The Dynamic Contribution of Neutrophils in the Chronic Respiratory Diseases

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ABSTRACT

Asthma, chronic obstructive pulmonary disease, and idiopathic pulmonary fibrosis are representative chronic respiratory diseases (CRDs). Although they differ in terms of disease presentation, they are all thought to arise from unresolved inflammation. Neutrophils are not only the first responders to acute inflammation, but they also help resolve the inflammation. Notably, emerging clinical studies show that CRDs are associated with systemic and local elevation of neutrophils. Moreover, murine studies suggest that airway-infiltrating neutrophils not only help initiate airway inflammation but also prolong the inflammation. Given this background, this review describes neutrophil-mediated immune responses in CRDs and summarizes the completed, ongoing, and potential clinical trials that test the therapeutic value of targeting neutrophils in CRDs. The review also clarifies the importance of understanding how neutrophils interact with other immune cells and how these interactions contribute to chronic inflammation in specific CRDs. This information may help identify future therapeutic strategies for CRDs.

Keywords: Neutrophils; asthma; chronic obstructive pulmonary diseases; idiopathic pulmonary fibrosis; lung; inflammation

INTRODUCTION

Chronic respiratory disease (CRD) is an umbrella term for chronic diseases affecting the lungs and airways. The most common include asthma, chronic obstructive pulmonary disease (COPD), and idiopathic pulmonary fibrosis (IPF). Asthma and COPD are both accompanied by airway obstruction and prolonged airway inflammation. Common asthma symptoms are coughing, shortness of breath, wheezing, and chest tightness. Approximately 4% of adults globally have asthma, which is increasing annually. COPD includes chronic bronchitis and emphysema and is marked by breathing difficulties. It is the third leading cause of death worldwide (3.23 million deaths annually). Allergens such as pollen, house dust mites (HDMs), cockroaches, mold, and animal dander trigger asthma and aggravate COPD symptoms. These airborne allergens irritate the airway epithelium, causing it to
secrete excessive mucus and chemokines. However, COPD is not caused by either allergies or asthma. Instead, long-term exposure to environmental hazards such as air pollution and cigarette smoke are the main causes of COPD. IPF is another progressive respiratory disease with a poor prognosis. It affects approximately 3 million people worldwide. Its cause is unknown, but risk factors include smoking, lung injury, a family history of the disease, abnormal acid reflux, environmental exposure, and chronic viral infections.

Significantly, despite their different pathologies, asthma, COPD, and IPF are all initiated by airway inflammation that fails to resolve. While the immunological mechanisms that initiate and maintain these CRDs remain fully unraveled, their importance in CRD pathogenesis means that a greater understanding of them may lead to therapeutics that will help control and prevent CRDs.

Neutrophils are the most abundant cell type in human blood and critical responders in the first line of defense against pathogens. In the context of pulmonary infection, neutrophils are recruited to the site of inflammation and initiate inflammation by secreting various pro-inflammatory cytokines and chemokines. Besides, neutrophils can promote damaged tissue repairing by clearing nuclear debris resulting from lung injuries. Due to their short life span, the activities of neutrophils were long thought to be restricted to countering acute infections and clearing pathogens. However, emerging evidence suggests that neutrophils have broader activities; in particular, they may play an important role in initiating and contributing to the unabating inflammation that is the hallmark of many chronic diseases. Specifically, there is evidence of continuous recruitment of neutrophils to sites of inflammation, where they add to the chronic inflammatory responses by releasing proteases, forming neutrophil extracellular traps (NETs), and activating other immune cells.

Since CRDs such as severe asthma, COPD, and IPF are all associated with continuously high neutrophil levels, and there is evidence that suppressing these cells or their functions ameliorates CRD symptoms and lung damage, it is of interest to re-evaluate the hitherto overlooked role of neutrophils in these diseases. Here, we will discuss the diverse roles of neutrophils in CRDs and elucidate the mechanisms by which neutrophils crosstalk with other immune cells. The research in these areas may help us identify strategies to counteract the adverse effects of neutrophils in CRDs.

