from non-allergic donors compared to allergic donors. D: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in CD14+ cells from non-allergic donors compared to allergic donors. E: TYK2 protein abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in neutrophils from non-allergic donors compared to allergic donors. F: TYK2 abundance and phosphorylation (pTYK2 (Tyr1054/Tyr1055)) in eosinophils from non-allergic donors compared to allergic

donors. Allergic donors were defined based on aeroallergen-associated symptoms and allergen-specific IgE positivity; non-allergic donors had no reported allergy or sensitization. For statistical analyses, Normal distribution was confirmed using the Shapiro–Wilk test, followed by either t-test or Mann-Whitney U test. ΔGeoMean= $GeoMean_{stained\ sample}$-$GeoMean_{FMO.}$ GeoMean-Geometric Mean. *$p < 0.05$, ns = not significant. Parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.10. JAK2 protein abundance and phosphorylation in immune cells from allergic and non-allergic individuals

Besides TYK2, we compared JAK2 abundance and phosphorylation in blood collected from allergic and non-allergic donors (see Materials and Methods table 3). The allergic cohort consisted of volunteers reporting symptoms in response to aeroallergens (house dust mite, grass, or tree pollen) with confirmed allergen-specific IgE positivity, whereas non-allergic donors had no reported allergic symptoms or sensitization. Using flow cytometry, we analyzed total JAK2 and pJAK2 (Tyr1007/Tyr1008) across immune cell types, including T lymphocytes (identified by CD3) (Fig. 10A), helper T cells (CD4+) (Fig. 10B), cytotoxic T cells (CD8+) (Fig. 10C), monocytes (CD14) (Fig. 10D), neutrophils (Fig. 10E), and eosinophils (Fig. 10F). This analysis enabled direct comparison of JAK2 pathway activity in type 2–biased allergic inflammation versus neutrophilic COPD-associated inflammation. Notably, total JAK2 was unaltered throughout all cell populations, while a significant decrease in pJAK2 (Tyr1007/Tyr1008) was observed in neutrophils, total T cells, CD4+, and CD8+ T cells, as well as in monocytes, from allergic donors. However, there was no significant change in pJAK2 (Tyr1007/Tyr1008) levels in eosinophils between allergic and non-allergic donors. Together with the TYK2 data, these findings suggest that JAK2 signaling is differentially regulated across inflammatory endotypes, being dampened in allergic conditions but enhanced in smoking-related and COPD-associated inflammation. However, these findings should be interpreted in light of cohort size, age, and potential effects of ongoing allergen exposure and medication use.

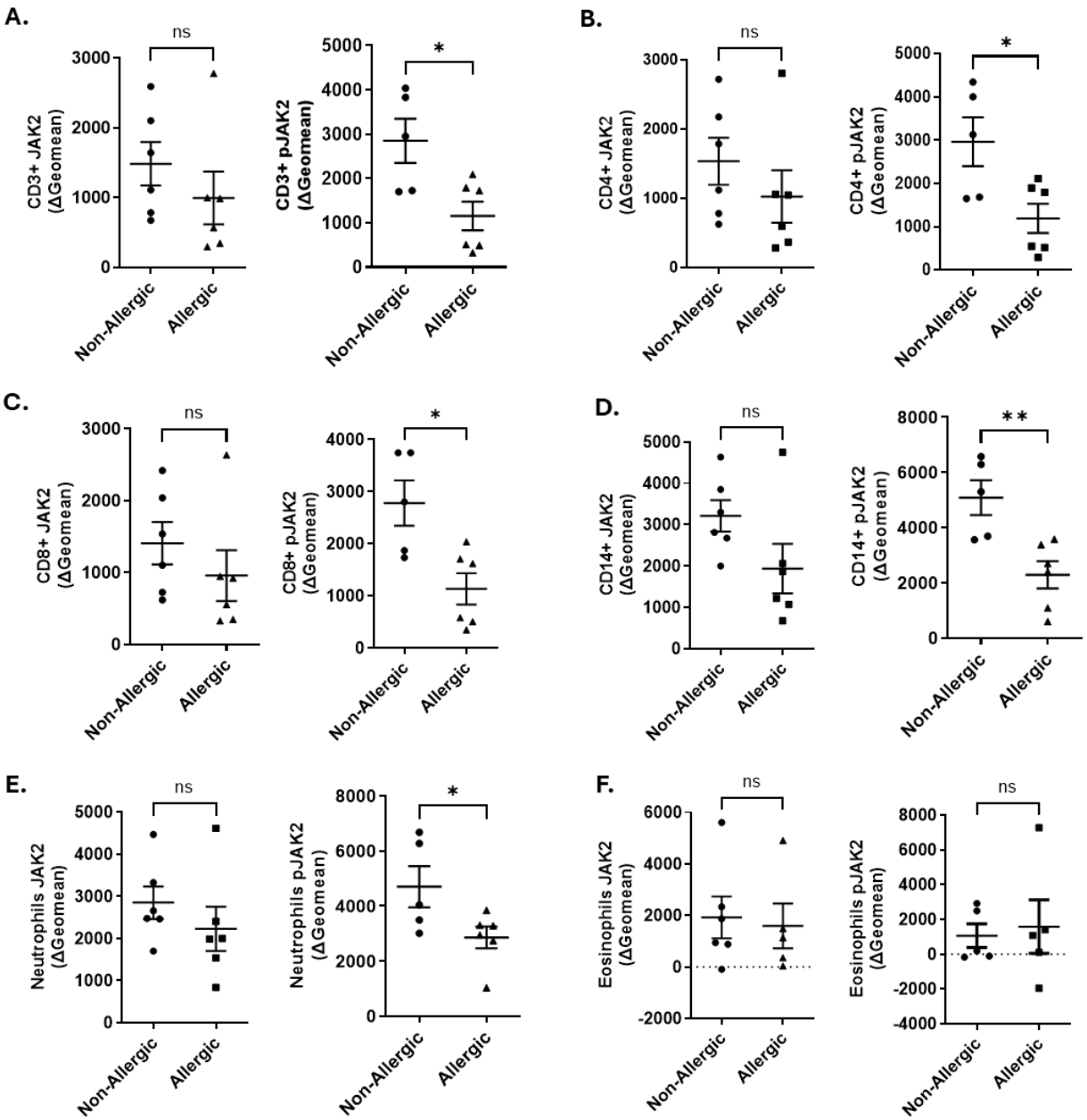


Figure 10. Differential JAK2 abundance and phosphorylation in the immune cells from non-allergic donors compared to allergic donors. A: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in CD3+ cells from non-allergic donors compared to allergic donors. B: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in CD4+ cells from non-allergic donors compared to allergic donors. C: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in CD8+ cells from non-allergic donors compared to allergic donors. D: TYK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in CD14+ cells from non-allergic donors compared to allergic donors. E: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in neutrophils from non-allergic donors compared to allergic

donors. F: JAK2 abundance and phosphorylation (pJAK2 (Tyr1007/Tyr1008)) in eosinophils from non-allergic donors compared to allergic donors. Allergic donors were defined based on aeroallergen-associated symptoms and allergen-specific IgE positivity; non-allergic donors had no reported allergy or sensitization. For statistical analyses, Normal distribution was confirmed using the Shapiro–Wilk test, followed by either t-test or Mann-Whitney U test. ΔGeoMean= $GeoMean_{stained\ sample}$-$GeoMean_{FMO}$. GeoMean-Geometric Mean. *$p < 0.05$, **$p < 0.01$, ns = not significant. Parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.11. The IL-23/IL-17A axis affects neutrophil effector function- Migration

Previous studies suggest that the IL-23/IL-17A axis mediates neutrophil recruitment indirectly, mainly through IL-8 secretion [160,161,163]. However, the direct effects of this axis on neutrophil effector functions remain poorly understood and require further investigation. In particular, whether IL-23 and IL-17A can directly modulate neutrophil chemotactic behavior independent of secondary chemokine production has remained unclear. Thus, we conducted a series of functional assays, starting with migration assays. To assess neutrophil migratory responses, we used a microBoyden chamber. In this setup, the chemoattractant or stimulus of interest was placed into the lower compartment, while cells were loaded into the upper compartment to establish a chemotactic gradient. The cells were allowed to migrate for 1 hour in this setup at 37°C.

Under these experimental conditions, we observed that neutrophils migrated towards IL-23 (10 ng/mL) and IL-17A (10 ng/mL) (Fig. 11A). This indicates that both cytokines can exert direct chemotactic or chemokinetic effects on neutrophils. In fact, the migratory response was further enhanced when the cells were exposed to the combination of both cytokines compared to each cytokine alone (Fig. 11A), indicating an additive effect that suggests cooperative signaling between IL-23– and IL-17A-driven pathways in regulating neutrophil motility.

Besides assessing the direct chemotactic effects, we also wanted to investigate the priming potential of IL-23 and IL-17A, as cytokine-mediated priming can enhance neutrophil responsiveness to classical chemoattractants such as IL-8. For this purpose, neutrophils were pretreated with IL-17A (10 ng/mL), IL-23 (10 ng/mL), or a vehicle control, for 30 minutes at 37°C, followed by migration toward IL-8 (10 nM) (Fig. 11B, 11C). Notably, pretreatment with IL-17A and IL-23 significantly increased neutrophil migration toward IL-8 compared to the

vehicle pretreated group. These findings demonstrate that IL-23 and IL-17A function as priming agents that heighten neutrophil sensitivity to canonical chemoattractant cues.

Furthermore, when we pretreated the cells with IL-17A (10 ng/mL), IL-23 (10 ng/mL), either alone or in combination, followed by subsequent stimulation with IL-8 (10 nM) (Fig. 11D), we observed that the combined pretreatment with IL-17A and IL-23 further amplified migration compared with pretreatment with either cytokine alone. This synergistic priming effect provides functional evidence for convergence of IL-23 and IL-17A signaling at the level of neutrophil effector programming, consistent with their cooperative role in neutrophilic inflammation in COPD.

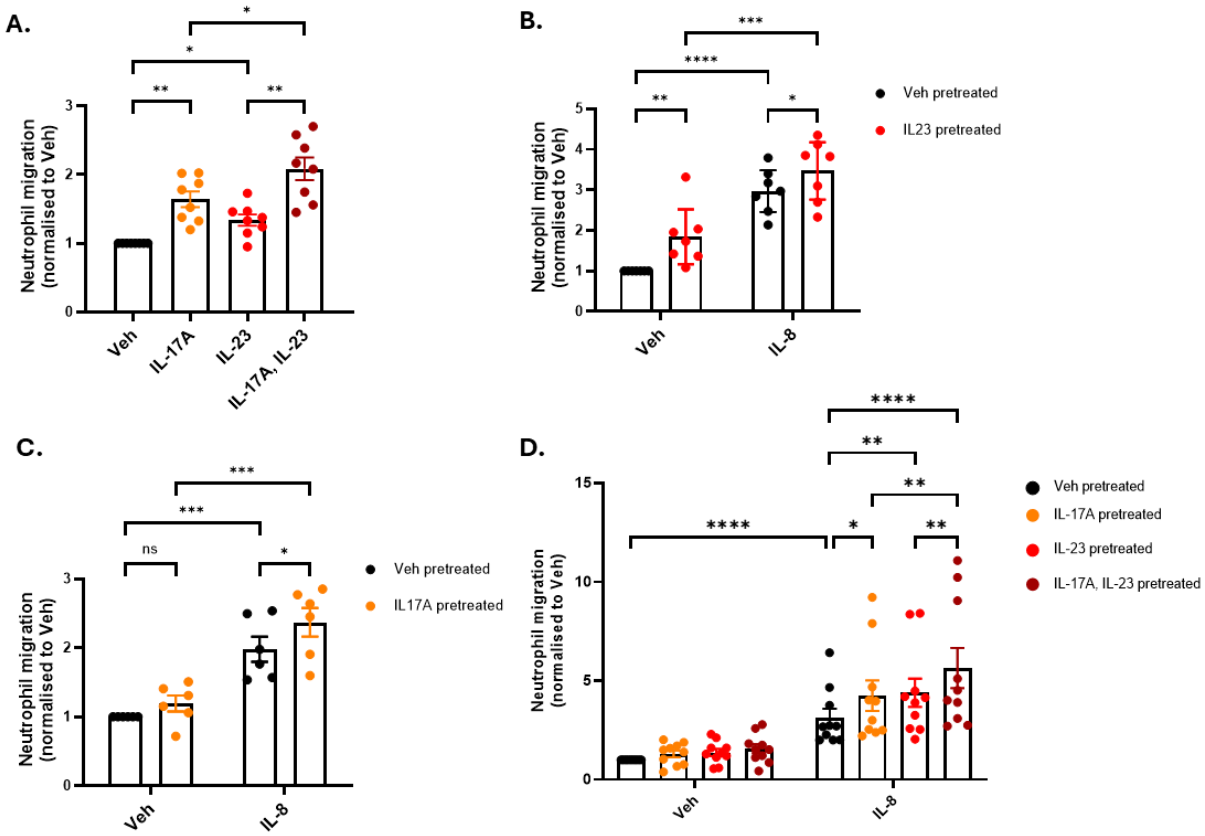


Figure 11. IL-23 and IL-17A enhance neutrophil migratory activity. A: PMNL were migrated towards Veh, IL-17A, IL-23, and IL-17A+IL-23 combined. B: PMNL were pretreated with IL-17A or Veh and then migrated towards IL-8. Results were normalised to the vehicle group. C: PMNL were pretreated with IL-23 or Veh and then migrated towards IL-8. D: PMNL were pretreated with IL-17A, IL-23, IL-17A+IL-23, or Veh and then migrated towards IL-8. Results were normalised to the vehicle group. Neutrophils were gated out of PMNL by excluding eosinophils based on their autofluorescence. Statistical analyses were performed- for A using One-way ANOVA followed by Tukey test for multiple comparisons; for B, C using 2way ANOVA followed by Fisher's Least Significant Difference test; for D, F using 2way

ANOVA followed by Tukey's test for multiple comparisons. Veh-Vehicle. $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***, $p < 0.0001$ = ****, ns = not significant. Parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.12. The IL-23/IL-17A axis affects neutrophil CD11b upregulation

In addition to neutrophil migration and ROS production, we further investigated the potential of IL-17A and IL-23 in CD11b upregulation, which is a neutrophil activation marker. CD11b upregulation reflects increased adhesive and migratory capacity of neutrophils and is indicative of functional priming. In this study, neutrophils were pretreated with IL-17A (10 ng/mL) (Fig. 12A) and IL-23 (10 ng/mL) (Fig. 12B) prior to C5a stimulation. Interestingly, we observed that neutrophils pretreated with IL-17A but not IL-23 showed a significant increase in CD11b surface expression. This indicates that IL-17A exerts a more direct priming effect on neutrophil CD11b upregulation than IL-23 in this context. As an integrin, CD11b is linked to the migratory response of cells. Higher CD11b expression in blood or airway neutrophils has been seen in COPD patients, especially during exacerbations [164,165]. These findings suggest that IL-17A-driven priming of CD11b may contribute to heightened neutrophil migratory activity and tissue infiltration during inflammatory flares in COPD.

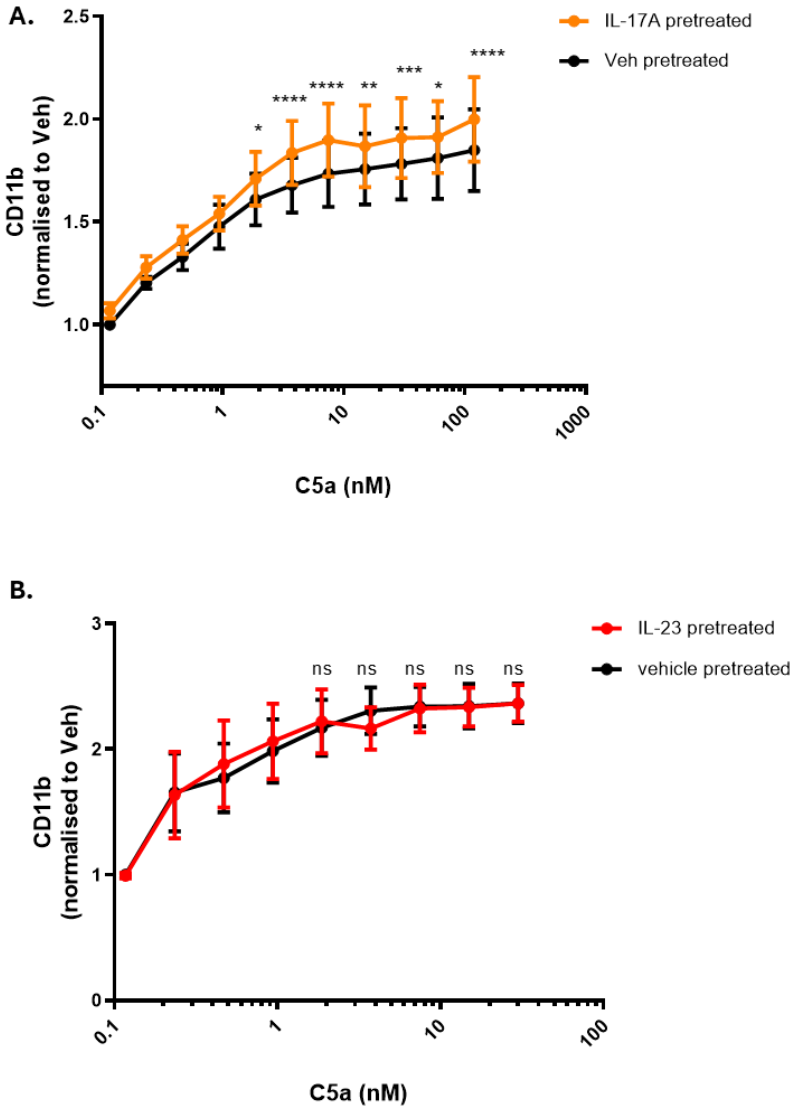


Figure 12. Effects of IL-23 and IL-17A on neutrophil CD11b upregulation. A: CD11b activation measured in PMNL pretreated with IL-17A or Veh and stimulated with C5a. B: CD11b upregulation measured in PMNL pretreated with IL-23 or Veh and stimulated with C5a. Results are normalised to the vehicle group. Neutrophils were gated out of PMNL by excluding eosinophils based on their autofluorescence. Statistical analyses were performed using 2way ANOVA followed by Sídák test for multiple comparisons. Veh-Vehicle. *p < 0.05, **p < 0.01, ***p < 0.001, ****p<0.0001, ns=not significant.

6.13. The IL-23/IL-17A axis affects neutrophil effector function – ROS production

Beyond migration, we examined the priming effects of IL-17A and IL-23 on additional neutrophil effector functions, specifically reactive oxygen species (ROS) production, which plays a crucial role in neutrophil-mediated inflammatory responses. Excessive ROS generation is a key contributor to oxidative tissue injury and impaired host defense in chronic lung disease. For this assay, neutrophils were pretreated with IL-17A (10 ng/mL) (Fig. 13A) or IL-23 (10 ng/mL) (Fig. 13B), followed by stimulation with serial dilutions of the complement-derived

peptide C5a, a known potent inflammatory mediator, which is associated with enhanced immune responses observed in chronic inflammatory diseases such as COPD [166]. This setup allowed assessment of cytokine-mediated priming of neutrophil oxidative burst in response to a classical inflammatory stimulus. Pretreatment with either IL-17A or IL-23 significantly increased ROS production. These results indicate that IL-23 and IL-17A lower the activation threshold of neutrophils, thereby amplifying inflammatory output upon secondary stimulation.

Together, these findings suggest that the IL-23/IL-17A axis contributes to neutrophilic inflammation by potentiating IL-8-mediated recruitment, as well as by directly enhancing neutrophil effector functions, such as ROS production. This dual effect on recruitment and activation provides a mechanistic explanation for how sustained IL-23/IL-17A signaling can drive persistent tissue damage in neutrophilic COPD.

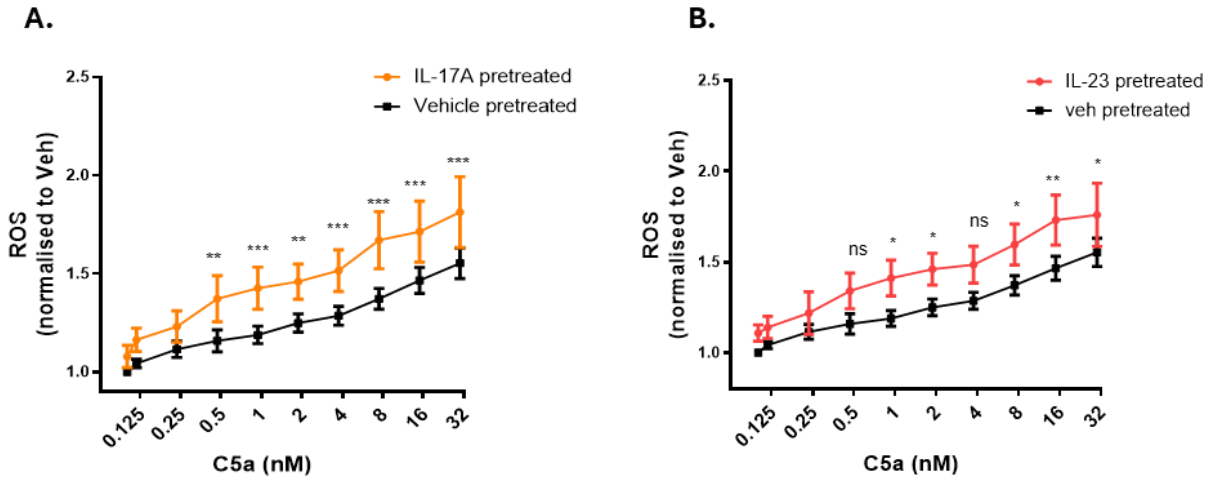


Figure 13. Effects of IL-23 and IL-17A on ROS production. A: ROS production measured in PMNL pretreated with IL-17A or Veh and stimulated with C5a. B: ROS production measured in PMNL pretreated with IL-23 or Veh and stimulated with C5a. Results are normalised to the vehicle group. Neutrophils were gated out of PMNL by excluding eosinophils based on their autofluorescence. Statistical analyses were performed using 2way ANOVA followed by Sídák test for multiple comparisons. Veh-Vehicle. *p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant. Parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.14. The IL-23/IL-17A axis does not affect calcium mobilization in neutrophils

Calcium mobilization is critical for IL-8–mediated neutrophil activation, enabling efficient chemotaxis, adhesion, degranulation, and ROS production. Accordingly, we investigated the impact of IL-23 and IL-17A on IL-8-induced calcium flux in neutrophils. This allowed us to determine whether cytokine-mediated priming operates upstream at the level of GPCR-triggered calcium signaling. Therefore, peripheral blood PMNLs were isolated and loaded with the calcium-sensitive fluorescent dye Fluo-3 and a dispersing agent to enhance dye uptake. Neutrophils within the PMNLs were stained with an anti-CD16 antibody before stimulation. The cells were pretreated with IL-17A, IL-23, or a vehicle control and calcium mobilization was induced with IL-8, while unstimulated cells served as a negative baseline. Calcium flux was continuously monitored by flow cytometry, and responses were compared to IL-8 stimulation. Under these conditions, pretreatment with IL-17A (Fig. 14A) or IL-23 (Fig. 14B) did not significantly change calcium mobilization compared to vehicle controls, indicating that neither cytokine influences IL-8–induced calcium responses in neutrophils. These data suggest that IL-23/IL-17A–mediated priming of neutrophil migration and effector function occurs downstream or independently of proximal calcium signaling.

