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Review

Neutrophil Extracellular Traps in Airway Diseases: Pathological Roles and Therapeutic Implications

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Abstract: Neutrophils are important effector cells of the innate immune response that fight pathogens by phagocytosis and degranulation. Neutrophil extracellular traps (NETs) are released into the extracellular space to defend against invading pathogens. Although NETs play a defensive role against pathogens, excessive NETs can contribute to the pathogenesis of airway diseases. NETs are known to be directly cytotoxic to the lung epithelium and endothelium, highly involved in acute lung injury, and implicated in disease severity and exacerbation. This review describes the role of NET formation in airway diseases, including chronic rhinosinusitis, and suggests that targeting NETs could be a therapeutic strategy for airway diseases.

Keywords: neutrophil extracellular traps; therapeutic targets; airway diseases; asthma; chronic rhinosinusitis; cystic fibrosis; chronic obstructive pulmonary disease; bronchiectasis; coronavirus disease 2019

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1. Introduction

Neutrophils, which are the most abundant innate immune cells, have a short lifespan and serve as the first defensive response against invading pathogens [1]. Neutrophils are produced in the bone marrow, and their release is regulated by C–X–C motif chemokine ligand 8 (CXCL8)-mediated neutrophil mobilization, which allows neutrophils to arrive at sites of inflammation [2]. They are characterized by a multilobed nucleus and granular cytoplasm, which are important for host defense. In the antibacterial response, neutrophils kill pathogens by degranulation, phagocytosis, cytokine production, and neutrophil extracellular trap (NET) formation [3,4]. Numerous studies have revealed that neutrophils play a central role in chronic inflammatory conditions, such as cancer [5,6], autoimmune diseases [7–9], and airway diseases [10,11]. NETs are net-like structures that kill pathogens by forming an extracellular structure with chromatin and granule proteins, such as neutrophil elastase (NE), myeloperoxidase (MPO), and calprotectin [12,13]. NET formation, also called NETosis, is a form of neutrophil cell death distinct from apoptosis and necroptosis, and its antimicrobial activity has been described [13,14]. NETs can trap almost any type of pathogens, even those that are too large to phagocytose, and they play an important role in the host's defense against infections that evade normal neutrophil killing [15,16]. Although NETs serve antibacterial functions, they are also known to be involved in the pathogenesis of multiple diseases and in disease severity [17–20]. This review describes the roles of NETs in airway diseases and suggests that a better understanding of their role may be crucial for therapeutic treatments. The systematic review was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Figure S1).

2. Neutrophil Extracellular Traps (NETs)

During NET formation, nuclear and granular membranes dissolve, and the chromatin decondenses in the cytoplasm. Subsequently, the plasma membrane is ruptured and

releases extracellular structures with DNA and several granule proteins, which serve to trap and kill pathogens (Figure 1). NETs are considered a double-edged sword of the immune system; they can play a beneficial role in innate immunity, but they can also exert proinflammatory effects and cause tissue damage [21,22].

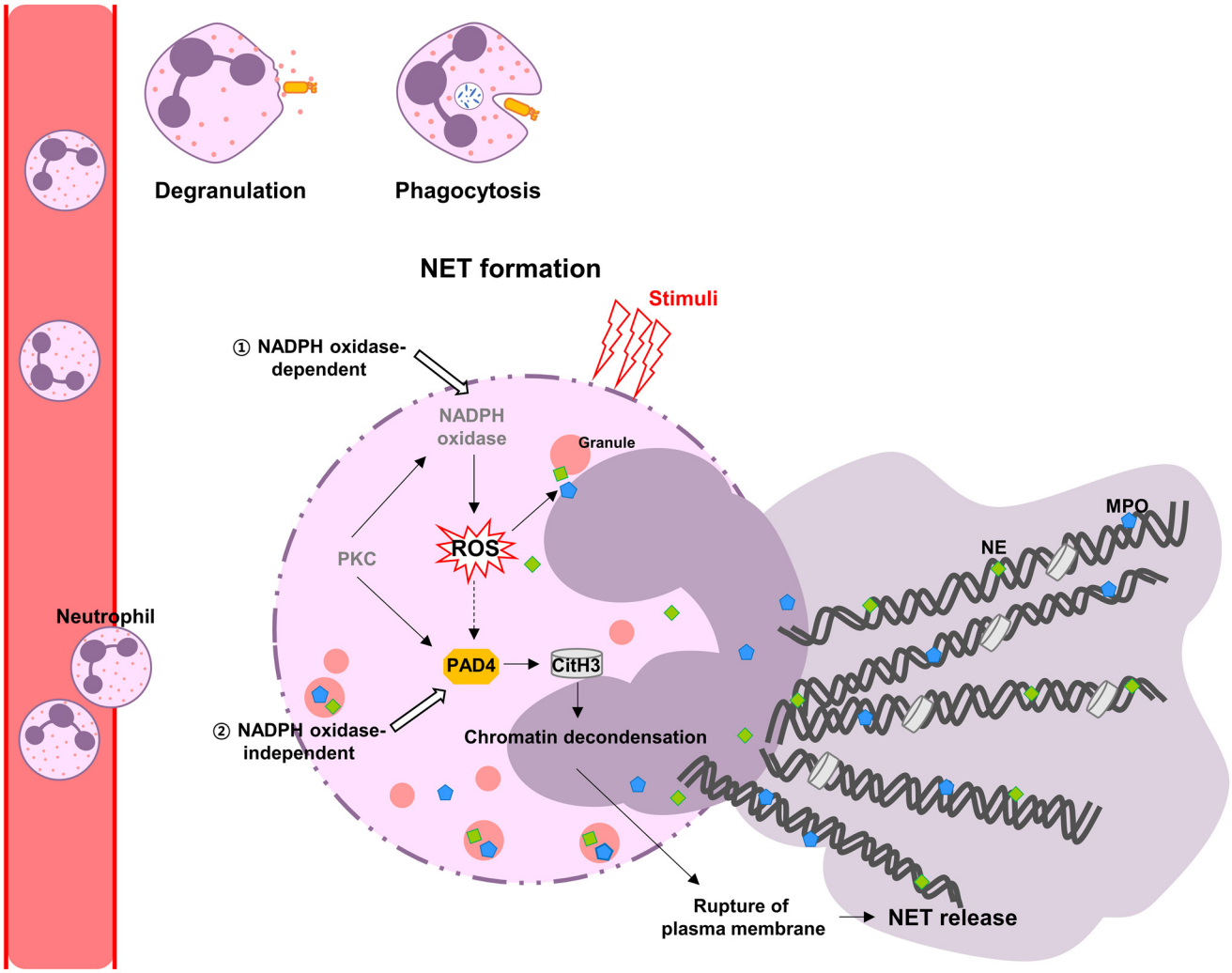

Figure 1. NADPH oxidase-dependent or -independent NET formation. Neutrophils eliminate invading pathogens through phagocytosis, degranulation, and NET formation. ① NADPH oxidase-mediated ROS stimulates MPO and NE to promote translocation from neutrophil granules into the nucleus and trigger the release of NETs. MPO binds chromatin with NE and contributes to the decondensation of chromatin, and then the nuclear membrane is disrupted. ② Activated PAD4 citrullinates histones, causing chromatin decondensation. NETs are released into the extracellular space to capture invading pathogens.

NETs are composed of extracellular DNA (eDNA) fibers with histones and granule proteins, including NE, MPO, calprotectin, and α-defensins. These components are released into the extracellular space after the cell membrane ruptures and induce the secretion of proinflammatory cytokines, which are implicated in airway neutrophilia and the airway inflammasome [23,24].

Histones are the major protein components of the nucleosome, which consists of an octamer of core histones (H2A, H2B, H3, and H4) wrapped in superhelical DNA. Histones can induce cytotoxicity through cell swelling and release of lactate dehydrogenase, cytokines, and chemokines, and may be involved in lung damage [25–27]. Some studies have reported that extracellular histones activate neutrophils to induce NET release [28,29]. NE is

a serine protease stored in the azurophilic granules of neutrophils and acts as a host defense mechanism in inflammation, the immune response, and coagulation [30]. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase generates reactive oxygen species (ROS) production. The generation of superoxide is mediated by NADPH oxidase complex and converted to hydrogen peroxide. MPO uses hydrogen peroxide as a substrate and promotes the release of NE from neutrophil granules. MPO and NE translocate from neutrophil granules into the nucleus and trigger the release of NETs [31]. MPO binds chromatin with NE and contributes to the decondensation of chromatin, and then the DNA is released into the extracellular space to capture invading pathogens [32,33]. NE produces several inflammatory chemokines, such as interleukin (IL)-8 and matrix metalloproteinase (MMP)-9 [34,35]. The inhibition of NE activity reduces tissue damage by attenuating inflammation and preventing NET formation [36]. Imbalances between NE and its inhibitors are implicated in the pathogenesis of several diseases [37,38]. MPO levels were elevated in patients with asthma and correlated with NET-derived proteins [23,39]. MPO/DNA complexes were also present at higher levels in patients with chronic inflammatory diseases and were associated with disease severity and clinical outcomes [40–42]. Calprotectin is a noncovalent heterodimer complex of calcium and zinc-binding protein of the S-100 family (S100A8 and S100A9) that is found primarily in neutrophils. Elevated calprotectin levels were found in inflammatory diseases and correlated strongly with disease severity [43,44]. α-Defensins, which are neutrophil granule proteins, are known to be neutrophil-associated antimicrobial peptides important for mucosal immune protection. Elevated levels of α-defensins and LL-37 were found in neutrophilic phenotype of airway diseases and were associated with cytotoxicity [45,46].

LL-37, the only type of cathelicidin present in humans [47], which is also an antimicrobial protein, contributes to disruption of the nuclear membrane and induces NET formation [48]. LL-37 was positively correlated with IL-8, tumor necrosis factor (TNF)-α, and the percentage of neutrophils [49]. Increased levels of LL-37 have been reported in severe disease and exacerbations [49,50]. MMPs are zinc-dependent endoproteases that are responsible for the degradation of extracellular matrix proteins, including collagen and fibronectin. MMP-8 (neutrophil collagenase) and MMP-9 (gelatinase B) are usually found in neutrophils, and their release induces NET formation [51]. MMP levels are elevated in neutrophilic airway diseases and are inversely correlated with disease severity and lung function [52–54]. Protein arginine deiminase type 4 (PAD4) is activated by protein kinase C (PKC) and translocates into the nucleus to induce histone citrullination [55,56]. It contributes to chromatin decondensation, leading to NET formation [57]. Excessive PAD4 activity has been related to airway disease. PAD4 inhibition or deficiency reduces citrullinated histones and NET-induced lung injury [58–60].

NETs are triggered by various stimuli, such as pathogens, proinflammatory cytokines, antibodies, and chemical stimuli, such as phorbol myristate acetate (PMA) [61–63]. Lipopolysaccharide (LPS) is an important outer membrane component of Gram-negative bacteria. LPS triggers inflammatory responses through Toll-like receptor 4 (TLR4), activates neutrophils, and promotes NET formation [13,64]. PMA is used to trigger NET formation. PMA activates PKC, which in turn activates NADPH oxidase and induces ROS production and calcium flux within the cell, leading to NET formation [65,66]. IL-8, also known as CXCL8, is a proinflammatory chemokine that attracts and activates neutrophils and triggers NET formation [13]. IL-8 is expressed in a variety of cell types, including neutrophils. Elevated IL-8 levels have been found in various airway diseases [67–69] and cause tissue damage and lung dysfunction [70].

Autophagy is primarily considered as a cell survival process to limit cellular damage. However, increased neutrophil autophagy and NET formation were associated with asthma severity and airway epithelial damage [21]. Qu et al. demonstrated that NET-treated alveolar epithelial cells induced in abnormal autophagy resulted in cellular damage [71]. A recent study reported that NETs translocate from the liver to the lungs by blood and promote acute lung injury [72]. NETs have been widely reported to induce chronic inflammation

and to be involved in disease pathogenesis. NETs appear to be a potential biomarker for patients with airway diseases, but more research is needed to understand this phenomenon.

3. NETs in Airway Diseases

Investigators have described mechanisms of NET formation, as well as the role of NETs in several diseases, such as chronic inflammatory diseases, autoimmune diseases, and metabolic disorders. Although NETs play a role in host defense, NETs induce direct cytotoxic effects on the lung epithelium and endothelium [73]. NET formation also causes many indirect complications, such as airway obstruction of the respiratory system. Excessive NETs are associated with numerous chronic inflammatory diseases, such as asthma [42,74,75], chronic obstructive pulmonary disease (COPD) [74–76], and cystic fibrosis (CF) [74,75,77] (Figure 2).

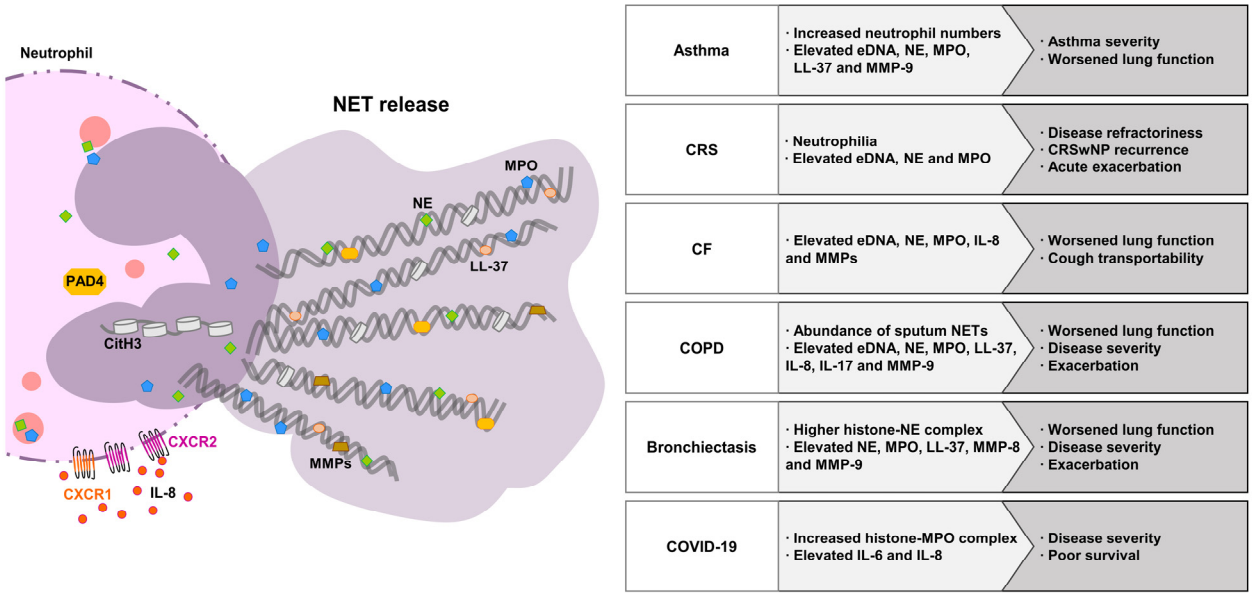

Figure 2. Clinical implications for neutrophil extracellular trap (NET) components in airway diseases. Elevated NET components have been implicated in the pathogenesis of airway diseases. NET: neutrophil extracellular trap; eDNA: extracellular DNA; NE: neutrophil elastase; MPO: myeloperoxidase; PAD4: protein arginine deiminase 4; CitH3: citrullination of histone H3; IL: interleukin; MMP: matrix metalloproteinase; CXCR: C–X–C motif chemokine receptor; CRS: chronic rhinosinusitis; CF: cystic fibrosis; COPD: chronic obstructive pulmonary disease; COVID-19: coronavirus disease 2019.

