Granulocyte-targeted therapies for airway diseases

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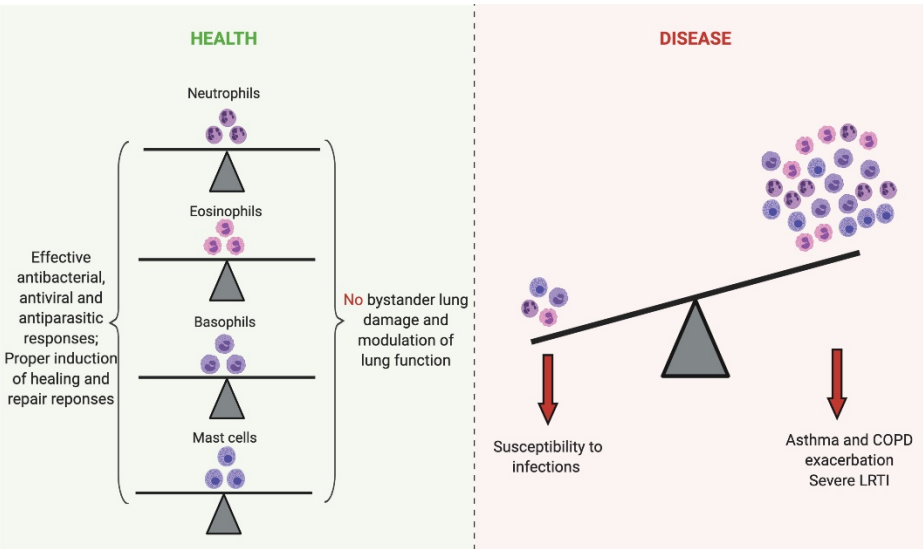
Table of contents:

Abstract

The average respiration rate for an adult is 12-20 breaths per minute, which constantly exposes the lungs to allergens and harmful particles. As a result, respiratory diseases, which includes asthma, chronic obstructive pulmonary disease (COPD) and acute lower respiratory tract infections (LTRI), are a major cause of death worldwide. Although asthma, COPD and LTRI are distinctly different diseases with separate mechanisms of disease progression, they do share a common feature – airway inflammation with intense recruitment and activation of granulocytes and mast cells. Neutrophils, eosinophils, basophils, and mast cells are crucial players in host defense against pathogens and maintenance of lung homeostasis. Upon contact with harmful particles, part of the pulmonary defense mechanism is to recruit these cells into the airways. Despite their protective nature, overactivation or accumulation of granulocytes and mast cells in the lungs results in unwanted chronic airway inflammation and damage. As such, understanding the bright and the dark side of these leukocytes in lung physiology paves the way for the development of therapies targeting this important mechanism of disease. Here we discuss the role of granulocytes in respiratory diseases and summarize therapeutic strategies focused on granulocyte recruitment and activation in the lungs.



Introduction

The lungs are constantly exposed to chemicals, particles, allergens and microorganisms from the external environment, resulting in their susceptibility to infections and injury. Respiratory diseases are known to be one of the leading causes of death worldwide, accounting for more than 10% of all disability-adjusted life-years (DALYs) – an estimate of the amount of active life lost due to a disease [1]. Common respiratory disorders can be grouped into allergic inflammation (e.g. asthma), destructive pathologies, such as chronic obstructive pulmonary disease (COPD) and acute lower respiratory infections. Among the chronic respiratory pathological conditions, COPD, asthma and pulmonary fibrosis still lack efficient treatment. Moreover, despite the availability of vaccines and antibiotics, acute lower respiratory tract infections are still the sixth leading cause of death among all diseases, and the first cause of death in children under the age of five [2]. Therefore, understanding the pathophysiology of these respiratory tract diseases is crucial for the development of new therapeutic strategies to decrease or prevent disease burden.

Inflammation is a common feature of several respiratory diseases. Of note, the influx of granulocytes – neutrophils, eosinophils, basophils – and mast cells activation are important to control proliferation of pathogens and induce tissue repair programs favoring the re-establishment of pulmonary homeostasis. On the other hand, the overactivation or the excessive recruitment of granulocytes in the lungs could lead to severe injury that may exacerbate the disease or worsen its prognosis (Figure 1). In this regard, several pharmacological approaches targeting the recruitment and/or the function of granulocytes have been suggested and, in many cases, implemented in the management of respiratory diseases.

Basophils, eosinophils and mast cells are classically considered as harmful components in allergies and asthma [3-5]. However, these cells also play important physiological roles in the coordination of defense responses against parasitic infections, tissue repair, tumor control,

angiogenesis, among others [6-9]. On the other hand, neutrophils are crucial to control bacterial and fungal infections but may also lead to tissue damage once dysregulated [10, 11]. Therefore, a challenging question remains: are granulocytes friends or foes of the inflammatory process? Here, we summarize the role of granulocytes and mast cells in asthma, COPD and acute respiratory tract infections and discuss the available and novel therapies targeting basophils, neutrophils, mast cells and eosinophils to control these lung diseases.