NEUTROPHILS IN CHRONIC RESPIRATORY DISEASES

Neutrophils in asthma

Asthma is one of the most prevalent CRDs. It is associated with airway obstruction and excessive mucus production and is generally considered an allergic disease caused by type-2 immune responses. Specifically, inhaled allergens disrupt the airway epithelium, which increases the production of alarmins, stimulating T helper (Th) 2 cells, type 2 innate lymphoid cells (ILC2s), eosinophils, and alveolar macrophages. The local and systemic augmentation of eosinophils is considered a key risk factor for asthma exacerbation. However, 10%–33% of asthmatics have normal ranges of serum immunoglobulin E (IgE) and a non-allergic phenotype that is less responsive to standard therapy and is marked by increased neutrophilic inflammation. Triggers for this non-allergic asthma phenotype include environmental factors, including ozone, air pollutants, and viral infection. In addition, many clinical studies have shown that increased neutrophil numbers are associated with greater asthma severity and steroid resistance.
Neutrophils secrete many different molecules, some of which have been found to contribute directly to airway inflammation and asthma exacerbation (Fig. 1). They include NETs, which are extracellular structures composed of chromatin backbone and granule proteins with antimicrobial activity. In healthy settings, NETs neutralize harmful pathogens in the lungs. However, when the NET formation is dysregulated and therefore excessive or continuous, it promotes tissue damage and inflammation. An example of this pathogenic role of NET in asthma is the study by He et al. They showed that when a murine model of asthma is challenged with intratracheal < 2.5-µm particulate matter (PM2.5), it increases asthma severity. Specifically, it elevates airway inflammation, mucus secretion, and neutrophil infiltration. Mechanistic analyses then showed that the lung-recruited neutrophils release NETs, which increases the local expression and activity of the oxidoreductase NQO1. This, in turn, upregulates the expression of the mucin gene \textit{MUC5AC} in airway epithelial cells, thereby aggravating mucus hypersecretion. Similarly, Shin et al. reported recently that...
NET-producing neutrophils contribute to air pollutant-induced asthma exacerbation. They observed that intratracheal instillation of diesel exhaust particle (DEP), as an asthma exacerbator, generates a novel neutrophil subset in the airway that expresses SiglecF. These cells produce more NETs than conventional neutrophils. Moreover, they produce more cysteinyl leukotrienes, which are known to exacerbate asthma. SiglecF⁺ neutrophils are upregulated in an asthma exacerbation model (i.e., HDM-induced asthma followed by DEP challenge), and the neutralization of their NET or cysteinyl leukotriene production blocks HDM+DEP-induced airway hyperresponsiveness (AHR).

Other potentially pathogenic neutrophil-derived molecules are transforming growth factor (TGF)-β. Haddad et al. showed that the blood neutrophils from severe asthmatic patients induced changes in morphology and epithelial-mesenchymal transition (EMT) of human bronchial epithelial cells more than healthy control neutrophils. They also found that the asthmatic neutrophils increased TGF-β productions and neutralizing TGF-β reduced EMT-related gene expressions from primary epithelial cells. In addition, increased TGF-β production could be associated with poor lung function. Torrego et al. demonstrated that the allergen challenge decreased forced expiratory volume in one second (FEV1) and augmented TGF-β secreting neutrophils in the bronchial mucosa. These two data suggested that the TGF-β-producing neutrophils destroy airway epithelium and worsen asthma phenotype.

Neutrophil-derived proteases could damage the airway and worsen asthma symptoms. One is neutrophil elastase (NE), which is stored in neutrophil azurophilic granules and is released when neutrophils are activated by exposure to cigarette smoke or inflammation. Suzuki et al. showed that when healthy rodents inhale human NE, they display increased immune cell infiltration, epithelial cell damage, and airway constriction. Moreover, patients with neutrophilic asthma exhibit high systemic expression of the elastase gene ELA2. In addition, mice with OVA-induced asthma exhibit attenuated AHR, airway immune cell recruitment, and type 2 inflammation when an NE inhibitor is administered prior to the OVA challenge. Another neutrophil-derived protease is matrix metallopeptidase (MMP)-9. It is secreted when neutrophils physically encounter allergens in the presence of IgE. Moreover, patients who have severe asthma despite receiving high-dose glucocorticoids display higher MMP9 levels in their bronchoalveolar lavage fluid (BALF) than healthy controls or steroid-untreated patients with moderate or mild asthma. Notably, the BALF MMP9 levels in the cohort correlate with BALF neutrophil counts. Moreover, in vitro analyses showed that steroids do not reduce MMP production by BALF neutrophils. These findings suggest that further studies on neutrophils may reveal mechanisms that can be targeted to control non-allergic and severe asthma.