3.1. Asthma

Asthma is a heterogeneous chronic inflammatory disease of the airways characterized by airway hyper-responsiveness, airflow obstruction, and airway remodeling. There are several subtypes of asthma, with different endotypes and phenotypes [78]. Asthma patients are divided into four inflammatory phenotypes according to the cellular composition of the sputum: eosinophilic, neutrophilic, paucigranulocytic, or mixed granulocytic. Adult asthma is primarily associated with type 2 inflammation, characterized by the expression of type 2 cytokines (IL-4, IL-5, and IL-13), eosinophilic airway infiltration, and mucus secretion [79]. On the other hand, type 2-low asthma is characterized by neutrophilic or paucigranulocytic inflammation and is associated with severity and corticosteroid resistance [80–82]. NETs are known to be beneficial for host defense, but they damage airway epithelial cells and induce autoantigen production in airway epithelial cells [21,83]. Several studies have demonstrated that NET levels were elevated in patients with severe asthma [23,42,45]. IL-8 is a potent NET inducer and has been found to be elevated in severe asthma [84–86]. Abnormally high levels of neutrophils, NE, and IL-8 were observed in patients with neutrophilic asthma [87]. Pham et al. reported that IL-8 induced neutrophil

autophagy and NET production in patients with severe asthma [21]. Neutrophil-derived MMP-9 is implicated in asthma severity [88]. Elevated levels of NET components, such as eDNA, LL-37, α-defensins, and NE, were found in patients with neutrophilic asthma and associated with worsened lung function and asthma severity [45]. eDNA was associated with elevated expression of IL-8 and IL-1β, as well as increased caspase-1 activity [45]. This may suggest that NETs can activate the inflammasome to trigger the secretion of IL-1β [23,89,90]. Increased calprotectin levels were detected in the serum of asthma patients and correlated with the neutrophil proportion and lung function decline [91]. Neutrophil cytoplasts, which are enucleated cell bodies released during NETosis, correlated positively with IL-17 levels and were detected in severe asthma with neutrophil inflammation [24]. In a neutrophilic asthma animal model, NETs caused severe airway inflammation, and inhibition of NET production reduced airway inflammation and hyperresponsiveness [92]. This evidence proves that NETs contribute to the pathophysiology of asthma; therefore, it is necessary to understand the various mechanisms of NET formation.

3.2. Chronic Rhinosinusitis (CRS)

CRS is a heterogeneous inflammatory disease characterized by persistent symptomatic inflammation of the nose and paranasal sinuses [93], caused by multifactorial factors [94,95]. CRS is classified into CRS with nasal polyps (CRSwNP) and without nasal polyps (CRSsNP) according to the phenotype, and type 2 and non-type 2 according to the endotype [96]. Type 2 inflammation with eosinophils is predominant in Western countries, whereas non-type 2 inflammation with neutrophils is found in Asian countries [97,98]. Neutrophil infiltration has been found in the sinus mucosa of CRS patients, suggesting that neutrophils may play an important role in the pathologic process of CRS [99]. In addition, neutrophilic inflammation is associated with corticosteroid resistance in CRSwNP [100,101], which may be accounted for by the IL-36γ/IL-36R pathway [101]. Mucus levels of IL-1β, IL-6, IL-8, and TNF-α were significantly higher in elderly (≥60 years) CRS patients and were positively correlated with neutrophilia [102]. It has been suggested that elderly CRS patients tend to have neutrophilic inflammation and may be less responsive to corticosteroids. Kim et al. demonstrated that tissue neutrophilia is a risk factor for refractory CRSwNP in an Asian population and found that elevated expression of NE and IL-36α was highly associated with refractoriness [103]. Furthermore, tissue neutrophil and NET expression were correlated with refractoriness in non-eosinophilic or neutrophilic CRSwNP [104]. NETs were present in the sinus mucosa and subepithelial layer, and were significantly higher in CRSwNP [46]. Neutrophilic granule proteins, such as MPO and NE, as well as antimicrobial proteins such as LL-37, showed significantly increased expression in CRSwNP patients [46,103,105]. MPO was significantly higher in tissues from patients with recurrent CRSwNP [105]. In addition, eDNA and NET-forming neutrophils were elevated in CRS regardless of the presence of nasal polyps; interestingly, their levels were significantly higher in nasal secretions from patients with exacerbated CRSwNP or exacerbated CRSsNP [106]. Calprotectin (S100A8/A9) levels were significantly higher in NP tissue from CRSwNP patients than in controls and were highly correlated with tissue IL-8 and MPO levels both in controls and CRSwNP patients [107]. Interestingly, Delemarre et al. reported that the number of neutrophils, the expression of MPO, IL-6, and IL-8, and the activity of cathepsin G/NE were elevated in patients with type 2 CRSwNP. They also observed the coexistence of eosinophilic and neutrophilic inflammation in severe type 2 CRSwNP [108]. Cluster analysis showed high expression levels of neutrophil-associated MPO, IL-8, IL-6, IL-1β, MMP-8, and MMP-9 in CRS with low type 2 inflammation [109]. NETs were also found to be abundant in the NP tissues of CRS patients with low type 2 inflammation, and eosinophil extracellular traps (EETs) were rarely observed. In cases of high type 2 inflammation with the highest levels of IL-5, eosinophil cationic protein, and total immunoglobulin E, eosinophil extracellular traps were predominant, and few NETs were observed [109]. Several reports have been published on NET formation in CRS, but there are fewer studies than on other diseases; thus, further research is needed.

3.3. Cystic Fibrosis (CF)

CF is an inherited chronic inflammatory disease characterized by neutrophilic-dominant inflammation. Free DNA and neutrophil counts were significantly higher in the bronchoalveolar lavage fluid (BALF) of CF patients compared to healthy controls [110], and other studies have suggested that this DNA is derived from NETs [111,112]. Elevated eDNA levels were found in the sputum and BALF from CF patients and were associated with worsened lung function [111,113,114]. Several studies have demonstrated that NET formation was increased in airway samples from CF patients and was associated with worsened lung function [77]. NET components, including NE and MPO, were present in the sputum of CF patients [113,115]. NE is a risk factor for severity and directly induces structural lung damage in CF patients [115,116]. MPO is associated with worse clinical outcomes and lung function decline in CF [117]. MMP-8 and MMP-9 levels were elevated in the BALF of CF patients [118] and may contribute to the progression of CF [119]. Another component of NETs, calprotectin (S100A8/A9), is negatively correlated with severity of obstruction, as measured by parameters such as the forced expiratory volume in 1 s (FEV1) [120,121]. Autoantibodies against bactericidal permeability-increasing protein (BPI), which is stored in azurophilic granules of neutrophils, are found in CF patients, associated with NETs and worsened lung function [122]. This may be further evidence that NETs are involved in autoimmunity in CF. Delayed neutrophil apoptosis led to a longer neutrophil lifespan and increased NET production [123]. Recent studies have suggested that NETs are a major factor in lung inflammation and damage in CF. Therefore, it is necessary to develop NET-targeted therapies that yield positive effects, while minimizing side-effects such as cytotoxicity.

3.4. Chronic Obstructive Pulmonary Disease (COPD)

Chronic obstructive pulmonary disease (COPD) is a progressive pulmonary disease that is characterized by progressive inflammation and airflow limitation. Neutrophilic inflammation is a hallmark of COPD after long-term exposure to external stresses, such as cigarette smoke, viruses, and oxidative stress [124,125]. Several studies have highlighted that airway neutrophil levels are associated with disease severity and exacerbations, as well as corticosteroid resistance in COPD [126–128]. Neutrophils are a major source of IL-17, and sputum IL-17 levels were associated with airflow limitation and obstruction in COPD [129,130]. NETs have also been found in sputum from both stable-state and exacerbated COPD patients [131–133], and their concentration was associated with disease severity in patients with COPD [19]. The expression of sputum IL-8, MMP-9, and NE showed positive correlations with the neutrophil proportion, while lung function showed a negative correlation [128,134,135]. NET components, including eDNA, LL-37, α-defensins, NE, and MPO were also significantly higher in neutrophilic COPD patients and were associated with disease severity [45,131,136]. Additionally, *PAD4* gene expression was significantly higher in neutrophils than in non-neutrophils in COPD patients [45]. This evidence could explain the increased levels of NETs and greater disease severity in the airways of patients with COPD.

3.5. Bronchiectasis

Bronchiectasis is a chronic inflammatory disease characterized by mild to moderate airflow obstruction. The pathophysiological hallmark of bronchiectasis is neutrophilic inflammation, which is related to airway infection, lung damage, and impaired mucociliary clearance [137,138]. Neutrophil counts were significantly higher in patients with bronchiectasis than in healthy controls and were positively correlated with the severity of bronchiectasis [139,140]. Neutrophil-derived proteins, including NE, MPO, and MMPs, were also increased in patients with bronchiectasis [54,140]. As a predictor of disease progression, NE showed greater activity in the sputum of patients with bronchiectasis and was associated with exacerbation frequency and decreased FEV1 [141–143]. Additionally, increased levels of antimicrobial peptide LL-37 and decreased levels of secretory leucocyte

protease inhibitor (SLPI) were associated with decreased FEV1 and exacerbation, indicating that the dysregulation of antimicrobial peptides is associated with disease severity [144]. IL-6 and IL-8 levels were significantly increased in the sputum and BALF of bronchiectasis patients, and neutrophil influx was induced [140,143]. MMP-8 and MMP-9 levels were also significantly higher in patients with bronchiectasis than in healthy controls and were strongly correlated with the progression of bronchiectasis [54,145]. Using sputum proteomics, Keir et al. demonstrated that differentially expressed proteins were associated with components of NETs, including NE, MPO, and neutrophil gelatinase-associated lipocalin (NGAL), and increased amounts of NETs were associated with bronchiectasis severity [20]. These studies demonstrated that NETs contribute to disease severity and lung inflammation.

3.6. Coronavirus Disease 2019 (COVID-19)

Coronavirus disease 2019 (COVID-19) is a multisystem inflammatory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), characterized by distinct patterns of disease progression that suggest diverse host immune responses. An uncontrolled inflammatory response can lead to cytokine storm syndrome and cause severe COVID-19 [146]. Proinflammatory cytokine levels were significantly higher in patients with severe COVID-19 [147,148]. Elevated neutrophils and a neutrophil-to-lymphocyte ratio were observed in patients with COVID-19 compared to healthy controls, predicting disease severity and poor clinical outcomes [148–150]. In addition, neutrophils from COVID patients exhibited a larger size and lower granularity than healthy cells [151], and low-density neutrophils were correlated with disease severity [147]. Several studies have reported that NETs were elevated in patients with COVID-19 and were correlated with disease severity [40,152]. In vitro studies have also demonstrated that SARS-CoV-2 induces the release of NETs [152]. NE and MPO levels were higher in the serum of COVID-19 patients [151,153]. eDNA, MPO-DNA, and CitH3 showed significant increases in sera from COVID-19 patients [153]. Serum IL-6, IL-8, and TNF-α levels were also significantly increased in COVID-19 and were associated with severity and poor survival [154].

4. Potential Therapeutic Targets of NETs

Therapeutic approaches such as inhibitors of NET formation can help attenuate chronic airway inflammation and reduce the severity of airway diseases, including CRS and asthma (Table 1).

4.1. DNase

NETs are degraded by DNase or a DNase-like enzyme targeting eDNA or histones [155,156]. Elevated eDNA has been associated with disease severity in chronic diseases and has become a therapeutic target. DNase I has been approved by the US Food and Drug Administration (FDA) and is commonly used for CF. Recombinant DNase reduces mucus viscosity and improves mucociliary function. DNase treatment improves lung function and reduces exacerbation in airway diseases, such as CF [157,158] and asthma [23,159]. DNase I significantly reduced the expression of MPO and CitH3, and alleviated airway hyper-responsiveness and airway mucus obstruction in a neutrophilic asthma model [92]. Lachowicz-Scroggins et al. demonstrated that NETs damage airway epithelial cells, which can be prevented by disrupting NETs with DNase [23]. DNase also reduced the activity of MPO and NE in CF [113] and COVID-19 patients [160,161]. However, in bronchiectasis studies, it had significant negative effects on patients [162].