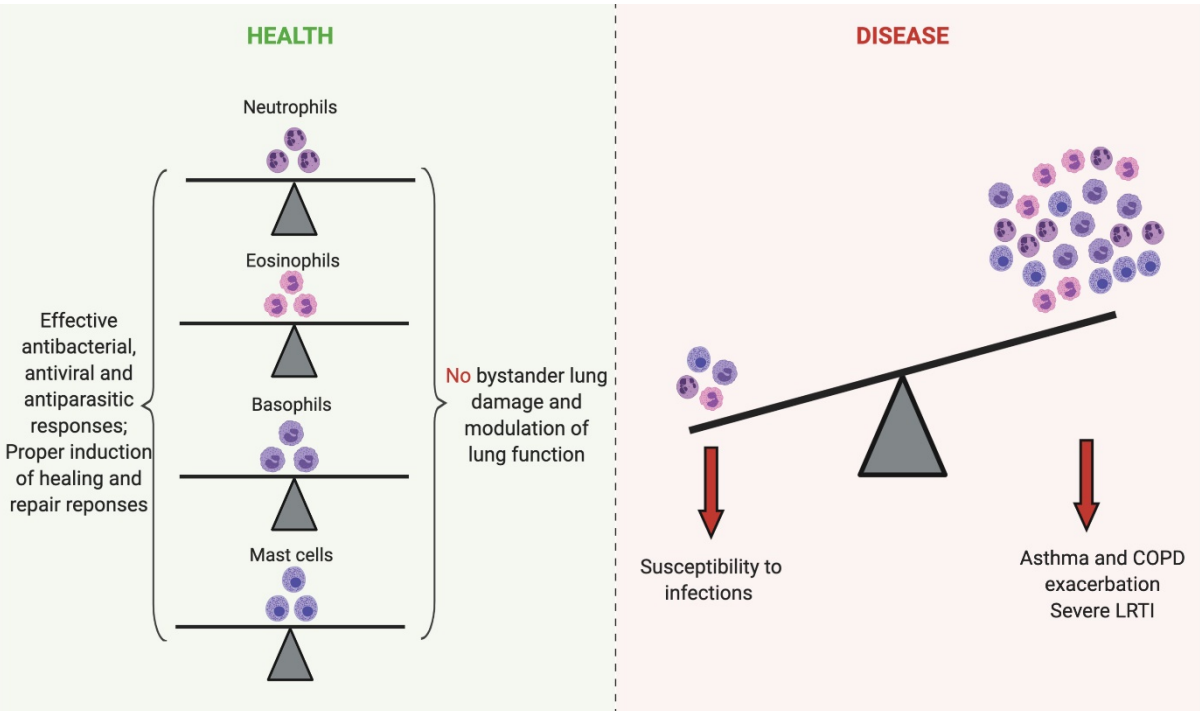


Figure 1 – Granulocytes in health and disease. Neutrophils, eosinophils, basophils and mast cells are crucial for the maintenance of lung health by preventing potential infections and inducing repair responses when needed. However, overactivation or recruitment of these cells can induce increased lung injury and bronchoconstriction, which aggravates and exacerbates respiratory diseases such as asthma, COPD, and lower respiratory tract infections. (Created with Biorender.com ®).

Types of granulocytes and their functions

In general, granulocytes are defined as immune cells that have specialized granules in the cytoplasm and traditionally include neutrophils, eosinophils, and basophils [12]. Mast cells

are also granule-containing immune cells, yet being tissue resident cells, they are not typically classified as granulocytes [13].

The granular content of each type of granulocyte is related to different cell functions and is used for the classification of these cells by light microscopy using cytochemical staining methods. Among others, various enzymes, inflammatory mediators and antimicrobial peptides are packed in the cytoplasmic granules of granulocytes and are released upon cell activation.

Granulocytes and mast cells are produced in the bone marrow through the process of haematopoiesis [12, 13]. The bone marrow provides suitable niches for the production of the pluripotent and self-renewed hematopoietic stem cells (HSCs) that give rise to the lymphoid or myeloid multipotent progenitors (MPPs) [14, 15]. MPPs eventually generate the lymphoid or myeloid lineages that include, among other cell types, lymphocytes and granulocytes, respectively [14]. Of interest, although the lineage origin of mast cells is still debated, mature mast cells arise from mast cell progenitors that circulate as agranular cells and enter the tissues where they complete their development into specific subsets of long-lived differentiated mast cells [15, 16].

The proliferation and maturation of granulocytes in the bone marrow requires approximately 7-12 days before their release into the bloodstream (circulating leukocytes) and their homing to different tissues (resident leukocytes), including the lungs [17, 18]. Resident eosinophils, neutrophils, basophils and mast cells, present in the lung mucosa in physiological conditions, are important for the mucosal immune surveillance and the maintenance of organ homeostasis [6, 7, 19]. A given insult in the lungs, such as infection, injury and allergens, induces the recruitment of mature granulocytes from the circulation through different chemokine gradients depending on the nature of the stimulus [20]. Upon activation, resident mast cells and granulocytes can also secrete cytokines and chemokines, thus enhancing the accumulation of leukocytes into the airways [21]. Different stimuli are detected by innate

immune receptors in pulmonary cells (e.g. pattern recognition receptors – PRRs) and trigger inflammatory signaling cascades that eventually result in the production of distinct repertoires of chemokines. Receptors on the surface of eosinophils, neutrophils and basophils can recognize specific chemokines mediating the recruitment of specific subsets of granulocytes in response to a given stimulus (Figure 2) [20]. Activated granulocytes and/or mast cells in the lungs mediate defense responses against invaders but can also contribute to immunopathology.

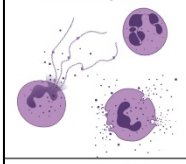
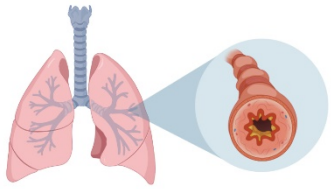
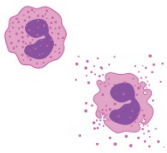
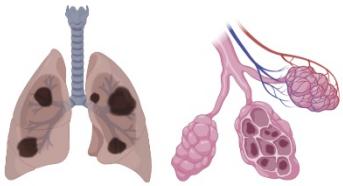
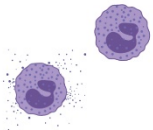
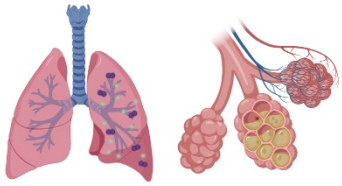
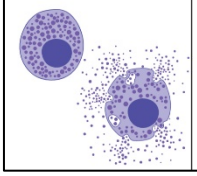
Cell type	Chemokine receptors	Mechanism of disease	Disease
Neutrophils 	**CXCR1 and CXCR2** CCR1, CCR2, CCR3, CCR5, CXCR3, and CXCR4	Overproduction of proteases, Neutrophil extracellular traps, proinflammatory cytokines and ROS ↑ Lung damage and edema	Asthma 
Eosinophils 	**CCR1 and CCR3** CXCR2 and CXCR4	Secretion of proteases, induction of type 2 responses, histamine and leukotrienes release, ↑ Mucus hypersecretion, bronchoconstriction and lung damage	COPD 
Basophils 	**CCR2 and CCR3** CCR1, CCR5, CXCR1 and CXCR4	Activation of inflammatory macrophages, induction of type 2 responses, release of histamine and leukotrienes ↑ Mucus hypersecretion, bronchoconstriction and lung damage	Pneumonia 
Mast cells 	CXCR1, CXCR2, CXCR3, CXCR4, CX3CR1, CCR1, CCR3, CCR4, CCR5	Release of histamine, prostaglandins, leukotrienes, TNF-α, IL-6, IL-8 and Th2 cytokines ↑ Mucus hypersecretion, bronchoconstriction and lung damage	

Figure 2 – Granulocyte Mechanisms of Disease. Neutrophil activation and degranulation lead to the release of proteases, antimicrobial peptides, peroxidases, cytokines, and ROS that increase lung edema and damage lung epithelial and endothelial cells. Eosinophil, basophil and mast cells activation magnify type 2 responses while the secretion of proteases, histamine, leukotrienes and prostaglandins increase mucus secretion, bronchoconstriction and damage to the epithelial cells. (Created with Biorender.com ®).