**Neutrophils in COPD**

COPD is characterized by irreversible airflow obstruction with a persistent cough, shortness of breath, and a decline in lung function. Since it has a deleterious effect on patient quality of life and is a leading cause of death, it is increasingly posing a significant social and economic burden. Smoking is a major risk factor for COPD, followed by air pollutants and occupational chemicals. Exposure to these agents induces local and systemic inflammation and subsequent pulmonary symptoms. Although bronchodilators can alleviate COPD symptoms, they cannot arrest or ameliorate the underlying disease. Therefore, the development of new therapeutic targets is urgently needed for COPD.

Compared to other CRDs, COPD is associated with consistently higher neutrophilic inflammation (Fig. 1). Indeed, neutrophils are the most abundant cells in the sputum or BALF.
of patients with both stable and exacerbated COPD. Moreover, neutrophil numbers in sputum correlate with the severity of airflow obstruction in COPD. Like in asthma, NE plays an important pathogenic role in COPD. First, by proteolyzing lung tissues, NE is a major cause of alveolar enlargement and destruction. Secondly, NE stimulates airway epithelial cells to express MUC5AC, thereby inducing hyperplasia of secretory cells and mucin production. Thirdly, NE activates macrophages, releasing active MMPs that induce lung destruction and emphysema. The macrophages also produce proinflammatory cytokines that trigger inflammation. Notably, neutrophils also secrete MMPs in COPD. In particular, neutrophils in the induced sputum of COPD patients display elevated levels of MMP8 and MMP9 that are associated with COPD exacerbations. Moreover, smoking (a major risk factor for COPD) increases the expression of MMP9 and MMP12 in lung neutrophils in mice. Smoking also has the same effect on neutrophils in the induced sputum of COPD patients compared to healthy non-smokers or smokers. The role of NETs in COPD is currently unclear: while some studies showed that high NET levels in the sputum of patients with COPD are associated with more frequent exacerbations, another observed that patients with COPD exhibit reduced NET formation. The latter study also suggested that this low NET formation is associated with higher frequencies of exacerbations due to defective bacterial clearance.

**Neutrophils in IPF**

IPF is a progressive respiratory disease with a poor prognosis. Unlike the other CRDs, the contribution of neutrophils to the pathogenesis of IPF and their clinical significance remains controversial. Nonetheless, several clinical investigations suggest neutrophil-mediated inflammation plays an essential role in the onset, progression, and acute exacerbation of IPF, as follows (Fig. 1). First, the lungs and sputum of IPF patients have higher numbers of neutrophils than the specimens from healthy controls. Secondly, compared to healthy controls, IPF patients have significantly higher levels of interleukin (IL)-8 in BALF. IL-8 is a potent chemokine that attracts neutrophils to sites of inflammation. Significantly, the numbers of neutrophils in the BALF from IPF patients also correlate with BALF IL-8 levels. Thirdly, compared to patients with other fibrotic interstitial pneumonia, IPF patients contain elevated levels of the calcium-binding S100 protein in their serum and BALF. S100A9 is mainly produced by neutrophils and macrophages. Notably, the serum and BALF levels of S100A9 in the IPF patients correlated positively with the neutrophil counts in BALF. Finally, patients with acute exacerbation of IPF demonstrate abnormally high serum NE levels.