4.2. Neutrophil Protease Inhibitors

As mentioned for NET formation, NE and MPO are involved in the decondensation of chromatin. Therefore, their inhibition could inhibit NET formation. Endogenous NE inhibitors, such as SERPINA1 (α-1 antitrypsin; AAT) and SLPI, mobilized NE activity. A mouse model with NE deficiency showed significantly reduced mucus hypersecretion

and lung damage [163]. AAT deficiency is associated with the prevalence of airway diseases, such as bronchiectasis [164] and CF [165]. SLPI is produced by airway epithelial cells and neutrophils and acts to maintain the protease/antiprotease balance, preventing protease-mediated tissue destruction. SLPI reduces NE activity and IL-8 expression in CF patients [166]. Studies found reduced SLPI levels in patients with exacerbated COPD [167] and in patients with severe asthma [168]. However, levels of the protease inhibitors TIMP-1, SLPI, and elafin were higher in severe COVID-19 patients than in influenza patients [169]. A selective NE inhibitor, GW311616A, prevented ROS production and NET formation and ameliorated lung function in a COPD model both in vitro and in vivo [170]. AZD9668, an oral NE inhibitor, has been tried in COPD [171], CF [172], and bronchiectasis [173]. AZD9668 treatment resulted in decreases in inflammatory markers, such as IL-6 and IL-8, but there was no significant change in NE activity and lung function in bronchiectasis [173] and CF [172]. In clinical trials of COPD patients, there were no significant differences in inflammatory markers, lung function, and patient symptoms [171,174]. An MPO inhibitor, aminobenzoic acid hydrazide (ABAH), delayed NET formation [175]. However, ABAH did not completely inhibit MPO. ABAH reduced airway hyper-responsiveness and oxidative stress in an asthma mouse model with mixed inflammation, but did not inhibit neutrophil and eosinophil infiltration [176]. MPO inhibitors, PF-1355 and AZM198, have been shown to attenuate neutrophil degranulation and NET formation by inhibiting MPO activity in vasculitis [177,178]. Various MPO inhibitors, such as a ferulic acid derivative (INV-315), a thiouracil derivative (OF-06282999), and triazolopyrimidine, are being developed and investigated [179]. As mentioned, although the clinical potential of NE and MPO inhibitors has been investigated, there are limitations such as poor preclinical results, uncertainty regarding the therapeutically effective dose, and potential toxicity. Therefore, more research on various diseases, including airway diseases, is needed.

4.3. CXCR2 Antagonists

The chemokine receptor CXCR2 mediates neutrophil migration and induces NET formation through IL-8. AZD5069 is an antagonist of CXCR2, and Pedersen et al. found that it inhibited NET formation in sputum and blood neutrophils from COPD patients [180,181]. AZD5069 has been tried in bronchiectasis [182] and asthma [181,183,184]. In these clinical studies, neutrophil counts were reduced, but AZD5069 treatment did not improve clinical outcomes. In a clinical study of CF with the selective CXCR2 antagonist SB-656933, neutrophils, NE, and free DNA levels were reduced, while IL-8 and fibrinogen levels were increased. However, there were no changes in lung function and symptoms, and side-effects occurred in some patients [185]. Danirixin demonstrated antagonism of CXCR2 activity and blockade of mediated neutrophil activation both in vitro and in vivo [186]. Clinical trials for COPD have shown no clinical benefits for patients [187,188]. Reparixin, an IL-8 receptor (CXCR1/2) blocker, inhibits CXCL8-induced neutrophil activation. Reparixin treatment significantly reduced neutrophil recruitment in a mouse model of LPS-induced pulmonary inflammation [189]. Neutralizing IL-8 using reparixin and an anti–IL-8 antibody reduced NET formation in the plasma of COVID-19 patients [67].

4.4. ROS Scavengers

ROS production is an important inducer of NET formation. Diphenyleneiodonium chloride (DPI) inhibits gluconeogenesis and oxidative stress by inhibiting NADPH oxidase. DPI also inhibits eDNA release and NET formation [190–192]. Nevertheless, N-acetyl-L-cysteine (NAC) and DPI decrease inflammatory cytokine and ROS production, thereby inhibiting EET formation and improving lung function in asthma [193,194]. NAC, an antioxidant, decreases mucous viscosity and indirectly inhibits NET formation. NAC treatment reduced PMA-induced NETs, but H_2O_2- and *Staphylococcus aureus*-induced NETs did not change [195]. NAC reduced the risk of acute exacerbations in patients with COPD [196,197]. In patients with bronchiectasis, NAC treatment was also associated with

a reduced incidence of exacerbations and sputum production, with no severe adverse effects [198].

4.5. Other Inhibitors

Inhibition of PAD4 prevents NET formation by reducing histone citrullination [199–201]. Cl-amidine, a PAD4 inhibitor, reduced the neutrophil count, decreased levels of citrullinated histone H3 (CitH3) and inflammatory cytokines, and inhibited NET formation and tissue damage [200–202]. This agent also inhibited EET formation [203,204]. Streptonigrin is also a selective inhibitor of PAD4 that led to decreased expression of CitH3 and proinflammatory cytokines [205]. A novel small-molecule PAD4 inhibitor (PAD4i) developed by AstraZeneca reduced eDNA, CitH3, and NET formation in *Haemophilus influenzae*-infected or ionomycin/calcium chloride-stimulated neutrophils [206].

Hydroxychloroquine (HCQ), an FDA-approved antimalarial drug, is a less potent derivative of chloroquine that inhibits activated neutrophils and NET formation by attenuating Toll-like receptor-9 (TLR-9) and ROS production [207]. It has also been reported that HCQ reduced the levels of proinflammatory cytokines [208], but did not affect the expression of NE, PAD4, and MPO [209]. Several studies have proven that HCQ attenuated NET formation in patients with systemic lupus erythematosus [210] and COVID-19 [211].

Metformin is one of the most widely prescribed antidiabetic drugs. Metformin activates AMP-activated protein kinase (AMPK) and inhibits the mTOR pathway. Metformin reduced neutrophil counts and NET formation in several diseases [212–214]. Metformin reduced the expression of NET components, such as NE, histones, and eDNA, as well as prevented PMA- and ionomycin-induced NET formation through inhibition of NADPH oxidase [214]. Several studies have shown that metformin reduced markers of inflammation and may be associated with attenuation of mortality and poor outcomes from COVID-19 [215,216]. However, Oh et al. reported that metformin reduced the risk of COVID-19 in patients with type 2 diabetes mellitus, but did not affect mortality [217].

In addition, there are many studies on various phytochemicals that inhibit NET formation. *Epilobium pyrricholophum* extract [218] and *Artemisia gmelinii* [219] extract significantly attenuated inflammatory cell recruitment, including neutrophil, and reduced IL6 and IL8 expression in a COPD mouse model. Moreover, *Artemisia gmelinii* extract also protected lung tissues from injury [219]. Monomeric epigallocatechin-3-gallate, a component of green tea, inhibited NE activity and reduced NET formation and tissue damage in vivo [220]. Gingerols [221] and zingerone [222], compounds derived from ginger, attenuates NET formation and ROS production. However, more research is needed to prove the therapeutic effects of phytochemicals in airway diseases.

Table 1. An overview of potential targets of neutrophil extracellular traps (NETs) in airway diseases.

Target Molecule	Inhibitor(s)	Clinical Trial	Disease(s)	Reference(s)
DNA	DNase I	FDA approved	Asthma CF Bronchiectasis COVID-19	[23,92,159] [113,157,158] [162] [160,161]
Neutrophil elastase (NE)	AZD9668	Phase IIa Phase IIb Phase II	CF COPD Bronchiectasis	[172] [171,174] [173]
	GW311616A	NA	COPD	[170]
Myeloperoxidase (MPO)	ABAH	NA	Asthma	[176]
CXCR2	AZD5069	Phase IIb Phase IIa [223] Phase IIa	Asthma COPD Bronchiectasis	[183,184] [180] [182]
	Danirixin	Phase IIb	COPD	[187,188]

Table 1. *Cont.*

Target Molecule	Inhibitor(s)	Clinical Trial	Disease(s)	Reference(s)
CXCR1/2	Reparixin	Phase II/III (COVID-19 pneumonia) [224]	COVID-19	[67]
Reactive oxygen species (ROS)	N-acetyl-L-cysteine (NAC)	FDA approved	COPD Bronchiectasis	[196,197] [198]
Multiple pathways	Hydroxychloroquine (HCQ)	FDA approved	COVID-19	[211]
	Metformin	FDA approved	COVID-19	[215–217]

5. Conclusions

Neutrophils are an essential component of the innate immune system and play an important role in airway diseases including CRS. In addition, neutrophils and NETs are involved in the progression and severity of several airway diseases. This review focused on understanding the mechanisms underlying NET formation and suggesting NET inhibitors. DNase, NE inhibitors, and ROS scavengers have been proposed as therapeutic approaches to inhibit NET formation, but limitations such as therapeutic efficacy or toxicity in preclinical or clinical stages remain to be resolved. Although many studies have been conducted on the pathophysiological relevance of NET formation, further research is still needed to investigate novel treatment strategies for airway diseases.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms24055034/s1. Reference [225] is cited in the supplementary materials.

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References

1. Hidalgo, A.; Chilvers, E.R.; Summers, C.; Koenderman, L. The Neutrophil Life Cycle. *Trends Immunol.* **2019**, *40*, 584–597. [CrossRef] [PubMed]
2. Cambier, S.; Gouwy, M.; Proost, P. The chemokines CXCL8 and CXCL12: Molecular and functional properties, role in disease and efforts towards pharmacological intervention. *Cell Mol. Immunol.* **2023**, *20*, 217–251. [CrossRef] [PubMed]
3. Mayadas, T.N.; Cullere, X.; Lowell, C.A. The multifaceted functions of neutrophils. *Annu. Rev. Pathol.* **2014**, *9*, 181–218. [CrossRef]
4. Liew, P.X.; Kubes, P. The Neutrophil's Role During Health and Disease. *Physiol. Rev.* **2019**, *99*, 1223–1248. [CrossRef]
5. Mollinedo, F. Neutrophil Degranulation, Plasticity, and Cancer Metastasis. *Trends Immunol.* **2019**, *40*, 228–242. [CrossRef] [PubMed]
6. Brostjan, C.; Oehler, R. The role of neutrophil death in chronic inflammation and cancer. *Cell Death Discov.* **2020**, *6*, 26. [CrossRef] [PubMed]
7. Glennon-Alty, L.; Hackett, A.P.; Chapman, E.A.; Wright, H.L. Neutrophils and redox stress in the pathogenesis of autoimmune disease. *Free Radic. Biol. Med.* **2018**, *125*, 25–35. [CrossRef] [PubMed]
8. Carmona-Rivera, C.; Carlucci, P.M.; Moore, E.; Lingampalli, N.; Uchtenhagen, H.; James, E.; Liu, Y.; Bicker, K.L.; Wahamaa, H.; Hoffmann, V.; et al. Synovial fibroblast-neutrophil interactions promote pathogenic adaptive immunity in rheumatoid arthritis. *Sci. Immunol.* **2017**, *2*, aag3358. [CrossRef]
9. Wigerblad, G.; Kaplan, M.J. Neutrophil extracellular traps in systemic autoimmune and autoinflammatory diseases. *Nat. Rev. Immunol.* **2022**, 1–15. [CrossRef]