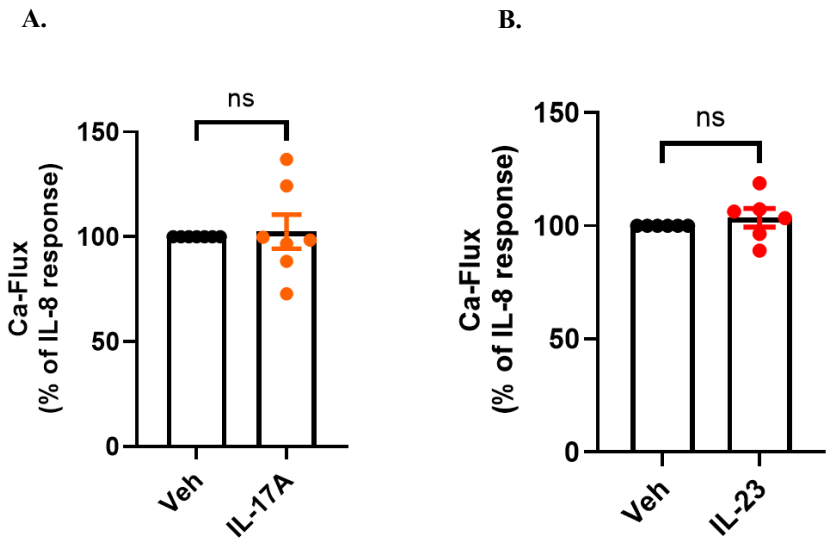


Figure 14. Effects of IL-23 and IL-17A on calcium flux in neutrophils. A: calcium flux measured in PMNL pretreated with IL-17A or Veh and stimulated with IL-8. B: calcium flux measured in PMNL pretreated with IL-23 or Veh and stimulated with IL-8. Results are measured

as percent of IL-8 response where the vehicle group is expressed at 100 %. Neutrophils were gated out of PMNL by excluding eosinophils based on their autofluorescence. Statistical analyses were performed using 2way ANOVA followed by Sídák test for multiple comparisons. Veh-Vehicle. ns = not significant

6.15. Brepocitinib blocks IL-23/IL-17A enhanced neutrophil migration in vitro

Over the years, a wide range of JAK inhibitors have been developed, tested and approved for clinical use in various immune-mediated inflammatory disease such as psoriasis, rheumatoid arthritis, and Crohn's disease. Since IL-23/IL-17A signaling is a crucial player in neutrophil-mediated inflammation and is known to be associated with TYK2 phosphorylation [167], we used brepocitinib, an investigational TYK2/JAK1 inhibitor [140]. This approach allowed us to interrogate the functional relevance of JAK1/TYK2 signaling downstream of the IL-23/IL-17A axis in neutrophils.

Here, we observed that neutrophils when pretreated with brepocitinib (300 nM) showed a significantly reduced migration towards IL-17A (10 ng/mL), IL-23 (10 ng/mL), and their combination (Fig. 15A). Beyond its inhibitory effects on IL-17A– and IL-23–stimulated migration, pretreatment with brepocitinib (300 nM) also led to a modest reduction in neutrophil migration toward IL-8 (10 nM) (Fig. 15B). This suggests partial modulation of downstream chemotactic signaling rather than complete suppression of classical chemokine responses. Additionally, brepocitinib (300 nM) pretreatment also inhibited the increased migratory response caused by the combined priming effects of IL-17A and IL-23 pretreatment, followed by subsequent stimulation towards IL-8 (Fig. 15C). Furthermore, brepocitinib (1µM) significantly reduced neutrophil migration toward conditioned media from cultured COPD lung tissue (Fig. 15D), which likely contains a wide variety of inflammatory mediators, enzymes, and collagens. This finding suggests that JAK1/TYK2 inhibition can dampen neutrophil responses to complex inflammatory milieus resembling the COPD lung microenvironment.

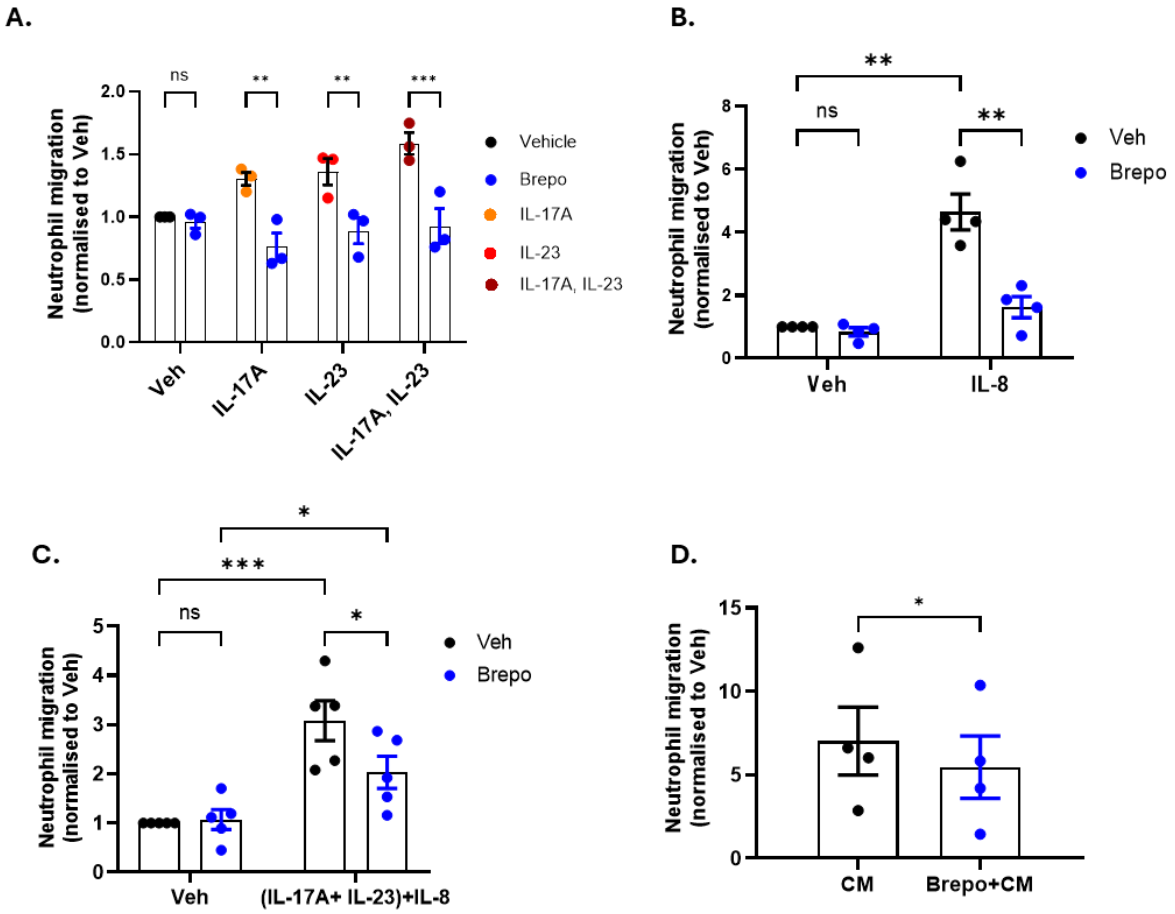


Figure 15. Brepocitinib inhibits IL-23, IL-17A, IL-8 driven neutrophil migration. A: Brepo or Veh pretreated PMNL were migrated towards IL-17A, IL-23, and their combination. B: Brepo or Veh pretreated PMNL were migrated towards IL-8. C: Brepo or Veh PMNL pretreated along with IL-17A and IL-23 pretreatments were migrated towards IL-8. D: Brepo or Veh pretreated PMNL were migrated towards conditioned media from COPD PCLS. Results were normalised to the vehicle group. Results were normalised to the vehicle group. Neutrophils were gated out of PMNL by excluding eosinophils based on their autofluorescence. Statistical analyses were performed for A, B, C, using 2way ANOVA followed by Holm-Sídák test or Fisher's Least Significant Difference test for multiple comparisons; for D, using the paired t-test. Veh-Vehicle; Brepo-Brepocitinib. *p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.16. Brepocitinib blocks IL-23/IL-17A-enhanced neutrophil ROS production in vitro

To further investigate the effects of brepocitinib on other neutrophil effector functions such as ROS production mediated by C5a as well as IL-17A (Fig. 16A) and IL-23 (Fig. 16B) priming,

we pretreated neutrophils with brepocitinib (300 nM) before stimulation with C5a. Brepocitinib significantly reduced ROS production induced by C5a stimulation and blocked the increased ROS production in neutrophils primed with either IL-17A or IL-23 prior to C5a stimulation.

These results show that brepocitinib not only restricts neutrophil migration but also significantly decreases their oxidative burst ability, which might lead to a reduction in neutrophil-associated inflammation.

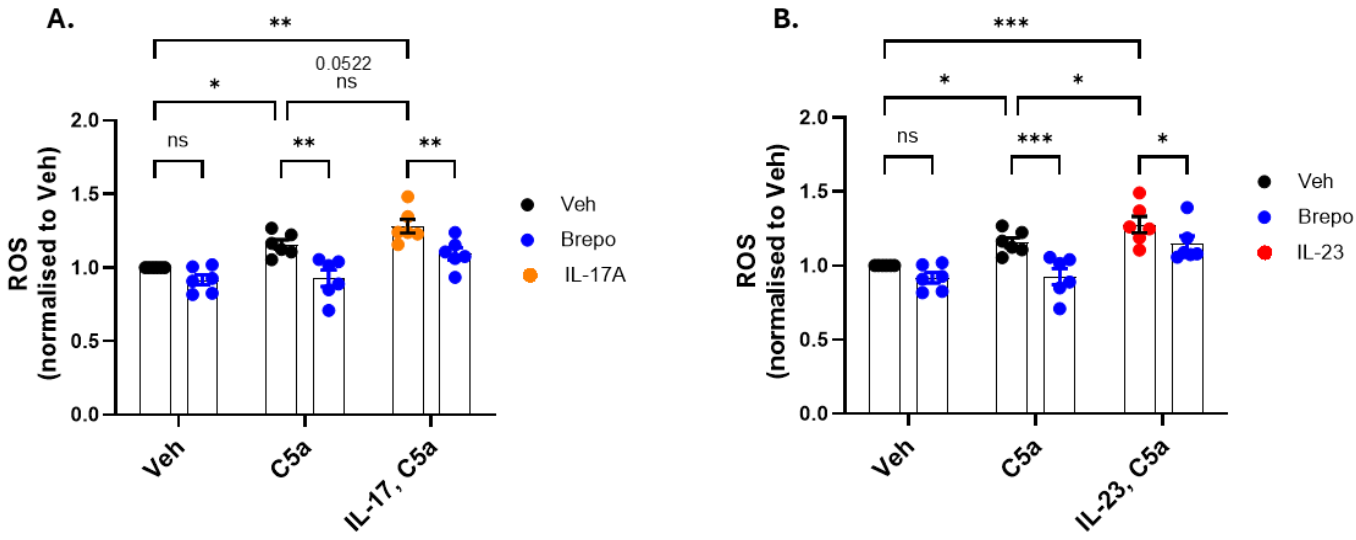


Figure 16. Brepocitinib inhibits IL-23, IL-17A, and C5A-induced neutrophil ROS production. A: ROS production measured in PMNL pretreated with Brepo or Veh, followed by IL-17A or Veh and stimulated with C5a. B: ROS production measured in PMNL pretreated with Brepo or Veh, followed by IL-23 or Veh and stimulated with C5a. Results were normalised to the vehicle group. Neutrophils were gated out of PMNL by excluding eosinophils based on their autofluorescence. Statistical analyses were performed using 2way ANOVA followed by Holm-Sídák test for multiple comparisons. Veh-Vehicle; Brepo-Brepocitinib. *p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.17. Brepocitinib blocked IL-23/IL-17A induced TYK2 and JAK1 phosphorylation

Our data so far clearly show that brepocitinib, a TYK2/JAK1 inhibitor, not only blocks IL-23-mediated effects, as expected, since IL-23 acts via TYK2, but also inhibits responses triggered by IL-17A. Since IL-17A has not been directly linked to JAK/STAT signaling, especially not with TYK2, we examined the effects of IL-17A and IL-23 on the phosphorylation of JAKs to

study their involvement in JAK/STAT signaling. For this purpose, we stimulated the cells with IL-23 (Fig. 17A, B) and IL-17A (Fig. 17C, D) each for 1 hour, in the presence or absence of brepocitinib, and then examined the phosphorylation of TYK2 and JAK1 (pTYK2 (Tyr1054/Tyr1055) and pJAK1 (Tyr1022/Tyr1023)) by flow cytometry. We observed that IL-23 treatment caused a significant increase in pTYK2 (Tyr1054/Tyr1055), as expected, but not in pJAK1 (Tyr1022/Tyr1023). Similarly, IL-17A stimulation was associated with increased phosphorylation of both pTYK2 (Tyr1054/Tyr1055) and pJAK1 (Tyr1022/Tyr1023). Again, the increase in phosphorylation was effectively reduced with brepocitinib co-treatment.

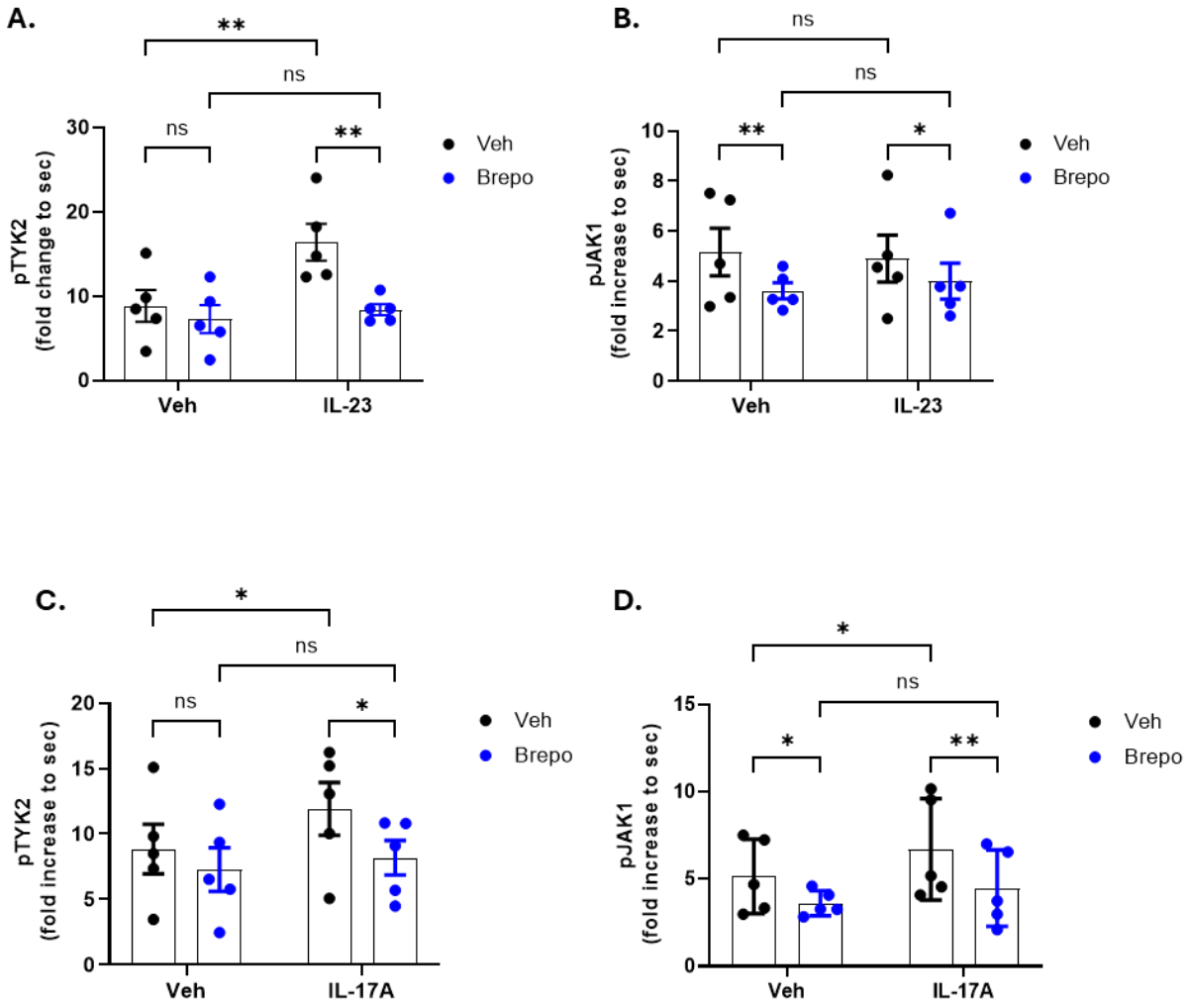


Figure 17. Brepocitinib inhibits IL-23 and IL-17A-stimulated TYK2 and JAK1 phosphorylation in neutrophils. A: PMNL pretreated with IL-23 or veh in the presence of Brepo or veh were measured for phosphorylation of TYK2 (pTYK2 (Tyr1054/Tyr1055)). B: PMNL pretreated with IL-23 or veh in the presence of Brepo or veh were measured for phosphorylation of JAK1 (pJAK1 (Tyr1022/Tyr1023)). C: PMNL pretreated with IL-17A or

veh in the presence of Brepo or veh were measured for phosphorylation of TYK2 (pTYK2 (Tyr1054/Tyr1055)). D: PMNL pretreated with IL-17A or veh in the presence of Brepo or veh were measured for phosphorylation of JAK1 (pJAK1 (Tyr1022/Tyr1023)). Results are reported as fold change to the secondary antibody. Neutrophils were gated out of PMNL by excluding eosinophils based on their autofluorescence. Statistical analyses were performed using 2-way ANOVA followed by Fisher's Least Significant Difference test. Veh-Vehicle; Brepo-Brepocitinib; sec-secondary antibody. *$p < 0.05$, **$p < 0.01$, ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.18. Western blot analysis confirmed TYK2 phosphorylation in response to IL-23/IL-17A

To investigate whether the IL-23/IL-17A axis activates TYK2 in human neutrophils, donor-derived neutrophils were stimulated with IL-17A or IL-23 for 15 minutes, and phosphorylation responses were assessed. Both cytokines caused a significant increase in TYK2 phosphorylation, confirmed by Western blot analysis (Fig. 18A). Densitometric analysis showed that pTYK2 (Tyr1054/Tyr1055) levels were significantly higher compared to controls, while total TYK2 levels stayed constant following stimulation with both IL-17A (Fig. 18B) and IL-23 (Fig. 18C). These results demonstrate a rapid and strong phosphorylation of the TYK2 pathway by both IL-17A and IL-23, aligning with our previously shown flow cytometry data showing increased pTYK2 (Tyr1054/Tyr1055) in human PMNL after stimulation with IL-17A and IL-23.

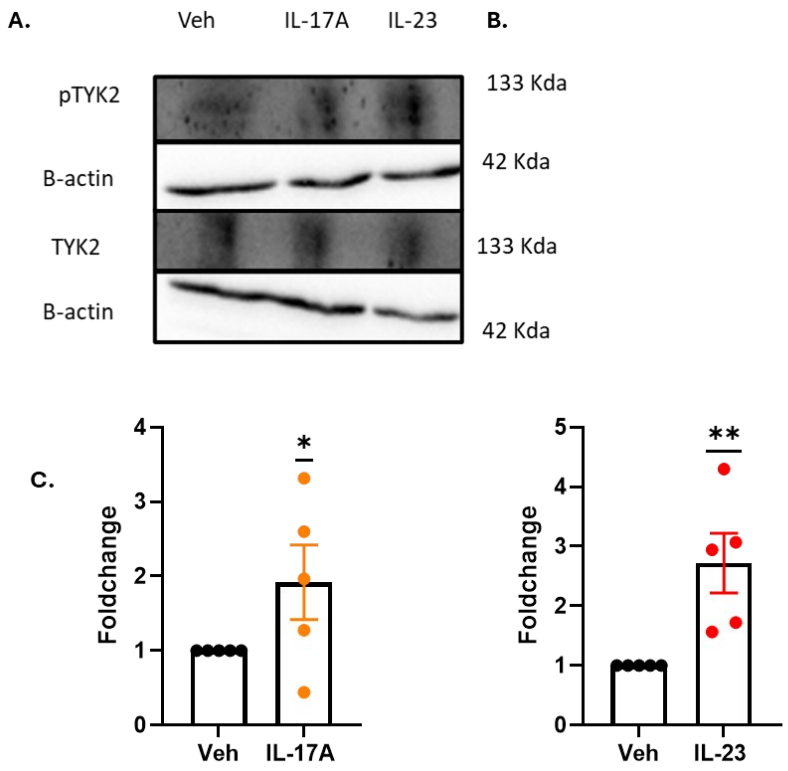


Figure 18. IL-17A and IL-23 induce phosphorylation of TYK2 in human PMNL. Isolated human PMNL were stimulated with IL-17A (10 ng/mL) or IL-23 (10 ng/mL) for 15 minutes. Whole-cell lysates were prepared and analyzed by Western blotting using antibodies against pTYK2 (Tyr1054/Tyr1055) and total TYK2. β-actin was used as a loading control. A: Representative Western blot showing phosphorylated TYK2 (pTYK2 (Tyr1054/Tyr1055)) and total TYK2 levels in vehicle and cytokine-stimulated PMNL. (B–C) Quantification of pTYK2 (Tyr1054/Tyr1055)/TYK2 ratios following stimulation with (B) IL-17A and (C) IL-23. Results were normalised to the vehicle group. Outliers were assessed using the Grubbs' test; no significant outliers were detected. Normal distribution was confirmed using the Shapiro–Wilk test. Statistical analyses were performed using a one-sample t-test. Veh-Vehicle. Veh-Vehicle. *p < 0.05, **p < 0.01, ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.19. Indirect phosphorylation of JAKs by IL-8 and C5a

We also observe that brepocitinib not only blocks IL-23 and IL-17A-mediated effects but also inhibits responses triggered by IL-8 and C5a. IL-8 and C5a have not been directly linked to JAK/STAT signaling; therefore, we examined the effects of these mediators on JAK phosphorylation to explore their involvement in JAK/STAT signaling. For this, we stimulated the cells with IL-8 (Fig. 19A) and C5a (Fig. 19B) for 1 hour and 20 minutes, respectively, then

examined the phosphorylation of TYK2, JAK1, and JAK2 by flow cytometry. We observed that IL-8 treatment caused a significant increase in pTYK2 (Tyr1054/Tyr1055), pJAK1 (Tyr1022/Tyr1023), and pJAK2 (Tyr1007/Tyr1008) in neutrophils. Conversely, C5a pretreatment significantly increased phosphorylation of pTYK2 (Tyr1054/Tyr1055) and pJAK1 (Tyr1022/Tyr1023), but not pJAK2 (Tyr1007/Tyr1008).