Multiple lines of evidence also suggest that neutrophil-secreted factors contribute to the fibrotic microenvironment in IPF, which is characterized by the formation of myofibroblasts that deposit collagen in the lung interstitial spaces. First, neutrophil-derived NETs induce fibroblasts to differentiate into myofibroblasts and are critical for developing bleomycin-induced lung fibrosis, an animal model of IPF. Secondly, a murine study showed that NE in the lung is upregulated during the onset of IPF and contributes to the pathogenesis of IPF by remodeling the extracellular matrix (ECM). Thirdly, neutrophil MMPs also remodel the ECM in the early stage of IPF and lung injury, thereby promoting disease exacerbation. Fourthly, several studies suggest that neutrophil-derived leukotrienes (LTs) stimulate the infiltration of other immune cells into the lung, thereby boosting IPF progression. Specifically, patients with IPF have significantly higher levels of LTC4 and LTB4 in BALF compared to non-asthmatic controls. Similarly, mice with bleomycin-induced fibrosis have higher LTB4 levels in BALF than vehicle-treated control mice. Moreover, LTC4 transgenic mice develop more severe IPF than wild-type mice. Interestingly, a recent study showed that senescent
lungs and fibroblasts could also secrete LTs, which induces naïve fibroblasts to synthesize collagen, thereby promoting lung fibrosis. Finally, neutrophil-derived cytokines may contribute to fibrosis in different ways depending on the stage of pulmonary fibrosis. Thus, in the early inflammatory phase, the infiltrating neutrophils may injure the lung by secreting proinflammatory cytokines such as IL-1β, tumor necrosis factor (TNF)α, IL-6, and IL-8. Conversely, neutrophils can secrete profibrotic mediators during the chronic fibrotic stage. Regarding this, TGF-β shifts the neutrophil phenotype to N2-type, increasing its profibrotic functions. This is significant because TGF-β is a potent pro-fibrotic mediator produced by various immune cells (especially macrophages) and strongly promotes pulmonary fibrosis.

CROSSTALK BETWEEN NEUTROPHILS AND IMMUNE CELLS IN THE AIRWAYS

As described above, neutrophils contribute to CRDs in many ways, particularly by secreting molecules that drive the pathogenic activities of other immune cells. Therefore, it is important to understand the interactions between neutrophils and other immune cells in the lungs since this will allow us to identify the cellular/molecular mechanisms that drive CRDs. This information may point to molecules that can be targeted for therapy. Here, we will discuss how neutrophils interact with other innate immune cells.

Interactions between neutrophils and macrophages

In the lungs, macrophages act as the foremost immune barrier against foreign particles or infection. When they encounter cigarette smoke, pollutants, or microorganisms, they become activated and secrete neutrophil chemoattractants, including CXCL1, CXCL2, IL-1α, and CCL2. They also promote neutrophil infiltration by releasing LTB₄. Moreover, they secrete various cytokines such as IL-1, IL-6, and IL-13 that regulate neutrophil functions. In addition, they produce granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF), which can increase the lifespan of neutrophils in the inflammatory site (Fig. 2).

In turn, the recruited neutrophils become activated and secrete interferon (IFN)-γ and macrophage inflammatory protein (MIP)-1α and MIP-1β, which recruit circulating monocytes to the site of inflammation. Neutrophils also produce granule proteins such as cathelicidin-related antimicrobial peptides, LL-37, azurocidin, cathepsin G (CTSG), and proteinases that increase the inflammatory responses of macrophages. The NE from neutrophils also causes macrophages to produce MMPs such as MMP2, MMP9, and MMP14. In addition, NE acts synergistically with lipopolysaccharide to induce macrophages to produce the inflammatory cytokines IL-8, IL-1β, and TNF-α. Neutrophils also shape the microbicidal capability and polarization of macrophages. Thus, when neutrophils undergo apoptosis, they release azurocidin and lactoferrin, which are taken up by macrophages. This enhances both the microbicidal activity and the M1 polarization of macrophages. Similarly, Soehnlein et al. showed that neutrophil granule proteins trigger the release by macrophages of TNF-α and IFN-γ, which then act in an autocrine loop to promote macrophage phagocytosis. Notably, there are reports that neutrophils can induce the opposite polarization of macrophages towards the M2 phenotype. This may be mediated by neutrophil microvesicles bearing annexin A1 and phosphatidylserine. Moreover, when M2 macrophages are co-cultured with NETs, their secretion of proinflammatory cytokines rises; by contrast, NET-treated M1 macrophages undergo cell death.
Thus, resident macrophages recruit neutrophils to the site of inflammation, after which the neutrophils recruit new macrophages. The two cell types then regulate each other’s functions. In particular, neutrophil-secreted granule proteins and NETs promote macrophage efferocytosis, pathogen phagocytosis, and the resolution of inflammation. This close reciprocal relationship warrants further investigation into macrophage-neutrophil interactions in chronic inflammation.