10. Barnes, P.J. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. *J. Allergy Clin. Immunol.* **2016**, *138*, 16–27. [CrossRef]
11. Crisford, H.; Sapey, E.; Rogers, G.B.; Taylor, S.; Nagakumar, P.; Lokwani, R.; Simpson, J.L. Neutrophils in asthma: The good, the bad and the bacteria. *Thorax* **2021**, *76*, 835–844. [CrossRef] [PubMed]
12. Kaplan, M.J.; Radic, M. Neutrophil extracellular traps: Double-edged swords of innate immunity. *J. Immunol.* **2012**, *189*, 2689–2695. [CrossRef] [PubMed]
13. Brinkmann, V.; Reichard, U.; Goosmann, C.; Fauler, B.; Uhlemann, Y.; Weiss, D.S.; Weinrauch, Y.; Zychlinsky, A. Neutrophil extracellular traps kill bacteria. *Science* **2004**, *303*, 1532–1535. [CrossRef]
14. Steinberg, B.E.; Grinstein, S. Unconventional roles of the NADPH oxidase: Signaling, ion homeostasis, and cell death. *Sci. STKE* **2007**, *2007*, pe11. [CrossRef]
15. Lu, T.; Kobayashi, S.D.; Quinn, M.T.; Deleo, F.R. A NET Outcome. *Front. Immunol.* **2012**, *3*, 365. [CrossRef] [PubMed]
16. Branzk, N.; Lubojemska, A.; Hardison, S.E.; Wang, Q.; Gutierrez, M.G.; Brown, G.D.; Papayannopoulos, V. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. *Nat. Immunol.* **2014**, *15*, 1017–1025. [CrossRef]
17. Lood, C.; Blanco, L.P.; Purmalek, M.M.; Carmona-Rivera, C.; De Ravin, S.S.; Smith, C.K.; Malech, H.L.; Ledbetter, J.A.; Elkon, K.B.; Kaplan, M.J. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. *Nat. Med.* **2016**, *22*, 146–153. [CrossRef]
18. Khandpur, R.; Carmona-Rivera, C.; Vivekanandan-Giri, A.; Gizinski, A.; Yalavarthi, S.; Knight, J.S.; Friday, S.; Li, S.; Patel, R.M.; Subramanian, V.; et al. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Sci. Transl. Med.* **2013**, *5*, 178ra40. [CrossRef]
19. Dicker, A.J.; Crichton, M.L.; Pumphrey, E.G.; Cassidy, A.J.; Suarez-Cuartin, G.; Sibila, O.; Furrie, E.; Fong, C.J.; Ibrahim, W.; Brady, G.; et al. Neutrophil extracellular traps are associated with disease severity and microbiota diversity in patients with chronic obstructive pulmonary disease. *J. Allergy Clin. Immunol.* **2018**, *141*, 117–127. [CrossRef]
20. Keir, H.R.; Shoemark, A.; Dicker, A.J.; Perea, L.; Pollock, J.; Giam, Y.H.; Suarez-Cuartin, G.; Crichton, M.L.; Lonergan, M.; Oriano, M.; et al. Neutrophil extracellular traps, disease severity, and antibiotic response in bronchiectasis: An international, observational, multicohort study. *Lancet Respir. Med.* **2021**, *9*, 873–884. [CrossRef]
21. Pham, D.L.; Ban, G.Y.; Kim, S.H.; Shin, Y.S.; Ye, Y.M.; Chwae, Y.J.; Park, H.S. Neutrophil autophagy and extracellular DNA traps contribute to airway inflammation in severe asthma. *Clin. Exp. Allergy* **2017**, *47*, 57–70. [CrossRef]
22. Papayannopoulos, V. Neutrophil extracellular traps in immunity and disease. *Nat. Rev. Immunol.* **2018**, *18*, 134–147. [CrossRef] [PubMed]
23. Lachowicz-Scroggins, M.E.; Dunican, E.M.; Charbit, A.R.; Raymond, W.; Looney, M.R.; Peters, M.C.; Gordon, E.D.; Woodruff, P.G.; Lefrancais, E.; Phillips, B.R.; et al. Extracellular DNA, Neutrophil Extracellular Traps, and Inflammasome Activation in Severe Asthma. *Am. J. Respir. Crit. Care Med.* **2019**, *199*, 1076–1085. [CrossRef]
24. Krishnamoorthy, N.; Douda, D.N.; Bruggemann, T.R.; Ricklefs, I.; Duvall, M.G.; Abdulnour, R.E.; Martinod, K.; Tavares, L.; Wang, X.; Cernadas, M.; et al. Neutrophil cytoplasts induce T(H)17 differentiation and skew inflammation toward neutrophilia in severe asthma. *Sci. Immunol.* **2018**, *3*, aao4747. [CrossRef]
25. Fattahi, F.; Grailer, J.J.; Lu, H.; Dick, R.S.; Parlett, M.; Zetoune, F.S.; Nunez, G.; Ward, P.A. Selective Biological Responses of Phagocytes and Lungs to Purified Histones. *J. Innate Immun.* **2017**, *9*, 300–317. [CrossRef]
26. Abrams, S.T.; Zhang, N.; Manson, J.; Liu, T.; Dart, C.; Baluwa, F.; Wang, S.S.; Brohi, K.; Kipar, A.; Yu, W.; et al. Circulating histones are mediators of trauma-associated lung injury. *Am. J. Respir. Crit. Care Med.* **2013**, *187*, 160–169. [CrossRef] [PubMed]
27. Bosmann, M.; Grailer, J.J.; Ruemmler, R.; Russkamp, N.F.; Zetoune, F.S.; Sarma, J.V.; Standiford, T.J.; Ward, P.A. Extracellular histones are essential effectors of C5aR- and C5L2-mediated tissue damage and inflammation in acute lung injury. *FASEB J.* **2013**, *27*, 5010–5021. [CrossRef]
28. Shrestha, B.; Ito, T.; Kakuuchi, M.; Totoki, T.; Nagasato, T.; Yamamoto, M.; Maruyama, I. Recombinant Thrombomodulin Suppresses Histone-Induced Neutrophil Extracellular Trap Formation. *Front. Immunol.* **2019**, *10*, 2535. [CrossRef]
29. Huang, H.; Tohme, S.; Al-Khafaji, A.B.; Tai, S.; Loughran, P.; Chen, L.; Wang, S.; Kim, J.; Billiar, T.; Wang, Y.; et al. Damage-associated molecular pattern-activated neutrophil extracellular trap exacerbates sterile inflammatory liver injury. *Hepatology* **2015**, *62*, 600–614. [CrossRef]
30. Massberg, S.; Grahl, L.; von Bruehl, M.L.; Manukyan, D.; Pfeiler, S.; Goosmann, C.; Brinkmann, V.; Lorenz, M.; Bidzhekov, K.; Khandagale, A.B.; et al. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat. Med.* **2010**, *16*, 887–896. [CrossRef] [PubMed]
31. Bjornsdottir, H.; Welin, A.; Michaelsson, E.; Osla, V.; Berg, S.; Christenson, K.; Sundqvist, M.; Dahlgren, C.; Karlsson, A.; Bylund, J. Neutrophil NET formation is regulated from the inside by myeloperoxidase-processed reactive oxygen species. *Free Radic. Biol. Med.* **2015**, *89*, 1024–1035. [CrossRef]
32. Metzler, K.D.; Goosmann, C.; Lubojemska, A.; Zychlinsky, A.; Papayannopoulos, V. A myeloperoxidase-containing complex regulates neutrophil elastase release and actin dynamics during NETosis. *Cell Rep.* **2014**, *8*, 883–896. [CrossRef] [PubMed]
33. Papayannopoulos, V.; Metzler, K.D.; Hakkim, A.; Zychlinsky, A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J. Cell Biol.* **2010**, *191*, 677–691. [CrossRef]

34. Devaney, J.M.; Greene, C.M.; Taggart, C.C.; Carroll, T.P.; O'Neill, S.J.; McElvaney, N.G. Neutrophil elastase up-regulates interleukin-8 via toll-like receptor 4. *FEBS Lett.* **2003**, *544*, 129–132. [CrossRef]
35. Pham, C.T. Neutrophil serine proteases: Specific regulators of inflammation. *Nat. Rev. Immunol.* **2006**, *6*, 541–550. [CrossRef] [PubMed]
36. Okeke, E.B.; Louttit, C.; Fry, C.; Najafabadi, A.H.; Han, K.; Nemzek, J.; Moon, J.J. Inhibition of neutrophil elastase prevents neutrophil extracellular trap formation and rescues mice from endotoxic shock. *Biomaterials* **2020**, *238*, 119836. [CrossRef]
37. Alam, S.; Li, Z.; Janciauskiene, S.; Mahadeva, R. Oxidation of Z alpha1-antitrypsin by cigarette smoke induces polymerization: A novel mechanism of early-onset emphysema. *Am. J. Respir. Cell Mol. Biol.* **2011**, *45*, 261–269. [CrossRef] [PubMed]
38. Dunlea, D.M.; Fee, L.T.; McEnery, T.; McElvaney, N.G.; Reeves, E.P. The impact of alpha-1 antitrypsin augmentation therapy on neutrophil-driven respiratory disease in deficient individuals. *J. Inflamm. Res.* **2018**, *11*, 123–134. [CrossRef]
39. Varricchi, G.; Modestino, L.; Poto, R.; Cristinziano, L.; Gentile, L.; Postiglione, L.; Spadaro, G.; Galdiero, M.R. Neutrophil extracellular traps and neutrophil-derived mediators as possible biomarkers in bronchial asthma. *Clin. Exp. Med.* **2022**, *22*, 285–300. [CrossRef]
40. Middleton, E.A.; He, X.Y.; Denorme, F.; Campbell, R.A.; Ng, D.; Salvatore, S.P.; Mostyka, M.; Baxter-Stoltzfus, A.; Borczuk, A.C.; Loda, M.; et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. *Blood* **2020**, *136*, 1169–1179. [CrossRef]
41. Tucker, S.L.; Sarr, D.; Rada, B. Neutrophil extracellular traps are present in the airways of ENaC-overexpressing mice with cystic fibrosis-like lung disease. *BMC Immunol.* **2021**, *22*, 7. [CrossRef]
42. Granger, V.; Taille, C.; Roach, D.; Letuve, S.; Dupin, C.; Hamidi, F.; Noel, B.; Neukirch, C.; Aubier, M.; Pretolani, M.; et al. Circulating neutrophil and eosinophil extracellular traps are markers of severe asthma. *Allergy* **2020**, *75*, 699–702. [CrossRef] [PubMed]
43. Vogl, T.; Eisenblatter, M.; Voller, T.; Zenker, S.; Hermann, S.; van Lent, P.; Faust, A.; Geyer, C.; Petersen, B.; Roebrock, K.; et al. Alarmin S100A8/S100A9 as a biomarker for molecular imaging of local inflammatory activity. *Nat. Commun.* **2014**, *5*, 4593. [CrossRef]
44. Shi, H.; Zuo, Y.; Yalavarthi, S.; Gockman, K.; Zuo, M.; Madison, J.A.; Blair, C.; Woodward, W.; Lezak, S.P.; Lugogo, N.L.; et al. Neutrophil calprotectin identifies severe pulmonary disease in COVID-19. *J. Leukoc. Biol.* **2021**, *109*, 67–72. [CrossRef]
45. Wright, T.K.; Gibson, P.G.; Simpson, J.L.; McDonald, V.M.; Wood, L.G.; Baines, K.J. Neutrophil extracellular traps are associated with inflammation in chronic airway disease. *Respirology* **2016**, *21*, 467–475. [CrossRef] [PubMed]
46. Cao, Y.; Chen, F.; Sun, Y.; Hong, H.; Wen, Y.; Lai, Y.; Xu, Z.; Luo, X.; Chen, Y.; Shi, J.; et al. LL-37 promotes neutrophil extracellular trap formation in chronic rhinosinusitis with nasal polyps. *Clin. Exp. Allergy* **2019**, *49*, 990–999. [CrossRef]
47. Durr, U.H.; Sudheendra, U.S.; Ramamoorthy, A. LL-37, the only human member of the cathelicidin family of antimicrobial peptides. *Biochim. Biophys. Acta* **2006**, *1758*, 1408–1425. [CrossRef] [PubMed]
48. Neumann, A.; Berends, E.T.; Nerlich, A.; Molhoek, E.M.; Gallo, R.L.; Meerloo, T.; Nizet, V.; Naim, H.Y.; von Kockritz-Blickwede, M. The antimicrobial peptide LL-37 facilitates the formation of neutrophil extracellular traps. *Biochem. J.* **2014**, *464*, 3–11. [CrossRef]
49. Persson, L.J.; Aanerud, M.; Hardie, J.A.; Miodini Nilsen, R.; Bakke, P.S.; Eagan, T.M.; Hiemstra, P.S. Antimicrobial peptide levels are linked to airway inflammation, bacterial colonisation and exacerbations in chronic obstructive pulmonary disease. *Eur. Respir. J.* **2017**, *49*, 1601328. [CrossRef] [PubMed]
50. Lande, R.; Gregorio, J.; Facchinetti, V.; Chatterjee, B.; Wang, Y.H.; Homey, B.; Cao, W.; Wang, Y.H.; Su, B.; Nestle, F.O.; et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* **2007**, *449*, 564–569. [CrossRef]
51. Nakamura, K.; Nakayama, H.; Sasaki, S.; Takahashi, K.; Iwabuchi, K. Mycobacterium avium-intracellulare complex promote release of pro-inflammatory enzymes matrix metalloproteinases by inducing neutrophil extracellular trap formation. *Sci. Rep.* **2022**, *12*, 5181. [CrossRef] [PubMed]
52. Baraldo, S.; Bazzan, E.; Zanin, M.E.; Turato, G.; Garbisa, S.; Maestrelli, P.; Papi, A.; Miniati, M.; Fabbri, L.M.; Zuin, R.; et al. Matrix metalloproteinase-2 protein in lung periphery is related to COPD progression. *Chest* **2007**, *132*, 1733–1740. [CrossRef]
53. Wells, J.M.; Parker, M.M.; Oster, R.A.; Bowler, R.P.; Dransfield, M.T.; Bhatt, S.P.; Cho, M.H.; Kim, V.; Curtis, J.L.; Martinez, F.J.; et al. Elevated circulating MMP-9 is linked to increased COPD exacerbation risk in SPIROMICS and COPD Gene. *JCI Insight* **2018**, *3*, 123614. [CrossRef] [PubMed]
54. Taylor, S.L.; Rogers, G.B.; Chen, A.C.; Burr, L.D.; McGuckin, M.A.; Serisier, D.J. Matrix metalloproteinases vary with airway microbiota composition and lung function in non-cystic fibrosis bronchiectasis. *Ann. Am. Thorac. Soc.* **2015**, *12*, 701–707. [CrossRef] [PubMed]
55. Wang, Y.; Wysocka, J.; Sayegh, J.; Lee, Y.H.; Perlin, J.R.; Leonelli, L.; Sonbuchner, L.S.; McDonald, C.H.; Cook, R.G.; Dou, Y.; et al. Human PAD4 regulates histone arginine methylation levels via demethylimination. *Science* **2004**, *306*, 279–283. [CrossRef]
56. Neeli, I.; Dwivedi, N.; Khan, S.; Radic, M. Regulation of extracellular chromatin release from neutrophils. *J. Innate Immun.* **2009**, *1*, 194–201. [CrossRef]
57. Knight, J.S.; Subramanian, V.; O'Dell, A.A.; Yalavarthi, S.; Zhao, W.; Smith, C.K.; Hodgin, J.B.; Thompson, P.R.; Kaplan, M.J. Peptidylarginine deiminase inhibition disrupts NET formation and protects against kidney, skin and vascular disease in lupus-prone MRL/lpr mice. *Ann. Rheum. Dis.* **2015**, *74*, 2199–2206. [CrossRef]