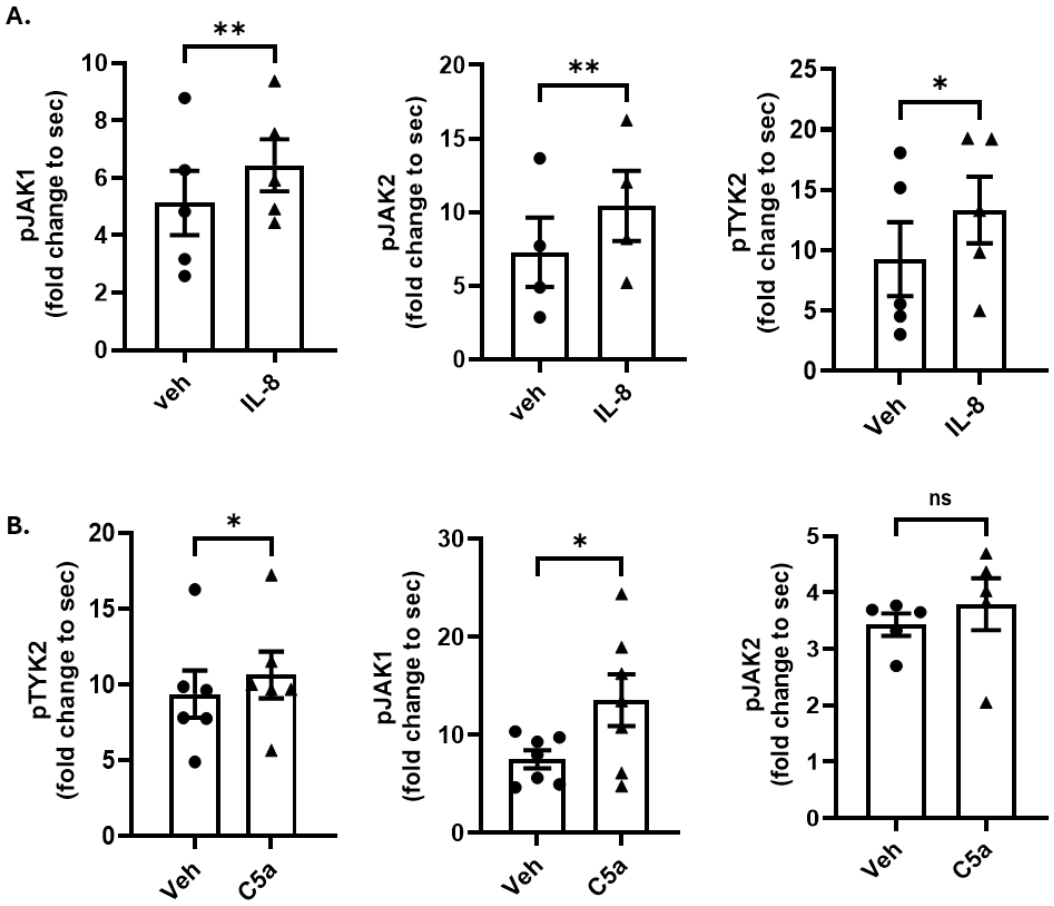
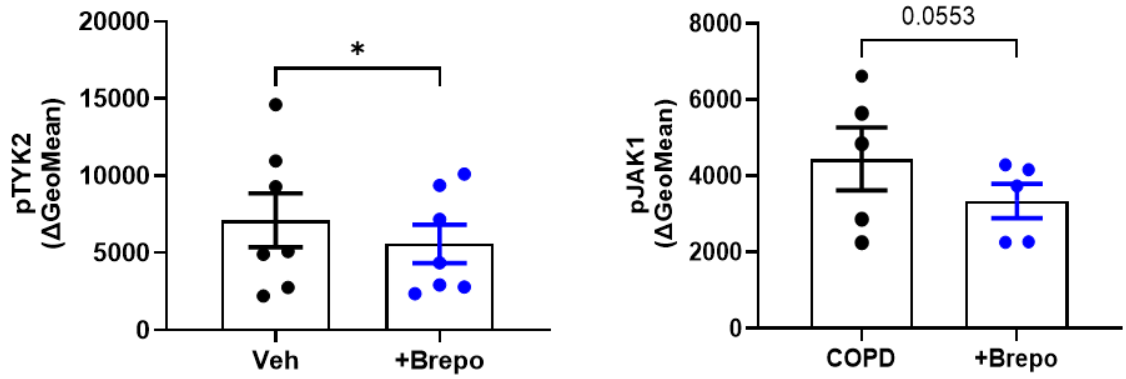


Figure 19. IL-8 and C5a stimulate phosphorylation of JAK1, JAK2 and TYK2 in neutrophils. A, B: PMNL stimulated with vehicle, IL-8, or C5a were measured for phosphorylation of JAK1 (pJAK1 (Tyr1022/Tyr1023)), JAK1 (pJAK1 (Tyr1022/Tyr1023)), and TYK2 (pTYK2 (Tyr1054/Tyr1055)). Results are reported as fold change relative to the secondary antibody. Outliers were assessed using the Grubbs' test. Normal distribution was confirmed using the Shapiro–Wilk test. Statistical analyses were performed using the paired t-test. Veh-Vehicle; Brepo-Brepocitinib; sec-secondary antibody. *$p < 0.05$, **$p < 0.01$, ns = not significant. Parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.20. Brepocitinib reduced pTYK2 (Tyr1054/Tyr1055) and pJAK1 (Tyr1022/Tyr1023) in neutrophils from COPD patients

We next aimed to confirm the inhibitory effects of brepocitinib on elevated phosphorylated TYK2 (pTYK2 (Tyr1054/Tyr1055)) and JAK1 (pJAK1 (Tyr1022/Tyr1023)) levels in COPD patients. In our previous data, we observed that brepocitinib reduced IL-23/IL-17A-mediated increases in pTYK2 (Tyr1054/Tyr1055) and pJAK1 (Tyr1022/Tyr1023). To validate these findings, whole blood from COPD patients was treated with brepocitinib (1µM) or vehicle for 1 hour at 37°C, followed by the assessment of pTYK2 (Tyr1054/Tyr1055) (Fig. 20A) and pJAK1 (Tyr1022/Tyr1023) (Fig. 20B) using flow cytometry. Consistent with our previous results, brepocitinib induced a marked reduction in pTYK2 (Tyr1054/Tyr1055) and a trend towards reduced pJAK1 (Tyr1022/Tyr1023) (p=0.055), demonstrating its direct inhibitory



effect on TYK2 and JAK1phosphorylation in COPD.

Figure 20. Brepocitinib inhibits TYK2 and JAK1 phosphorylation in neutrophils. Whole blood from COPD patients treated with Brepo or vehicle for 1 hour and then were measured for phosphorylation of A: TYK2 (pTYK2 (Tyr1054/Tyr1055)) and B: JAK1(pJAK1 (Tyr1022/Tyr1023)). For statistical analysis was performed by paired t-test.The results are expressed as ΔGeoMean. ΔGeoMean= $GeoMean_{stained\ sample}$-$GeoMean_{FMO}$. GeoMean-Geometric Mean. Veh-Vehicle; Brepo-Brepocitinib. *p < 0.05, ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.21. Brepocitinib inhibits IL-17A and IL-23-induced IL-8 production in PBECs

To investigate the potential role of the epithelium in IL-23/IL-17A axis-mediated neutrophil responses, we employed primary bronchial epithelial cells (PBECs) differentiated at an air-liquid interface (ALI). These cells were stimulated with either IL-17A (10 ng/mL) or IL-23 (10 ng/mL). IL-17A is known to directly influence epithelial cells, inducing the secretion of IL-8 [168], a major neutrophil chemoattractant [168] and its effects can be further amplified by the presence of additional pro-inflammatory cytokines such as TNF-α and IL-1β [169].

In our experimental setup, ALI-PBECs were treated with a positive control consisting of TNF-α + IL-1β (20 ng/mL) alone or in combination with IL-17A or IL-23, with or without brepocitinib (300 nM) as a pharmacological intervention. After this stimulation, conditioned medium was collected, and then freshly isolated neutrophils were co-cultured with the treated PBECs for 2 hours at 37°C. Neutrophil activation was assessed by measuring CD11b surface expression by flow cytometry (Fig. 21A).

Analysis of IL-8 protein levels in conditioned media collected from stimulated PBEC cultures revealed that exposure to the positive control stimulus (TNF-α + IL-1β) caused a significant increase in IL-8 secretion compared to unstimulated cells, confirming the strong pro-inflammatory nature of this cytokine combination [169–171] . Notably, when IL-17A (Fig. 21A) was added to the positive control, we observed a synergistic rise in IL-8 production. A similar pattern appeared when IL-23 (Fig. 21B) was combined with the positive control, again resulting in an increased IL-8 response with borderline significance ($p = 0.0503$). These results suggest that both IL-17A and IL-23 can enhance epithelial-derived IL-8 secretion in a pro-inflammatory environment. Importantly, when brepocitinib (300 nM) was included as a treatment, IL-8 levels were significantly lowered across all conditions—both in the positive control alone and with IL-17A or IL-23. This demonstrates that brepocitinib effectively reduces epithelial IL-8 production.

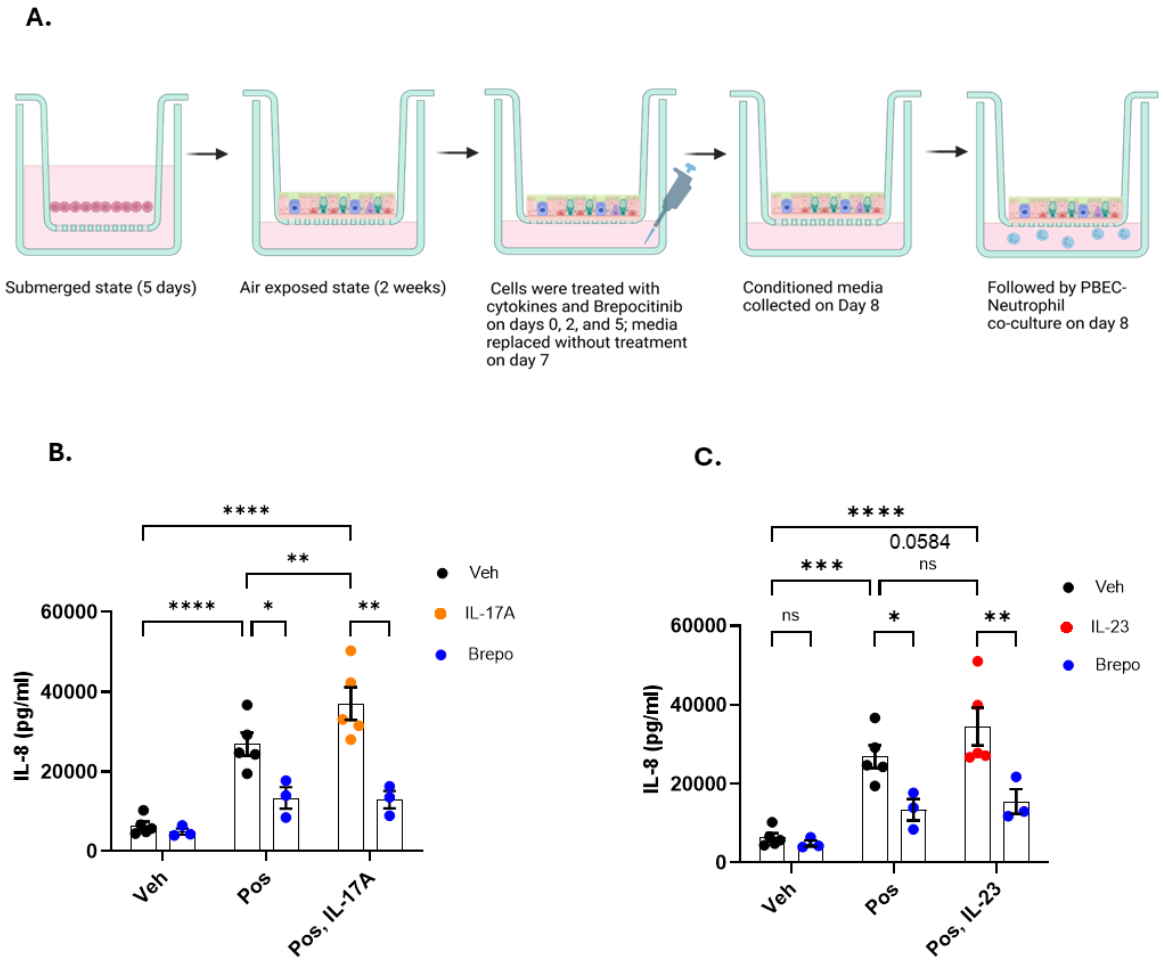


Figure 21. Brepocitinib inhibits IL-17A and IL-23-induced IL-8 production in PBECs. A: Schematic representation of the ALI PBEC experimental setup. The PBECs in submerged state for 5 days; air exposed for 2 weeks; now fully differentiated PBECs were treated with media containing IL-17A or IL-23 (at 10 ng/mL) or Veh, either alone or in combination with a positive control (TNFα + IL-1β at 20 ng/mL), with or without Brepo for a week (day 0, 2, 5). On day 7, fresh medium was added and incubated for 24 hours. On day 8, conditioned media is collected before the co-culture for ELISA and migration assays, followed by neutrophil co-culture on the basal side of the inserts for 2 hours. B and C: Conditioned media from PBECs treated with IL-17A and IL-23, respectively, were measured for IL-8 secretion using ELISA. Statistical analyses were performed using mixed-effect analysis followed by the Holm-Sídák test for multiple comparisons. Veh-Vehicle; Brepo-Brepocitinib; Pos- Positive control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.22. Brepocitinib inhibits IL-17A and IL-23-induced IL-8 in PBECs, reducing neutrophil migration

Furthermore, to directly assess the functional consequences of epithelial stimulation on neutrophil behaviour, we performed migration assays using conditioned media collected from PBECs subjected to the same treatment conditions described above. When neutrophils were exposed to conditioned media from IL-17A-treated (Fig. 22A) or IL-23-treated (Fig. 22B) PBECs, there was a significant increase in neutrophil chemotaxis. Notably, including brepocitinib during epithelial cell stimulation resulted in conditioned media that significantly reduced neutrophil migration across all sample conditions. These results show that pharmacological inhibition with brepocitinib not only suppresses epithelial cytokine release but also effectively reduces the downstream chemotactic signals that promote neutrophil recruitment.

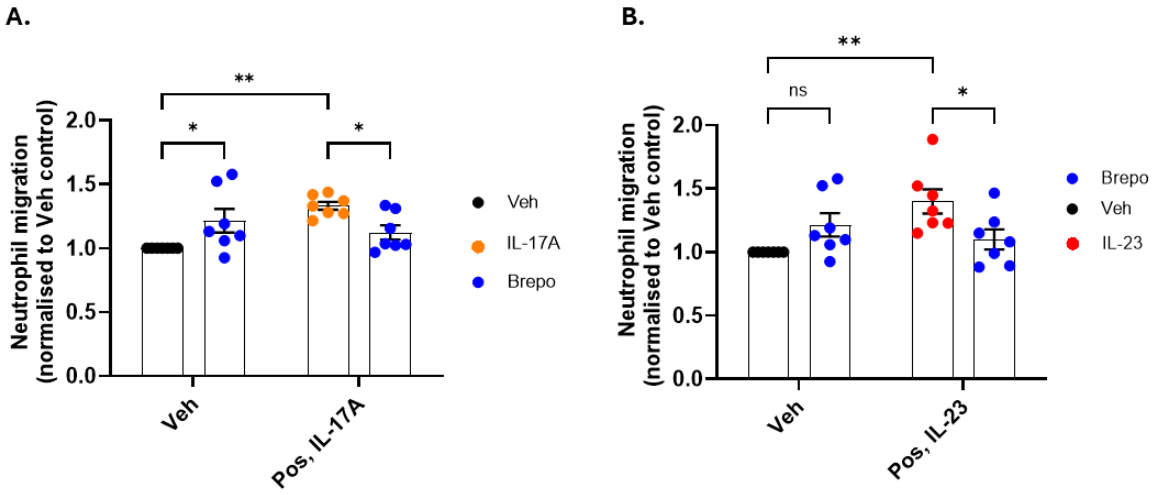


Figure 22. Brepocitinib inhibits IL-17A- and IL-23-induced IL-8 production in PBECs, thereby inhibiting neutrophil recruitment. A and B: PMNL migrated towards the conditioned media from PBECs treated with IL-17A and IL-23, respectively. Results are normalised to the vehicle-treated group, which is expressed as 1. Statistical analyses were performed using 2-way ANOVA followed by Holm-Sídák test for multiple comparisons. Veh-Vehicle; Brepo-Brepocitinib; Pos- Positive control. *$p < 0.05$, **$p < 0.01$, ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.23. Brepocitinib inhibits IL-17A and IL-23-induced CD11b in PBEC-neutrophil co-culture

Using the same experimental setup as described earlier (Fig. 21A), freshly isolated neutrophils were co-cultured with treated PBECs for 2 hours at 37°C. Subsequently, neutrophil activation was then assessed by measuring CD11b surface expression via flow cytometry. Neutrophils co-cultured with IL-17A (Fig. 23A) and IL-23 (Fig. 23A) stimulated PBECs showed a distinct increase in CD11b, indicating enhanced activation. Notably, this increase was significantly reduced when epithelial cells were co-treated with brepocitinib. Overall, these results indicate that epithelial cells stimulated by IL-17A and IL-23 promote neutrophil activation, and that brepocitinib effectively reduces this epithelial-driven neutrophil priming.

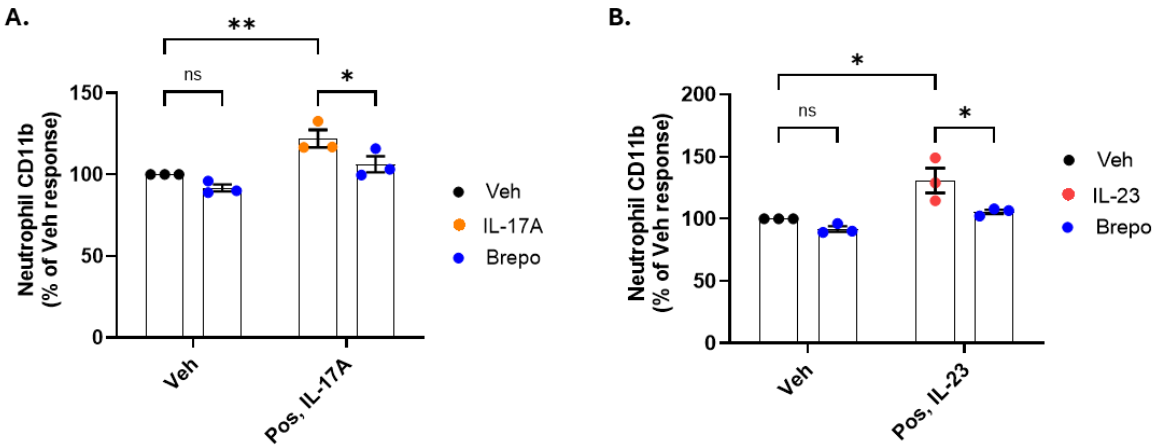


Figure 23. Brepocitinib inhibits IL-17A and IL-23-induced IL-8 production in PBECs, decreasing neutrophil CD11b upregulation in the coculture. A and B: PMNL co-cultured with PBECs treated with IL-17A and IL-23, respectively. Neutrophils were gated out of PMNL using CD16 and were measured for CD11b upregulation. Results are expressed as a percentage of the response in the vehicle-treated control group, expressed as 100 %. Statistical analyses were performed using 2-way ANOVA followed by Fisher's Least Significant Difference test. Veh-Vehicle; Brepo-Brepocitinib; Pos- Positive control. *p < 0.05, **p < 0.01, ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.24. Effects of brepocitinib on IL-8 production in smoke-induced PBECs

In a separate experiment, air–liquid interface (ALI)-differentiated primary bronchial epithelial cells (PBECs) were exposed to cigarette smoke daily for seven days starting from day 0. Brepocitinib (300 nM) was added to the basal medium during this period in the experimental

groups, while controls received only basal medium. On day 7, the medium was replaced with fresh basal medium without smoke or inhibitor, and cultures were incubated for additional 24 hours. Conditioned media collected on day 8 were analyzed for IL-8 levels by ELISA (Fig. 24). Consistent with the pro-inflammatory effects of smoke, smoke-exposed PBECs showed slightly higher IL-8 levels than controls. Treatment with brepocitinib caused a mild, non-significant reduction in IL-8 secretion in smoke-exposed PBECs.

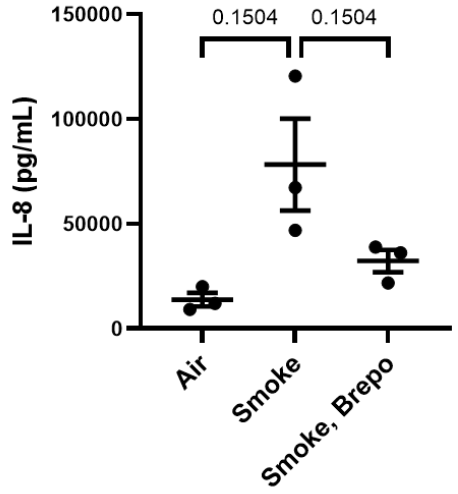


Figure 24. Effect of brepocitinib on IL-8 release from smoke activated PBECs. The fully differentiated PBEC were treated with air, smoke, and smoke+brepo for a week (day 0, 2, 5). On day 7, fresh medium was added and incubated for 24 hours. On day 8, conditioned media is collected. Conditioned media from PBECs treated with air, smoke, and smoke + brepo, respectively, were measured for IL-8 secretion by ELISA. Statistical analyses were performed using one-way ANOVA followed by Holm-Sídák test for multiple comparisons. Brepo-Brepocitinib. ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.25. Deucravacitinib, a TYK2 specific inhibitor blocks IL-17A and IL-23-induced IL-8 production in PBECs

In an experimental setup similar to the previous one (refer to Fig. 21A), ALI-differentiated PBECs were treated for 7 days starting on day 0, with a positive control (TNFα + IL-1β at 20 ng/mL), either alone or combined with the cytokines IL-17A or IL-23 at 10 ng/mL, with or without deucravacitinib (300 nM) added to the basal medium. On day 7, the medium was replaced with fresh medium and incubated for 24 hours. On day 8, conditioned medium was collected for ELISA.

Consistent with our previous findings with brepocitinib, blocking TYK2 signaling with deucravacitinib also led to a significant decrease in IL-8 secretion from activated PBECs. Importantly, this inhibitory effect was observed both in cultures treated with the positive control alone and in those stimulated with the positive control along with IL-17A (Fig. 25A) or IL-23

(Fig. 25B). These results further emphasize the role of TYK2 in IL-8 release in response to pro-inflammatory signals in PBECs.

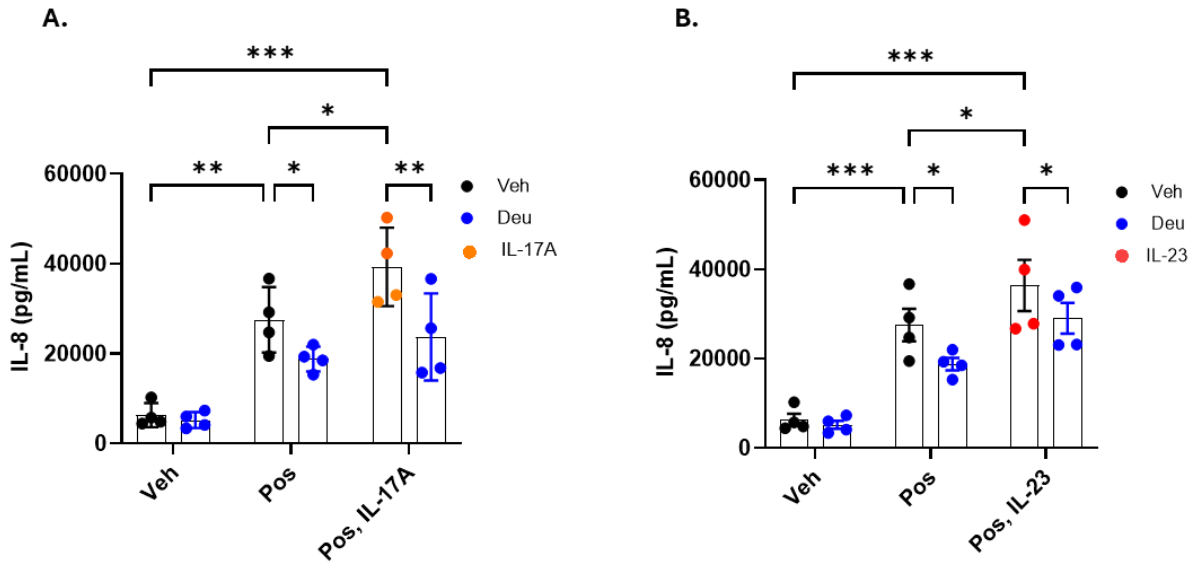


Figure 25. The effect of deucravacitinib on IL-8 release from activated PBECs. The fully differentiated PBEC were treated with media containing IL-17A or IL-23 (at 10 ng/mL) or Veh, either alone or in combination with a positive control (TNFα + IL-1β at 20 ng/mL), with or without Deu for a week (day 0, 2,5). On day 7, fresh medium was added and incubated for 24 hours. On day 8, conditioned media was collected. A and B: Conditioned media from PBEC treated with IL-17A and IL-23, respectively, were measured for IL-8 secretion using ELISA. Statistical analyses were performed using 2-way ANOVA followed by the Holm-Sídák test for multiple comparisons. Veh-Vehicle; Deu-Deucravacitinib; Pos- Positive control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.26. GR (Glucocorticoid Receptor) protein abundance in COPD patients

A proposed mechanism for corticosteroid resistance involves the potential overexpression of the glucocorticoid receptor beta (GRβ) isoform, which acts as a dominant-negative variant that inhibits GRα, the functional receptor. This isoform is produced through alternative splicing of the NR3C1 gene [172,173]. Previous research has linked neutrophilic inflammation, the IL-23/IL-17A axis, and corticosteroid resistance in patients with chronic lung diseases, especially regarding corticosteroid insensitivity of PBMCs and airway epithelium [114,174]. Nonetheless, the precise mechanisms are not fully understood. Our microarray analysis revealed a significant

increase in NR3C1 (Fig. 26A) mRNA abundance in sputum cells from severe COPD patients compared to those with moderate disease. While this may indicate upregulation of the inflammation-related GRβ isoform, the overall increase in NR3C1 level does not clearly differentiate between alternative splicing into GRα or GRβ. Proper identification of these isoforms requires isoform-specific protein analyses.