Neutrophils

Neutrophils are the most abundant granulocytes in the blood and rapidly respond to viral, bacterial and fungal infections [20]. Morphologically, human neutrophils have a multilobe nucleus and numerous, rather heterogeneous, cytoplasmic granules that are small and stain light-pink or purple-blue following treatment with chemically neutral dyes. The granular content of mature neutrophils include a number of antimicrobial peptides, myeloperoxidase (MPO), the serine proteases proteinase 3 (PR3), cathepsin G, neutrophil elastase (NE), the enzymatic inactive protease cationic antimicrobial protein of 37 kd (CAP37, aka azurocidin) and the neutrophil serine protease 4 (NSP4), cysteine proteases, lactoferrin, lipocalin, metalloproteinases and gelatinase [22]. In health, circulating neutrophils infiltrate the lungs, in a diurnal pattern coordinating physiological surveillance responses [10] and regulating the numbers of alveolar macrophages [19].

The recognition of a given inflammatory stimulus by resident alveolar macrophages and pulmonary parenchyma cells lead to the production of chemokines (e.g. CXCL1, CXCL2, CXCL8), cytokines (e.g. IL-6, TNF-α) and other pro-inflammatory mediators (e.g. leukotriene $(LT)B_4$) that promote a significant and rapid recruitment of neutrophils into the lungs [23]. Neutrophil inflammatory chemokine receptors are classically CXCR1 and CXCR2 [24], however, infiltrating neutrophils can increase expression of other receptors such as CCR1, CCR2, CCR3, CCR5, CXCR3, and CXCR4 [25]. Gradients of proinflammatory mediators increase neutrophil stiffness and expression of adhesion molecules, leading to their consequent retention in pulmonary capillary beds and subsequent transmigration to the sites of infection/injury in the lungs [26]. Activated neutrophils degranulate and release antimicrobial peptides and other preformed mediators that mediate pathogen clearance at the site of infection. Additionally, neutrophils can kill potential pathogens by the extrusion of neutrophil extracellular traps (NETs) and by phagocytosis [20]. Therefore, neutrophils are considered the

first line of defence against infections in the lungs [23]. Besides their classical described functions, neutrophils were recently shown to mediate tissue repair [27] and anti-tumour responses [28].

In contrast, the permanence and overactivation of neutrophils in the lungs can cause injury, pulmonary dysfunction and even death [29, 30]. Several airway diseases including COPD, bronchiolitis, pneumonia, asthma and acute lung injury are characterized by neutrophil infiltration of the airway wall [31]. Both clinical and experimental data have shown an association between neutrophil numbers and the severity and progression of these airway diseases [32-34]. This dual "face" of neutrophils is further evidenced by the different phenotypes of these cells (N1 *versus* N2) that can either promote or inhibit lung cancer [35]. In view of the opposing roles of neutrophils in airway diseases, a finely tuned response is ideal to promote an efficient clearance of potential invaders while preventing immune mediated lung injury.

Eosinophils

Eosinophils are not as abundant as neutrophils in the blood and are characterized by basic granules that are stained in pink or orange with the classical cytology dyes such as eosin. The granular content of eosinophils includes cationic proteins such as eosinophil cationic protein (ECP, aka RNase3), eosinophil-derived neurotoxin (EDN, also known as RNase2), major basic protein (MBP), eosinophil peroxidase (EPO), hydrolytic enzymes and a diverse repertoire of preformed cytokines, chemokines, and growth factors [36]. Eosinophils are recruited into the lungs mostly in response to IL-5, eotaxin or histamine production [37, 38]. Moreover, IL-5 is crucial for the production, maturation and survival of eosinophils [39]. Among the inflammatory chemokine receptors, CCR1 and CCR3 are highly expressed in eosinophils [24]. Historically, eosinophils are thought to promote defences against parasitic infections, through the release of their cytoplasmic granular content. However, some studies

have shown that eosinophils can also enhance antiviral responses [40, 41] and have immunomodulatory functions [42].

The "dark" side of eosinophils is evidenced by their role in asthma. Eosinophil recruitment and activation in the lungs are associated with asthma severity and, therefore, are targets for the development of therapeutic strategies [43]. Because of the contrasting roles of eosinophils in homeostasis and disease, it has been suggested that different phenotypes are associated to distinct contexts. Indeed, the lungs contain a morphological and functional distinct population of resident eosinophils that are important regulators of the T helper (Th) 2 responses during asthma, in contrast to the inflammatory recruited eosinophils [6]. Thus, new eosinophil-directed therapies are expected to consider targeting selected eosinophil phenotypes that are associated with disease development, rather than with their protective functions.

Basophils

Basophils are easily recognized by their numerous metachromatic-stained granules. Basic pigments such as methylene and toluidine blue stain basophil granules dark purple, in contrast to the blue colour seen with mast cells granules [44]. Basophils constitute a relatively rare population in the bloodstream, thus making them difficult to isolate and study [45]. As such, the immunological roles of basophils have been neglected in comparison to other leukocytes. Basophil recruitment is mainly associated with the activation of CCR2 and CCR3 by inflammatory CCL chemokines (CCL2, CCL5, CCL7, CCL8, CCL11 CCL12, CCL13) [24]. Based on their granular content, basophils share some functions with mast cells. Histamine, chondroitin sulphate, proteolytic enzymes, cysteinyl leukotrienes (cysLTs), PAF and cytokines are released upon basophil activation, and similarly to mast cells, they are related to the pathophysiology of allergies and asthma [46, 47]. Moreover, basophils can act as antigen presenting cells and may induce Th2 responses, thus contributing to both the host defence against helminth parasites and chronic allergic inflammation [48].

Interestingly, basophils were also recently shown to regulate alveolar macrophage function and development through the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) [7]. While the physiological actions of basophils are poorly described, their contribution to airway diseases, such as asthma, has been relatively more widely explored. Together with mast cells, activated basophils contribute to type 2 inflammation by secretion of cytokines such as IL-5, IL4, IL-13 and thymic stromal lymphopoietin (TSLP), among others [44]. However, basophils can be distinguished from mast cells by not only their morphological characteristics, but also by their tissue distribution, lifespan and ontogeny. In contrast to mast cells, basophils complete their maturation in the bone marrow and are short-lived cells in the circulation (2-3 days), being recruited to the lungs during inflammation [44]. Yet, new approaches of cell isolation and signalling mapping are required in order to shed light on the function of basophils in both health and disease.