**Interactions between neutrophils and dendritic cells**

Dendritic cells (DCs) are professional antigen-presenting cells that can initiate T-cell and B-cell responses. Numerous studies have shown that neutrophil-DC interactions can modulate adaptive immune responses. Thus, when DCs are co-cultured with human neutrophils, their expression of CD86 and HLA-DR is upregulated via cell contact-dependent mechanisms and promotes antigen-specific T-cell responses. Moreover, neutrophils that express macrophage antigen-1 (Mac-1) can bind to the C-type lectin receptor DC-SIGN.
on DCs, thereby generating mature DCs that trigger strong T-cell proliferation. This DC-maturing effect of neutrophils is also mediated by their production of TNF-α. Moreover, neutrophils shape DC migration from sites of infection to the lymph nodes, where they activate T cells. Specifically, when neutrophils in Mycobacterium tuberculosis (Mt)-infected mice are depleted, DCs are more likely to be infected rather than acquiring Mt antigens by ingesting infected neutrophils. This downregulates DC migration to the mediastinal lymph nodes and delays the activation and proliferation of antigen-specific CD4+ T cells.

Neutrophils also promote DC migration to inflamed sites by releasing the chemokines CCL3, CCL5, and CCL20. This may be mediated by the effect of environmental agents on neutrophils. For example, when peripheral blood neutrophils from smokers with or without COPD and healthy non-smokers were cultured with lipopolysaccharide or organic dust, they all produced the DC chemokine CCL3. However, while monoclonal antibody (mAb)-mediated neutralization of the TNF-α produced by the neutrophils blocked neutrophil production of CCL3 in healthy smokers and non-smokers, it was less effective in smokers with COPD. This suggests that while inflammatory responses or foreign molecules can activate neutrophils to recruit DCs into the lungs, the COPD environment may hamper this DC-recruiting function of neutrophils. Neutrophils may also promote DC recruitment by releasing proteases such as CTSG and NE that process the inactive precursors of DC chemoattractants (e.g., chemerin) into their highly active forms. In addition, neutrophils can activate DCs. One mechanism involves the secretion of the granule proteins α-defensins, cathelicidins, and lactoferrin, which bind to their corresponding receptors on the DC surface. Another mechanism involves NETs: a study with an in vivo model of emphysema showed that cigarette smoke promotes NET formation and that these NETs activate plasmacytoid DCs (pDCs) to produce IFN-α, a type I IFN. This, in turn, promotes the maturation and antigen presentation of the pDCs, which then strongly activate Th1 and Th17 differentiation. Notably, IFN-α from pDCs also stimulates neutrophils to form NETs. Thus, neutrophils and DCs may reciprocally augment each other’s functions, potentially creating vicious circles. There is also evidence that, conversely, DCs promote neutrophil recruitment: Kim et al. showed with a murine model of emphysema that DCs and monocytes recruited to the lungs promote emphysema by secreting serum amyloid A, which has chemotactic activity and induces neutrophil recruitment.

Thus, while there is clear evidence that neutrophils can regulate DCs and adaptive immune responses, there is also some evidence of the converse relationship. Further investigations into neutrophil-DC relationships in the context of chronic inflammation may prove fruitful in identifying therapeutic targets.

### Interactions between neutrophils and ILCs

ILCs are innate immune cells ubiquitously distributed throughout various tissues but are enriched at the mucosal and barrier surfaces. ILCs were first identified in 2010 and have now been categorized as ILC1, ILC2, and ILC3 based on their hallmark cytokines and transcription factors.