Thus, we examined the levels of GRα (Fig. 26B) and GRβ (Fig. 26C) proteins in peripheral blood neutrophils from COPD patients, smokers without COPD, and healthy controls using flow cytometry. Results indicated that COPD patients exhibited significantly higher GRβ levels compared to healthy controls, while GRα levels were similar across groups. Notably, the GRα to GRβ ratio (Fig. 26D), an important marker of corticosteroid sensitivity [175] was significantly decreased in COPD patients relative to controls.

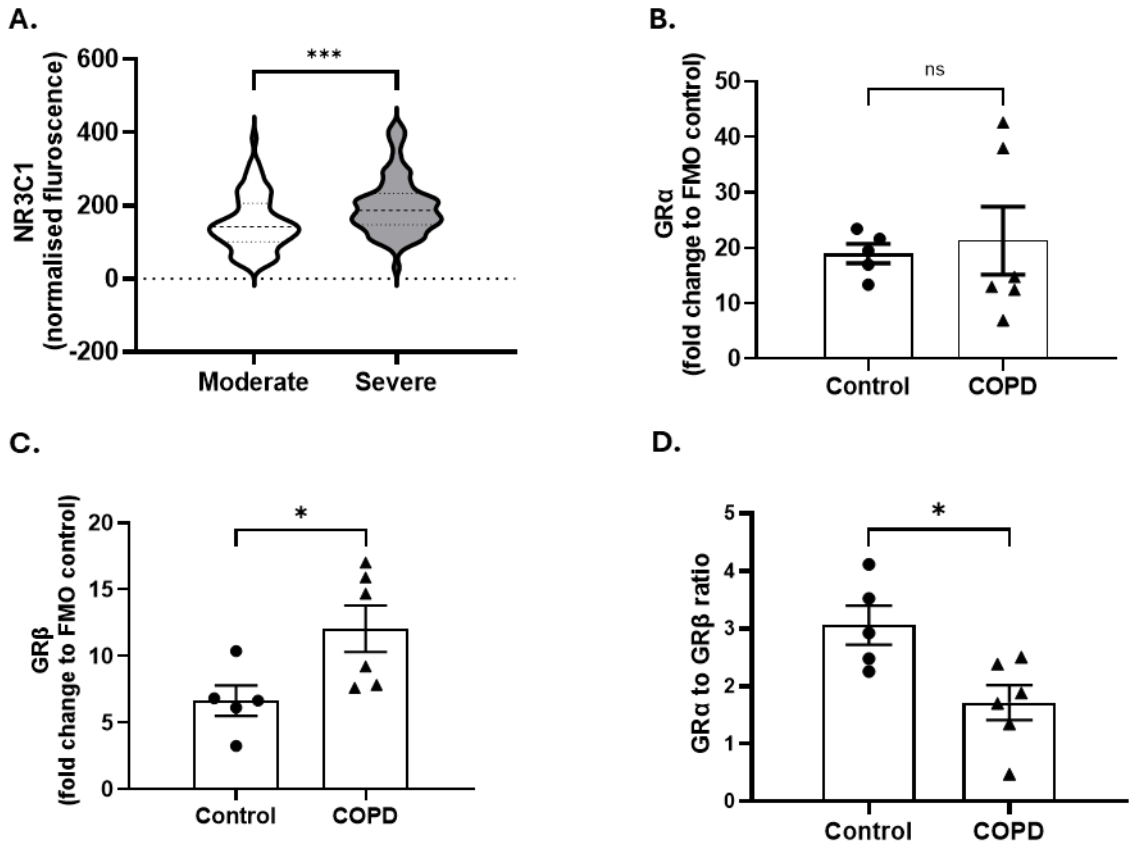


Figure 26. NR3C1 mRNA levels in moderate versus severe COPD, and Glucocorticoid receptor protein levels in healthy donors versus COPD. A: NR3C1 mRNA levels in sputum-derived immune cells (neutrophils and macrophages) from patients with moderate (stage II) and severe (stages III and IV) COPD, based on microarray data. B: Protein abundance levels of GRα in neutrophils from healthy donors compared to COPD patients. C: Protein abundance levels of

GRβ in neutrophils from healthy donors compared to COPD patients. D: GRα to GRβ ratio in neutrophils from healthy donors compared to COPD patients. Statistical analyses were performed using either unpaired t-test or Mann-Whitney U test based on normal distribution. *p < 0.05, ***p < 0.001, ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.27. GR (Glucocorticoid Receptor) protein abundance in smokers

Similarly, neutrophils from smokers showed a trend toward higher levels of the glucocorticoid receptor isoform GRβ (Fig. 27B) compared to those from healthy non-smokers. However, there was no significant difference in GRα (Fig. 27A) protein abundance between the groups. Consistently, the GRα to GRβ ratio (Fig. 27C) was significantly higher in healthy controls than in smokers, indicating potentially greater glucocorticoid sensitivity in non-smokers. Overall, these findings suggest that increased GRβ protein abundance may already be present in smokers before the onset of clinically apparent disease. This supports the idea that GRβ could serve as an early molecular marker of corticosteroid resistance, emphasizing its potential as a predictive biomarker for smoking-related airway inflammation.

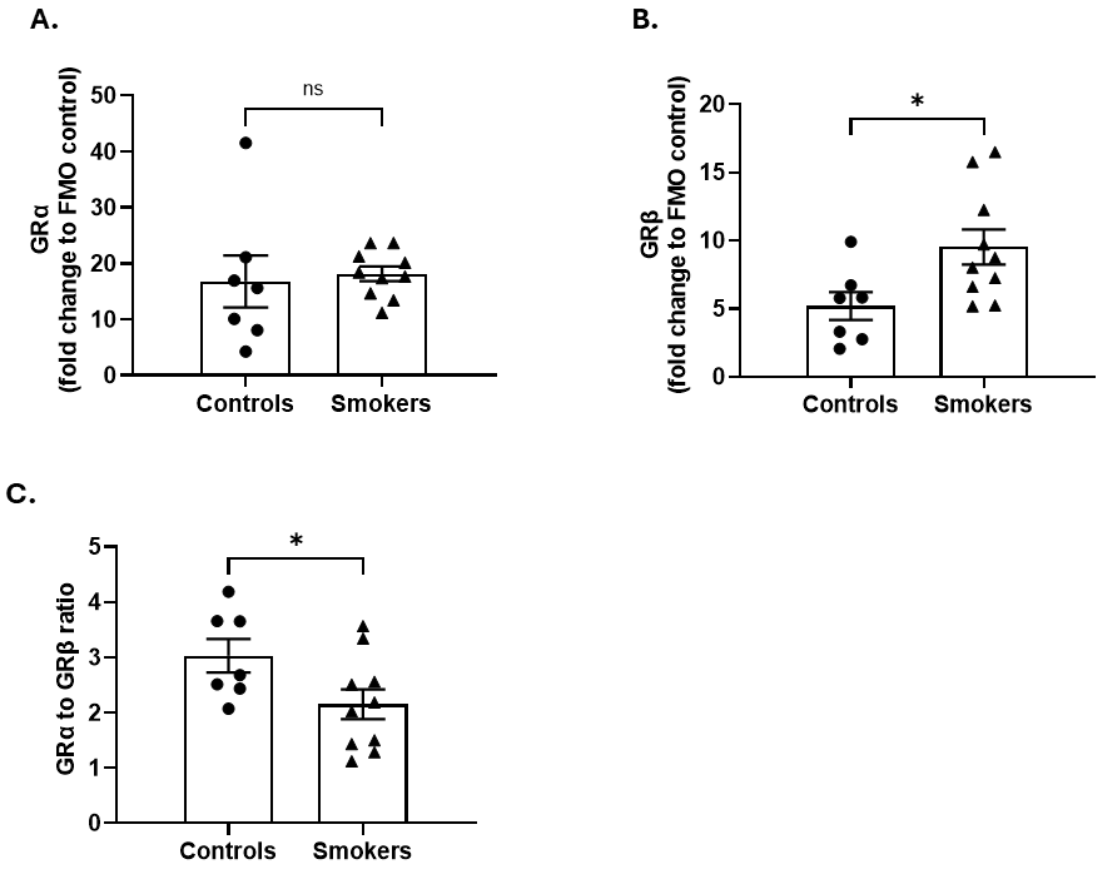


Figure 27. Glucocorticoid receptor expression in smokers versus non-smokers. A: Protein abundance levels of GRα in neutrophils from healthy non-smokers compared to smokers. B: Protein abundance levels of GRβ in neutrophils from healthy non-smokers compared to smokers. C: GRα GRβ to ratio in neutrophils from healthy non-smokers compared to smokers. Statistical analyses were performed using either unpaired t-test based on normal distribution. *p < 0.05, ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.28. IL-23 and IL-17A induce GRβ upregulation in neutrophils, mimicking disease patterns, which can be mitigated with Brepocitinib

To further explore the role of IL-23 and IL-17A in corticosteroid resistance, PMNL were pretreated with a combination of IL-17A and IL-23 (10 ng/mL each) (Fig. 28A) for 3 hours at 37°C, then examined for glucocorticoid receptor isoforms using flow cytometry. This cytokine stimulation caused a significant shift in receptor protein abundance, marked by a notable

decrease in GRα and an increase in GRβ. As a result, the GRα to GRβ ratio was significantly lowered under these conditions, mimicking key molecular features of corticosteroid resistance commonly seen in patients with COPD and in smokers.

When cells were co-treated with brepocitinib (300 nM) (Fig. 28B), a dual TYK2/JAK1 inhibitor, a clear modulatory effect on receptor protein abundance was observed. Specifically, brepocitinib treatment caused a significant decrease in GRβ levels, while GRα protein abundance remained consistent. This selective effect on GRβ resulted in a notable increase in the GRα to GRβ ratio compared to cytokine treatment alone. These results emphasize the ability of brepocitinib to counteract cytokine-induced alterations in glucocorticoid receptor balance and highlight its potential to restore the GRα to GRβ ratio, an important factor in corticosteroid sensitivity, in inflammatory conditions such as COPD.

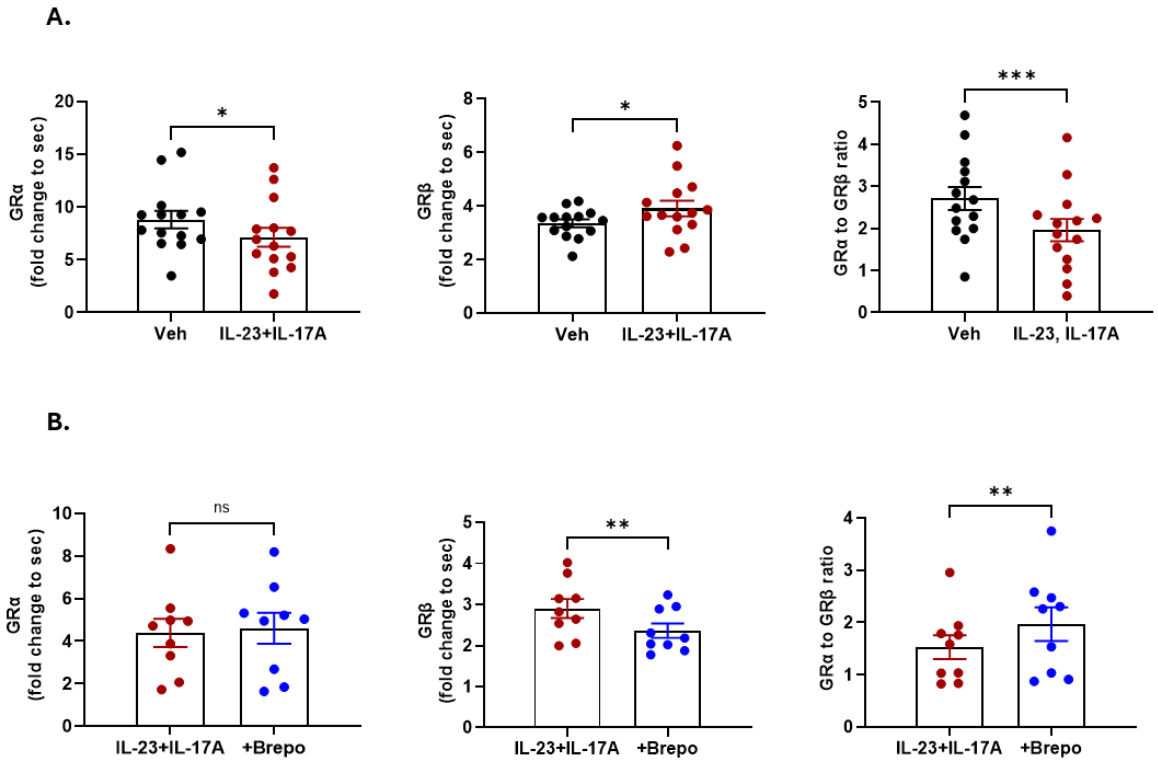


Figure 28. Effect of Brepocitinib on IL-17A/IL-23-induced upregulation of GRβ in neutrophils. A: PMNL were pretreated with IL-17A and IL-23 for 3 hours and stained for GRα and GRβ, and the protein abundance levels of GRα, GRβ, and their ratio in neutrophils are shown. B: PMNL were pretreated with IL-17A and IL-23, with or without Brepo, for 3 hours, then stained for GRα and GRβ and the protein abundance levels of GRα, GRβ, and their ratio in neutrophils are shown. Results are expressed as fold change relative to the secondary antibody. Neutrophils were gated out from PMNL by excluding eosinophils based on autofluorescence. Statistical analyses were performed using a paired t-test. GRα-Glucocorticoid

receptor alpha; GRβ - Glucocorticoid receptor beta; Veh - Vehicle; Brepo - Brepocitinib. *p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.29. Brepocitinib blocks IL-23-induced neutrophil recruitment into the airways

Since our initial experiments demonstrated that IL-23 stimulation leads to a marked increase in neutrophil migration in vitro, we sought to confirm and validate this effect in corresponding in vivo models. Specifically, when IL-23 was administered intranasally at a dose of 2 µg to C57Bl/6 mice, there was a significant and noticeable rise in the number of neutrophils present in the bronchoalveolar lavage (BAL) fluid, as depicted in Fig. 29A. Building on our in vitro data, which indicated that brepocitinib can effectively counteract neutrophil activation induced by IL-23, our in vivo experiments revealed that co-treatment with brepocitinib at a dose of 3 mg/kg, delivered orally, resulted in a significant reduction of neutrophil recruitment into the airways that was initially prompted by IL-23 administration (Fig. 29B). It is also noteworthy that other immune cell populations within the BAL fluid, such as alveolar macrophages (Fig. 29D), and lymphocytes (Fig. 29C), did not show any significant changes in their numbers following these treatments. However, IL-23 treatment increased the migration of eosinophils, which opens another important avenue to further investigate the role of IL-23 in eosinophil recruitment (Fig. 29E).

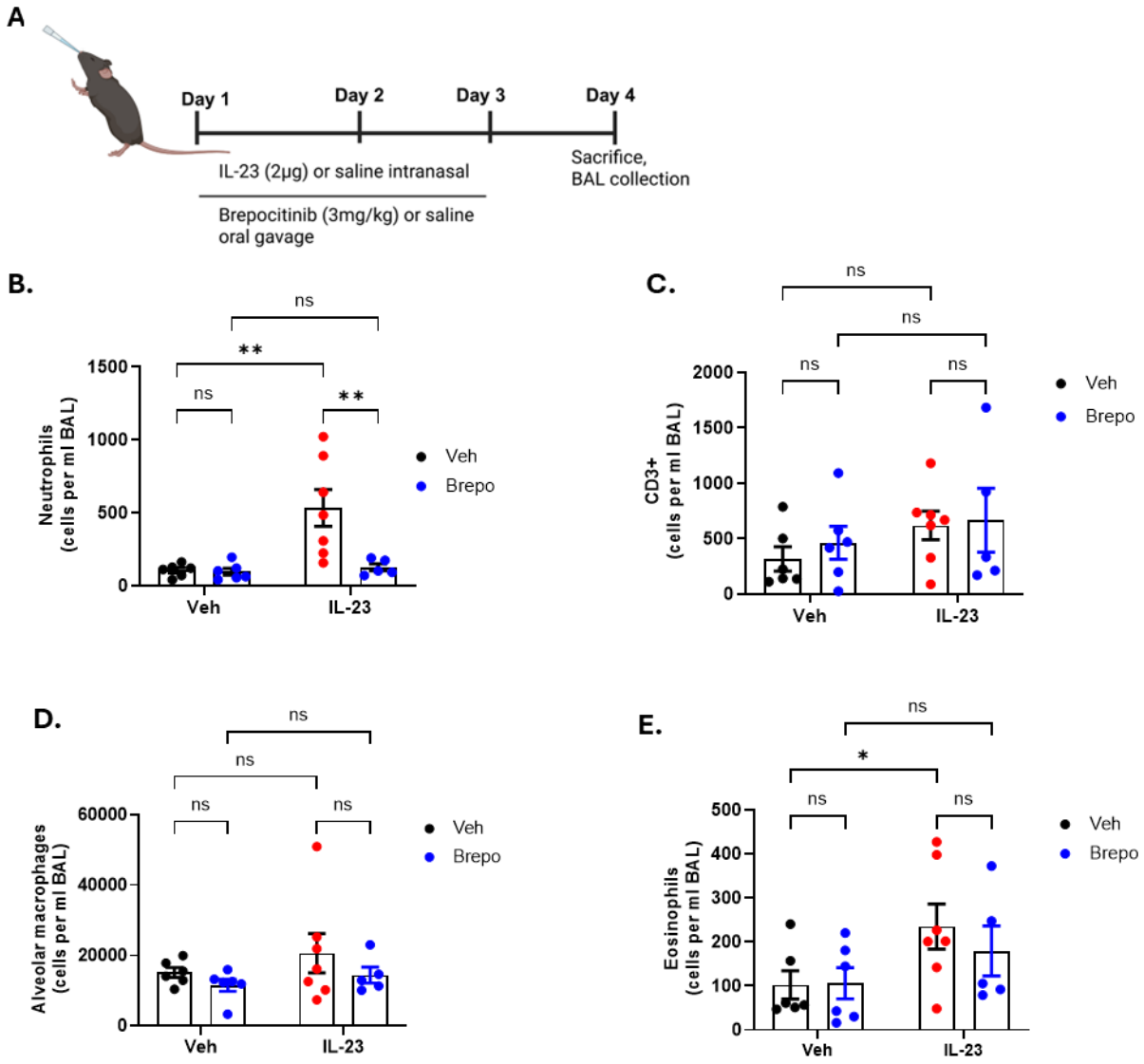


Figure 29. Effect of Brepocitinib on the airway neutrophil recruitment by IL-23 in vivo. A: Schematic representation of in vivo experimental setup for intranasal administration of IL-23 (2µg) or vehicle (saline) along with Brepo (3 mg/kg) or vehicle (saline) through oral gavage for 3 days, followed by sacrifice and BAL collection on day 4 and immune cells in the BAL was measured using flow cytometry. B: Neutrophil recruitment in the BAL. C: CD3+ cells in the BAL. D: Alveolar macrophages in the BAL. E: Eosinophils in the BAL. Statistical analyses were performed using mixed-effect analysis followed by Fisher's Least Significant Difference test. Veh-Vehicle; Brepo-Brepocitinib. *p < 0.05, **p < 0.01, ns = not significant. Parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.30. Brepocitinib mitigates LPS-induced airway neutrophil inflammation in vivo

Ivanov et al. previously revealed that IL-23 enhances LPS-induced IL-17A production in vivo and thereby promotes neutrophilic inflammation in the airways [157].

To expand on our findings and assess whether brepocitinib can reduce neutrophilic inflammation in a broader physiological setting, we used an acute inflammation model triggered by intranasal administration of lipopolysaccharide (LPS, 5 µg) in the presence of brepocitinib (10 mg/kg) or vehicle co-treatment (Fig. 30A). As expected, LPS challenge caused a significant influx of neutrophils into the bronchoalveolar lavage (BAL), confirming the strong neutrophilic response typical of this model. Importantly, co-treatment with brepocitinib significantly reduced the extent of LPS-induced neutrophil infiltration into the airways (Fig. 30B), demonstrating its anti-inflammatory efficacy in vivo.

Other immune cell populations in the BAL, such as alveolar macrophages (Fig. 30D), showed no significant changes in response to either LPS or brepocitinib treatment. Interestingly, the proportion of CD3+ T cells (Fig. 30C) and eosinophils (Fig. 30E) increased after LPS administration. This increase could not be statistically reduced by brepocitinib co-treatment alongside LPS, although a decreasing trend was observed in CD3+ and eosinophil populations with brepocitinib co-treatment.