Mast cells

Mast cells are highly granulated, mononucleated cells developed from the CD34+ progenitors. Once expanded in the bone marrow, these progenitors circulate in the bloodstream to become tissue resident cells. In the tissues mast cell progenitors mature under the influence of cytokines, especially by stem cell factor (SCF), the high affinity ligand of the cKit [49]. Mast cells are mostly known to be activated by the immunoglobulin (Ig) E-dependent pathway. However, they can also be stimulated by several IgE-independent triggers that activate different surface receptors, such as toll-like receptors (TLR), G protein-coupled receptors (GPCRs), complement receptors, lectin receptors and the recently identified human Mas-related G-protein coupled receptor member X2 (MGRX2) [50]. Mast cell chemokine receptors such as CXCR1–4, CX3CR1, CCR1, CCR3–5 are important for mast cell activation [51]. Upon activation, mast cells degranulate and release highly bioactive mediators that are grouped into three main categories: the granule-associated pre-formed mediators, such as tryptase, chymase,

histamine, heparin and TNF-α; the *de-novo* synthesized lipid mediators such as LTC_4, D_4, E_4 and B_4, platelet activating factor (PAF) and prostaglandin (PG)D_2; and last but not least, an array of cytokines and chemokines, including IL-6, IL-8, IL-4, IL-5, IL-10 and eotaxin, among others [52]. While preformed and the lipid mediators are released within minutes, cytokines and chemokines are released several hours after mast cell activation, during the late phase of the response. Notably, depending on a specific stimulus, mast cells can release all kinds of mediator, or they may differentially release certain kinds of mediators [53].

Human mast cells are typically distinguished by their protease content, being referred to as tryptase-containing (MC_T), chymase-containing (MC_C) and tryptase/chymase-containing (MC_{TC}) mast cells [52]. In addition, there is strong evidence of further mast cell heterogeneity with respect to their receptors, mediators and their consequent functional tissue specificity. For instance, lung mast cells express high levels of TLRs, while skin mast cells exhibit low levels of this class of receptors [54]. Moreover, anti-IgE-mediated activation was recently shown to cause higher release of LTC_4 and PGD_2 from isolated lung mast cells, compared to their skin, heart and synovial cavity counterparts [55]. On the other hand, substance P, a MRGPRX2 agonist, failed to induce lipid mediator production from lung mast cells and caused no histamine and tryptase release from both lung and heart mast cells, whereas it induced a significant concentration-dependent release of these mediators from skin mast cells [55]. In the lungs, the main population of mast cells is of the MC_T type, which is located mainly in the bronchial and bronchiolar lamina propria [54, 56]. Mast cells have also been shown to be activated by several respiratory pathogens, such as *Mycobacterium tuberculosis*, *Mycoplasma pneumonia*, influenza virus and the respiratory syncytial virus (RSV) [57, 58] and were recently shown to act as antigen presenting cells [59]. Thus, in the lungs, mast cells play a dual role: hey contribute to allergic asthma when activated mainly by IgE-mechanisms, and they also seem to be sentinel cells against different lung pathogens.

Granulocytes in Airway Diseases

Asthma

Asthma is a chronic inflammatory disease of the airways, affecting over 235 million people worldwide [60, 61] and classically characterized by inflammation, mucus hypersecretion, airway hyper-responsiveness (AHR) and airway remodeling [62]. This heterogeneous chronic disorder is orchestrated by various inflammatory cells and cellular components to mount diverse clinical phenotypes and complex underlying endotypes [63]. Granulocytes play a major role in the development of asthma, and distinct granulocyte populations seem to be associated with particular phenotypes and endotypes. Thus, the inflammatory and immune processes in the allergic phenotype are linked to the recruitment of eosinophils into the airways, a cardinal feature of the Th2 response in the lung [64]. On the other hand, there is a sub-population of asthmatic patients that are considered as non-Th2 immunologic responsive, where neutrophils are the main inflammatory cells involved in the pathogenesis. Additional phenotypes/endotypes of asthma include Th1 skewed responses, Th17-high inflammation, obesity– and smoking-associated asthma, exercise-induced bronchoconstriction, and the very late-onset asthma that is associated with the decreased function of the ageing lung [44, 63].

Allergic asthma is commonly initiated by an inappropriate immune response towards inhaled allergens [65], such as house dust mite, spores, pollens, animal dander, etc. [66]. The exposure of allergens in the airways can activate receptors on the airway epithelium, such as TLRs and PRRs, to initiate allergic responses [67]. This results in the capture and processing of these antigens by dendritic cells in the basement membrane of the airway epithelium, where they mature and migrate to lymph nodes to present the processed antigen to naïve $CD4^+$ T cells [68, 69]. The presence of IL-4 drives the differentiation of naïve $CD4^+$ T cells into Th2 cells. Transmigration of eosinophils into the airway involves a cascade of signaling pathways, where

302 the priming and activation of Th2 cells releases cytokines, including IL-5. Besides mediating
303 eosinophil maturation, migration and survival [39], IL-5, in combination with chemokines like
304 eotaxin, adhesion molecules, such as ICAM-1, VCAM-1, E-selectin and the integrin VLA-4,
305 induces eosinophilia in the airways [70, 71]. In fact, the pivotal role of IL-5 in asthma has been
306 evidenced in earlier studies in IL-5-deficient mice showing reduced eosinophil trafficking into
307 the airways and decreased AHR [72]. When activated, eosinophils secrete cytotoxic granule
308 proteins like MBP and EPO, which result in the damage of airway epithelial cells and induce
309 histamine release from mast cells and basophils [73]. Besides eliciting damage to the airways
310 directly through the production of granular proteins, studies have shown that eosinophils are
311 the source of LTC_4, which, following conversion to LTD_4 and LTE_4, is involved in the AHR,
312 mucus hypersecretion and bronchoconstriction in asthma [74].
313 Mast cells and basophils are also involved in the pathogenesis of asthma [48]. The
314 cytokines IL-4 and IL-13, generated mostly from Th2 cells, induce B cells to undergo isotype
315 class switching from IgM to IgE [75]. The interaction between Th2 and B cells (through CD40
316 – CD40L signaling) activates B cells to produce IgE antibodies into the bloodstream, which
317 bind to the high affinity IgE receptors (FcεRI) on the surface of mast cells and basophils [76].
318 When the lungs are re-exposed to the allergen, the resulting IgE-allergen complexes lead to
319 FcεRI cross-linking, thus triggering mast cell activation and degranulation [76]. The release of
320 pre-formed histamine and TNF-α and the newly-formed arachidonic acid metabolites are
321 largely responsible for the early symptoms of the asthmatic reaction including
322 bronchoconstriction [5, 77]. Mast cells also have an active role in the late phase reaction that
323 is mediated via the release of cytokines and chemokines [78]. Another type of histamine-
324 producing cell would be the basophil, although it is not as prominent as mast cells in the IgE-
325 driven responses that is characteristic of allergic asthma [79]. Basophils produce cytokines IL-
326 4 and IL-13 that contribute to the shift towards Th2 inflammation in asthma [80]. While there

is evidence showing that the lifespan of basophil can be extended by the binding of IgE to the FcεRI [81], the full extent of basophil contribution to innate immunity and asthma remains to be elucidated.