The ILC1 group, characterized by high production of IFN-γ and TNF-α, includes natural killer (NK) cells. There is evidence that neutrophils and NK cells can shape each other’s functions during bacterial infection. Thus, in Legionella pneumophila infection, neutrophils release IL-18, which is essential for the MyD88-mediated production of IFN-γ by NK cells and the clearance of the bacteria. However, in Mt infection, the IFN-γ produced by NK cells suppresses the influx of neutrophils into the lungs, thereby inhibiting host defense against the bacterium.
Also, inflammatory cytokines from ILC1s could induce myeloid cells (including neutrophils) to produce MMPs, disrupting the epithelial barrier and rendering it vulnerable to the ingress of infectious agents and their products.

The ILC2 group produces GM-CSF (which induces neutrophil differentiation) and the type 2 cytokines IL-4, IL-5, IL-13, and IL-9. Several lines of evidence suggest that ILC2s can regulate neutrophils. First, ILC2s may be elicited soon after severe traumatic injuries since such injuries are associated with a rapid elevation in circulating IL-4, IL-5, and IL-13 levels. Xu et al. showed recently that patients with multiple injury trauma also quickly exhibit high serum levels of IL-33, an alarmin released after barrier integrity has been breached and is a known stimulator of ILC2s. The authors then showed with a mouse model of hemorrhagic shock and tissue trauma that post-traumatic IL-33 induces ILC2s to produce IL-5 and that this, in turn, causes neutrophils to produce more IL-5. This ultimately results in early lung injury, a common consequence of polytrauma. Secondly, when a cigarette smoke-induced COPD model is conducted in mice that lack ILC2s, the neutrophil numbers in the lung are lower. This effect results in significantly greater collagen deposition in the airways compared to wild-type mice. Thirdly, ILC2s in the bone marrow help hematopoietic and progenitor cells to recover from 5-fluorouracil-induced stress by producing GM-CSF, which induces the differentiation of myeloid cells (including neutrophils). This role of ILC2s is mediated by IL-33 produced by the stressed progenitor cells. Finally, Zhang et al. showed that lung ILC2s differ from ILC2s in other tissues by expressing neuropilin-1 (NRP-1), a transmembrane glycoprotein that is upregulated by TGF-β. The authors then observed that knocking out NRP-1 in the bleomycin-induced model of lung fibrosis suppresses IL-5 and IL-13 production by lung ILC2s and protects the mice from lung fibrosis. Importantly, they observed that NRP-1 knockout reduces neutrophil recruitment to the lung. Since neutrophils are potent producers of TGF-β, these findings suggest that 1) ILC2s can induce neutrophil recruitment, and 2) conversely, neutrophils can promote profibrotic ILC2 functions in the lungs. Interestingly, a recent study by Patel et al. asked why neutrophil depletion worsens airway damage in an HDM-induced model of allergic inflammation. They then found that the upregulation of G-CSF, induced by neutropenia, directly increases ILC2 production of IL-5 and IL-13. The latter study shows how specific mechanisms that regulate either ILC2s or neutrophils can also shape functions of other cells.

The group 3 ILCs also express GM-CSF along with the Th17-associated cytokines IL-17A, IL-17F, and IL-22. Several studies showed that ILC3s regulate neutrophils and vice versa. First, ILC3s are the most abundant lung ILC subset in COPD, and in COPD patients with emphysema, their numbers in the lung correlate significantly with lung neutrophil frequencies. Notably, both ILC3 and neutrophil frequencies in the lung also correlate with decreased lung function. These correlations between ILC3 and neutrophil numbers reflect the fact that IL-17 produced by ILC3s is a key driver of neutrophil recruitment to the lung in COPD. Moreover, ILC3s can induce neutrophil migration and survival by secreting the neutrophil chemoattractant CXCL8 and GM-CSF, respectively. Also, analyses of the murine model of emphysema showed that neutrophil-derived IL-1 causes ILC3s to proliferate and produce IL-17A and that the IL-17A from ILC3s, in turn, induces the neutrophils to produce MMP12, which generates emphysema. Thus, ILC3s and neutrophils can cross-regulate each other in CRDs.