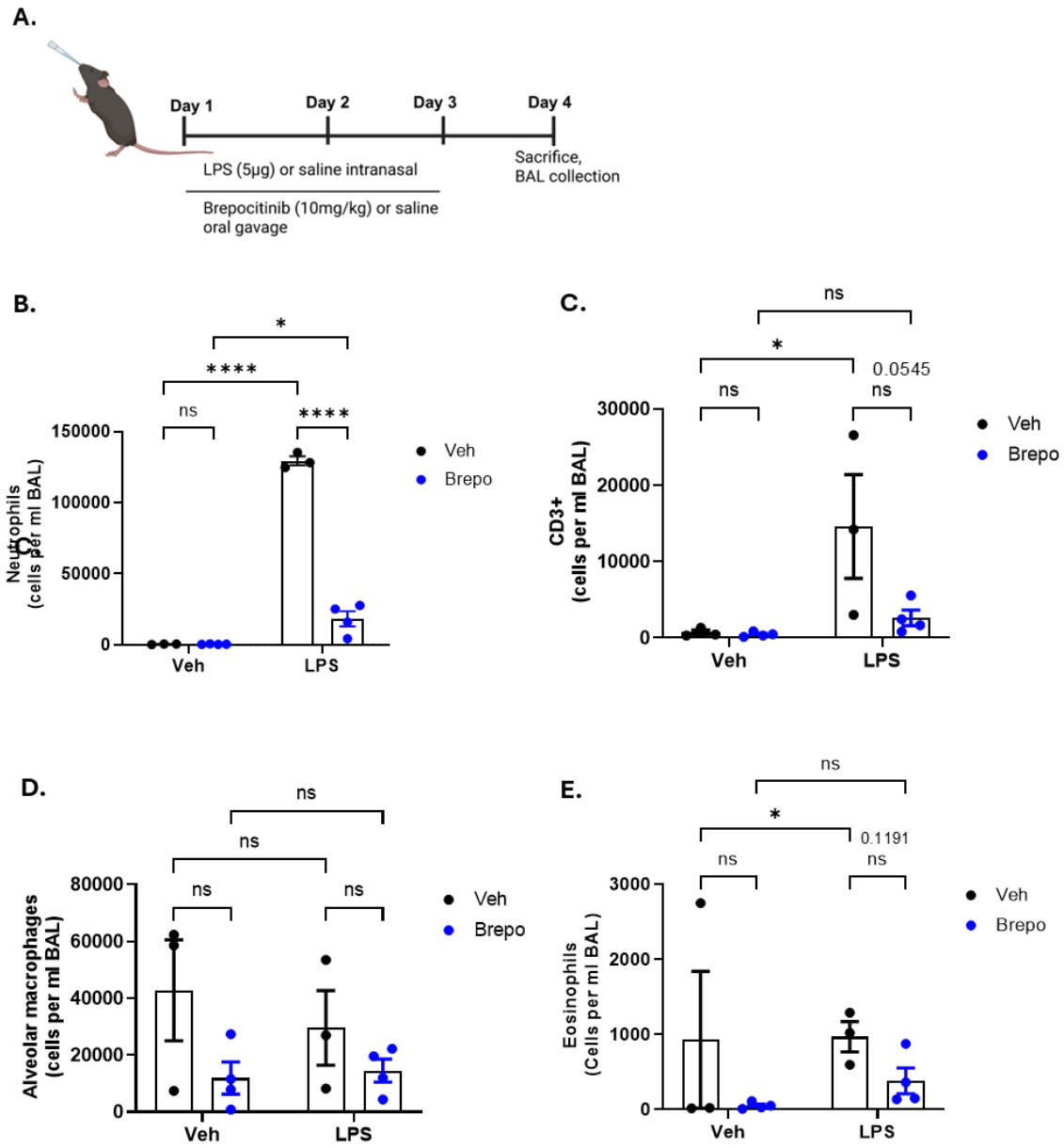


Figure 30. Effect of Brepocitinib on LPS-induced airway neutrophil inflammation in vivo. A: Schematic representation of the in vivo experimental setup for LPS-induced inflammation. Intranasal administration of LPS (5 µg) or vehicle (saline) along with Brepo (10 mg/kg) or vehicle (saline) through oral gavage for 3 days, followed by sacrifice and BAL collection on day 4 and immune cells in the BAL was measured using flow cytometry. B: Neutrophil recruitment in the BAL. C: CD3+ cells in the BAL. D: Alveolar macrophages in the BAL. E: Eosinophils in the BAL. Statistical analyses were performed using mixed-effect analysis followed by Fisher's Least Significant Difference test. Veh-Vehicle; Brepo-Brepocitinib. *$p < 0.05$, ****$p < 0.0001$, ns = not significant. Parts of this figure are adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

6.31. Brepocitinib mitigates TYK2 phosphorylation in the lungs of LPS-induced neutrophil inflammation

In the acute neutrophilic airway inflammation model using LPS (Fig. 31A), we examined pTYK2 (Tyr1054/Tyr1055)-positive cells in the lung tissues of mice that received LPS or vehicle, with or without brepocitinib, using immunofluorescence microscopy (Fig. 31B). When quantifying the immunofluorescence (IF) images, we observed a significant increase in pTYK2 (Tyr1054/Tyr1055)-positive cells in the lung tissue of mice treated with lipopolysaccharide (LPS) (Fig. 31C). Notably, this increase in pTYK2 (Tyr1054/Tyr1055)-positive cells was effectively reversed in the group of mice that received combined brepocitinib and LPS treatment (Fig. 31C).

In summary, these results highlight the major experimental findings of our study and provide evidence for the crucial involvement of the IL-23/IL-17A axis in neutrophilic airway inflammation and inducing corticosteroid resistance in the context of COPD. These effects may be amenable to therapeutic modulation by targeting TYK2 and JAK1. The implications of these observations are further discussed in the following Discussion section.

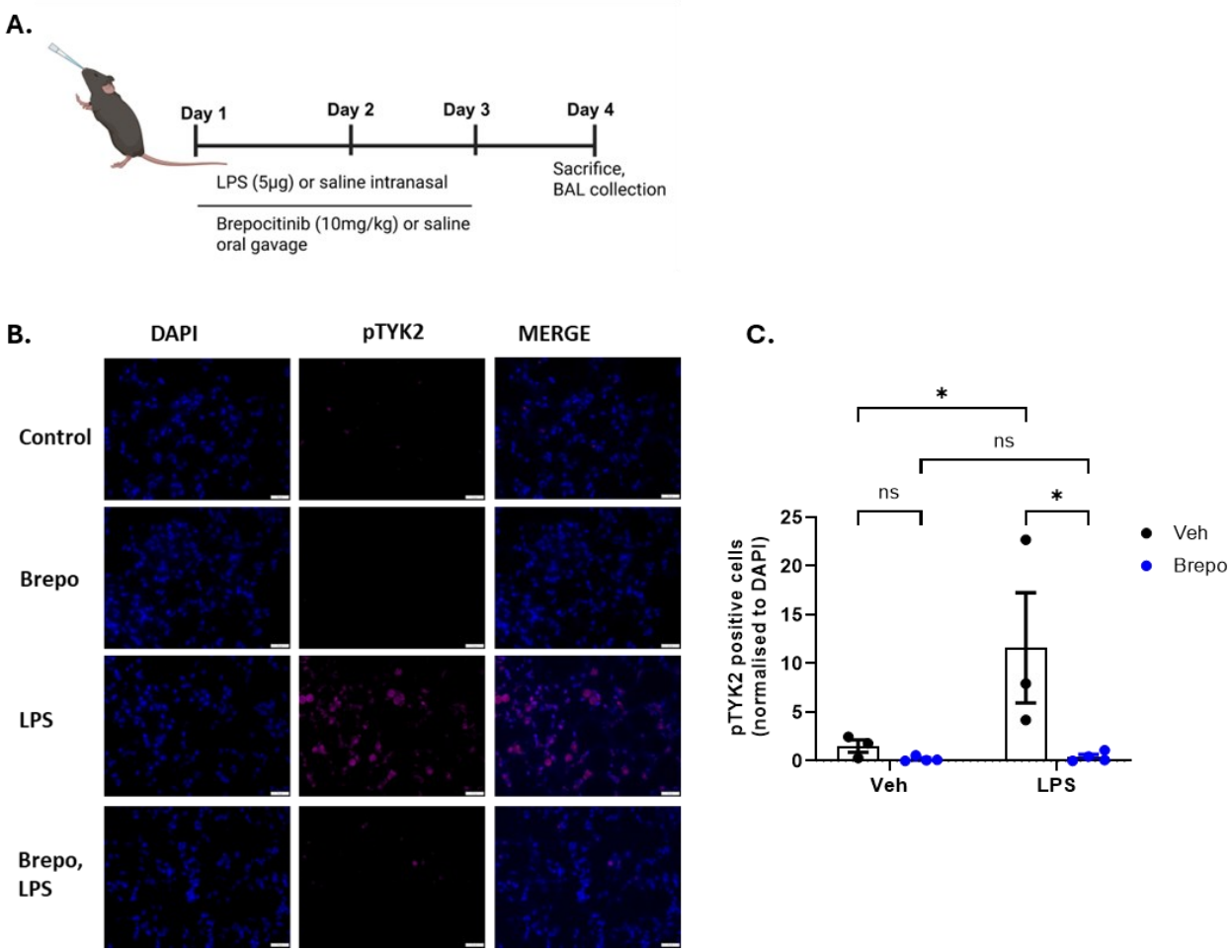


Figure 31. Effect of Brepocitinib on pTYK2 (Tyr1054/Tyr1055)-positive cells in the lung tissues in in vivo LPS-induced neutrophilic airway. A: Schematic representation of the in vivo experimental setup for LPS-induced inflammation. B: IF analysis of lung tissue from the LPS-induced inflammation model. C: Quantification of IF images from E. Statistical analyses were performed using mixed-effect analysis followed by Fisher's Least Significant Difference test. Veh-Vehicle; Brepo-Brepocitinib, IF-Immunofluorescence. *$p < 0.05$, ns = not significant. Adapted from Nayak BB et al., Am J Physiol Lung Cell Mol Physiol, 2026.

7. Discussion

In this study, we demonstrated that the IL-23 / IL-17A axis plays a crucial role in regulating neutrophil effector functions and recruitment, and that TYK2/JAK1 inhibition offers a promising therapeutic strategy to target IL-23/IL-17A-driven neutrophilic inflammation, especially in severe, exacerbation-prone COPD.

Our data clearly show how neutrophil effector functions, activation markers, and recruitment are influenced by the IL-23/IL-17A cytokines both in vitro and in vivo. These findings are essential for understanding the functional contribution of the IL-23/IL-17A axis in neutrophilic inflammation. The inhibition of these responses by brepocitinib, a TYK2/JAK1 inhibitor, highlights the potential of targeting the JAK/STAT pathway, particularly TYK2/JAK1, to reduce this neutrophilic inflammation. In vivo, brepocitinib significantly reduced neutrophil recruitment induced by IL-23 or LPS challenge.

Our findings further support this concept, as we observe differences in TYK2 and JAK1 phosphorylation in patient samples compared to healthy controls. This phenotype was reproduced through IL-23/IL-17A stimulation and was reversed with brepocitinib treatment. Additionally, our data indicate that overprotein abundance of GRβ in neutrophils acts as an early, predictive biomarker for corticosteroid resistance, even before clear clinical signs of COPD appear. This phenotype was also reproduced by IL-23/IL-17A stimulation and reversed by brepocitinib treatment. Collectively, these results support TYK2/JAK1 as a central signaling node linking cytokine-driven immune dysregulation to neutrophil-mediated pathology in COPD and highlight its translational potential as both a biomarker and therapeutic target.

7.1. IL-23/IL-17A axis; JAKs and neutrophilic inflammation in COPD, smokers and allergic donors compared to healthy controls

Our study shows that the IL-23/IL-17A axis, which so far has been considered a central regulator of neutrophilic inflammation as a modulator of the adaptive immune response (the non-Th2 endotype), can also directly contribute to neutrophil activation, further supporting neutrophilic inflammation. In COPD, neutrophilic inflammation is a hallmark of disease heterogeneity, especially in severe COPD patients with frequent exacerbations. BAL and blood neutrophil counts have been shown to predict exacerbation frequency and mortality in COPD

cohorts, underscoring the clinical relevance of neutrophil-driven pathology [103,104]. Studies have shown that IL-23 and IL-17A levels are higher in the serum and sputum of COPD patients compared to healthy controls [145,176]. Additionally, elevated IL-23 and IL-17A levels have been reported in the serum and sputum of patients with severe COPD compared to those with milder forms of the disease, indicating a correlation with disease severity [103,104,159]. Since IL-23 signals through TYK2 and JAK2, we examined the protein abundance of both kinases in neutrophils under pathological conditions. We also analyzed JAK1, as many IL-17-driven autoimmune diseases suggest JAK1/JAK2 involvement [177–179]. With this in mind, we studied the protein abundance of TYK2, JAK2, and JAK1 to expand our understanding of JAK kinase-related pathways. Analysis of publicly available microarray data [159] showed that mRNA levels of *TYK2* and *JAK1* were markedly higher in sputum cells from patients with severe COPD (stages III and IV) compared to those with moderate COPD (stage II). This suggests a possible link between the increased abundance of these genes and disease severity. Conversely, *JAK2* mRNA abundance did not show significant differences between the groups, highlighting the specific role of TYK2 and JAK1 phosphorylation in advanced COPD stages, which may be associated with more pronounced inflammatory responses. These observations align with our serum cytokine measurements in patients with severe COPD and support previous reports showing that IL-23 levels are higher in individuals with severe COPD compared to those with milder disease [56]. Elevated IL-23 levels have also been observed in the alveolar macrophages of smokers who are HIV-1 positive [180], and smokers—with or without COPD—show higher numbers of IL-23–positive cells in the bronchial epithelium and submucosa than non-smokers [99]. Similarly, cigarette smoke has been connected to higher IL-17A levels in airway tissues [181,182], and elevated circulating levels of IL-17A have been reported in both the serum and sputum of COPD patients [145,176], with further increases observed during exacerbations [103,104]. Interestingly, although IL-17A levels showed no difference between moderate and severe COPD in the microarray data, they were significantly higher in the serum of our COPD patients, particularly among those with severe disease. This discrepancy could be due to technical variations, patient heterogeneity, or might suggest that systemic IL-17A is a more sensitive indicator of disease severity. Overall, these results reinforce the ongoing role of the IL-23/IL-17A axis in COPD development and, for the first time, reveal a disease-severity-dependent rise in TYK2 and JAK1.

Further the flow cytometry analysis of whole blood was done to measure the protein abundance of TYK2, JAK1 and JAK2 and their phosphorylation (pTYK2, pJAK1, pJAK2) respectively. Phosphorylation of TYK2 at Tyr1054/Tyr1055, JAK2 at Tyr1007/Tyr1008, and JAK1 at Tyr1022/Tyr1023 reflects activation of these kinases following cytokine receptor engagement. Increased phosphorylation at these activation-associated tyrosine residues suggests enhanced downstream inflammatory signaling, particularly through JAK/STAT-associated pathways involved in IL-23- and IL-17-mediated responses. Conversely, reduced phosphorylation following inhibitor treatment indicates suppression of kinase activation and attenuation of inflammatory signaling cascades. Our flow cytometric data further supported the mRNA abundance of *TYK2/JAK1* associated with COPD disease severity, by revealing increased pTYK2 (Tyr1054/Tyr1055) levels in neutrophils from COPD patients compared to healthy controls, indicating systemic pathway activation. Interestingly, elevated pTYK2 (Tyr1054/Tyr1055) and pJAK1 (Tyr1022/Tyr1023) levels were also observed in younger smokers without COPD compared to non-smoking healthy individuals, implying early pathway activation before clinical symptoms appear. As smoking is a key risk factor for COPD, these findings suggest that early pathway activation may contribute to disease development. This points to the involvement of TYK2/JAK1-driven neutrophilic inflammation in COPD pathogenesis and underscores the potential of pTYK2 (Tyr1054/Tyr1055) as an early biomarker for smoke-related diseases, aiding early diagnosis and treatment. Overall, the data emphasize the role of TYK2/JAK1 signaling in COPD and its promise as a therapeutic target and early indicator of disease development. Along with neutrophils, the flow cytometric analysis also showed a significant or trending increase in the protein abundance and phosphorylation of TYK2, JAK1, and JAK2 in some other immune populations in COPD patients or smokers compared to matched healthy controls. This further supports the idea of enhanced JAK phosphorylation in various immune cells during COPD pathogenesis and associated inflammation. In contrast, when comparing blood donors with allergic conditions to those without allergies, we observed significantly lower levels of phosphorylated JAK2 ($p=0.0476$) and phosphorylated TYK2 ($p=0.164$) in neutrophils isolated from allergic individuals. This reduced phosphorylation of JAK2 and TYK2 in allergic donors may reflect a response skewed towards Th2-mediated inflammation, which is characterized by cytokines such as IL-4 and IL-13 and involves distinct downstream signaling pathways from the non-Th2, neutrophilic inflammation typically observed in COPD. Overall, these findings highlight differences in

immune signaling pathways between allergic and COPD conditions, emphasizing distinct molecular mechanisms underlying each inflammatory endotype [183,184].

Some broader reviews and mechanistic studies on JAK/STAT signaling in airway inflammation support the idea of targeting TYK2 in neutrophilic conditions [185]. Meanwhile, in dermatology and autoimmune disease research, TYK2 inhibitors that target the IL-12 / IL-23 axis are becoming increasingly prominent, highlighting the practical potential of this approach [186,187].

7.2. IL-23/IL-17A axis role in neutrophil effector function, recruitment, activation

Besides the JAKs, the microarray data analysis revealed and reconfirmed that IL-23 gene abundance was increased in severe COPD patients compared to moderate ones. Despite previous studies, the IL-17A gene abundance in this microarray dataset was not statistically significantly different between the severe and moderate groups, despite showing an increasing trend in severe COPD patients.

We further examined how the IL-23/IL-17A axis influences neutrophil effector functions, specifically migration and ROS production. Both cytokines, whether alone or together, significantly increased neutrophil migration in vitro, with their combined effect being more substantial than either cytokine alone. Besides directly promoting migration, they also primed neutrophils to respond more strongly to chemoattractants like IL-8. These priming effects extended to ROS production, a crucial factor in disease progression and flare-ups in COPD. Our results align with previous research suggesting that during colitis onset, high IL-23 levels shift neutrophils into an IL-17A-producing phenotype and enhance the abundance of genes related to neutrophil activation and recruitment [171]. Similarly, IL-17A is known to recruit neutrophils into the airway lumen, triggering the release of chemokines like CXCL1 and CXCL8/IL-8 from airway epithelial cells, as demonstrated in our ALI cultures. Regarding ROS production, murine models with neutrophils lacking IL-17A have exhibited reduced ROS release [188]. Likewise, there is evidence that IL-23 promotes NETs formation, a process associated with ROS production [189]. IL-17A, though not IL-23 priming, also enhanced CD11b upregulation in neutrophils stimulated with C5a. This further supports IL17A's

contribution to neutrophil activation. This has been previously addressed in a study on a mouse arthritis model [190]. A lack of calcium flux after IL-17A or IL-23 stimulation is expected, as their receptors are not known to be connected to the PLC–IP_3–Ca^{2+} signaling pathway. Instead, these cytokines are known to activate transcriptional pathways: IL-17A through the Act1–TRAF6–NF-κB/MAPK axis, and IL-23 via JAK2/TYK2–STAT3/STAT4 [75,79,191].

Overall, these findings support our observations on ROS production in neutrophils primed with these cytokines. Our findings go beyond this traditional view, demonstrating that IL-23 and IL-17A not only indirectly stimulate chemokine production via epithelial cells but also directly promote neutrophil migration, enhance neutrophil responsiveness to chemoattractants such as IL-8, augment inflammatory mediators like C5a, and increase reactive oxygen species (ROS) production. To our knowledge, this dual role as both a chemotactic signal and a functional activator represents a novel extension of the IL-23/IL-17A axis in neutrophil biology and inflammation.

7.3. TYK2/JAK1 inhibition as a therapeutic strategy:

a. In vitro studies

Since IL-23 receptors interact with TYK2, and based on our previous observations regarding Tyk2 and JAK1 phosphorylation in COPD and smokers we investigated whether blocking TYK2, and JAK1 in neutrophils could stop IL-23-driven effects. We employed brepocitinib, an experimental TYK2/JAK1 inhibitor [192].

In COPD, antigen-presenting cells (APCs) are strongly activated by cigarette smoke, oxidative stress, and microbial stimuli [26,93]. APC-derived IL-23 is essential for promoting and maintaining Th17 cell polarization and ILC3 stabilization[19]. Th17 cells mainly produce IL-17A and IL-17F, which stimulate epithelial cells to release neutrophil-attracting chemokines like CXCL1 and IL-8 (CXCL8) [193], thereby promoting neutrophil recruitment to the airways [19]. To further assess the therapeutic potential of brepocitinib, we studied its ability to inhibit IL-23/IL-17A–stimulated IL-8 release from primary bronchial epithelial cells (PBECs) cultured at the air–liquid interface (ALI) and stimulated with TNF-α/IL-1β. Notably, brepocitinib significantly suppressed IL-17A and IL-23–induced IL-8 secretion. This was supported by reduced neutrophil migration toward media conditioned by brepocitinib-treated PBECs and

lower CD11b surface expression on neutrophils co-cultured with these cells. Additionally, the selective TYK2 inhibitor deucravacitinib also decreased IL-23 and IL-17A–stimulated IL-8 release, though less effectively than brepocitinib, suggesting that both TYK2 and JAK1 contribute to this mechanism.

Besides that, brepocitinib inhibits IL-17A and IL-23-induced neutrophil migration, as well as neutrophils primed with IL-17A or IL-23 migrating toward IL-8. Brepocitinib also reduces ROS in IL-23 and IL-17A-primed neutrophils induced with C5a. It is notable that brepocitinib decreases neutrophil migration and/or ROS production triggered by IL-8, C5a, and IL-17A, as well as by conditioned media from COPD lung tissue cultures (of unknown composition) besides IL-23, even though these mediators are not directly linked to the JAK/STAT pathway. These broader inhibitory effects suggest intriguing mechanistic hypotheses, leading us to discuss TYK2 and JAK1 phosphorylation in the context of these cytokines, given that brepocitinib is a TYK2/JAK1 inhibitor.

b. TYK2/JAK1 phosphorylation

We observed that all three mediators induced TYK2 phosphorylation, suggesting either signaling cross-talk with the JAK/STAT pathway or phosphorylation of TYK2 as well as JAK1, probably via alternative signaling pathways. This suggests that cross-talk or noncanonical phosphorylation routes may engage TYK2 even beyond classical cytokine receptor–JAK coupling.

Our results align with previous research showing that in monocytic U937 cells, IL-17A activates TYK2, indicating signaling through the JAK/STAT pathway [84]. Although IL-8 mainly triggers downstream pathways like PI3K and MAPK, it has also been shown to stimulate JAK3 activity in neutrophils, which suggests an indirect mechanism potentially leading to TYK2 or JAK1 phosphorylation [194]. Similarly, C5a signals via PI3K, ERK, and p38 MAPK, which may interact with the JAK/STAT pathway [195]. An additional possible mechanism for increased TYK2 phosphorylation after stimulation with IL-17A, IL-8, and C5a is the release of secondary cytokines from neutrophils, such as IL-6, a key cytokine involved in COPD. It is known that IL-17A [196–198], IL-8 [199], and C5a [200–202] can quickly induce IL-6 mRNA expression and secretion. IL-6 can then activate TYK2, and JAK1 through its receptor complex [203–205]. These findings suggest potential indirect pathways for TYK2 phosphorylation, either through pathway cross-talk or by releasing secondary cytokines that engage the

JAK/STAT pathway. Thus, rather than acting purely upstream, the TYK2/JAK1 axis may represent a convergent point for multiple inflammatory signals, making it a particularly attractive target.

To further confirm the effectiveness of brepocitinib in inhibiting the IL-23 and IL-17A-mediated effects observed in neutrophils, we co-treated the cells with brepocitinib while stimulating them with these cytokines. The increase in pTYK2 (Tyr1054/Tyr1055) levels caused by IL-23 or IL-17A stimulation was reduced by brepocitinib co-treatment. Brepocitinib also mitigated the rise in pJAK1 (Tyr1022/Tyr1023) observed with IL-17A stimulation. The effectiveness of brepocitinib in the context of COPD was further confirmed when blood from COPD patients treated with brepocitinib showed lower pTYK2 (Tyr1054/Tyr1055) and pJAK1 (Tyr1022/Tyr1023) abundance compared to vehicle treatments. These results clarify that brepocitinib blocks IL-23 and IL-17A mediated functional effects and reduces JAK activation in neutrophils stimulated with IL-23/IL-17A stimulated as well as in COPD blood, suggesting the involvement of JAKs, especially TYK2 and JAK1, in the IL-23/IL-17A axis of neutrophilic airway inflammation in COPD.

c. In vivo studies

In an in vivo setting, brepocitinib reduced neutrophilic recruitment to the airways induced by IL-23, which aligns with our previous in vitro findings, demonstrating the priming role of IL-23 in neutrophil migration. Besides that, brepocitinib also blocked neutrophilic infiltration in the LPS-induced acute lung inflammation model. LPS is known to trigger neutrophil recruitment via two mechanisms: the canonical pathway and the non-canonical pathway [206,207]. The canonical pathway operates through the TLR4 activation on macrophages leading to activation of downstream MyD88/TRIF pathways, which causes the cytokine/chemokine release [206]. The non-canonical inflammasome pathway is activated by LPS binding to caspase-11 leading to IL-1β / IL-18 release [207–209]. Although there is no clear evidence to date supporting the idea that TYK2 might directly participate in canonical LPS signaling, previous studies indicate that knocking out or inhibiting TYK2 can decrease caspase-11 induction, lower IL-1β and IL-18 levels, and reduce neutrophil recruitment in mice. Therefore, blocking TYK2 could impair neutrophilic inflammation through suppression of cytokine networks and modulation of inflammasomes [210].