58. Savchenko, A.S.; Borissoff, J.I.; Martinod, K.; De Meyer, S.F.; Gallant, M.; Erpenbeck, L.; Brill, A.; Wang, Y.; Wagner, D.D. VWF-mediated leukocyte recruitment with chromatin decondensation by PAD4 increases myocardial ischemia/reperfusion injury in mice. *Blood* **2014**, *123*, 141–148. [CrossRef]
59. Suzuki, M.; Ikari, J.; Anazawa, R.; Tanaka, N.; Katsumata, Y.; Shimada, A.; Suzuki, E.; Tatsumi, K. PAD4 Deficiency Improves Bleomycin-induced Neutrophil Extracellular Traps and Fibrosis in Mouse Lung. *Am. J. Respir. Cell Mol. Biol.* **2020**, *63*, 806–818. [CrossRef]
60. Lefrancais, E.; Mallavia, B.; Zhuo, H.; Calfee, C.S.; Looney, M.R. Maladaptive role of neutrophil extracellular traps in pathogen-induced lung injury. *JCI Insight* **2018**, *3*, e98178. [CrossRef] [PubMed]
61. Rodriguez-Espinosa, O.; Rojas-Espinosa, O.; Moreno-Altamirano, M.M.; Lopez-Villegas, E.O.; Sanchez-Garcia, F.J. Metabolic requirements for neutrophil extracellular traps formation. *Immunology* **2015**, *145*, 213–224. [CrossRef] [PubMed]
62. Keshari, R.S.; Jyoti, A.; Dubey, M.; Kothari, N.; Kohli, M.; Bogra, J.; Barthwal, M.K.; Dikshit, M. Cytokines induced neutrophil extracellular traps formation: Implication for the inflammatory disease condition. *PLoS ONE* **2012**, *7*, e48111. [CrossRef]
63. Kenny, E.F.; Herzig, A.; Kruger, R.; Muth, A.; Mondal, S.; Thompson, P.R.; Brinkmann, V.; Bernuth, H.V.; Zychlinsky, A. Diverse stimuli engage different neutrophil extracellular trap pathways. *Elife* **2017**, *6*, 24437. [CrossRef]
64. Clark, S.R.; Ma, A.C.; Tavener, S.A.; McDonald, B.; Goodarzi, Z.; Kelly, M.M.; Patel, K.D.; Chakrabarti, S.; McAvoy, E.; Sinclair, G.D.; et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat. Med.* **2007**, *13*, 463–469. [CrossRef] [PubMed]
65. Fuchs, T.A.; Abed, U.; Goosmann, C.; Hurwitz, R.; Schulze, I.; Wahn, V.; Weinrauch, Y.; Brinkmann, V.; Zychlinsky, A. Novel cell death program leads to neutrophil extracellular traps. *J. Cell Biol.* **2007**, *176*, 231–241. [CrossRef] [PubMed]
66. Gupta, A.K.; Giaglis, S.; Hasler, P.; Hahn, S. Efficient neutrophil extracellular trap induction requires mobilization of both intracellular and extracellular calcium pools and is modulated by cyclosporine A. *PLoS ONE* **2014**, *9*, e97088. [CrossRef] [PubMed]
67. Kaiser, R.; Leunig, A.; Pekayvaz, K.; Popp, O.; Joppich, M.; Polewka, V.; Escaig, R.; Anjum, A.; Hoffknecht, M.L.; Gold, C.; et al. Self-sustaining IL-8 loops drive a prothrombotic neutrophil phenotype in severe COVID-19. *JCI Insight* **2021**, *6*, 150862. [CrossRef]
68. Hosoki, K.; Ying, S.; Corrigan, C.; Qi, H.; Kurosky, A.; Jennings, K.; Sun, Q.; Boldogh, I.; Sur, S. Analysis of a Panel of 48 Cytokines in BAL Fluids Specifically Identifies IL-8 Levels as the Only Cytokine that Distinguishes Controlled Asthma from Uncontrolled Asthma, and Correlates Inversely with FEV1. *PLoS ONE* **2015**, *10*, e0126035. [CrossRef]
69. Gibson, P.G.; Simpson, J.L.; Saltos, N. Heterogeneity of airway inflammation in persistent asthma: Evidence of neutrophilic inflammation and increased sputum interleukin-8. *Chest* **2001**, *119*, 1329–1336. [CrossRef] [PubMed]
70. Reynolds, C.J.; Quigley, K.; Cheng, X.; Suresh, A.; Tahir, S.; Ahmed-Jushuf, F.; Nawab, K.; Choy, K.; Walker, S.A.; Mathie, S.A.; et al. Lung Defense through IL-8 Carries a Cost of Chronic Lung Remodeling and Impaired Function. *Am. J. Respir Cell Mol. Biol.* **2018**, *59*, 557–571. [CrossRef]
71. Qu, M.; Chen, Z.; Qiu, Z.; Nan, K.; Wang, Y.; Shi, Y.; Shao, Y.; Zhong, Z.; Zhu, S.; Guo, K.; et al. Neutrophil extracellular traps-triggered impaired autophagic flux via METTL3 underlies sepsis-associated acute lung injury. *Cell Death Discov.* **2022**, *8*, 375. [CrossRef]
72. Vats, R.; Kaminski, T.W.; Brzoska, T.; Leech, J.A.; Tutuncuoglu, E.; Katoch, O.; Jonassaint, J.; Tejero, J.; Novelli, E.M.; Pradhan-Sundd, T.; et al. Liver-to-lung microembolic NETs promote gasdermin D-dependent inflammatory lung injury in sickle cell disease. *Blood* **2022**, *140*, 1020–1037. [CrossRef] [PubMed]
73. Saffarzadeh, M.; Juenemann, C.; Queisser, M.A.; Lochnit, G.; Barreto, G.; Galuska, S.P.; Lohmeyer, J.; Preissner, K.T. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: A predominant role of histones. *PLoS ONE* **2012**, *7*, e32366. [CrossRef] [PubMed]
74. Keir, H.R.; Chalmers, J.D. Neutrophil extracellular traps in chronic lung disease: Implications for pathogenesis and therapy. *Eur. Respir. Rev.* **2022**, *31*, 210241. [CrossRef] [PubMed]
75. Porto, B.N.; Stein, R.T. Neutrophil Extracellular Traps in Pulmonary Diseases: Too Much of a Good Thing? *Front. Immunol.* **2016**, *7*, 311. [CrossRef] [PubMed]
76. Trivedi, A.; Khan, M.A.; Bade, G.; Talwar, A. Orchestration of Neutrophil Extracellular Traps (Nets), a Unique Innate Immune Function during Chronic Obstructive Pulmonary Disease (COPD) Development. *Biomedicines* **2021**, *9*, 53. [CrossRef]
77. Dwyer, M.; Shan, Q.; D'Ortona, S.; Maurer, R.; Mitchell, R.; Olesen, H.; Thiel, S.; Huebner, J.; Gadjeva, M. Cystic fibrosis sputum DNA has NETosis characteristics and neutrophil extracellular trap release is regulated by macrophage migration-inhibitory factor. *J. Innate Immun.* **2014**, *6*, 765–779. [CrossRef]
78. Moore, W.C.; Bleecker, E.R. Asthma heterogeneity and severity-why is comprehensive phenotyping important? *Lancet Respir Med.* **2014**, *2*, 10–11. [CrossRef]
79. Wynn, T.A. Type 2 cytokines: Mechanisms and therapeutic strategies. *Nat. Rev. Immunol.* **2015**, *15*, 271–282. [CrossRef]
80. Ray, A.; Kolls, J.K. Neutrophilic Inflammation in Asthma and Association with Disease Severity. *Trends Immunol.* **2017**, *38*, 942–954. [CrossRef]
81. Kuruvilla, M.E.; Lee, F.E.; Lee, G.B. Understanding Asthma Phenotypes, Endotypes, and Mechanisms of Disease. *Clin. Rev. Allergy Immunol.* **2019**, *56*, 219–233. [CrossRef]

82. Moore, W.C.; Hastie, A.T.; Li, X.; Li, H.; Busse, W.W.; Jarjour, N.N.; Wenzel, S.E.; Peters, S.P.; Meyers, D.A.; Bleecker, E.R.; et al. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. *J. Allergy Clin. Immunol.* **2014**, *133*, 1557–1563.e5. [CrossRef] [PubMed]
83. Choi, Y.; Pham, L.D.; Lee, D.H.; Ban, G.Y.; Lee, J.H.; Kim, S.H.; Park, H.S. Neutrophil Extracellular DNA Traps Induce Autoantigen Production by Airway Epithelial Cells. *Mediators Inflamm.* **2017**, *2017*, 5675029. [CrossRef] [PubMed]
84. Jatakanon, A.; Uasuf, C.; Maziak, W.; Lim, S.; Chung, K.F.; Barnes, P.J. Neutrophilic inflammation in severe persistent asthma. *Am. J. Respir Crit. Care Med.* **1999**, *160 Pt 1*, 1532–1539. [CrossRef] [PubMed]
85. Silvestri, M.; Bontempelli, M.; Giacomelli, M.; Malerba, M.; Rossi, G.A.; Di Stefano, A.; Rossi, A.; Ricciardolo, F.L. High serum levels of tumour necrosis factor-alpha and interleukin-8 in severe asthma: Markers of systemic inflammation? *Clin. Exp. Allergy* **2006**, *36*, 1373–1381. [CrossRef]
86. Bonnans, C.; Vachier, I.; Chavis, C.; Godard, P.; Bousquet, J.; Chanez, P. Lipoxins are potential endogenous antiinflammatory mediators in asthma. *Am. J. Respir Crit. Care Med.* **2002**, *165*, 1531–1535. [CrossRef]
87. Baines, K.J.; Simpson, J.L.; Wood, L.G.; Scott, R.J.; Gibson, P.G. Systemic upregulation of neutrophil alpha-defensins and serine proteases in neutrophilic asthma. *Thorax* **2011**, *66*, 942–947. [CrossRef]
88. Cundall, M.; Sun, Y.; Miranda, C.; Trudeau, J.B.; Barnes, S.; Wenzel, S.E. Neutrophil-derived matrix metalloproteinase-9 is increased in severe asthma and poorly inhibited by glucocorticoids. *J. Allergy Clin. Immunol.* **2003**, *112*, 1064–1071. [CrossRef]
89. Kim, R.Y.; Pinkerton, J.W.; Essilfie, A.T.; Robertson, A.A.B.; Baines, K.J.; Brown, A.C.; Mayall, J.R.; Ali, M.K.; Starkey, M.R.; Hansbro, N.G.; et al. Role for NLRP3 Inflammasome-mediated, IL-1beta-Dependent Responses in Severe, Steroid-Resistant Asthma. *Am. J. Respir Crit. Care Med.* **2017**, *196*, 283–297. [CrossRef]
90. Simpson, J.L.; Phipps, S.; Baines, K.J.; Oreo, K.M.; Gunawardhana, L.; Gibson, P.G. Elevated expression of the NLRP3 inflammasome in neutrophilic asthma. *Eur. Respir. J.* **2014**, *43*, 1067–1076. [CrossRef]
91. Lee, Y.G.; Hong, J.; Lee, P.H.; Lee, J.; Park, S.W.; Kim, D.; Jang, A.S. Serum Calprotectin Is a Potential Marker in Patients with Asthma. *J. Korean Med. Sci.* **2020**, *35*, e362. [CrossRef]
92. Xia, M.; Xu, F.; Ni, H.; Wang, Q.; Zhang, R.; Lou, Y.; Zhou, J. Neutrophil activation and NETosis are the predominant drivers of airway inflammation in an OVA/CFA/LPS induced murine model. *Respir Res.* **2022**, *23*, 289. [CrossRef]
93. Khalmuratova, R.; Shin, H.W. Crosstalk Between Mucosal Inflammation and Bone Metabolism in Chronic Rhinosinusitis. *Clin. Exp. Otorhinolaryngol.* **2021**, *14*, 43–49. [CrossRef] [PubMed]
94. Ko, Y.K.; Zhang, Y.L.; Wee, J.H.; Han, D.H.; Kim, H.J.; Rhee, C.S. Human Rhinovirus Infection Enhances the Th2 Environment in Allergic and Non-allergic Patients with Chronic Rhinosinusitis. *Clin. Exp. Otorhinolaryngol.* **2021**, *14*, 217–224. [CrossRef]
95. Lee, H.S.; Volpe, S.J.; Chang, E.H. The Role of Viruses in the Inception of Chronic Rhinosinusitis. *Clin. Exp. Otorhinolaryngol.* **2022**, *15*, 310–318. [CrossRef]
96. Kim, D.H.; Kim, S.W.; Basurrah, M.A.; Hwang, S.H. Clinical and Laboratory Features of Various Criteria of Eosinophilic Chronic Rhinosinusitis: A Systematic Review and Meta-Analysis. *Clin. Exp. Otorhinolaryngol.* **2022**, *15*, 230–246. [CrossRef] [PubMed]
97. Kim, D.K.; Eun, K.M.; Kim, M.K.; Cho, D.; Han, S.A.; Han, S.Y.; Seo, Y.; Lee, D.H.; Cho, S.H.; Kim, D.W. Comparison Between Signature Cytokines of Nasal Tissues in Subtypes of Chronic Rhinosinusitis. *Allergy Asthma Immunol. Res.* **2019**, *11*, 201–211. [CrossRef] [PubMed]
98. Yao, Y.; Zeng, M.; Liu, Z. Revisiting Asian chronic rhinosinusitis in the era of type 2 biologics. *Clin. Exp. Allergy* **2022**, *52*, 231–243. [CrossRef]
99. Fokkens, W.J.; Lund, V.J.; Hopkins, C.; Hellings, P.W.; Kern, R.; Reitsma, S.; Toppila-Salmi, S.; Bernal-Sprekelsen, M.; Mullol, J.; Alobid, I.; et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2020. *Rhinology* **2020**, *58* (Suppl. S29), 1–464. [CrossRef]
100. Wen, W.; Liu, W.; Zhang, L.; Bai, J.; Fan, Y.; Xia, W.; Luo, Q.; Zheng, J.; Wang, H.; Li, Z.; et al. Increased neutrophilia in nasal polyps reduces the response to oral corticosteroid therapy. *J. Allergy Clin. Immunol.* **2012**, *129*, 1522–1528.e5. [CrossRef]
101. Wang, H.; Li, Z.Y.; Jiang, W.X.; Liao, B.; Zhai, G.T.; Wang, N.; Zhen, Z.; Ruan, J.W.; Long, X.B.; Wang, H.; et al. The activation and function of IL-36gamma in neutrophilic inflammation in chronic rhinosinusitis. *J. Allergy Clin. Immunol.* **2018**, *141*, 1646–1658. [CrossRef] [PubMed]
102. Morse, J.C.; Li, P.; Ely, K.A.; Shilts, M.H.; Wannemuehler, T.J.; Huang, L.C.; Sheng, Q.; Chowdhury, N.I.; Chandra, R.K.; Das, S.R.; et al. Chronic rhinosinusitis in elderly patients is associated with an exaggerated neutrophilic proinflammatory response to pathogenic bacteria. *J. Allergy Clin. Immunol.* **2019**, *143*, 990–1002.e6. [CrossRef] [PubMed]
103. Kim, D.K.; Kim, J.Y.; Han, Y.E.; Kim, J.K.; Lim, H.S.; Eun, K.M.; Yang, S.K.; Kim, D.W. Elastase-Positive Neutrophils Are Associated with Refractoriness of Chronic Rhinosinusitis with Nasal Polyps in an Asian Population. *Allergy Asthma Immunol. Res.* **2020**, *12*, 42–55. [CrossRef]
104. Cha, H.; Lim, H.S.; Park, J.A.; Jo, A.; Ryu, H.T.; Kim, D.W.; Kim, J.K.; Hong, S.N.; Shin, H.W.; Kim, D.W. Effects of Neutrophil and Eosinophil Extracellular Trap Formation on Refractoriness in Chronic Rhinosinusitis with Nasal Polyps. *Allergy Asthma Immunol. Res.* **2023**, *15*, 94–108. [CrossRef]
105. Poposki, J.A.; Klingler, A.I.; Stevens, W.W.; Suh, L.A.; Tan, B.K.; Peters, A.T.; Abdala-Valencia, H.; Grammer, L.C.; Welch, K.C.; Smith, S.S.; et al. Elevation of activated neutrophils in chronic rhinosinusitis with nasal polyps. *J. Allergy Clin. Immunol.* **2022**, *149*, 1666–1674. [CrossRef]