Neutrophils are also known to play a role in the pathogenesis of asthma. Minimal infiltration of neutrophils is observed in the airways of patients with mild-to-moderate asthma, but it is noticeable in the airways of patients with severe asthma and acute asthma exacerbations [82, 83]. Neutrophils can attract eosinophils through IL-8 (or *via* CXCL1/2 in mice), and induce eosinophil degranulation by secreting neutrophilic lactoferrin and elastase [84]. In addition, augmented levels of IL-17 were shown to correlate with increased neutrophil recruitment and disease severity in asthma patients [85]. Matrix metalloproteinase (MMP)-9 is primarily produced by neutrophils and also promotes eosinophil migration and airway remodeling [86]. Dendritic cell presentation requires MMP-9 for antigen uptake in the airway; therefore, MMP-9 knockout mice display a reduction in allergic airway inflammation [87].

Granulocyte-targeted therapies for Asthma:

Corticosteroids (CS) are the gold standard in asthma therapy, and although they are effective at abating airway eosinophilia, it has been consistently shown that they are ineffective against neutrophilic inflammation [88, 89]. Furthermore, neutrophilic asthma is generally seen in patients treated with CS, as these drugs can decrease apoptosis of neutrophils and potentially contribute to neutrophil activation [64]. Of note, different studies have evaluated the effect of macrolides, anti-IL17 therapy and CXCR1/2 antagonism in reducing neutrophil recruitment in asthma [90-92]. However, no significant improvement was shown, and further research is needed to evaluate the effect of these treatment strategies in different asthma subpopulations.

Current therapies of asthma can be grouped into those targeting allergic asthma (high serum IgE and atopy), eosinophilic asthma (exacerbations, sputum eosinophils and steroid-dependent asthma), neutrophilic asthma (sputum neutrophils, steroid-resistant and/or non-Th2

phenotype) and chronic airflow obstruction (low lung function and high serum periostin) [93]. Inhaled selective β_2-agonists are the most common medication that provides rapid relief of asthma symptoms [94]. They are effective bronchodilators, but they are unable to suppress the ongoing airway inflammation. Short-acting β_2-agonists (SABA) provide short-term relief (onset of action in 5 min, duration 4-6 h), while long-acting β_2-agonists (LABA) deliver a longer (more than 12 h) bronchodilation [62]. The reduction of AHR by LABA without abating the airway inflammation, leads to false perception of controlling the disease, and result in uncontrolled progression of the inflammatory process [95].

For controlling airway inflammation and preventing damage/remodeling of the airways, inhaled corticosteroids (ICS) are the first line of treatment and, in combination with LABA, they are regarded as the gold standard in the management of asthma. ICS reduce inflammation-associated leukocyte infiltration into the airways and suppress airway inflammation, thus leading to asthma relief and more effective management of the disease [62, 96]. Although ICS suppress airway inflammation, they do not cure the disease. Another major drawback is the development of resistance against ICS in a subset of patients, which necessitates the use of higher doses, and, eventually, oral CS administration is needed to systemically suppress the uncontrolled inflammation [62, 97, 98]. Prolonged oral CS treatment is not ideal due to their numerous side effects, including water retention, lipid and cortisol metabolism dysfunction, cataracts, glaucoma, osteoporosis and increased risk of opportunistic infections [62, 96]. It is of note that approximately 10% of the asthmatic patients respond poorly or do not respond to CS at all, accounting for about 50% of the total healthcare cost in managing asthma [99].

An alternative approach to reduce bronchoconstriction and eosinophilia is the use of leukotriene receptor antagonists, such as montelukast and pranlukast. They bind to $CysLT_1$ receptors expressed on the airway smooth muscle and block the action of LTC_4, LTD_4 and

LTE_4, resulting in bronchodilation and reduction of circulating eosinophils in the blood [100]. While $CysLT_1$ receptor antagonists display clinical improvements in asthmatic symptoms and lung function, they are less effective than ICS. However, they are still in use, as they are orally effective, have less unwanted side effects than CS, and provide an alternative treatment for patients who are resistant to CS [101]. Furthermore, it was recently shown that the activation of the epithelial P2Y receptors can induce bronchoconstriction, whereas montelukast acts as an antagonist for the $P2Y_6$ receptor, suggesting an additional potential action of montelukast in asthma [102].

Rather than broadly suppressing systemic inflammation, recent asthma therapies adopt newer targeted approaches and use therapeutic monoclonal antibodies (mAbs) to manage the disease [103]. The first-in-class biologic intended for the treatment of persistent allergic asthma in patients with high serum IgE and mast cell levels is the IgE-neutralizing humanized mAb omalizumab, which was approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in 2003 and 2005, respectively [99]. In addition to its binding to the portion of IgE that interacts with FcεRI on the surface of mast cells and basophils, omalizumab can down-regulate the expression of FcεRI, thus moderating IgE-mediated responses in asthma [62].

For targeted therapies against eosinophilic asthma, mepolizumab and reslizumab are mAbs that target IL-5 to reduce eosinophilia. Both antibodies neutralize circulating IL-5 and inhibit its binding to the IL-5 receptor (IL-5R), thus resulting in decreased blood eosinophils to be trafficked to the lungs. They were initially shown to be ineffective in clinical trials of unselected asthma patients but they proved effective against placebo when patients with severe eosinophilic asthma were selected [104, 105]. Both drugs were approved by the FDA and EMA in 2015 and 2016, respectively. In addition, instead of targeting IL-5 itself, benralizumab is a humanized mAb that binds to the IL-5Rα subunit on human eosinophils and basophils and

blocks ligand-independent IL-5R signaling. It also acts via antibody-dependent cell-mediated cytotoxicity (ADCC) and consequently depletes IL-5Rα-expressing cells [106]. Several Phase IIb and III clinical trials had shown the clinical efficacy of benralizumab in reducing exacerbations and controlling severe asthma [107, 108], prior to its approval by the FDA in 2017 and by the EMA in 2018 for the treatment of patients with severe eosinophilic asthma.

Besides IL-5, the cytokines IL-4 and IL-13 are also important drivers of Th2-mediated inflammation and B-cell differentiation, as well as supporting the recruitment of eosinophils and inducing airway bronchoconstriction [75]. It was hypothesized that targeting IL-4 and/or IL-13 would benefit the downregulation of type 2 inflammation, eosinophils trafficking, and AHR [109, 110].While both IL-4 and IL-13 can activate the heterodimeric IL-4R complexes, IL-4 preferentially binds to the IL-4Rα subunit and regulates Th2 function and IgE class switching, whereas IL-13 induces chemokines for the recruitment of eosinophils into the lungs [76, 93]. The anti-IL-4 mAb pascolizumab showed some promise in preclinical studies [111], but it was not further developed due to lack of clinical benefit in a pilot study in patients with symptomatic steroid-naive asthma (ClinicalTrials.gov Identifier: NCT00024544). In addition, pitrakinra (Aerovant) is a recombinant human IL-4 variant that binds to the IL-4Rα subunit and acts as a dual IL-4/IL-13 antagonist, it was initially reported to lack efficacy in a clinical trial that recruited not fully controlled asthmatic patients with ICS and LABA (ClinicalTrials.gov Identifier: NCT00801853). Yet, further analysis revealed that pitrakinra was able to reduce asthma exacerbations in selected trial participants with eosinophilic asthma (ClinicalTrials.gov Identifier: NCT00801853). Similarly, the fully human anti-IL-4Rα mAb AMG 317 also failed to demonstrate clinical efficacy across the entire group of patients with moderate to severe asthma in a Phase II clinical trial [112]. On the other hand, dupilumab, a human mAb targeting the IL-4Rα subunit, blocking both IL-4 and IL-13 pathways, and approved since 2017 for the treatment of moderate-to-severe atopic dermatitis, has been shown to reduce exacerbations and