7.4. GRβ as a biomarker of steroid resistance in neutrophils

Our study offers new insights into the neutrophil-intrinsic mechanisms of corticosteroid resistance and potential therapeutic approaches in COPD. This was addressed through overexpression of the glucocorticoid receptor β isoform (GRβ), thus lowering the GRα-to-GRβ, which correlates with corticosteroid resistance. Under homeostatic conditions, human neutrophils show higher baseline levels of GRβ compared to other leukocytes, which might explain their reduced sensitivity to glucocorticoid-induced apoptosis [211]. Previous studies have indicated that IL-17A and IL-23 can induce corticosteroid resistance in PBMC and airway structural cells [115]. In line with this, we now demonstrate that IL-23 and IL-17A further increase GRβ abundance in neutrophils from healthy donors, reflecting the levels observed in COPD patients. Even smokers without COPD showed higher GRβ abundance compared to healthy non-smoking controls, suggesting that GRβ abundance occurs before the disease develops and could thus serve as an early biomarker of corticosteroid resistance in the context of smoke-induced airway inflammation. Additionally, the increased GRβ abundance induced by IL-17A and IL-23 was reversed with brepocitinib treatment.

Mechanistically, activation of NF-κB has been linked to the selective increase of GRβ in epithelial and lymphoid cells during inflammatory conditions thereby contributing to steroid insensitivity [212]. Similarly, exposure to IL-17A and IL-23 might also elevate GRβ levels through the NF-κB pathway [213]. IL-23 has been shown to induce receptor activator of NF-κB ligand (RANKL) abundance, which can be mediated through both the NF-κB and TYK2/JAK2-STAT3/4 pathways [214]. It has also been reported that IL-23 can promote secondary cytokine induction by releasing IL-17A and IL-6, which further enhances NF-κB activation in epithelial and stromal cells [215]. Furthermore, in keratinocytes and epithelial cells, TYK2 inhibition may decrease IL-23-induced NF-κB activity [115,216]. Similarly, IL-17A is known to signal via the canonical ACT1 / TRAF6 pathway, activating IkB kinase, leading to IκB degradation and NF-κB translocation into the nucleus [217].

Besides TYK2, activation of JAK1 can also trigger NF-κB signaling through phosphorylation of STAT1 and STAT3. In airway epithelial cells, it has been reported that IFN-γ or IL-6 signals via JAK1-STAT1 and increases NF-κBp65 activity, thereby contributing to heightened expression of proinflammatory cytokines like CXCL10 and CCL5, even in the presence of steroids, whereas knocking down STAT1 restores steroid insensitivity [218,219]. Similarly, it

has been reported that activation of JAK1 leads to phosphorylation of STAT3, which works together with NF-κB to sustain the transcription of inflammatory mediators, contributing to glucocorticoid-resistant inflammation [220,221]. Since NF-κB signaling has been associated with glucocorticoid insensitivity, TYK2, JAK2, and JAK1 direct or indirect interactions with NF-κB offer a mechanistic pathway through which JAK signaling can facilitate or sustain steroid resistance.

From a translational perspective, measuring GRβ levels in neutrophils could aid in categorizing COPD or at-risk individuals into groups that respond to steroids versus those resistant to them—facilitating more tailored anti-inflammatory treatments. For patients prone to exacerbations with high neutrophilic GRβ levels, using TYK2/JAK1 inhibitors or other non-steroidal anti-inflammatory methods may prove more effective than increasing corticosteroid doses.

7.5. Integrative findings, implications, and limitations

Our research highlights that IL-23/IL-17A, through TYK2/JAK1 signaling, drives neutrophil activation pathways as a key factor contributing to neutrophilic inflammation and corticosteroid resistance in COPD, with significant implications for treatment. The emerging conceptual framework suggests that the IL-23/IL-17A axis mimics the pattern seen in COPD and non-COPD smokers, triggering TYK2 and JAK1 activation in neutrophils and promoting local recruitment of neutrophils while enhancing their effector functions. These activated neutrophils further amplify inflammation through the release of ROS, NETs, proteases, and by promoting additional chemokine production. This also contributes to the development of steroid resistance via GRβ upregulation. Over time, this cycle may lead to airway remodeling, increased mucus production, a higher risk of exacerbations, and declining lung function. From a translational perspective, an important implication is that TYK2 and JAK1 activation in neutrophils could serve as biomarkers for early smoke-induced airway inflammation. In exacerbation-prone COPD patients with steroid resistance, inhibitors like brepocitinib could be used as an additional treatment alongside existing therapies.

Due to their immunosuppressive nature, JAK inhibitors can reduce phagocytosis by decreasing receptor abundance and adhesion molecules required for the process, impairing oxidative burst, or dampening cytokine-induced activation, thereby increasing susceptibility to infections [222,223]. Thus, similar to corticosteroids, a major challenge of using JAK inhibitors in COPD

patients is the increased risk of respiratory infections, making careful patient selection and monitoring essential. Although our study shows promising results and offers new important insights into COPD management, it is limited by a small sample size and patient heterogeneity in terms of disease stage, comorbidities, and medication, which introduce significant variability among COPD patients. The limited sample size also restricted sex-disaggregated analyses, particularly in female subgroups, thereby limiting the statistical power to draw definitive conclusions regarding sex-dependent signaling responses. Another limitation of our study is that we used brepocitinib as a pretreatment or co-treatment in both in vitro and in vivo models, which does not precisely mimic the clinical setting, where treatment usually starts in an already inflamed environment. Even though brepocitinib treatment reduced increased pTYK2 (Tyr1054/Tyr1055) and pJAK1 (Tyr1022/Tyr1023) in neutrophils from smokers, this could not be fully validated in the smoke exposure model using PBEC, where IL-8 production was only partly brepocitinib. Using JAK inhibitors to treat COPD requires careful assessment of their advantages and potential risks. JAK inhibitors serve as effective immunomodulators, but they can cause side effects like higher infection risk, blood clots, cardiovascular issues, and off-target effects resulting from limited selectivity in certain JAK inhibitors [224]. Since neutrophil function is also critical for host defense (via phagocytosis, oxidative burst, microbial killing), potent TYK2 / JAK1 inhibition might compromise bacterial clearance. In the COPD context, where frequent bacterial colonization and exacerbation risk are already high, caution is necessary when immunomodulating COPD patients, as this may increase the risk of pneumonia. Like corticosteroids, a significant challenge when using JAK inhibitors in COPD patients is the heightened risk of respiratory infections, which necessitates careful patient selection and monitoring.

7.6. Future perspectives

In future studies we should address the following challenges and questions:

1. Our models involved concurrent or prophylactic inhibitor treatment. Further testing is needed to determine whether brepocitinib can reverse established neutrophilic inflammation, reduce lung remodeling, or synergize with existing COPD treatments, such as inhaled corticosteroids, bronchodilators, phosphodiesterase inhibitors, and macrolides.

2. In our study, inflammatory mediators such as IL-8 and C5a, which are not traditionally linked to JAK/STAT signaling, still induced TYK2 and JAK1 phosphorylation, which requires further investigation and analysis of their crosstalk with the JAK/STAT signaling pathway for a clearer understanding.
3. To enhance therapy effectiveness and minimize risk, patients might be chosen based on neutrophilic phenotype, increased IL-23/IL-17A levels, and steroid resistance. Additional long-term studies are necessary to confirm that pTYK2 (Tyr1054/Tyr1055)/pJAK1 (Tyr1022/Tyr1023) and GRβ are reliable markers.
4. Due to the complex nature of COPD inflammatory milieu, consisting of IL-23, IL-17A, IL-6, TNF, IL-1β, IL-8, complement, microbes, and oxidative stress. Therefore, brepocitinib may be an adjunct rather than a monotherapy. Exploring synergistic effects of brepocitinib with existing therapies is crucial.
5. Further research is needed to understand the detailed mechanisms of the JAKs signaling cascade activated by ligands like IL-23, IL-17A, IL-8, and C5a in neutrophils, and to map their interactions with NF-κB, MAPK, PI3K, and inflammasome pathways—the role of neutrophil-intrinsic versus paracrine signaling warrants further investigation.

In conclusion, our findings position the IL-23/IL-17A–TYK2/JAK1 axis as a key driver of neutrophil recruitment, activation, and steroid resistance in COPD. We show that this pathway is already activated in smokers prior to overt disease, supporting its role in early pathogenic priming, and that pharmacological inhibition with brepocitinib effectively dampens neutrophil effector functions, epithelial chemokine release, and airway neutrophilia in vivo. Together with the identification of GRβ upregulation as a neutrophil-intrinsic marker of corticosteroid resistance, these data provide a mechanistic framework linking IL-17 cytokine signaling to disease progression and therapeutic refractoriness in COPD. While careful patient selection and safety considerations will be essential given the immunosuppressive potential of JAK inhibition, targeting TYK2/JAK1 may represent a rational adjunctive strategy for exacerbation-prone, neutrophilic, steroid-resistant COPD and offers a foundation for future biomarker-guided precision approaches.

8. Bibliography

[1] Holt PG, Strickland DH, Wikström ME, Jahnsen FL. Regulation of immunological homeostasis in the respiratory tract. Nat Rev Immunol 2008;8:142–52. https://doi.org/10.1038/nri2236.

[2] Iwasaki A, Foxman EF, Molony RD. Early local immune defences in the respiratory tract. Nat Rev Immunol 2017;17:7–20. https://doi.org/10.1038/nri.2016.117.

[3] Janbazacyabar H, van Bergenhenegouwen J, Varasteh S, Garssen J, Folkerts G, Braber S. Repeated exposure of bronchial epithelial cells to particular matter increases allergen-induced cytokine release and permeability. Cytokine 2022;154:155878. https://doi.org/10.1016/j.cyto.2022.155878.

[4] Moldoveanu B, Otmishi P, Jani P, Walker J, Sarmiento X, Guardiola J, et al. Inflammatory mechanisms in the lung. J Inflamm Res 2009;2:1–11. https://doi.org/10.2147/JIR.S4385.

[5] Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 2016;138:16–27. https://doi.org/10.1016/j.jaci.2016.05.011.

[6] Eckhardt CM, Wu H. Environmental Exposures and Lung Aging: Molecular Mechanisms and Implications for Improving Respiratory Health. Curr Environ Health Rep 2021;8:281–93. https://doi.org/10.1007/s40572-021-00328-2.

[7] Bouma F, Nyberg F, Olin A-C, Carlsen HK. Genetic susceptibility to airway inflammation and exposure to short-term outdoor air pollution. Environ Health 2023;22:50. https://doi.org/10.1186/s12940-023-00996-7.

[8] Adler KB, Fischer BM, Wright DT, Cohn LA, Becker S. Interactions between respiratory epithelial cells and cytokines: relationships to lung inflammation. Ann N Y Acad Sci 1994;725:128–45. https://doi.org/10.1111/j.1749-6632.1994.tb00275.x.

[9] Ponce-Gallegos MA, Ramírez-Venegas A, Falfán-Valencia R. Th17 profile in COPD exacerbations. Int J Chron Obstruct Pulmon Dis 2017;12:1857–65. https://doi.org/10.2147/COPD.S136592.

[10] Kotrba J, Müller I, Pausder A, Hoffmann A, Camp B, Boehme JD, et al. Innate players in Th2 and non-Th2 asthma: emerging roles for the epithelial cell, mast cell, and monocyte/macrophage network. American Journal of Physiology-Cell Physiology 2024;327:C1373–83. https://doi.org/10.1152/ajpcell.00488.2024.

[11] Reza MI, Ambhore NS. Inflammation in Asthma: Mechanistic Insights and the Role of Biologics in Therapeutic Frontiers. Biomedicines 2025;13:1342. https://doi.org/10.3390/biomedicines13061342.

[12] Wan XC, Woodruff PG. Biomarkers in Severe Asthma. Immunol Allergy Clin North Am 2016;36:547–57. https://doi.org/10.1016/j.iac.2016.03.004.

[13] Xie C, Yang J, Gul A, Li Y, Zhang R, Yalikun M, et al. Immunologic aspects of asthma: from molecular mechanisms to disease pathophysiology and clinical translation. Front Immunol 2024;15. https://doi.org/10.3389/fimmu.2024.1478624.

[14] Hsu AT, Gottschalk TA, Tsantikos E, Hibbs ML. The Role of Innate Lymphoid Cells in Chronic Respiratory Diseases. Front Immunol 2021;12. https://doi.org/10.3389/fimmu.2021.733324.

[15] Ray A, Kolls JK. Neutrophilic Inflammation in Asthma and Association with Disease Severity. Trends Immunol 2017;38:942–54. https://doi.org/10.1016/j.it.2017.07.003.

[16] Liu T, Woodruff PG, Zhou X. Advances in non-type 2 severe asthma: from molecular insights to novel treatment strategies. Eur Respir J 2024;64:2300826. https://doi.org/10.1183/13993003.00826-2023.
[17] Alcorn JF, Crowe CR, Kolls JK. TH17 Cells in Asthma and COPD. Annual Review of Physiology 2010;72:495–516. https://doi.org/10.1146/annurev-physiol-021909-135926.
[18] Xie C, Wang K, Yang K, Zhong Y, Gul A, Luo W, et al. Toward precision medicine in COPD: phenotypes, endotypes, biomarkers, and treatable traits. Respir Res 2025;26:274. https://doi.org/10.1186/s12931-025-03356-w.
[19] Rouzic OL, Pichavant M, Frealle E, Guillon A, Si-Tahar M, Gosset P. Th17 cytokines: novel potential therapeutic targets for COPD pathogenesis and exacerbations. European Respiratory Journal 2017;50. https://doi.org/10.1183/13993003.02434-2016.
[20] Wang Z, Lin J, Liang L, Huang F, Yao X, Peng K, et al. Global, regional, and national burden of chronic obstructive pulmonary disease and its attributable risk factors from 1990 to 2021: an analysis for the Global Burden of Disease Study 2021. Respiratory Research 2025;26:2. https://doi.org/10.1186/s12931-024-03051-2.
[21] 2024 GOLD Report. Global Initiative for Chronic Obstructive Lung Disease - GOLD n.d. https://goldcopd.org/2024-gold-report/ (accessed June 3, 2025).
[22] Hooper R, Burney P, Vollmer WM, McBurnie M, Gislason T, Tan WC, et al. Risk factors for COPD spirometrically defined from the lower limit of normal in the BOLD project. Eur Respir J 2012;39:1343–53. https://doi.org/10.1183/09031936.00002711.
[23] Cioboata R, Balteanu MA, Mitroi DM, Vrabie SC, Vlasceanu SG, Andrei GM, et al. Beyond Smoking: Emerging Drivers of COPD and Their Clinical Implications in Low- and Middle-Income Countries: A Narrative Review. Journal of Clinical Medicine 2025;14. https://doi.org/10.3390/jcm14134633.
[24] Cao Z, Tong X, He L, Luo Y, Huang K, Li W, et al. Burden of chronic obstructive pulmonary disease and its attributable risk factors in 204 countries and territories, 1990-2021: results from the Global Burden of Disease Study 2021. BMJ Public Health 2026;4:e002489. https://doi.org/10.1136/bmjph-2024-002489.
[25] Brinchault G, Diot P, Dixmier A, Goupil F, Guillais P, Gut-Gobert C, et al. [Comorbidities of COPD]. Rev Pneumol Clin 2015;71:342–9. https://doi.org/10.1016/j.pneumo.2015.09.009.
[26] Barnes PJ. Inflammatory endotypes in COPD. Allergy 2019;74:1249–56. https://doi.org/10.1111/all.13760.
[27] Barnes PJ. Asthma-COPD Overlap. Chest 2016;149:7–8. https://doi.org/10.1016/j.chest.2015.08.017.
[28] David B, Bafadhel M, Koenderman L, De Soyza A. Eosinophilic inflammation in COPD: from an inflammatory marker to a treatable trait. Thorax 2021;76:188–95. https://doi.org/10.1136/thoraxjnl-2020-215167.
[29] Baltazar-García EA, Vargas-Guerrero B, Gasca-Lozano LE, Gurrola-Díaz CM. Molecular changes underlying pulmonary emphysema and chronic bronchitis in Chronic Obstructive Pulmonary Disease: An updated review. Histol Histopathol 2024;39:805–16. https://doi.org/10.14670/HH-18-699.
[30] Ferrera MC, Labaki WW, Han MK. Advances in Chronic Obstructive Pulmonary Disease. Annu Rev Med 2021;72:119–34. https://doi.org/10.1146/annurev-med-080919-112707.
[31] Beasley V, Joshi PV, Singanayagam A, Molyneaux PL, Johnston SL, Mallia P. Lung microbiology and exacerbations in COPD. Int J Chron Obstruct Pulmon Dis 2012;7:555–69. https://doi.org/10.2147/COPD.S28286.

[32] Wedzicha JA, Donaldson GC. Exacerbations of chronic obstructive pulmonary disease. Respir Care 2003;48:1204–13; discussion 1213-1215.
[33] Sapey E, Stockley RA. COPD exacerbations . 2: aetiology. Thorax 2006;61:250–8. https://doi.org/10.1136/thx.2005.041822.
[34] Sarir H, Henricks PAJ, van Houwelingen AH, Nijkamp FP, Folkerts G. Cells, mediators and Toll-like receptors in COPD. European Journal of Pharmacology 2008;585:346–53. https://doi.org/10.1016/j.ejphar.2008.03.009.
[35] Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. Nat Rev Immunol 2008;8:183–92. https://doi.org/10.1038/nri2254.
[36] Freeman CM, Han MK, Martinez FJ, Murray S, Liu LX, Chensue SW, et al. Cytotoxic potential of lung CD8(+) T cells increases with chronic obstructive pulmonary disease severity and with in vitro stimulation by IL-18 or IL-15. J Immunol 2010;184:6504–13. https://doi.org/10.4049/jimmunol.1000006.
[37] Brusselle GG, Demoor T, Bracke KR, Brandsma C-A, Timens W. Lymphoid follicles in (very) severe COPD: beneficial or harmful? Eur Respir J 2009;34:219–30. https://doi.org/10.1183/09031936.00150208.
[38] Flayer CH, Linderholm AL, Ge MQ, Juarez M, Franzi L, Tham T, et al. COPD with elevated sputum group 2 innate lymphoid cells is characterized by severe disease. medRxiv 2024:2023.11.21.23298837. https://doi.org/10.1101/2023.11.21.23298837.
[39] Borger JG, Lau M, Hibbs ML. The Influence of Innate Lymphoid Cells and Unconventional T Cells in Chronic Inflammatory Lung Disease. Front Immunol 2019;10. https://doi.org/10.3389/fimmu.2019.01597.
[40] Herro R, Grimes HL. The diverse roles of neutrophils from protection to pathogenesis. Nat Immunol 2024;25:2209–19. https://doi.org/10.1038/s41590-024-02006-5.
[41] Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol 2013;13:159–75. https://doi.org/10.1038/nri3399.
[42] Torfs K, Vermeersch G, Gouwy M, Devos T, Proost P, Struyf S. Neutrophils as critical orchestrators of chronic inflammation. Cell Mol Immunol 2026;23:123–49. https://doi.org/10.1038/s41423-025-01380-w.
[43] Nauseef WM, Borregaard N. Neutrophils at work. Nat Immunol 2014;15:602–11. https://doi.org/10.1038/ni.2921.
[44] Lacy P. Mechanisms of Degranulation in Neutrophils. All Asth Clin Immun 2006;2:98. https://doi.org/10.1186/1710-1492-2-3-98.
[45] Bedard K, Krause K-H. The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology. Physiological Reviews 2007;87:245–313. https://doi.org/10.1152/physrev.00044.2005.
[46] Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil Extracellular Traps Kill Bacteria. Science 2004;303:1532–5. https://doi.org/10.1126/science.1092385.
[47] Shim HB, Deniset JF, Kubes P. Neutrophils in homeostasis and tissue repair. Int Immunol 2022;34:399–407. https://doi.org/10.1093/intimm/dxac029.
[48] Christoffersson G, Vågesjö E, Vandooren J, Lidén M, Massena S, Reinert RB, et al. VEGF-A recruits a proangiogenic MMP-9-delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissue. Blood 2012;120:4653–62. https://doi.org/10.1182/blood-2012-04-421040.
[49] Ozel I, Duerig I, Domnich M, Lang S, Pylaeva E, Jablonska J. The Good, the Bad, and the Ugly: Neutrophils, Angiogenesis, and Cancer. Cancers 2022;14. https://doi.org/10.3390/cancers14030536.

[50] Shrestha S, Hong C-W. Extracellular Mechanisms of Neutrophils in Immune Cell Crosstalk. Immune Netw 2023;23:e38. https://doi.org/10.4110/in.2023.23.e38.
[51] Sreeramkumar V, Adrover JM, Ballesteros I, Cuartero MI, Rossaint J, Bilbao I, et al. Neutrophils scan for activated platelets to initiate inflammation. Science 2014;346:1234–8. https://doi.org/10.1126/science.1256478.
[52] Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR, Mercat A, et al. Acute respiratory distress syndrome. Nat Rev Dis Primers 2019;5:18. https://doi.org/10.1038/s41572-019-0069-0.
[53] McIntyre KW, Shuster DJ, Gillooly KM, Warrier RR, Connaughton SE, Hall LB, et al. Reduced incidence and severity of collagen-induced arthritis in interleukin-12-deficient mice. Eur J Immunol 1996;26:2933–8. https://doi.org/10.1002/eji.1830261219.
[54] Constantinescu CS, Wysocka M, Hilliard B, Ventura ES, Lavi E, Trinchieri G, et al. Antibodies against IL-12 prevent superantigen-induced and spontaneous relapses of experimental autoimmune encephalomyelitis. J Immunol 1998;161:5097–104. https://doi.org/10.4049/jimmunol.161.9.5097.
[55] Segal BM, Dwyer BK, Shevach EM. An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease. J Exp Med 1998;187:537–46. https://doi.org/10.1084/jem.187.4.537.
[56] Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature 2003;421:744–8. https://doi.org/10.1038/nature01355.
[57] Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity 2000;13:715–25. https://doi.org/10.1016/s1074-7613(00)00070-4.
[58] Rouvier E, Luciani MF, Mattéi MG, Denizot F, Golstein P. CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a herpesvirus saimiri gene. J Immunol 1993;150:5445–56. https://doi.org/10.4049/jimmunol.150.12.5445.
[59] Kennedy J, Rossi DL, Zurawski SM, Vega F, Kastelein RA, Wagner JL, et al. Mouse IL-17: a cytokine preferentially expressed by alpha beta TCR + CD4-CD8-T cells. J Interferon Cytokine Res 1996;16:611–7. https://doi.org/10.1089/jir.1996.16.611.
[60] Yao Z, Painter SL, Fanslow WC, Ulrich D, Macduff BM, Spriggs MK, et al. Human IL-17: a novel cytokine derived from T cells. J Immunol 1995;155:5483–6. https://doi.org/10.4049/jimmunol.155.12.5483.
[61] Fossiez F, Djossou O, Chomarat P, Flores-Romo L, Ait-Yahia S, Maat C, et al. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J Exp Med 1996;183:2593–603. https://doi.org/10.1084/jem.183.6.2593.
[62] Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005;6:1123–32. https://doi.org/10.1038/ni1254.
[63] Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol 2009;27:485–517. https://doi.org/10.1146/annurev.immunol.021908.132710.
[64] Wilson NJ, Boniface K, Chan JR, McKenzie BS, Blumenschein WM, Mattson JD, et al. Development, cytokine profile and function of human interleukin 17-producing helper T cells. Nat Immunol 2007;8:950–7. https://doi.org/10.1038/ni1497.