106. Hwang, J.W.; Kim, J.H.; Kim, H.J.; Choi, I.H.; Han, H.M.; Lee, K.J.; Kim, T.H.; Lee, S.H. Neutrophil extracellular traps in nasal secretions of patients with stable and exacerbated chronic rhinosinusitis and their contribution to induce chemokine secretion and strengthen the epithelial barrier. *Clin. Exp. Allergy* **2019**, *49*, 1306–1320. [CrossRef] [PubMed]
107. Van Crombruggen, K.; Vogl, T.; Perez-Novo, C.; Holtappels, G.; Bachert, C. Differential release and deposition of S100A8/A9 proteins in inflamed upper airway tissue. *Eur. Respir. J.* **2016**, *47*, 264–274. [CrossRef]
108. Delemarre, T.; Holtappels, G.; De Ruyck, N.; Zhang, N.; Nauwynck, H.; Bachert, C.; Gevaert, E. A substantial neutrophilic inflammation as regular part of severe type 2 chronic rhinosinusitis with nasal polyps. *J. Allergy Clin. Immunol.* **2021**, *147*, 179–188.e2. [CrossRef]
109. Wang, X.; Sima, Y.; Zhao, Y.; Zhang, N.; Zheng, M.; Du, K.; Wang, M.; Wang, Y.; Hao, Y.; Li, Y.; et al. Endotypes of chronic rhinosinusitis based on inflammatory and remodeling factors. *J. Allergy Clin. Immunol.* **2022**, *151*, 458–468. [CrossRef]
110. Kirchner, K.K.; Wagener, J.S.; Khan, T.Z.; Copenhaver, S.C.; Accurso, F.J. Increased DNA levels in bronchoalveolar lavage fluid obtained from infants with cystic fibrosis. *Am. J. Respir. Crit. Care Med.* **1996**, *154*, 1426–1429. [CrossRef]
111. Marcos, V.; Zhou-Suckow, Z.; Onder Yildirim, A.; Bohla, A.; Hector, A.; Vitkov, L.; Krautgartner, W.D.; Stoiber, W.; Griese, M.; Eickelberg, O.; et al. Free DNA in cystic fibrosis airway fluids correlates with airflow obstruction. *Mediators Inflamm.* **2015**, *2015*, 408935. [CrossRef]
112. Dubois, A.V.; Gauthier, A.; Brea, D.; Varaigne, F.; Diot, P.; Gauthier, F.; Attucci, S. Influence of DNA on the activities and inhibition of neutrophil serine proteases in cystic fibrosis sputum. *Am. J. Respir. Cell Mol. Biol.* **2012**, *47*, 80–86. [CrossRef]
113. Papayannopoulos, V.; Staab, D.; Zychlinsky, A. Neutrophil elastase enhances sputum solubilization in cystic fibrosis patients receiving DNase therapy. *PLoS ONE* **2011**, *6*, e28526. [CrossRef]
114. Piva, T.C.; Luft, C.; Antunes, K.H.; Marostica, P.J.C.; Pinto, L.A.; Donadio, M.V.F. Extracellular DNA in sputum is associated with pulmonary function and hospitalization in patients with cystic fibrosis. *Respir. Med.* **2020**, *172*, 106144. [CrossRef]
115. Dittrich, A.S.; Kuhbandner, I.; Gehrig, S.; Rickert-Zacharias, V.; Twigg, M.; Wege, S.; Taggart, C.C.; Herth, F.; Schultz, C.; Mall, M.A. Elastase activity on sputum neutrophils correlates with severity of lung disease in cystic fibrosis. *Eur. Respir. J.* **2018**, *51*, 1701910. [CrossRef]
116. Rosenow, T.; Mok, L.C.; Turkovic, L.; Berry, L.J.; Sly, P.D.; Ranganathan, S.; Tiddens, H.; Stick, S.M. The cumulative effect of inflammation and infection on structural lung disease in early cystic fibrosis. *Eur. Respir. J.* **2019**, *54*, 1801771. [CrossRef] [PubMed]
117. Kim, J.S.; Okamoto, K.; Rubin, B.K. Pulmonary function is negatively correlated with sputum inflammatory markers and cough clearability in subjects with cystic fibrosis but not those with chronic bronchitis. *Chest* **2006**, *129*, 1148–1154. [CrossRef] [PubMed]
118. Ratjen, F.; Hartog, C.M.; Paul, K.; Wermelt, J.; Braun, J. Matrix metalloproteases in BAL fluid of patients with cystic fibrosis and their modulation by treatment with dornase alpha. *Thorax* **2002**, *57*, 930–934. [CrossRef] [PubMed]
119. Gaggar, A.; Hector, A.; Bratcher, P.E.; Mall, M.A.; Griese, M.; Hartl, D. The role of matrix metalloproteinases in cystic fibrosis lung disease. *Eur. Respir. J.* **2011**, *38*, 721–727. [CrossRef]
120. Gray, R.D.; Imrie, M.; Boyd, A.C.; Porteous, D.; Innes, J.A.; Greening, A.P. Sputum and serum calprotectin are useful biomarkers during CF exacerbation. *J. Cyst Fibros* **2010**, *9*, 193–198. [CrossRef]
121. Reid, P.A.; McAllister, D.A.; Boyd, A.C.; Innes, J.A.; Porteous, D.; Greening, A.P.; Gray, R.D. Measurement of serum calprotectin in stable patients predicts exacerbation and lung function decline in cystic fibrosis. *Am. J. Respir. Crit. Care Med.* **2015**, *191*, 233–236. [CrossRef]
122. Skopelja, S.; Hamilton, B.J.; Jones, J.D.; Yang, M.L.; Mamula, M.; Ashare, A.; Gifford, A.H.; Rigby, W.F. The role for neutrophil extracellular traps in cystic fibrosis autoimmunity. *JCI Insight* **2016**, *1*, e88912. [CrossRef] [PubMed]
123. Gray, R.D.; Hardisty, G.; Regan, K.H.; Smith, M.; Robb, C.T.; Duffin, R.; Mackellar, A.; Felton, J.M.; Paemka, L.; McCullagh, B.N.; et al. Delayed neutrophil apoptosis enhances NET formation in cystic fibrosis. *Thorax* **2018**, *73*, 134–144. [CrossRef] [PubMed]
124. Rutgers, S.R.; Timens, W.; Kaufmann, H.F.; van der Mark, T.W.; Koeter, G.H.; Postma, D.S. Comparison of induced sputum with bronchial wash, bronchoalveolar lavage and bronchial biopsies in COPD. *Eur. Respir. J.* **2000**, *15*, 109–115. [CrossRef] [PubMed]
125. Pesci, A.; Majori, M.; Cuomo, A.; Borciani, N.; Bertacco, S.; Cacciani, G.; Gabrielli, M. Neutrophils infiltrating bronchial epithelium in chronic obstructive pulmonary disease. *Respir. Med.* **1998**, *92*, 863–870. [CrossRef] [PubMed]
126. Culpitt, S.V.; Maziak, W.; Loukidis, S.; Nightingale, J.A.; Matthews, J.L.; Barnes, P.J. Effect of high dose inhaled steroid on cells, cytokines, and proteases in induced sputum in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **1999**, *160 Pt 1*, 1635–1639. [CrossRef]
127. Parr, D.G.; White, A.J.; Bayley, D.L.; Guest, P.J.; Stockley, R.A. Inflammation in sputum relates to progression of disease in subjects with COPD: A prospective descriptive study. *Respir. Res.* **2006**, *7*, 136. [CrossRef]
128. Baines, K.J.; Simpson, J.L.; Gibson, P.G. Innate immune responses are increased in chronic obstructive pulmonary disease. *PLoS ONE* **2011**, *6*, e18426. [CrossRef]
129. Doe, C.; Bafadhel, M.; Siddiqui, S.; Desai, D.; Mistry, V.; Rugman, P.; McCormick, M.; Woods, J.; May, R.; Sleeman, M.A.; et al. Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. *Chest* **2010**, *138*, 1140–1147. [CrossRef]
130. Roos, A.B.; Sanden, C.; Mori, M.; Bjermer, L.; Stampfli, M.R.; Erjefalt, J.S. 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*, 1232–1241. [CrossRef] [PubMed]

131. Pedersen, F.; Marwitz, S.; Holz, O.; Kirsten, A.; Bahmer, T.; Waschki, B.; Magnussen, H.; Rabe, K.F.; Goldmann, T.; Uddin, M.; et al. Neutrophil extracellular trap formation and extracellular DNA in sputum of stable COPD patients. *Respir. Med.* **2015**, *109*, 1360–1362. [CrossRef] [PubMed]
132. Grabcanovic-Musija, F.; Obermayer, A.; Stoiber, W.; Krautgartner, W.D.; Steinbacher, P.; Winterberg, N.; Bathke, A.C.; Klappacher, M.; Studnicka, M. Neutrophil extracellular trap (NET) formation characterises stable and exacerbated COPD and correlates with airflow limitation. *Respir. Res.* **2015**, *16*, 59. [CrossRef] [PubMed]
133. Obermayer, A.; Stoiber, W.; Krautgartner, W.D.; Klappacher, M.; Kofler, B.; Steinbacher, P.; Vitkov, L.; Grabcanovic-Musija, F.; Studnicka, M. New aspects on the structure of neutrophil extracellular traps from chronic obstructive pulmonary disease and in vitro generation. *PLoS ONE* **2014**, *9*, e97784. [CrossRef]
134. Wilkinson, T.M.; Patel, I.S.; Wilks, M.; Donaldson, G.C.; Wedzicha, J.A. Airway bacterial load and FEV1 decline in patients with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **2003**, *167*, 1090–1095. [CrossRef]
135. Keatings, V.M.; Collins, P.D.; Scott, D.M.; Barnes, P.J. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am. J. Respir. Crit. Care Med.* **1996**, *153*, 530–534. [CrossRef]
136. Thulborn, S.J.; Mistry, V.; Brightling, C.E.; Moffitt, K.L.; Ribeiro, D.; Bafadhel, M. Neutrophil elastase as a biomarker for bacterial infection in COPD. *Respir. Res.* **2019**, *20*, 170. [CrossRef]
137. Shoemark, A.; Cant, E.; Carreto, L.; Smith, A.; Oriano, M.; Keir, H.R.; Perea, L.; Canto, E.; Terranova, L.; Vidal, S.; et al. A point-of-care neutrophil elastase activity assay identifies bronchiectasis severity, airway infection and risk of exacerbation. *Eur. Respir. J.* **2019**, *53*, 1900303. [CrossRef] [PubMed]
138. Loukides, S.; Bouros, D.; Papatheodorou, G.; Lachanis, S.; Panagou, P.; Siafakas, N.M. Exhaled H(2)O(2) in steady-state bronchiectasis: Relationship with cellular composition in induced sputum, spirometry, and extent and severity of disease. *Chest* **2002**, *121*, 81–87. [CrossRef] [PubMed]
139. Bedi, P.; Davidson, D.J.; McHugh, B.J.; Rossi, A.G.; Hill, A.T. Blood Neutrophils Are Reprogrammed in Bronchiectasis. *Am. J. Respir. Crit. Care Med.* **2018**, *198*, 880–890. [CrossRef]
140. Angrill, J.; Agusti, C.; De Celis, R.; Filella, X.; Rano, A.; Elena, M.; De La Bellacasa, J.P.; Xaubet, A.; Torres, A. Bronchial inflammation and colonization in patients with clinically stable bronchiectasis. *Am. J. Respir. Crit. Care Med.* **2001**, *164*, 1628–1632. [CrossRef]
141. Sly, P.D.; Gangell, C.L.; Chen, L.; Ware, R.S.; Ranganathan, S.; Mott, L.S.; Murray, C.P.; Stick, S.M.; Investigators, A.C. Risk factors for bronchiectasis in children with cystic fibrosis. *N. Engl. J. Med.* **2013**, *368*, 1963–1970. [CrossRef]
142. Chalmers, J.D.; Moffitt, K.L.; Suarez-Cuartin, G.; Sibila, O.; Finch, S.; Furrie, E.; Dicker, A.; Wrobel, K.; Elborn, J.S.; Walker, B.; et al. Neutrophil Elastase Activity Is Associated with Exacerbations and Lung Function Decline in Bronchiectasis. *Am. J. Respir. Crit. Care Med.* **2017**, *195*, 1384–1393. [CrossRef]
143. Simpson, J.L.; Grissell, T.V.; Douwes, J.; Scott, R.J.; Boyle, M.J.; Gibson, P.G. Innate immune activation in neutrophilic asthma and bronchiectasis. *Thorax* **2007**, *62*, 211–218. [CrossRef] [PubMed]
144. Sibila, O.; Perea, L.; Canto, E.; Shoemark, A.; Cassidy, D.; Smith, A.H.; Suarez-Cuartin, G.; Rodrigo-Troyano, A.; Keir, H.R.; Oriano, M.; et al. Antimicrobial peptides, disease severity and exacerbations in bronchiectasis. *Thorax* **2019**, *74*, 835–842. [CrossRef] [PubMed]
145. Garratt, L.W.; Sutanto, E.N.; Ling, K.M.; Looi, K.; Iosifidis, T.; Martinovich, K.M.; Shaw, N.C.; Kicic-Starcevich, E.; Knight, D.A.; Ranganathan, S.; et al. Matrix metalloproteinase activation by free neutrophil elastase contributes to bronchiectasis progression in early cystic fibrosis. *Eur. Respir. J.* **2015**, *46*, 384–394. [CrossRef]
146. Vaninov, N. In the eye of the COVID-19 cytokine storm. *Nat. Rev. Immunol.* **2020**, *20*, 277. [CrossRef] [PubMed]
147. Lucas, C.; Wong, P.; Klein, J.; Castro, T.B.R.; Silva, J.; Sundaram, M.; Ellingson, M.K.; Mao, T.; Oh, J.E.; Israelow, B.; et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature* **2020**, *584*, 463–469. [CrossRef]
148. Zhou, Z.; Ren, L.; Zhang, L.; Zhong, J.; Xiao, Y.; Jia, Z.; Guo, L.; Yang, J.; Wang, C.; Jiang, S.; et al. Heightened Innate Immune Responses in the Respiratory Tract of COVID-19 Patients. *Cell Host Microbe* **2020**, *27*, 883–890.e2. [CrossRef]
149. Giamarellos-Bourboulis, E.J.; Netea, M.G.; Rovina, N.; Akinosoglou, K.; Antoniadou, A.; Antonakos, N.; Damoraki, G.; Gkavogianni, T.; Adami, M.E.; Katsaounou, P.; et al. Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure. *Cell Host Microbe* **2020**, *27*, 992–1000.e3. [CrossRef]
150. Schulte-Schrepping, J.; Reusch, N.; Paclik, D.; Bassler, K.; Schlickeiser, S.; Zhang, B.W.; Kramer, B.; Krammer, T.; Brumhard, S.; Bonaguro, L.; et al. Severe COVID-19 Is Marked by a Dysregulated Myeloid Cell Compartment. *Cell* **2020**, *182*, 1419–1440. [CrossRef] [PubMed]
151. Parackova, Z.; Zentsova, I.; Bloomfield, M.; Vrabcova, P.; Smetanova, J.; Klocperk, A.; Meseznikov, G.; Casas Mendez, L.F.; Vymazal, T.; Sediva, A. Disharmonic Inflammatory Signatures in COVID-19: Augmented Neutrophils' but Impaired Monocytes' and Dendritic Cells' Responsiveness. *Cells* **2020**, *9*, 2206. [CrossRef]
152. Veras, F.P.; Pontelli, M.C.; Silva, C.M.; Toller-Kawahisa, J.E.; de Lima, M.; Nascimento, D.C.; Schneider, A.H.; Caetite, D.; Tavares, L.A.; Paiva, I.M.; et al. SARS-CoV-2-triggered neutrophil extracellular traps mediate COVID-19 pathology. *J. Exp. Med.* **2020**, *217*, e20201129. [CrossRef] [PubMed]