to improve lung function in patients with uncontrolled asthma [113]. Being effective in asthmatic patients that had withdrawn ICS and LABA [114], dupilumab was approved by FDA and EMA in 2018 and 2019, respectively as add-on maintenance therapy for patients with severe uncontrolled asthma characterized by raised blood eosinophils.

Considering the activation of the heterodimeric type II IL-4R complexes by IL-13, this cytokine is also a candidate target for the development of treatment options for chronic airway disease [93]. However, lebrikizumab, a human monoclonal antibody against IL-13, did not significantly improve lung function in patients with severe uncontrolled asthma [115]. Interestingly, patients with higher serum levels of the matricellular protein periostin that may be implicated in asthma pathophysiology, responded better to lebrikizumab than the patients with low periostin levels [115]. The drug was eventually discontinued when another phase III trial displayed lack of efficacy in reducing asthma exacerbations in patients. Lebrikizumab was repositioned by being granted Fast Track designation by the FDA in 2019 for the treatment of atopic dermatitis. Tralokinumab, another anti-IL-13 mAb, also showed inconsistent effects on asthma exacerbation rate in patients with severe, uncontrolled asthma [116] and, similarly to lebrikizumab, was recently been reported to benefit patients with moderate-to-severe atopic dermatitis in a phase III clinical trial [117].

The sustained granulocytic inflammation in asthma suggests that part of asthma pathobiology may be related to an impairment of resolution of inflammation [118]. Resolution of inflammation is an active process coordinated by specialized pro-resolving mediators (SPMs) that leads to termination of inflammation [119]. SPMs mainly act through binding to receptors in cell surface. Of note, eosinophils, neutrophils, mast cells and lung epithelial cells express SPM receptors being able to produce and respond to these molecules [120]. A proper resolution of inflammation assures the reduction of secretion of pro-inflammatory mediators and recruitment of granulocytes while increases clearance of apoptotic cells and induces tissue

repair responses [119]. Severity of asthma has been correlated with reduction in the levels of SPMs in bronchoalveolar lavage fluid of patients [121] and treatment strategies that induce resolution have been protective in several preclinical studies by decreasing eosinophil counts in the lungs and preventing degranulation of mast cells [121-123].

Evidently, the outcomes of both preclinical and clinical investigations point to the urgent need to appropriately identify the various phenotypes and endotypes of asthma that may guide the development of more beneficial targeted therapeutic interventions.

Chronic obstructive pulmonary disease (COPD)

COPD is a chronic inflammatory lung disease with persistent airflow obstruction that is presented clinically as emphysema, obstructive bronchitis, exacerbation and lung function decline [124]. COPD continues to be a leading cause of morbidity and mortality worldwide, with an estimated prevalence of 328 million people already being the third leading cause of death worldwide [125]. Neutrophils are the major contributor to the pathogenesis of COPD in which heightened airway neutrophilia is observed in patients, correlating very well with disease severity [32]. This could in part be due to the proteases released by neutrophils that can cause host damage and lead to emphysema [126]. Smoking can release both GM-CSF and granulocyte colony-stimulating factor (G-CSF) from epithelial cells and macrophages, which can stimulate granulocyte production, release and survival [127]. Neutrophils in the peripheral blood bind to endothelial cells via E-selectin and are drawn to the airway by neutrophil chemoattractants, such as CXC ligand-1 (CXCL1), CXCL5, CXCL8 and LTB_4 [124]. Secreted granule proteins and serine proteases from neutrophils contribute towards alveolar destruction, inflammation and oxidative stress. Cathepsin G, NE and PR3 have also been reported to contribute towards mucus hypersecretion [128, 129]. NETs secreted by activated neutrophils, contain histones, NE and MPO, and are found to be excessive in COPD patients, contributing to disease progression [130].

Granulocyte-targeted therapies for COPD:

Targeting neutrophilic inflammation in COPD is a potential treatment for the disease. Tyrphostin AG825, an inhibitor of the ErbB family of receptor tyrosine kinases (RTKs), was shown to prevent GM-CSF-mediated survival of isolated neutrophils from the blood of COPD patients. This was further validated in mice nebulized with lipopolysaccharide (LPS) and immediately given Tyrphostin AG825 via i.p., in which Tyrphostin AG825 increased percentage of neutrophil apoptosis [131]. Matrine, a bioactive component of *Sophora flavescens* Ait (Kushen), was administered daily via gavage in mice exposed to 4 days of cigarette smoke. Matrine was observed to reduce lung neutrophilia and NE activity, mainly through the apoptosis of neutrophils [132]. AZD8999, a novel muscarinic acetylcholine receptor antagonist and β2-adrenoceptor agonist (MABA), was shown to inhibit IL-8, IL-1β and MMP-9 release from human peripheral blood neutrophils stimulated with LPS [133]. AZD5069, a chemokine (C-X-C motif) receptor 2 (CXCR2) antagonist, was shown to reduce NET formation and levels of IL-8/CXCL-8 in sputum and blood neutrophils isolated from COPD patients [134]. Galectin (Gal)-9, a beta-galactoside lectin protein, was able to reduce the number of neutrophils and the levels of MMP-2 and MMP-9 in the bronchoalveolar lavage fluid (BALF) in a porcine pancreatic elastase (PPE)-induced emphysema model. Gal-9 was also able to inhibit the chemotactic activity of neutrophils *in vitro* [135].

Another candidate for neutrophil-targeted therapy may be IL-26, which is a neutrophil mobilizing cytokine that is increased in BALF of COPD patients and long term smokers [136]. In addition, an alternative treatment strategy may be the use of AZD7986, a competitive and reversible inhibitor of dipeptidyl peptidase 1 (DPP1), which was shown to inhibit whole blood NE activity in healthy volunteers after once-daily dosing for 21 – 28 days with no serious side effects [137]. Nanomedicine is also opening new avenues for cell-targeted drug treatments

[138]. PEGylated immune-conjugated poly(lactic-co-glycolic acid) (PLGA)-nanoparticle (PINP) have been used to deliver drugs specifically to neutrophils [139].