[65] Teng MWL, Bowman EP, McElwee JJ, Smyth MJ, Casanova J-L, Cooper AM, et al. IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. Nat Med 2015;21:719–29. https://doi.org/10.1038/nm.3895.
[66] Lee HS, Park D-E, Lee J-W, Chang Y, Kim HY, Song W-J, et al. IL-23 secreted by bronchial epithelial cells contributes to allergic sensitization in asthma model: role of IL-23 secreted by bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 2017;312:L13–21. https://doi.org/10.1152/ajplung.00114.2016.
[67] Yoon J, Leyva-Castillo JM, Wang G, Galand C, Oyoshi MK, Kumar L, et al. IL-23 induced in keratinocytes by endogenous TLR4 ligands polarizes dendritic cells to drive IL-22 responses to skin immunization. J Exp Med 2016;213:2147–66. https://doi.org/10.1084/jem.20150376.
[68] Wang Z, Shi D. Research progress on the neutrophil components and their interactions with immune cells in the development of psoriasis. Skin Research and Technology 2023;29:e13404. https://doi.org/10.1111/srt.13404.
[69] Kvedaraite E, Lourda M, Ideström M, Chen P, Olsson S, Forkel M, et al. Tissue-infiltrating neutrophils represent the main source of IL-23 in the colon of patients with IBD. Gut 2016;65:1632–41. https://doi.org/10.1136/gutjnl-2014-309014.
[70] Taylor PR, Roy S, Leal SM, Sun Y, Howell SJ, Cobb BA, et al. Activation of neutrophils by autocrine IL-17A–IL-17RC interactions during fungal infection is regulated by IL-6, IL-23, RORγt and dectin-2. Nat Immunol 2014;15:143–51. https://doi.org/10.1038/ni.2797.
[71] Eustace A, Smyth LJC, Mitchell L, Williamson K, Plumb J, Singh D. Identification of cells expressing IL-17A and IL-17F in the lungs of patients with COPD. Chest 2011;139:1089–100. https://doi.org/10.1378/chest.10-0779.
[72] Hong J, Kang M-H, Lee J, Cha M-S, Bae Y-S, Kim HY, et al. γδ+ T-cell-derived IL-17A stimulates airway epithelial/stromal cells to secrete G-CSF, promoting lung-specific pathogenic Siglec-F+ neutrophil development in PPE-induced emphysema. Cell Mol Immunol 2025;22:791–805. https://doi.org/10.1038/s41423-025-01301-x.
[73] Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, et al. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. J Immunol 2002;168:5699–708. https://doi.org/10.4049/jimmunol.168.11.5699.
[74] Hunter CA. New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. Nat Rev Immunol 2005;5:521–31. https://doi.org/10.1038/nri1648.
[75] Gaffen SL, Jain R, Garg AV, Cua DJ. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. Nat Rev Immunol 2014;14:585–600. https://doi.org/10.1038/nri3707.
[76] Dong C. TH17 cells in development: an updated view of their molecular identity and genetic programming. Nat Rev Immunol 2008;8:337–48. https://doi.org/10.1038/nri2295.
[77] Yoshimura A, Naka T, Kubo M. SOCS proteins, cytokine signalling and immune regulation. Nat Rev Immunol 2007;7:454–65. https://doi.org/10.1038/nri2093.
[78] Chen Z, Laurence A, Kanno Y, Pacher-Zavisin M, Zhu B-M, Tato C, et al. Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. Proc Natl Acad Sci U S A 2006;103:8137–42. https://doi.org/10.1073/pnas.0600666103.
[79] Gaffen SL. Structure and signalling in the IL-17 receptor family. Nat Rev Immunol 2009;9:556–67. https://doi.org/10.1038/nri2586.

[80] Shen F, Gaffen SL. Structure-function relationships in the IL-17 receptor: implications for signal transduction and therapy. Cytokine 2008;41:92–104. https://doi.org/10.1016/j.cyto.2007.11.013.
[81] Feng Y, Chen Z, Tu S-Q, Wei J-M, Hou Y-L, Kuang Z-L, et al. Role of Interleukin-17A in the Pathomechanisms of Periodontitis and Related Systemic Chronic Inflammatory Diseases. Front Immunol 2022;13:862415. https://doi.org/10.3389/fimmu.2022.862415.
[82] Li X, Bechara R, Zhao J, McGeachy MJ, Gaffen SL. IL-17 receptor-based signaling and implications for disease. Nat Immunol 2019;20:1594–602. https://doi.org/10.1038/s41590-019-0514-y.
[83] Huangfu L, Li R, Huang Y, Wang S. The IL-17 family in diseases: from bench to bedside. Signal Transduct Target Ther 2023;8:402. https://doi.org/10.1038/s41392-023-01620-3.
[84] Subramaniam SV, Cooper RS, Adunyah SE. Evidence for the Involvement of JAK/STAT Pathway in the Signaling Mechanism of Interleukin-17. Biochemical and Biophysical Research Communications 1999;262:14–9. https://doi.org/10.1006/bbrc.1999.1156.
[85] Li Y, Guo N, Zhao Y, Chen J, Zhao J, Bian J, et al. IL-17A activates JAK/STAT signaling to affect drug metabolizing enzymes and transporters in HepaRG cells. Mol Immunol 2024;175:55–62. https://doi.org/10.1016/j.molimm.2024.09.008.
[86] Agalioti T, Cortesi F, Gagliani N. TH17 cell immune adaptation. Curr Opin Immunol 2023;83:102333. https://doi.org/10.1016/j.coi.2023.102333.
[87] Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 2006;126:1121–33. https://doi.org/10.1016/j.cell.2006.07.035.
[88] McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, et al. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. Nat Immunol 2007;8:1390–7. https://doi.org/10.1038/ni1539.
[89] Lubberts E. The IL-23–IL-17 axis in inflammatory arthritis. Nat Rev Rheumatol 2015;11:415–29. https://doi.org/10.1038/nrrheum.2015.53.
[90] Al-Ramli W, Préfontaine D, Chouiali F, Martin JG, Olivenstein R, Lemière C, et al. T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. J Allergy Clin Immunol 2009;123:1185–7. https://doi.org/10.1016/j.jaci.2009.02.024.
[91] Moschen AR, Tilg H, Raine T. IL-12, IL-23 and IL-17 in IBD: immunobiology and therapeutic targeting. Nat Rev Gastroenterol Hepatol 2019;16:185–96. https://doi.org/10.1038/s41575-018-0084-8.
[92] Abdo AIK, Tye GJ. Interleukin 23 and autoimmune diseases: current and possible future therapies. Inflamm Res 2020;69:463–80. https://doi.org/10.1007/s00011-020-01339-9.
[93] Vassallo R, Walters PR, Lamont J, Kottom TJ, Yi ES, Limper AH. Cigarette smoke promotes dendritic cell accumulation in COPD; a Lung Tissue Research Consortium study. Respiratory Research 2010;11:45. https://doi.org/10.1186/1465-9921-11-45.
[94] Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-β induces development of the TH17 lineage. Nature 2006;441:231–4. https://doi.org/10.1038/nature04754.
[95] Fujii U, Miyahara N, Taniguchi A, Waseda K, Morichika D, Kurimoto E, et al. IL-23 Is Essential for the Development of Elastase-Induced Pulmonary Inflammation and

Emphysema. Am J Respir Cell Mol Biol 2016;55:697–707. https://doi.org/10.1165/rcmb.2016-0015OC.
[96] Rong B, Fu T, Rong C, Liu W, Li K, Liu H. Association between serum CCL-18 and IL-23 concentrations and disease progression of chronic obstructive pulmonary disease. Scientific Reports 2020;10:17756. https://doi.org/10.1038/s41598-020-73903-6.
[97] Moermans C, Damas K, Guiot J, Njock MS, Corhay JL, Henket M, et al. Sputum IL-25, IL-33 and TSLP, IL-23 and IL-36 in airway obstructive diseases. Reduced levels of IL-36 in eosinophilic phenotype. Cytokine 2021;140:155421. https://doi.org/10.1016/j.cyto.2021.155421.
[98] Roy MG, Livraghi-Butrico A, Fletcher AA, McElwee MM, Evans SE, Boerner RM, et al. Muc5b Is Required for Airway Defense. Nature 2014;505:412–6. https://doi.org/10.1038/nature12807.
[99] Di Stefano A, Caramori G, Gnemmi I, Contoli M, Vicari C, Capelli A, et al. T helper type 17-related cytokine expression is increased in the bronchial mucosa of stable chronic obstructive pulmonary disease patients. Clinical and Experimental Immunology 2009;157:316–24. https://doi.org/10.1111/j.1365-2249.2009.03965.x.
[100] Liang C, Shen Y, Xu Y, Liang Y, Qiu S, Tang H, et al. Dendritic Cells Promote the Differentiation of ILCs into NCR−ILC3s in the Lungs of Mice Exposed to Cigarette Smoke. COPD: Journal of Chronic Obstructive Pulmonary Disease 2024;21:2389909. https://doi.org/10.1080/15412555.2024.2389909.
[101] Tian X, Wang S, Zhang C, Prakash YS, Vassallo R. Blocking IL-23 Signaling Mitigates Cigarette Smoke-Induced Murine Emphysema. Environ Toxicol 2024;39:5334–46. https://doi.org/10.1002/tox.24405.
[102] Roos AB, Sandén C, Mori M, Bjermer L, Stampfli MR, Erjefält JS. IL-17A Is Elevated in End-Stage Chronic Obstructive Pulmonary Disease and Contributes to Cigarette Smoke-induced Lymphoid Neogenesis. Am J Respir Crit Care Med 2015;191:1232–41. https://doi.org/10.1164/rccm.201410-1861OC.
[103] Roos AB, Sethi S, Nikota J, Wrona CT, Dorrington MG, Sandén C, et al. IL-17A and the Promotion of Neutrophilia in Acute Exacerbation of Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med 2015;192:428–37. https://doi.org/10.1164/rccm.201409-1689OC.
[104] Ma R, Su H, Jiao K, Liu J. Association Between IL-17 and Chronic Obstructive Pulmonary Disease: A Systematic Review and Meta-Analysis. Int J Chron Obstruct Pulmon Dis 2023;18:1681–90. https://doi.org/10.2147/COPD.S412626.
[105] Yanagisawa H, Hashimoto M, Minagawa S, Takasaka N, Ma R, Moermans C, et al. Role of IL-17A in murine models of COPD airway disease. Am J Physiol Lung Cell Mol Physiol 2017;312:L122–30. https://doi.org/10.1152/ajplung.00301.2016.
[106] Pichavant M, Sharan R, Le Rouzic O, Olivier C, Hennegrave F, Rémy G, et al. IL-22 Defect During Streptococcus pneumoniae Infection Triggers Exacerbation of Chronic Obstructive Pulmonary Disease. EBioMedicine 2015;2:1686–96. https://doi.org/10.1016/j.ebiom.2015.09.040.
[107] Mardi A, Abdolmohammadi-Vahid S, Sadeghi SA, Jafarzadeh S, Abbaspour-Aghdam S, Hazrati A, et al. Nanocurcumin modulates Th17 cell responses in moderate and severe COPD patients. Heliyon 2024;10:e30025. https://doi.org/10.1016/j.heliyon.2024.e30025.
[108] Christenson SA, van den Berge M, Faiz A, Inkamp K, Bhakta N, Bonser LR, et al. An airway epithelial IL-17A response signature identifies a steroid-unresponsive COPD patient subgroup. J Clin Invest 2019;129:169–81. https://doi.org/10.1172/JCI121087.

[109] Ouyang S, Liu C, Xiao J, Chen X, Lui AC, Li X. Targeting IL-17A/glucocorticoid synergy to CSF3 expression in neutrophilic airway diseases. JCI Insight 2020;5. https://doi.org/10.1172/jci.insight.132836.
[110] Ramos-Ramírez P, Tliba O. Glucocorticoid Receptor β (GRβ): Beyond Its Dominant-Negative Function. International Journal of Molecular Sciences 2021;22:3649. https://doi.org/10.3390/ijms22073649.
[111] Huang H, Wang W. Molecular mechanisms of glucocorticoid resistance. European Journal of Clinical Investigation 2023;53:e13901. https://doi.org/10.1111/eci.13901.
[112] Min J, Perera L, Krahn JM, Jewell CM, Moon AF, Cidlowski JA, et al. Probing Dominant Negative Behavior of Glucocorticoid Receptor β through a Hybrid Structural and Biochemical Approach. Mol Cell Biol 2018;38:e00453-17. https://doi.org/10.1128/MCB.00453-17.
[113] Kelly A, Bowen H, Jee Y-K, Mahfiche N, Soh C, Lee T, et al. The glucocorticoid receptor β isoform can mediate transcriptional repression by recruiting histone deacetylases. Journal of Allergy and Clinical Immunology 2008;121:203-208.e1. https://doi.org/10.1016/j.jaci.2007.09.010.
[114] Vazquez-Tello A, Halwani R, Hamid Q, Al-Muhsen S. Glucocorticoid receptor-beta up-regulation and steroid resistance induction by IL-17 and IL-23 cytokine stimulation in peripheral mononuclear cells. J Clin Immunol 2013;33:466–78. https://doi.org/10.1007/s10875-012-9828-3.
[115] Halwani R, Sultana A, Al-Kufaidy R, Jamhawi A, Vazquez-Tello A, Al-Muhsen S. Th-17 regulatory cytokines inhibit corticosteroid induced airway structural cells apoptosis. Respir Res 2016;17:6. https://doi.org/10.1186/s12931-015-0307-2.
[116] Lo C-Y, Wang C-H, Wang C-W, Chen C-J, Huang H-Y, Chung F-T, et al. Increased Interleukin-17 and Glucocorticoid Receptor-β Expression in Interstitial Lung Diseases and Corticosteroid Insensitivity. Front Immunol 2022;13:905727. https://doi.org/10.3389/fimmu.2022.905727.
[117] Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report. GOLD Executive Summary. American Journal of Respiratory and Critical Care Medicine 2017;195:557–82. https://doi.org/10.1164/rccm.201701-0218PP.
[118] Rabe KF, Bateman ED, O'Donnell D, Witte S, Bredenbröker D, Bethke TD. Roflumilast–an oral anti-inflammatory treatment for chronic obstructive pulmonary disease: a randomised controlled trial. Lancet (London, England) 2005;366:563–71. https://doi.org/10.1016/S0140-6736(05)67100-0.
[119] Hakim A, Adcock IM, Usmani OS. Corticosteroid resistance and novel anti-inflammatory therapies in chronic obstructive pulmonary disease: current evidence and future direction. Drugs 2012;72:1299–312. https://doi.org/10.2165/11634350-000000000-00000.
[120] Suissa S, Patenaude V, Lapi F, Ernst P. Inhaled corticosteroids in COPD and the risk of serious pneumonia. Thorax 2013;68:1029–36. https://doi.org/10.1136/thoraxjnl-2012-202872.
[121] Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. N Engl J Med 2008;359:2355–65. https://doi.org/10.1056/NEJMra0800353.

[122] Cui Y, Luo L, Li C, Chen P, Chen Y. Long-term macrolide treatment for the prevention of acute exacerbations in COPD: a systematic review and meta-analysis. Int J Chron Obstruct Pulmon Dis 2018;13:3813–29. https://doi.org/10.2147/COPD.S181246.
[123] Vollenweider DJ, Frei A, Steurer-Stey CA, Garcia-Aymerich J, Puhan MA. Antibiotics for exacerbations of chronic obstructive pulmonary disease. Cochrane Database Syst Rev 2018;2018:CD010257. https://doi.org/10.1002/14651858.CD010257.pub2.
[124] Pailhoriès H, Herrmann J-L, Velo-Suarez L, Lamoureux C, Beauruelle C, Burgel P-R, et al. Antibiotic resistance in chronic respiratory diseases: from susceptibility testing to the resistome. Eur Respir Rev 2022;31:210259. https://doi.org/10.1183/16000617.0259-2021.
[125] Megna M, Balato A, Raimondo A, Balato N. Guselkumab for the treatment of psoriasis. Expert Opinion on Biological Therapy 2018;18:459–68. https://doi.org/10.1080/14712598.2018.1445223.
[126] Gordon KB, Strober B, Lebwohl M, Augustin M, Blauvelt A, Poulin Y, et al. Efficacy and safety of risankizumab in moderate-to-severe plaque psoriasis (UltIMMa-1 and UltIMMa-2): results from two double-blind, randomised, placebo-controlled and ustekinumab-controlled phase 3 trials. The Lancet 2018;392:650–61. https://doi.org/10.1016/S0140-6736(18)31713-6.
[127] Reich K, Papp KA, Blauvelt A, Tyring SK, Sinclair R, Thaçi D, et al. Tildrakizumab versus placebo or etanercept for chronic plaque psoriasis (reSURFACE 1 and reSURFACE 2): results from two randomised controlled, phase 3 trials. Lancet (London, England) 2017;390:276–88. https://doi.org/10.1016/S0140-6736(17)31279-5.
[128] Jairath V, D'Haens G, Sands BE, Travis S, Chaparro M, Peyrin-Biroulet L, et al. Bowel Urgency in Crohn's Disease: Effects of Mirikizumab in a Randomized Controlled Phase 3 Study. Clin Gastroenterol Hepatol 2026;24:463-473.e5. https://doi.org/10.1016/j.cgh.2025.07.003.
[129] Leonardi CL, Kimball AB, Papp KA, Yeilding N, Guzzo C, Wang Y, et al. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). Lancet (London, England) 2008;371:1665–74. https://doi.org/10.1016/S0140-6736(08)60725-4.
[130] Tan H-L, Rosenthal M. IL-17 in lung disease: friend or foe? Thorax 2013;68:788–90. https://doi.org/10.1136/thoraxjnl-2013-203307.
[131] Riani Moreira A, Uchoa da Silva C, de Paula Costa Mattos L, Jesus Simão J, Camargo Berti V, Henrique Vasconcelos Alves L, et al. IL-17-neutralizing antibody mitigates functional and structural changes in cigarette smoke-induced COPD model. Front Immunol 2025;16:1641300. https://doi.org/10.3389/fimmu.2025.1641300.
[132] Busse WW, Holgate S, Kerwin E, Chon Y, Feng J, Lin J, et al. Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. Am J Respir Crit Care Med 2013;188:1294–302. https://doi.org/10.1164/rccm.201212-2318OC.
[133] Novartis Pharmaceuticals. A Randomized, Double-blind, Placebo Controlled, Multiple Dose Study to Evaluate the Safety, Tolerability, and Efficacy of Intravenous Administration of Secukinumab (AIN457) in Patients With Asthma Not Adequately Controlled With Inhaled Corticosteroids and Long Acting Beta-agonists. clinicaltrials.gov; 2015.
[134] Caramori G, Adcock IM, Stefano AD, Chung KF. Cytokine inhibition in the treatment of COPD. COPD 2014;9:397–412. https://doi.org/10.2147/COPD.S42544.

[135] Christopoulou ME, Papakonstantinou E, Gonsard A, Stolz D. Differential effects of Ruxolitinib on airway remodeling in asthma and COPD. Eur Respir J n.d.;64:PA2976. https://doi.org/10.1183/13993003.congress-2024.PA2976.
[136] Fenwick PS, Macedo P, Kilty IC, Barnes PJ, Donnelly LE. Effect of JAK Inhibitors on Release of CXCL9, CXCL10 and CXCL11 from Human Airway Epithelial Cells. PLoS One 2015;10:e0128757. https://doi.org/10.1371/journal.pone.0128757.
[137] Milara J, Ballester B, de Diego A, Calbet M, Ramis I, Miralpeix M, et al. The pan-JAK inhibitor LAS194046 reduces neutrophil activation from severe asthma and COPD patients in vitro. Sci Rep 2022;12:5132. https://doi.org/10.1038/s41598-022-09241-6.
[138] Morgan F, O'Brien C, Young J, Burgess G, Leaker B, Singh D. Late Breaking Abstract - The safety and PK of an inhaled pan JAK inhibitor (KN-002) in subjects with COPD. Eur Respir J n.d.;64:PA3928. https://doi.org/10.1183/13993003.congress-2024.PA3928.
[139] Chikhoune L, Poggi C, Moreau J, Dubucquoi S, Hachulla E, Collet A, et al. JAK inhibitors (JAKi): Mechanisms of action and perspectives in systemic and autoimmune diseases. La Revue de Médecine Interne 2025;46:89–106. https://doi.org/10.1016/j.revmed.2024.10.452.
[140] Mease P, Helliwell P, Silwinska-Stanczyk P, Miakisz M, Ostor A, Peeva E, et al. Efficacy and Safety of the TYK2/JAK1 Inhibitor Brepocitinib for Active Psoriatic Arthritis: A Phase IIb Randomized Controlled Trial. Arthritis Rheumatol 2023;75:1370–80. https://doi.org/10.1002/art.42519.
[141] Luschnig P, Kienzl M, Roula D, Pilic J, Atallah R, Heinemann A, et al. The JAK1/2 inhibitor baricitinib suppresses eosinophil effector function and restricts allergen-induced airway eosinophilia. Biochemical Pharmacology 2021;192:114690. https://doi.org/10.1016/j.bcp.2021.114690.
[142] Teppan J, Schwanzer J, Rittchen S, Bärnthaler T, Lindemann J, Nayak B, et al. The disrupted molecular circadian clock of monocytes and macrophages in allergic inflammation. Front Immunol 2024;15:1408772. https://doi.org/10.3389/fimmu.2024.1408772.
[143] Derycke L, Zhang N, Holtappels G, Dutré T, Bachert C. IL-17A as a regulator of neutrophil survival in nasal polyp disease of patients with and without cystic fibrosis. J Cyst Fibros 2012;11:193–200. https://doi.org/10.1016/j.jcf.2011.11.007.
[144] Werner JL, Gessner MA, Lilly LM, Nelson MP, Metz AE, Horn D, et al. Neutrophils produce interleukin 17A (IL-17A) in a dectin-1- and IL-23-dependent manner during invasive fungal infection. Infect Immun 2011;79:3966–77. https://doi.org/10.1128/IAI.05493-11.
[145] Zhang L, Cheng Z, Liu W, Wu K. Expression of interleukin (IL)-10, IL-17A and IL-22 in serum and sputum of stable chronic obstructive pulmonary disease patients. COPD 2013;10:459–65. https://doi.org/10.3109/15412555.2013.770456.
[146] Goepfert A, Barske C, Lehmann S, Wirth E, Willemsen J, Gudjonsson JE, et al. IL-17-induced dimerization of IL-17RA drives the formation of the IL-17 signalosome to potentiate signaling. Cell Rep 2022;41:111489. https://doi.org/10.1016/j.celrep.2022.111489.
[147] Lay CS, Bridges A, Goulding J, Briddon SJ, Soloviev Z, Craggs PD, et al. Probing the binding of interleukin-23 to individual receptor components and the IL-23 heteromeric receptor complex in living cells using NanoBRET. Cell Chem Biol 2022;29:19-29.e6. https://doi.org/10.1016/j.chembiol.2021.05.002.