153. Zuo, Y.; Yalavarthi, S.; Shi, H.; Gockman, K.; Zuo, M.; Madison, J.A.; Blair, C.; Weber, A.; Barnes, B.J.; Egeblad, M.; et al. Neutrophil extracellular traps in COVID-19. *JCI Insight* **2020**, *5*, e138999. [CrossRef] [PubMed]
154. Del Valle, D.M.; Kim-Schulze, S.; Huang, H.H.; Beckmann, N.D.; Nirenberg, S.; Wang, B.; Lavin, Y.; Swartz, T.H.; Madduri, D.; Stock, A.; et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nat. Med.* **2020**, *26*, 1636–1643. [CrossRef] [PubMed]
155. Sumby, P.; Barbian, K.D.; Gardner, D.J.; Whitney, A.R.; Welty, D.M.; Long, R.D.; Bailey, J.R.; Parnell, M.J.; Hoe, N.P.; Adams, G.G.; et al. Extracellular deoxyribonuclease made by group A Streptococcus assists pathogenesis by enhancing evasion of the innate immune response. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 1679–1684. [CrossRef]
156. Hosseinnejad, A.; Ludwig, N.; Wienkamp, A.K.; Rimal, R.; Bleilevens, C.; Rossaint, R.; Rossaint, J.; Singh, S. DNase I functional microgels for neutrophil extracellular trap disruption. *Biomater. Sci.* **2021**, *10*, 85–99. [CrossRef]
157. Sawicki, G.S.; Chou, W.; Raimundo, K.; Trzaskoma, B.; Konstan, M.W. Randomized trial of efficacy and safety of dornase alfa delivered by eRapid nebulizer in cystic fibrosis patients. *J. Cyst. Fibros.* **2015**, *14*, 777–783. [CrossRef]
158. Fuchs, H.J.; Borowitz, D.S.; Christiansen, D.H.; Morris, E.M.; Nash, M.L.; Ramsey, B.W.; Rosenstein, B.J.; Smith, A.L.; Wohl, M.E. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. *N. Engl. J. Med.* **1994**, *331*, 637–642. [CrossRef]
159. Durward, A.; Forte, V.; Shemie, S.D. Resolution of mucus plugging and atelectasis after intratracheal rhDNase therapy in a mechanically ventilated child with refractory status asthmaticus. *Crit. Care Med.* **2000**, *28*, 560–562. [CrossRef]
160. Park, H.H.; Park, W.; Lee, Y.Y.; Kim, H.; Seo, H.S.; Choi, D.W.; Kwon, H.K.; Na, D.H.; Kim, T.H.; Choy, Y.B.; et al. Bioinspired DNase-I-Coated Melanin-Like Nanospheres for Modulation of Infection-Associated NETosis Dysregulation. *Adv. Sci.* **2020**, *7*, 2001940. [CrossRef]
161. Lee, Y.Y.; Park, H.H.; Park, W.; Kim, H.; Jang, J.G.; Hong, K.S.; Lee, J.Y.; Seo, H.S.; Na, D.H.; Kim, T.H.; et al. Long-acting nanoparticulate DNase-1 for effective suppression of SARS-CoV-2-mediated neutrophil activities and cytokine storm. *Biomaterials* **2021**, *267*, 120389. [CrossRef]
162. O'Donnell, A.E.; Barker, A.F.; Ilowite, J.S.; Fick, R.B. Treatment of idiopathic bronchiectasis with aerosolized recombinant human DNase I. rhDNase Study Group. *Chest* **1998**, *113*, 1329–1334. [CrossRef] [PubMed]
163. Gehrig, S.; Duerr, J.; Weitnauer, M.; Wagner, C.J.; Graeber, S.Y.; Schatterny, J.; Hirtz, S.; Belaaouaj, A.; Dalpke, A.H.; Schultz, C.; et al. Lack of neutrophil elastase reduces inflammation, mucus hypersecretion, and emphysema, but not mucus obstruction, in mice with cystic fibrosis-like lung disease. *Am. J. Respir. Crit. Care Med.* **2014**, *189*, 1082–1092. [CrossRef] [PubMed]
164. Parr, D.G.; Guest, P.G.; Reynolds, J.H.; Dowson, L.J.; Stockley, R.A. Prevalence and impact of bronchiectasis in alpha1-antitrypsin deficiency. *Am. J. Respir. Crit. Care Med.* **2007**, *176*, 1215–1221. [CrossRef] [PubMed]
165. McElvaney, N.G. Alpha-1 Antitrypsin Therapy in Cystic Fibrosis and the Lung Disease Associated with Alpha-1 Antitrypsin Deficiency. *Ann. Am. Thorac. Soc.* **2016**, *13* (Suppl. 2), S191–S196.
166. Vogelmeier, C.; Gillissen, A.; Buhl, R. Use of secretory leukoprotease inhibitor to augment lung antineutrophil elastase activity. *Chest* **1996**, *110* (Suppl. 6), 261S–266S. [CrossRef] [PubMed]
167. Zani, M.L.; Tanga, A.; Saidi, A.; Serrano, H.; Dallet-Choisy, S.; Baranger, K.; Moreau, T. SLPI and trappin-2 as therapeutic agents to target airway serine proteases in inflammatory lung diseases: Current and future directions. *Biochem. Soc. Trans.* **2011**, *39*, 1441–1446. [CrossRef]
168. Raundhal, M.; Morse, C.; Khare, A.; Oriss, T.B.; Milosevic, J.; Trudeau, J.; Huff, R.; Pilewski, J.; Holguin, F.; Kolls, J.; et al. High IFN-gamma and low SLPI mark severe asthma in mice and humans. *J. Clin. Investig.* **2015**, *125*, 3037–3050. [CrossRef]
169. Cambier, S.; Metzemaekers, M.; de Carvalho, A.C.; Nooyens, A.; Jacobs, C.; Vanderbeke, L.; Malengier-Devlies, B.; Gouwy, M.; Heylen, E.; Meersseman, P.; et al. Atypical response to bacterial coinfection and persistent neutrophilic bronchoalveolar inflammation distinguish critical COVID-19 from influenza. *JCI Insight* **2022**, *7*, e155055. [CrossRef]
170. Wang, K.; Liao, Y.; Li, X.; Wang, R.; Zeng, Z.; Cheng, M.; Gao, L.; Xu, D.; Wen, F.; Wang, T.; et al. Inhibition of neutrophil elastase prevents cigarette smoke exposure-induced formation of neutrophil extracellular traps and improves lung function in a mouse model of chronic obstructive pulmonary disease. *Int. Immunopharmacol.* **2023**, *114*, 109537. [CrossRef]
171. Vogelmeier, C.; Aquino, T.O.; O'Brien, C.D.; Perrett, J.; Gunawardena, K.A. A randomised, placebo-controlled, dose-finding study of AZD9668, an oral inhibitor of neutrophil elastase, in patients with chronic obstructive pulmonary disease treated with tiotropium. *COPD* **2012**, *9*, 111–120. [CrossRef] [PubMed]
172. Elborn, J.S.; Perrett, J.; Forsman-Semb, K.; Marks-Konczalik, J.; Gunawardena, K.; Entwistle, N. Efficacy, safety and effect on biomarkers of AZD9668 in cystic fibrosis. *Eur. Respir. J.* **2012**, *40*, 969–976. [CrossRef]
173. Stockley, R.; De Soyza, A.; Gunawardena, K.; Perrett, J.; Forsman-Semb, K.; Entwistle, N.; Snell, N. Phase II study of a neutrophil elastase inhibitor (AZD9668) in patients with bronchiectasis. *Respir. Med.* **2013**, *107*, 524–533. [CrossRef]
174. Kuna, P.; Jenkins, M.; O'Brien, C.D.; Fahy, W.A. AZD9668, a neutrophil elastase inhibitor, plus ongoing budesonide/formoterol in patients with COPD. *Respir. Med.* **2012**, *106*, 531–539. [CrossRef]
175. Metzler, K.D.; Fuchs, T.A.; Nauseef, W.M.; Reumaux, D.; Roesler, J.; Schulze, I.; Wahn, V.; Papayannopoulos, V.; Zychlinsky, A. Myeloperoxidase is required for neutrophil extracellular trap formation: Implications for innate immunity. *Blood* **2011**, *117*, 953–959. [CrossRef] [PubMed]