As mentioned above, neutrophils play a major role in microbial killing [20, 23], and inhibiting their function may leave the patient susceptible to future infections. Most of the novel therapies highlighted above for neutrophilic inflammation in COPD involve either the apoptosis of neutrophils or inhibition of chemotactic factors modulating neutrophil migration.

Although COPD is mainly observed to have neutrophilic inflammation, eosinophilic inflammation is enhanced during periods of severe exacerbations. Therefore, the eosinophil is a potential target for managing COPD exacerbation [140]. Increased numbers of eosinophils have been reported in the airway of COPD patients, with 40% of them having eosinophilic airway inflammation, predicting a more favorable response towards bronchodilators and CS therapy [141]. Heightened level of blood eosinophil is also associated to the risk of COPD exacerbation, increased mortality or decreased lung function [141]. The increased eosinophils in COPD could be recruited by IL-5 and type 2 innate lymphoid cells (ILC2), in which ILC2 is recruited by epithelial mediators (e.g. IL-33) [127].

Generally, established anti-eosinophilic therapy using mAbs against IL-5 or IL-5Rα are safe, albeit an increased susceptibility to helminthic infection [142]. Benralizumab, was tested in patients with eosinophilic COPD (ClinicalTrials.gov Identifier: NCT01227278) administered every 4 weeks (Q4W) (weeks 1, 4 and 8), and then Q8W (weeks 16, 24, 32, 40 and 48). It was observed that genes related to basophil or eosinophil, such as the serine protease PRSS33 were downregulated by benralizumab [143]. SB010, a GATA3-specific DNAzyme, was tested in COPD patients with sputum eosinophilia (DRKS00006087), in which patients inhaled SB010 for 28 days. SB010 was observed to decrease sputum eosinophil and IL-5 whilst increasing blood IFN-γ [144]. In general, anti-neutrophilic and anti-eosinophilic therapies are highly relevant to COPD and will play a major role in alleviating disease burden.

Mast cells increase numbers can be observed in COPD patients with centrilobular emphysema, and most likely contribute to airway hyperresponsiveness [127]. Moreover, disease severity has been found to be correlated with increased numbers of MC_T in the sputum and serum of COPD patients. However, a recent publication has shown that MC_{TC} numbers are increasing in COPD patients and linked this class of mast cells to improved lungs functions (reviewed in [145]).

Basophils currently play an obscure role in COPD, however a recent study showed that basophil-derived IL-4 plays a crucial role in the initiation of emphysema in a murine COPD model, driving the production of MMP-12 by interstitial macrophages [146]. This highlights that basophils may play a bigger role in COPD and this is worth further investigation.

Acute Lower respiratory tract Infections

For several years, acute lower respiratory tract infections (LRTI) have been one the main causes of death worldwide [2]. According to the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD), pneumonia and bronchiolitis are considered as LTRI, which caused nearly 2.38 million deaths in 2016 [1]. The broad spectrum of pathogens that can cause LTRI –bacteria, viruses and/or fungi- in addition to the crescent antibiotic resistance rates, create challenges for the diagnosis, prevention and treatment of these diseases [147]. Moreover, the pathogenesis of respiratory infections involves a complex interplay between pathogen virulence factors and the host defense responses. Treatment guidelines for LRTI vary among countries, but in general, the recommendation is the use of different pharmacological classes of antimicrobial agents depending on the etiology and severity [147]. Of note, in severe cases of LRTI, antibiotic therapy does not reduce mortality as the exuberant inflammatory responses triggered in the lungs, rather than pathogen proliferation, lead to severe lung injury and respiratory failure [148]. Therefore, the immune responses triggered by infection must be efficient, yet regulated, to assure proper pathogen clearance and minimum tissue damage.

Inflammation is a common feature of LTRI. Potential pathogens that evade the physical barriers of the respiratory tract and reach the lungs, will be detected by immune and non-immune resident cells via PRRs that bind to microbial associated molecular patterns (MAMPs) [149]. Different families of PRRs, such as TLRs, nucleotide-binding and oligomerization-domain proteins, and caspase-recruitment domain helicases recognize several MAMPs of any one microbe. Activation of signaling pathways by PRRs converge to the transcription of several pro-inflammatory genes as adhesion molecules, chemokines and other cytokines. The production of pro-phlogistic mediators culminates with the recruitment of leukocytes into the airways, specially neutrophils [150]. The recruitment of these cells into the airways occurs rapidly, and is mediated by the chemokine gradient produced by activated lung epithelial cells, macrophages and dendritic cells [33]. At the site of infection, neutrophils mediate important host defense functions such as phagocytosis of microorganisms, production of reactive oxygen species (ROS), antimicrobial peptides and proteases, and extrusion of NETs. The crucial role of neutrophils is evidenced by the higher susceptibility of patients to lung infections with deficits in neutrophil quantity (neutropenia) or defects in function (chronic granulomatous disease) [151, 152]. Furthermore, neutrophils were shown to be important for the development of adaptive immune responses following viral lung infections [153].

Besides the crucial role of neutrophils conferring protection against pulmonary infections, the exacerbated activation or prolonged recruitment of these cells into the airways may also be detrimental as it can damage the lungs, resulting in lung injury [154]. There are several microorganisms that had evolved strategies to neutralize the neutrophil responses in the lungs by evading phagocytosis, or blocking/cleaving proteases, antimicrobial peptides and ROS [11]. The vast accumulation of activated neutrophils is often associated with a worse prognosis during severe LTRI [33].

Highly virulent influenza A virus (IAV) infections lead to massive recruitment and activation of neutrophils in the airways [155, 156]. In this context, instead of being protective, neutrophils are the main inducers of lung injury through the release of tissue-destructive proteases, cytokines and ROS. In other viral lung infections, such as the one caused by RSV, neutrophils are related to severe bronchiolitis, tissue injury and correlated with disease severity [157, 158]. On the other hand, the depletion of neutrophils before or during the early-stages of an ongoing viral infection, leads to increased proliferation of the virus which contributes to higher morbidity and mortality [159]. The complex, often contrasting functions of neutrophils during respiratory viral infections are also highlighted by their putative dual contribution in the pulmonary inflammatory responses in rats infected with the rat coronavirus (rCoV)[160], as well as by the identification of the high ratio of neutrophils to lymphocytes as an independent risk factor for severe pneumonia during the coronavirus disease 2019 (COVID-19) pandemic [161, 162]. Indeed, the neutrophilic infiltration and increased secretion of pro-inflammatory cytokines has been shown to contribute with the severity of COVID-19 demanding efforts to reduce inflammation in the advanced stage of disease [163].