Given that ILCs are tissue-resident cells and there is considerable crosstalk and cross-regulation between ILCs and neutrophils, it is possible that novel therapeutics could prevent the onset of adaptive immune activation in CRDs by altering pathogenic ILC3-neutrophil interactions and thereby reprogramming the local cytokine milieu.
TARGETING NEUTROPHILS IN CHRONIC RESPIRATORY DISEASES

Since neutrophils participate in the initiation and aggravation of chronic lung inflammation, many preclinical studies have examined the therapeutic possibilities of targeting neutrophils. Neutrophilic proteases are key mediators of tissue damage and inflammation in CRDs, therefore, it has been suggested that proteases from neutrophils may be a suitable target for asthma and COPD. A potential neutrophil protease inhibitor may be α1-antitrypsin (A1AT), which is a protease inhibitor that is endogenously synthesized in the liver and released into the bloodstream. Clinical studies suggest that A1AT deficiency is associated with asthma-like phenotypes and that A1AT-deficient patients are more susceptible to asthma. Therefore, augmenting A1AT production may be a potent therapy for relieving neutrophil-mediated lung inflammation. Indeed, when patients with severe congenital A1AT deficiency are infused with A1AT, their emphysema improves (as measured by computed tomography). Other possibilities are Alvelestat (AZD9886) and Sivelestat, which are chemical inhibitors of NE. However, their clinical effects on COPD remain to be investigated.

Other targets are the MMPs, which also play a key role in the tissue damage in CRDs. They also have endogenous inhibitors, namely, the tissue inhibitors of metalloproteinases (TIMPs). However, clinical trials targeting TIMPs have not yet been conducted. Another possibility is AZD1236, which selectively inhibits MMP9 and MMP12. A short-term trial indicated that it is safe in patients with COPD but may lack therapeutic efficacy. Yet another candidate is FP-025, an inhibitor of MMP-12. It is currently being tested for efficacy in asthma and COPD by a phase 2 trial (NCT03858686).

Neutrophil chemokines or chemokine receptors may also be candidate therapeutic targets for CRDs. One is CXCR2, which is critical for the influx of neutrophils into inflamed tissues. A study with a murine model of asthma showed that the CXCR2 antagonist ladarixin reduced acute and chronic neutrophil influx and attenuated AHR. Moreover, administration of ladarixin decreased bleomycin-induced lung fibrosis and significantly increased survival rates in mice with cigarette smoke-induced exacerbation of influenza A infection. Several clinical trials have also shown that CXCR2 antagonists have potential in CRDs. Thus, the CXCR1/2 antagonist MK-7123 effectively improves lung function in patients with COPD, and this is associated with reduced neutrophil counts in sputum and lower plasma MMP9 levels. Similarly, oral treatment with the CXCR2 antagonist AZD5069 reduces blood neutrophil counts in COPD patients without serious adverse effects. A phase 2 clinical trial (NCT00688467) evaluating the efficacy of MK-7123 in mild asthma has been conducted. However, the results have not yet been published. Finally, there are two phase-2 trials on the ability of BIIL 284 to treat COPD (NCT02249338 and NCT02249247). It is an LTB4 receptor antagonist that blocks neutrophil infiltration. The results of these studies have also not been published to date.

CONCLUSIONS

This review explored the role of neutrophils in various inflammatory lung diseases. The evidence to date suggests that neutrophils not only mediate acute lung inflammation caused by acute infection but also play essential roles in chronic inflammatory diseases such as...
asthma, COPD, and IPF. These roles involve neutrophil-derived proteases, cytokines, and NET formation, which induce airway epithelial cell remodeling and hyperplasia. Moreover, neutrophils can exacerbate CRD severity by interacting with many different immune cells (here, we mainly dealt with innate immune cells, namely, macrophages, DCs, and ILCs). Thus, these interactions and cross-communication may be novel therapeutic targets, especially because neutrophils contribute to the etiology of chronic inflammatory settings via similar mechanisms; this suggests that the neutrophil-targeting treatment strategies that work in one disease may also be similarly beneficial in other chronic diseases.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea (2022R1A2C3007730 and SRC 2017R1A5A1014560) and the Cooperative Research Program of Basic Medical Science and Clinical Science from Seoul National University College of Medicine (800-2021288).

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