176. Ogawa, H.; Azuma, M.; Umeno, A.; Shimizu, M.; Murotomi, K.; Yoshida, Y.; Nishioka, Y.; Tsuneyama, K. Singlet oxygen -derived nerve growth factor exacerbates airway hyperresponsiveness in a mouse model of asthma with mixed inflammation. *Allergol. Int.* **2022**, *71*, 395–404. [CrossRef]
177. Zheng, W.; Warner, R.; Ruggeri, R.; Su, C.; Cortes, C.; Skoura, A.; Ward, J.; Ahn, K.; Kalgutkar, A.; Sun, D.; et al. PF-1355, a mechanism-based myeloperoxidase inhibitor, prevents immune complex vasculitis and anti-glomerular basement membrane glomerulonephritis. *J. Pharmacol. Exp. Ther.* **2015**, *353*, 288–298. [CrossRef]
178. Antonelou, M.; Michaelsson, E.; Evans, R.D.R.; Wang, C.J.; Henderson, S.R.; Walker, L.S.K.; Unwin, R.J.; Salama, A.D.; Investigators, R.-I. Therapeutic Myeloperoxidase Inhibition Attenuates Neutrophil Activation, ANCA-Mediated Endothelial Damage, and Crescentic GN. *J. Am. Soc. Nephrol.* **2020**, *31*, 350–364. [CrossRef]
179. Chaikijurajai, T.; Tang, W.H.W. Myeloperoxidase: A potential therapeutic target for coronary artery disease. *Expert Opin. Ther. Targets* **2020**, *24*, 695–705. [CrossRef]
180. Pedersen, F.; Waschki, B.; Marwitz, S.; Goldmann, T.; Kirsten, A.; Malmgren, A.; Rabe, K.F.; Uddin, M.; Watz, H. Neutrophil extracellular trap formation is regulated by CXCR2 in COPD neutrophils. *Eur. Respir. J.* **2018**, *51*, 1700970. [CrossRef] [PubMed]
181. Uddin, M.; Watz, H.; Malmgren, A.; Pedersen, F. NETopathic Inflammation in Chronic Obstructive Pulmonary Disease and Severe Asthma. *Front. Immunol.* **2019**, *10*, 47. [CrossRef]
182. De Soyza, A.; Pavord, I.; Elborn, J.S.; Smith, D.; Wray, H.; Puu, M.; Larsson, B.; Stockley, R. A randomised, placebo-controlled study of the CXCR2 antagonist AZD5069 in bronchiectasis. *Eur. Respir. J.* **2015**, *46*, 1021–1032. [CrossRef] [PubMed]
183. Watz, H.; Uddin, M.; Pedersen, F.; Kirsten, A.; Goldmann, T.; Stellmacher, F.; Groth, E.; Larsson, B.; Bottcher, G.; Malmgren, A.; et al. Effects of the CXCR2 antagonist AZD5069 on lung neutrophil recruitment in asthma. *Pulm. Pharmacol. Ther.* **2017**, *45*, 121–123. [CrossRef] [PubMed]
184. O'Byrne, P.M.; Metev, H.; Puu, M.; Richter, K.; Keen, C.; Uddin, M.; Larsson, B.; Cullberg, M.; Nair, P. Efficacy and safety of a CXCR2 antagonist, AZD5069, in patients with uncontrolled persistent asthma: A randomised, double-blind, placebo-controlled trial. *Lancet Respir. Med.* **2016**, *4*, 797–806. [CrossRef]
185. Moss, R.B.; Mistry, S.J.; Konstan, M.W.; Pilewski, J.M.; Kerem, E.; Tal-Singer, R.; Lazaar, A.L.; Investigators, C.F. Safety and early treatment effects of the CXCR2 antagonist SB-656933 in patients with cystic fibrosis. *J. Cyst Fibros* **2013**, *12*, 241–248. [CrossRef]
186. Busch-Petersen, J.; Carpenter, D.C.; Burman, M.; Foley, J.; Hunsberger, G.E.; Kilian, D.J.; Salmon, M.; Mayer, R.J.; Yonchuk, J.G.; Tal-Singer, R. Danirixin: A Reversible and Selective Antagonist of the CXC Chemokine Receptor 2. *J. Pharmacol. Exp. Ther* **2017**, *362*, 338–346. [CrossRef] [PubMed]
187. Lazaar, A.L.; Miller, B.E.; Donald, A.C.; Keeley, T.; Ambery, C.; Russell, J.; Watz, H.; Tal-Singer, R.; For, I. CXCR2 antagonist for patients with chronic obstructive pulmonary disease with chronic mucus hypersecretion: A phase 2b trial. *Respir. Res.* **2020**, *21*, 149. [CrossRef]
188. Keir, H.R.; Richardson, H.; Fillmore, C.; Shoemark, A.; Lazaar, A.L.; Miller, B.E.; Tal-Singer, R.; Chalmers, J.D.; Mohan, D. CXCL-8-dependent and -independent neutrophil activation in COPD: Experiences from a pilot study of the CXCR2 antagonist danirixin. *ERJ Open Res.* **2020**, *6*, 23120541. [CrossRef] [PubMed]
189. Zarbock, A.; Allegretti, M.; Ley, K. Therapeutic inhibition of CXCR2 by Reparixin attenuates acute lung injury in mice. *Br. J. Pharmacol* **2008**, *155*, 357–364. [CrossRef] [PubMed]
190. Ostafin, M.; Pruchniak, M.P.; Ciepiela, O.; Reznick, A.Z.; Demkow, U. Different procedures of diphenyleneiodonium chloride addition affect neutrophil extracellular trap formation. *Anal. Biochem.* **2016**, *509*, 60–66. [CrossRef]
191. Wang, C.; Wei, Z.; Han, Z.; Wang, J.; Zhang, X.; Wang, Y.; Liu, Q.; Yang, Z. Neutrophil extracellular traps promote cadmium chloride-induced lung injury in mice. *Environ. Pollut.* **2019**, *254 Pt A*, 113021. [CrossRef]
192. Bonilla, M.C.; Quiros, O.N.; Wendt, M.; Hennig-Pauka, I.; Morgelin, M.; von Kockritz-Blickwede, M.; de Buhr, N. New Insights into Neutrophil Extracellular Trap (NETs) Formation from Porcine Neutrophils in Response to Bacterial Infections. *Int J. Mol. Sci.* **2022**, *23*, 8953. [CrossRef] [PubMed]
193. Silveira, J.S.; Antunes, G.L.; Kaiber, D.B.; da Costa, M.S.; Marques, E.P.; Ferreira, F.S.; Gassen, R.B.; Breda, R.V.; Wyse, A.T.S.; Pitrez, P.; et al. Reactive oxygen species are involved in eosinophil extracellular traps release and in airway inflammation in asthma. *J. Cell Physiol* **2019**, *234*, 23633–23646. [CrossRef] [PubMed]
194. Kim, H.J.; Sim, M.S.; Lee, D.H.; Kim, C.; Choi, Y.; Park, H.S.; Chung, I.Y. Lysophosphatidylserine induces eosinophil extracellular trap formation and degranulation: Implications in severe asthma. *Allergy* **2020**, *75*, 3159–3170. [CrossRef] [PubMed]
195. Zawrotniak, M.; Kozik, A.; Rapala-Kozik, M. Selected mucolytic, anti-inflammatory and cardiovascular drugs change the ability of neutrophils to form extracellular traps (NETs). *Acta Biochim. Pol.* **2015**, *62*, 465–473. [CrossRef]
196. Rogliani, P.; Matera, M.G.; Page, C.; Puxeddu, E.; Cazzola, M.; Calzetta, L. Efficacy and safety profile of mucolytic/antioxidant agents in chronic obstructive pulmonary disease: A comparative analysis across erdosteine, carbocysteine, and N-acetylcysteine. *Respir. Res.* **2019**, *20*, 104. [CrossRef]
197. Cazzola, M.; Calzetta, L.; Page, C.; Jardim, J.; Chuchalin, A.G.; Rogliani, P.; Matera, M.G. Influence of N-acetylcysteine on chronic bronchitis or COPD exacerbations: A meta-analysis. *Eur. Respir. Rev.* **2015**, *24*, 451–461. [CrossRef]
198. Qi, Q.; Ailiyaer, Y.; Liu, R.; Zhang, Y.; Li, C.; Liu, M.; Wang, X.; Jing, L.; Li, Y. Effect of N-acetylcysteine on exacerbations of bronchiectasis (BENE): A randomized controlled trial. *Respir. Res.* **2019**, *20*, 73. [CrossRef]

199. Lewis, H.D.; Liddle, J.; Coote, J.E.; Atkinson, S.J.; Barker, M.D.; Bax, B.D.; Bicker, K.L.; Bingham, R.P.; Campbell, M.; Chen, Y.H.; et al. Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation. *Nat. Chem. Biol.* **2015**, *11*, 189–191. [CrossRef]
200. Biron, B.M.; Chung, C.S.; O'Brien, X.M.; Chen, Y.; Reichner, J.S.; Ayala, A. Cl-Amidine Prevents Histone 3 Citrullination and Neutrophil Extracellular Trap Formation, and Improves Survival in a Murine Sepsis Model. *J. Innate Immun.* **2017**, *9*, 22–32. [CrossRef]
201. Knight, J.S.; Luo, W.; O'Dell, A.A.; Yalavarthi, S.; Zhao, W.; Subramanian, V.; Guo, C.; Grenn, R.C.; Thompson, P.R.; Eitzman, D.T.; et al. Peptidylarginine deiminase inhibition reduces vascular damage and modulates innate immune responses in murine models of atherosclerosis. *Circ. Res.* **2014**, *114*, 947–956. [CrossRef]
202. Zhang, T.; Mei, Y.; Dong, W.; Wang, J.; Huang, F.; Wu, J. Evaluation of protein arginine deiminase-4 inhibitor in TNBS- induced colitis in mice. *Int. Immunopharmacol.* **2020**, *84*, 106583. [CrossRef] [PubMed]
203. Fukuchi, M.; Kamide, Y.; Ueki, S.; Miyabe, Y.; Konno, Y.; Oka, N.; Takeuchi, H.; Koyota, S.; Hirokawa, M.; Yamada, T.; et al. Eosinophil ETosis-Mediated Release of Galectin-10 in Eosinophilic Granulomatosis with Polyangiitis. *Arthritis Rheumatol.* **2021**, *73*, 1683–1693. [CrossRef]
204. Lu, Y.; Huang, Y.; Li, J.; Huang, J.; Zhang, L.; Feng, J.; Li, J.; Xia, Q.; Zhao, Q.; Huang, L.; et al. Eosinophil extracellular traps drive asthma progression through neuro-immune signals. *Nat. Cell Biol.* **2021**, *23*, 1060–1072. [CrossRef]
205. Dinallo, V.; Marafini, I.; Di Fusco, D.; Laudisi, F.; Franze, E.; Di Grazia, A.; Figliuzzi, M.M.; Caprioli, F.; Stolfi, C.; Monteleone, I.; et al. Neutrophil Extracellular Traps Sustain Inflammatory Signals in Ulcerative Colitis. *J. Crohns. Colitis* **2019**, *13*, 772–784. [CrossRef] [PubMed]
206. Winslow, S.; Odqvist, L.; Diver, S.; Riise, R.; Abdillahi, S.; Wingren, C.; Lindmark, H.; Wellner, A.; Lundin, S.; Yrlid, L.; et al. Multi-omics links IL-6 trans-signalling with neutrophil extracellular trap formation and Haemophilus infection in COPD. *Eur. Respir. J.* **2021**, *58*, 2003312. [CrossRef] [PubMed]
207. Zhang, S.; Zhang, Q.; Wang, F.; Guo, X.; Liu, T.; Zhao, Y.; Gu, B.; Chen, H.; Li, Y. Hydroxychloroquine inhibiting neutrophil extracellular trap formation alleviates hepatic ischemia/reperfusion injury by blocking TLR9 in mice. *Clin. Immunol.* **2020**, *216*, 108461. [CrossRef] [PubMed]
208. Schrezenmeier, E.; Dorner, T. Mechanisms of action of hydroxychloroquine and chloroquine: Implications for rheumatology. *Nat. Rev. Rheumatol.* **2020**, *16*, 155–166. [CrossRef]
209. Mazetto, B.M.; Hounkpe, B.W.; Saraiva, S.; Bizzacchi, J.M.A.; De Paula, E.V.; Orsi, F.A. Hydroxychloroquine Therapy and Netosis Regulators Expression in Patients with Primary Antiphospholipid Syndrome. *Blood* **2018**, *132*, 5049. [CrossRef]
210. Jung, H.; Bobba, R.; Su, J.; Shariati-Sarabi, Z.; Gladman, D.D.; Urowitz, M.; Lou, W.; Fortin, P.R. The protective effect of antimalarial drugs on thrombovascular events in systemic lupus erythematosus. *Arthritis. Rheum.* **2010**, *62*, 863–868. [CrossRef]
211. Skendros, P.; Mitsios, A.; Chrysanthopoulou, A.; Mastellos, D.C.; Metallidis, S.; Rafailidis, P.; Ntinopoulou, M.; Sertaridou, E.; Tsironidou, V.; Tsigalou, C.; et al. Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. *J. Clin. Investig.* **2020**, *130*, 6151–6157. [CrossRef] [PubMed]
212. Ibanez, L.; Jaramillo, A.M.; Ferrer, A.; de Zegher, F. High neutrophil count in girls and women with hyperinsulinaemic hyperandrogenism: Normalization with metformin and flutamide overcomes the aggravation by oral contraception. *Hum. Reprod.* **2005**, *20*, 2457–2462. [CrossRef] [PubMed]
213. Cameron, A.R.; Morrison, V.L.; Levin, D.; Mohan, M.; Forteath, C.; Beall, C.; McNeilly, A.D.; Balfour, D.J.; Savinko, T.; Wong, A.K.; et al. Anti-Inflammatory Effects of Metformin Irrespective of Diabetes Status. *Circ. Res.* **2016**, *119*, 652–665. [CrossRef]
214. Menegazzo, L.; Scattolini, V.; Cappellari, R.; Bonora, B.M.; Albiero, M.; Bortolozzi, M.; Romanato, F.; Ceolotto, G.; Vigili de Kreutzeberg, S.; Avogaro, A.; et al. The antidiabetic drug metformin blunts NETosis in vitro and reduces circulating NETosis biomarkers in vivo. *Acta Diabetol.* **2018**, *55*, 593–601. [CrossRef]
215. Usman, A.; Bliden, K.P.; Cho, A.; Walia, N.; Jerjian, C.; Singh, A.; Kundan, P.; Duhan, S.; Tantry, U.S.; Gurbel, P.A. Metformin use in patients hospitalized with COVID-19: Lower inflammation, oxidative stress, and thrombotic risk markers and better clinical outcomes. *J. Thromb. Thrombolysis* **2022**, *53*, 363–371. [CrossRef]
216. Bramante, C.T.; Ingraham, N.E.; Murray, T.A.; Marmor, S.; Hovertsen, S.; Gronski, J.; McNeil, C.; Feng, R.; Guzman, G.; Abdelwahab, N.; et al. Observational Study of Metformin and Risk of Mortality in Patients Hospitalized with COVID-19. *medRxiv* **2020**.
217. Oh, T.K.; Song, I.A. Metformin use and risk of COVID-19 among patients with type II diabetes mellitus: An NHIS-COVID-19 database cohort study. *Acta Diabetol.* **2021**, *58*, 771–778. [CrossRef] [PubMed]
218. Jung, S.Y.; Kim, G.D.; Choi, D.W.; Shin, D.U.; Eom, J.E.; Kim, S.Y.; Chai, O.H.; Kim, H.J.; Lee, S.Y.; Shin, H.S. Epilobiumpyrricholophum Extract Suppresses Porcine Pancreatic Elastase and Cigarette Smoke Extract-Induced Inflammatory response in a Chronic Obstructive Pulmonary Disease Model. *Foods* **2021**, *10*, 2929. [CrossRef]
219. Kim, S.Y.; Shin, D.U.; Eom, J.E.; Jung, S.Y.; Song, H.J.; Lim, K.M.; Kim, G.D.; Yun, S.I.; Kim, M.Y.; Shin, H.S.; et al. Artemisia gmelinii Attenuates Lung Inflammation by Suppressing the NF-kappaB/MAPK Pathway. *Antioxidants* **2022**, *11*, 568. [CrossRef]
220. Li, H.; Qiao, C.; Zhao, L.; Jing, Q.; Xue, D.; Zhang, Y. Epigallocatechin-3-gallate reduces neutrophil extracellular trap formation and tissue injury in severe acute pancreatitis. *J. Leukoc. Biol.* **2022**, *112*, 1427–1443. [CrossRef] [PubMed]
221. Ali, R.A.; Gandhi, A.A.; Dai, L.; Weiner, J.; Estes, S.K.; Yalavarthi, S.; Gockman, K.; Sun, D.; Knight, J.S. Antineutrophil properties of natural gingerols in models of lupus. *JCI Insight* **2021**, *6*, 138385. [CrossRef] [PubMed]

222. Zhu, Y.; Wang, D.; Luo, J.; Jie, J.; Liu, H.; Peng, L.; Bai, X.; Li, D. Zingerone Inhibits the Neutrophil Extracellular Trap Formation and Protects against Sepsis via Nrf2-Mediated ROS Inhibition. *Oxid Med. Cell Longev.* **2022**, *2022*, 3990607. [CrossRef] [PubMed]
223. Kirsten, A.M.; Forster, K.; Radeczky, E.; Linnhoff, A.; Balint, B.; Watz, H.; Wray, H.; Salkeld, L.; Cullberg, M.; Larsson, B. The safety and tolerability of oral AZD5069, a selective CXCR2 antagonist, in patients with moderate-to-severe COPD. *Pulm. Pharmacol. Ther.* **2015**, *31*, 36–41. [CrossRef]
224. Landoni, G.; Piemonti, L.; Monforte, A.D.; Grossi, P.; Zangrillo, A.; Bucci, E.; Allegretti, M.; Goisis, G.; Gavioli, E.M.; Patel, N.; et al. A Multicenter Phase 2 Randomized Controlled Study on the Efficacy and Safety of Reparixin in the Treatment of Hospitalized Patients with COVID-19 Pneumonia. *Infect. Dis. Ther.* **2022**, *11*, 1559–1574. [CrossRef] [PubMed]
225. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ* **2021**, *372*, n71. [CrossRef]