[148] Liu S, Desharnais J, Sahasrabudhe PV, Jin P, Li W, Oates BD, et al. Inhibiting complex IL-17A and IL-17RA interactions with a linear peptide. Sci Rep 2016;6:26071. https://doi.org/10.1038/srep26071.
[149] Vámos E, Kálmán N, Sturm EM, Nayak BB, Teppan J, Vántus VB, et al. Highly Selective MIF Ketonase Inhibitor KRP-6 Diminishes M1 Macrophage Polarization and Metabolic Reprogramming. Antioxidants (Basel) 2023;12:1790. https://doi.org/10.3390/antiox12101790.
[150] Knuplez E, Kienzl M, Trakaki A, Schicho R, Heinemann A, Sturm EM, et al. The anti-parasitic drug miltefosine suppresses activation of human eosinophils and ameliorates allergic inflammation in mice. Br J Pharmacol 2021;178:1234–48. https://doi.org/10.1111/bph.15368.
[151] Sturm EM, Parzmair GP, Radnai B, Frei RB, Sturm GJ, Hammer A, et al. Phosphoinositide-dependent protein kinase 1 (PDK1) mediates potent inhibitory effects on eosinophils. European Journal of Immunology 2015;45:1548–59. https://doi.org/10.1002/eji.201445196.
[152] Heinemann A, Schuligoi R, Sabroe I, Hartnell A, Peskar BA. Delta 12-prostaglandin J2, a plasma metabolite of prostaglandin D2, causes eosinophil mobilization from the bone marrow and primes eosinophils for chemotaxis. J Immunol 2003;170:4752–8. https://doi.org/10.4049/jimmunol.170.9.4752.
[153] Ninaber DK, Does AM van der, Hiemstra PS. Isolating Bronchial Epithelial Cells from Resected Lung Tissue for Biobanking and Establishing Well-Differentiated Air-Liquid Interface Cultures. JoVE (Journal of Visualized Experiments) 2023:e65102. https://doi.org/10.3791/65102.
[154] Schrumpf JA, Ninaber DK, van der Does AM, Hiemstra PS. TGF-β1 Impairs Vitamin D-Induced and Constitutive Airway Epithelial Host Defense Mechanisms. J Innate Immun 2020;12:74–89. https://doi.org/10.1159/000497415.
[155] Amatngalim GD, Schrumpf JA, Dishchekenian F, Mertens TCJ, Ninaber DK, Linden AC van der, et al. Aberrant epithelial differentiation by cigarette smoke dysregulates respiratory host defence. European Respiratory Journal 2018;51. https://doi.org/10.1183/13993003.01009-2017.
[156] Amatngalim GD, van Wijck Y, de Mooij-Eijk Y, Verhoosel RM, Harder J, Lekkerkerker AN, et al. Basal cells contribute to innate immunity of the airway epithelium through production of the antimicrobial protein RNase 7. J Immunol 2015;194:3340–50. https://doi.org/10.4049/jimmunol.1402169.
[157] Ivanov S, Bozinovski S, Bossios A, Valadi H, Vlahos R, Malmhäll C, et al. Functional relevance of the IL-23-IL-17 axis in lungs in vivo. Am J Respir Cell Mol Biol 2007;36:442–51. https://doi.org/10.1165/rcmb.2006-0020OC.
[158] Bärnthaler T, Maric J, Platzer W, Konya V, Theiler A, Hasenöhrl C, et al. The Role of PGE2 in Alveolar Epithelial and Lung Microvascular Endothelial Crosstalk. Sci Rep 2017;7:7923. https://doi.org/10.1038/s41598-017-08228-y.
[159] Singh D, Fox SM, Tal-Singer R, Plumb J, Bates S, Broad P, et al. Induced sputum genes associated with spirometric and radiological disease severity in COPD ex-smokers. Thorax 2011;66:489–95. https://doi.org/10.1136/thx.2010.153767.
[160] Chang HS, Lee T-H, Jun JA, Baek AR, Park J-S, Koo S-M, et al. Neutrophilic inflammation in asthma: mechanisms and therapeutic considerations. Expert Rev Respir Med 2017;11:29–40. https://doi.org/10.1080/17476348.2017.1268919.
[161] Syabbalo N. Role of IL-17 and IL-23 in the Pathogenesis of Neutrophilic Asthma. Int J Immunol Immunother 2020;7:049. https://doi.org/10.23937/2378-3672/1410049.

[162] Sun R, Hedl M, Abraham C. IL23 induces IL23R recycling and amplifies innate receptor-induced signalling and cytokines in human macrophages, and the IBD-protective IL23R R381Q variant modulates these outcomes. Gut 2020;69:264–73. https://doi.org/10.1136/gutjnl-2018-316830.
[163] Lemos HP, Grespan R, Vieira SM, Cunha TM, Verri WA, Fernandes KSS, et al. Prostaglandin mediates IL-23/IL-17-induced neutrophil migration in inflammation by inhibiting IL-12 and IFNgamma production. Proc Natl Acad Sci U S A 2009;106:5954–9. https://doi.org/10.1073/pnas.0812782106.
[164] Mallia P, Message SD, Contoli M, Gray KK, Telcian A, Laza-Stanca V, et al. Neutrophil adhesion molecules in experimental rhinovirus infection in COPD. Respiratory Research 2013;14:72. https://doi.org/10.1186/1465-9921-14-72.
[165] Yamagata T, Sugiura H, Yokoyama T, Yanagisawa S, Ichikawa T, Ueshima K, et al. Overexpression of CD-11b and CXCR1 on Circulating Neutrophils: Its Possible Role in COPD. CHEST 2007;132:890–9. https://doi.org/10.1378/chest.07-0569.
[166] Marc MM, Kristan SS, Rozman A, Kern I, Flezar M, Kosnik M, et al. Complement factor C5a in acute exacerbation of Chronic Obstructive Pulmonary Disease. Scand J Immunol 2010;71:386–91. https://doi.org/10.1111/j.1365-3083.2010.02385.x.
[167] Banerjee S, Biehl A, Gadina M, Hasni S, Schwartz DM. JAK-STAT Signaling as a Target for Inflammatory and Autoimmune Diseases: Current and Future Prospects. Drugs 2017;77:521–46. https://doi.org/10.1007/s40265-017-0701-9.
[168] Lindén A, Laan M, Anderson GP. Neutrophils, interleukin-17A and lung disease. European Respiratory Journal 2005;25:159–72. https://doi.org/10.1183/09031936.04.00032904.
[169] Honda K, Wada H, Nakamura M, Nakamoto K, Inui T, Sada M, et al. IL-17A synergistically stimulates TNF-α-induced IL-8 production in human airway epithelial cells: A potential role in amplifying airway inflammation. Exp Lung Res 2016;42:205–16. https://doi.org/10.1080/01902148.2016.1190796.
[170] Griffin GK, Newton G, Tarrio ML, Bu D, Maganto-Garcia E, Azcutia V, et al. IL-17 and TNF-α sustain neutrophil recruitment during inflammation through synergistic effects on endothelial activation. J Immunol 2012;188:6287–99. https://doi.org/10.4049/jimmunol.1200385.
[171] Li Y, Zhu L, Chu Z, Yang T, Sun H-X, Yang F, et al. Characterization and biological significance of IL-23-induced neutrophil polarization. Cell Mol Immunol 2018;15:518–30. https://doi.org/10.1038/cmi.2017.39.
[172] Oakley RH, Cidlowski JA. The biology of the glucocorticoid receptor: New signaling mechanisms in health and disease. Journal of Allergy and Clinical Immunology 2013;132:1033–44. https://doi.org/10.1016/j.jaci.2013.09.007.
[173] Barnes PJ. Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease. Journal of Allergy and Clinical Immunology 2013;131:636–45. https://doi.org/10.1016/j.jaci.2012.12.1564.
[174] Zijlstra GJ, Ten Hacken NHT, Hoffmann RF, van Oosterhout AJM, Heijink IH. Interleukin-17A induces glucocorticoid insensitivity in human bronchial epithelial cells. Eur Respir J 2012;39:439–45. https://doi.org/10.1183/09031936.00017911.
[175] Longui CA, Vottero A, Adamson PC, Cole DE, Kino T, Monte O, et al. Low glucocorticoid receptor alpha/beta ratio in T-cell lymphoblastic leukemia. Horm Metab Res 2000;32:401–6. https://doi.org/10.1055/s-2007-978661.

[176] Zou Y, Chen X, Liu J, Zhou DB, Kuang X, Xiao J, et al. Serum IL-1β and IL-17 levels in patients with COPD: associations with clinical parameters. Int J Chron Obstruct Pulmon Dis 2017;12:1247–54. https://doi.org/10.2147/COPD.S131877.
[177] Wei Y, Zhang T, Wei Y, Zhang T. Janus Kinases and Autoimmunity: Bridging Pathways to Therapy. IJDDP 2024;3:100007. https://doi.org/10.53941/ijddp.2024.100007.
[178] Hammitzsch A, Chen L, de Wit J, Al-Mossawi MH, Ridley A, Sekine T, et al. Inhibiting ex-vivo Th17 responses in Ankylosing Spondylitis by targeting Janus kinases. Sci Rep 2018;8:15645. https://doi.org/10.1038/s41598-018-34026-1.
[179] Seif F, Khoshmirsafa M, Aazami H, Mohsenzadegan M, Sedighi G, Bahar M. The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells. Cell Commun Signal 2017;15:23. https://doi.org/10.1186/s12964-017-0177-y.
[180] Barjaktarevic IZ, Crystal RG, Kaner RJ. The Role of Interleukin-23 in the Early Development of Emphysema in HIV1^{+} Smokers. Journal of Immunology Research 2016;2016:1–14. https://doi.org/10.1155/2016/3463104.
[181] Chang Y, Al-Alwan L, Alshakfa S, Audusseau S, Mogas AK, Chouiali F, et al. Upregulation of IL-17A/F from human lung tissue explants with cigarette smoke exposure: implications for COPD. Respir Res 2014;15:145. https://doi.org/10.1186/s12931-014-0145-7.
[182] Levänen B, Glader P, Dahlén B, Billing B, Qvarfordt I, Palmberg L, et al. Impact of tobacco smoking on cytokine signaling via interleukin-17A in the peripheral airways. Int J Chron Obstruct Pulmon Dis 2016;11:2109–16. https://doi.org/10.2147/COPD.S99900.
[183] Tomita K, Caramori G, Ito K, Sano H, Lim S, Oates T, et al. STAT6 expression in T cells, alveolar macrophages and bronchial biopsies of normal and asthmatic subjects. Journal of Inflammation 2012;9:5. https://doi.org/10.1186/1476-9255-9-5.
[184] Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. Science 1998;282:2258–61. https://doi.org/10.1126/science.282.5397.2258.
[185] Zhang F, Xia Y, Su J, Quan F, Zhou H, Li Q, et al. Neutrophil diversity and function in health and disease. Sig Transduct Target Ther 2024;9:343. https://doi.org/10.1038/s41392-024-02049-y.
[186] Catlett IM, Gao L, Hu Y, Banerjee S, Krueger JG. Pharmacodynamic Response to Deucravacitinib, an Oral, Selective, Allosteric TYK2 Inhibitor, in a Global, Phase 2, Randomized, Double-Blind, Placebo-Controlled Psoriasis Trial. Dermatol Ther (Heidelb) 2024;14:2827–39. https://doi.org/10.1007/s13555-024-01262-5.
[187] Gonciarz M, Pawlak-Buś K, Leszczyński P, Owczarek W. TYK2 as a therapeutic target in the treatment of autoimmune and inflammatory diseases. Immunotherapy 2021;13:1135–50. https://doi.org/10.2217/imt-2021-0096.
[188] Hu S, He W, Du X, Yang J, Wen Q, Zhong X-P, et al. IL-17 Production of Neutrophils Enhances Antibacteria Ability but Promotes Arthritis Development During Mycobacterium tuberculosis Infection. eBioMedicine 2017;23:88–99. https://doi.org/10.1016/j.ebiom.2017.08.001.
[189] Li X, Luck ME, Herrnreiter CJ, Cannon AR, Choudhry MA. IL-23 Promotes Neutrophil Extracellular Trap Formation and Bacterial Clearance in a Mouse Model of Alcohol and Burn Injury. ImmunoHorizons 2022;6:64–75. https://doi.org/10.4049/immunohorizons.2100109.
[190] Milanova V, Ivanovska N, Dimitrova P. The effect of interleukin 17 and Toll-like receptor 2 on CD11b expression and apoptosis of neutrophils in zymosaninduced

arthritis and paw oedema. Cent Eur J Immunol 2014;39:131–41. https://doi.org/10.5114/ceji.2014.43712.
[191] Dragotto M, D'Onghia M, Trovato E, Tognetti L, Rubegni P, Calabrese L. Therapeutic Potential of Targeting the JAK/STAT Pathway in Psoriasis: Focus on TYK2 Inhibition. Journal of Clinical Medicine 2024;13:3091. https://doi.org/10.3390/jcm13113091.
[192] Paik JJ, Vencovský J, Charles-Schoeman C, Wright GC, Vleugels RA, Goriounova AS, et al. Brepocitinib, a potent and selective TYK2/JAK1 inhibitor: scientific and clinical rationale for dermatomyositis. Clin Exp Rheumatol 2025;43:354–63. https://doi.org/10.55563/clinexprheumatol/eeglsa.
[193] Jones CE, Chan K. Interleukin-17 stimulates the expression of interleukin-8, growth-related oncogene-alpha, and granulocyte-colony-stimulating factor by human airway epithelial cells. Am J Respir Cell Mol Biol 2002;26:748–53. https://doi.org/10.1165/ajrcmb.26.6.4757.
[194] Henkels KM, Frondorf K, Gonzalez-Mejia ME, Doseff AL, Gomez-Cambronero J. IL-8-INDUCED NEUTROPHIL CHEMOTAXIS IS MEDIATED BY JANUS KINASE 3 (JAK3). FEBS Lett 2011;585:159–66. https://doi.org/10.1016/j.febslet.2010.11.031.
[195] Hao J, Meng L-Q, Xu P-C, Chen M, Zhao M-H. p38MAPK, ERK and PI3K Signaling Pathways Are Involved in C5a-Primed Neutrophils for ANCA-Mediated Activation. PLoS One 2012;7:e38317. https://doi.org/10.1371/journal.pone.0038317.
[196] Ogura H, Murakami M, Okuyama Y, Tsuruoka M, Kitabayashi C, Kanamoto M, et al. Interleukin-17 Promotes Autoimmunity by Triggering a Positive-Feedback Loop via Interleukin-6 Induction. Immunity 2008;29:628–36. https://doi.org/10.1016/j.immuni.2008.07.018.
[197] Gray EH, Srenathan U, Durham LE, Lalnunhlimi S, Steel KJA, Catrina A, et al. Human in vitro-induced IL-17A+ CD8+ T-cells exert pro-inflammatory effects on synovial fibroblasts. Clinical and Experimental Immunology 2023;214:103–19. https://doi.org/10.1093/cei/uxad068.
[198] Ma X, Reynolds SL, Baker BJ, Li X, Benveniste EN, Qin H. IL-17 Enhancement of the IL-6 Signaling Cascade in Astrocytes. The Journal of Immunology 2010;184:4898–906. https://doi.org/10.4049/jimmunol.1000142.
[199] Meier C, Brieger A. The role of IL-8 in cancer development and its impact on immunotherapy resistance. European Journal of Cancer 2025;218:115267. https://doi.org/10.1016/j.ejca.2025.115267.
[200] Ji M, Lu Y, Zhao C, Gao W, He F, Zhang J, et al. C5a Induces the Synthesis of IL-6 and TNF-α in Rat Glomerular Mesangial Cells through MAPK Signaling Pathways. PLOS ONE 2016;11:e0161867. https://doi.org/10.1371/journal.pone.0161867.
[201] Li XX, Gorman DM, Lee JD, Clark RJ, Woodruff TM. Unexpected Off-Target Activities for Recombinant C5a in Human Macrophages. The Journal of Immunology 2022;208:133–42. https://doi.org/10.4049/jimmunol.2100444.
[202] Karasu E, Demmelmaier J, Kellermann S, Holzmann K, Köhl J, Schmidt CQ, et al. Complement C5a Induces Pro-inflammatory Microvesicle Shedding in Severely Injured Patients. Front Immunol 2020;11. https://doi.org/10.3389/fimmu.2020.01789.
[203] Thuya WL, Cao Y, Ho PC-L, Wong AL-A, Wang L, Zhou J, et al. Insights into IL-6/JAK/STAT3 signaling in the tumor microenvironment: Implications for cancer therapy. Cytokine & Growth Factor Reviews 2025;85:26–42. https://doi.org/10.1016/j.cytogfr.2025.01.003.

[204] Johnson DE, O'Keefe RA, Grandis JR. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. Nat Rev Clin Oncol 2018;15:234–48. https://doi.org/10.1038/nrclinonc.2018.8.
[205] Heinrich PC, Behrmann I, Müller-Newen G, Schaper F, Graeve L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem J 1998;334:297–314. https://doi.org/10.1042/bj3340297.
[206] Sánchez-Tarjuelo R, Cortegano I, Manosalva J, Rodríguez M, Ruíz C, Alía M, et al. The TLR4-MyD88 Signaling Axis Regulates Lung Monocyte Differentiation Pathways in Response to Streptococcus pneumoniae. Front Immunol 2020;11. https://doi.org/10.3389/fimmu.2020.02120.
[207] Huang X, Feng Y, Xiong G, Whyte S, Duan J, Yang Y, et al. Caspase-11, a specific sensor for intracellular lipopolysaccharide recognition, mediates the non-canonical inflammatory pathway of pyroptosis. Cell & Bioscience 2019;9:31. https://doi.org/10.1186/s13578-019-0292-0.
[208] van de Veerdonk FL, Netea MG, Dinarello CA, Joosten LAB. Inflammasome activation and IL-1β and IL-18 processing during infection. Trends in Immunology 2011;32:110–6. https://doi.org/10.1016/j.it.2011.01.003.
[209] Karasawa T, Komada T, Baatarjav C, Aizawa E, Mizushina Y, Fujimura K, et al. Caspase-11 deficiency attenuates neutrophil recruitment into the atherosclerotic lesion in apolipoprotein E-deficient mice. Biochemical and Biophysical Research Communications 2023;686:149158. https://doi.org/10.1016/j.bbrc.2023.149158.
[210] Poelzl A, Lassnig C, Tangermann S, Hromadová D, Reichart U, Gawish R, et al. TYK2 licenses non-canonical inflammasome activation during endotoxemia. Cell Death Differ 2021;28:748–63. https://doi.org/10.1038/s41418-020-00621-x.
[211] Strickland I, Kisich K, Hauk PJ, Vottero A, Chrousos GP, Klemm DJ, et al. High constitutive glucocorticoid receptor beta in human neutrophils enables them to reduce their spontaneous rate of cell death in response to corticosteroids. J Exp Med 2001;193:585–93. https://doi.org/10.1084/jem.193.5.585.
[212] Webster JC, Oakley RH, Jewell CM, Cidlowski JA. Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative β isoform: A mechanism for the generation of glucocorticoid resistance. Proc Natl Acad Sci U S A 2001;98:6865–70. https://doi.org/10.1073/pnas.121455098.
[213] Vazquez-Tello A, Semlali A, Chakir J, Martin JG, Leung DY, Eidelman DH, et al. Induction of glucocorticoid receptor-beta expression in epithelial cells of asthmatic airways by T-helper type 17 cytokines. Clin Exp Allergy 2010;40:1312–22. https://doi.org/10.1111/j.1365-2222.2010.03544.x.
[214] Li X, Kim K-W, Cho M-L, Ju J-H, Kang C-M, Oh H-J, et al. IL-23 induces receptor activator of NF-κB ligand expression in fibroblast-like synoviocytes via STAT3 and NF-κB signal pathways. Immunology Letters 2010;127:100–7. https://doi.org/10.1016/j.imlet.2009.10.012.
[215] Liu T, Li S, Ying S, Tang S, Ding Y, Li Y, et al. The IL-23/IL-17 Pathway in Inflammatory Skin Diseases: From Bench to Bedside. Front Immunol 2020;11. https://doi.org/10.3389/fimmu.2020.594735.
[216] Works MG, Yin F, Yin CC, Yiu Y, Shew K, Tran T-T, et al. Inhibition of TYK2 and JAK1 Ameliorates Imiquimod-Induced Psoriasis-like Dermatitis by Inhibiting IL-22 and the IL-23/IL-17 Axis. The Journal of Immunology 2014;193:3278–87. https://doi.org/10.4049/jimmunol.1400205.

[217] Liu Y, Meng Y, Zhou C, Yan J, Guo C, Dong W. Activation of the IL-17/TRAF6/NF-κB pathway is implicated in Aβ-induced neurotoxicity. BMC Neuroscience 2023;24:14. https://doi.org/10.1186/s12868-023-00782-8.
[218] O'Connell D, Bouazza B, Kokalari B, Amrani Y, Khatib A, Ganther JD, et al. IFN-γ-induced JAK/STAT, but not NF-κB, signaling pathway is insensitive to glucocorticoid in airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 2015;309:L348-359. https://doi.org/10.1152/ajplung.00099.2015.
[219] Britt RD, Thompson MA, Sasse S, Pabelick CM, Gerber AN, Prakash YS. Th1 cytokines TNF-α and IFN-γ promote corticosteroid resistance in developing human airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 2019;316:L71–81. https://doi.org/10.1152/ajplung.00547.2017.
[220] Zegeye MM, Lindkvist M, Fälker K, Kumawat AK, Paramel G, Grenegård M, et al. Activation of the JAK/STAT3 and PI3K/AKT pathways are crucial for IL-6 trans-signaling-mediated pro-inflammatory response in human vascular endothelial cells. Cell Commun Signal 2018;16:55. https://doi.org/10.1186/s12964-018-0268-4.
[221] Ageeva T, Rizvanov A, Mukhamedshina Y. NF-κB and JAK/STAT Signaling Pathways as Crucial Regulators of Neuroinflammation and Astrocyte Modulation in Spinal Cord Injury. Cells 2024;13:581. https://doi.org/10.3390/cells13070581.
[222] Matsumoto H, Sudo R, Fujita Y, Onizawa M, Saito K, Sumichika Y, et al. Inhibition of CEACAM1 expression in cytokine-activated neutrophils using JAK inhibitors. BMC Immunology 2024;25:63. https://doi.org/10.1186/s12865-024-00656-6.
[223] Furuya MY, Asano T, Sumichika Y, Sato S, Kobayashi H, Watanabe H, et al. Tofacitinib inhibits granulocyte-macrophage colony-stimulating factor-induced NLRP3 inflammasome activation in human neutrophils. Arthritis Res Ther 2018;20:196. https://doi.org/10.1186/s13075-018-1685-x.
[224] Administration (TGA) TG. Important safety information for Janus kinase (JAK) inhibitors | Therapeutic Goods Administration (TGA) 2023. https://www.tga.gov.au/news/safety-updates/important-safety-information-janus-kinase-jak-inhibitors (accessed June 23, 2025).

Appendix

Buffer composition/preparations:

Assay buffer

90 mg Glucose

50 mg BSA

500µL HEPES

Fill up to 50 mL with PBS ($+Ca^{2+}/+Mg^{2+}$)

FACS staining buffer-

1 ml FBS (heat-inactivated)

Fill up to 50 with mL PBS ($–Ca^{2+}/–Mg^{2+}$)

FACS fix solution/ Buffer

30 mL FACS flow

4 mL cell fix

Fill up to 40 mL with Aquadest

Cell Fix-

13,51 mL Formaldehyde

500 mg N3Na

Fill up to 50 ml with PBS ($–Ca^{2+}/–Mg^{2+}$)

In vivo experiments:

BAL buffer-

29 mg EDTA

250 µL HEPES

Fill up to 100 mL PBS ($–Ca^{2+}/–Mg^{2+}$)

Staining buffer

4 % FCS

BAL buffer

Hemolysis buffer (10X stock)

9 g Ammonium Chloride

1 g KHCO3

37 mg EDTA

In 100 mL Aquadest

Hemolysis buffer (1X) (BD lysis solution)

1 mL 10X stock

9 mL Aquadest