Therefore, a timely regulated neutrophilic response must be achieved to confer proper clearance of virus without being harmful to the host. In order to assess whether modulating neutrophilic inflammation during viral lung infections would improve disease outcome, several preclinical studies have been done [164]. Treatment strategies that decrease, rather than block, the recruitment of neutrophils shows interesting potential in controlling lung viral infections [33]. Similarly, bacterial and fungal pulmonary infections are often associated with a pronounced recruitment of neutrophils. The accumulation and over activation of these cells impair the gaseous exchange in the lungs and increase cell wall disruption [165]. Interestingly, the unregulated inflammatory responses in the lungs can result in bacteria overgrowth and

599 dissemination [166, 167]. The production of proteases and cytokines by neutrophils contributes
600 to the lung epithelial barrier disruption that results in bacteremia [168].
601 *Granulocyte-targeted therapies for LRTI:*
602 Drugs that are used to impact neutrophil function or numbers in inflammatory diseases,
603 such as the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors
604 (statins), CSs and macrolides, are considered potential adjunctive therapy strategy targeting the
605 inflammatory exacerbations in severe pulmonary infections [169-171]. However, associative
606 and clinical studies evaluating the impact of these drugs are not consistent, as larger number of
607 patients is required for clearer results. Based on their pleiotropic anti-inflammatory and
608 immunomodulatory effects, HMG-CoA reductase inhibitors were tested as potential therapies
609 for severe pneumonia [172, 173]. In experimental models of pulmonary infection, statins were
610 shown to diminish lung injury, neutrophilic inflammation and improve survival, with greater
611 response when combined with antibiotics [174]. However, numerous retrospective clinical
612 studies have obtained conflicting results while evaluating the impact of statins on respiratory
613 tract infections. While most of these studies observed some beneficial effects of statins on
614 pneumonia severity [175-178], others found no significant disease improvement in the treated
615 group against placebo [179]. Of note, while the reduced recruitment of neutrophils into the
616 lungs after treatment with statins might decrease post-infection lung injury [180], changes in
617 neutrophil function, rather than numbers, were shown to be crucial for the beneficial role of
618 statins during pneumonia. A recent clinical trial showed that treatment of pneumonia patients
619 with HMG-CoA reductase inhibitors was protective by improving neutrophil function and
620 chemotaxis [181]. Unfortunately, it is unclear whether the protective effects observed would
621 be seen in other populations (such as young adults, or patients taking statins chronically). In
622 addition, the treatment schedules, route of administrations and the potential interactions of
623 drugs used to treat pneumonia (such as clarithromycin) are of concern [182].

Similar controversies have been shown when using CSs to treat severe LRTI patients. Current clinical data suggest a potential beneficial effect in the management of severe pneumonia characterized by a "hyper inflammatory" state [183-187]. The mechanisms underlying this protective role of CSs as adjunctive therapy for pneumonia and bronchiolitis include the decreased production of pro-inflammatory cytokines and the consequent decreased recruitment of neutrophils [188]. Nevertheless, there is no consensus in the dosage and type of CSs therapy among different clinical trials, and the induction of immunosuppression by steroids might worsen, instead of treating LRTI.

Recently, new therapeutic strategies directly targeting the recruitment of neutrophils have been carried out. Drugs developed to block the receptor CXCR2 which serves as a binding site for neutrophil-related chemokines, had been tested to treat pulmonary infections caused by influenza and *S. pneumoniae* [189-191], those included danirixin (GSK1325756) and DF2162. Preclinical studies have shown that inhibition of CXCR2 during influenza infection reduced neutrophil numbers in the airways, and prevented lung injury and mortality, with additive effects when combined with antiviral drugs [191, 192]. Phase II clinical studies were recently completed, and safety was not compromised since the viral clearance was not impaired. However, the assessment of treatment efficacy in reducing the severity of disease outcomes was limited due to the small number of patients evaluated (ClinicalTrials.gov Identifier: NCT02927431) [193].

Ideally, therapeutic strategies for the exacerbations of the neutrophilic responses during LRTI should target the harmful potentials of neutrophils while maintaining or enhancing their capacity to kill invading pathogens. Targeting specific neutrophil functions, rather than completely blocking their recruitment, might yield some benefit. In this regard, inhibiting NET release [194] or neutrophil serine proteases [195] have been shown to attenuate the deleterious role of inflammation during pneumonia. In addition, induction of resolution of inflammation

649 has been pointed as a remarkably interesting strategy to reduce disease severity during
650 pneumonia [119]. One pivotal feature of immunoresolvents is enhancing antimicrobial
651 responses while modulating neutrophilic inflammation, therefore, the harmful
652 immunosuppression caused by anti-inflammatory drugs is prevented [196]. Annexin A1 and
653 SPMs such as lipoxins and resolvins have been shown to be protective during viral and bacterial
654 pneumonia reducing neutrophil recruitment and activation while preventing lung damage [197-
655 200]. Of interest, part of the immunomodulatory effects of statins might be related to SPM
656 induction [201]. In order to effectively translate these findings to new therapeutics
657 development, proof of concept studies in humans are necessary.

658 In addition to neutrophils, other granulocytes also play a role in the defense responses
659 during LRTI. Eosinophils can be recruited into the airways during viral infections and elicit a
660 protective response against IAV [41], RSV [202], parainfluenza [203], rhinovirus [204] and
661 the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; COVID-19) [205]. Among
662 the different eosinophil granule proteins, ribonucleic acid degrading enzymes (RNases) have
663 antiviral properties and, together with the production of nitric oxide (NO) from eosinophils,
664 can contribute to virus clearance in the lungs [206, 207]. This protective role also extends for
665 bacterial infections: eosinophils can recognize, ingest and kill the bacteria [208].

666 Eosinophil can extrude DNA extracellular traps to entrap bacteria and other
667 extracellular pathogens [209]. However, their exact functions during an *in vivo* bacterial
668 infection in the lungs are yet to be elucidated. Besides their roles in host defenses, eosinophils
669 can also be detrimental during LRTI induced by RSV. The heightened eosinophilia observed
670 in the lungs of RSV infected patients is correlated with both airway obstructions and severity
671 of the disease. In this aspect, therapies targeting eosinophils are proposed to control airway
672 hyperresponsiveness post-RSV infection [210]. Such strategies intend to control asthma
673 symptoms that may arise after infection and were discussed before. Because of the limited

knowledge on the contribution of eosinophils to disease development in most viral and bacterial lung infections, this granulocyte has not been considered as potential therapeutic target for the majority of LRTI.

A similar antimicrobial role is played by mast cells [145] and basophils [211]. Mast cells express several PRRs that enable their activation following viral or bacterial infections in the lungs [145]. The antimicrobial peptides of mast cells granules enhanced the killing of *Klebsiella pneumoniae* [212] and *Streptococcus pneumoniae* [213]. Interestingly, mast cells can indirectly execute phagocytosis of bacteria by secreting cytokines such as TNF-α and IL-6, which recruit neutrophils and macrophages during pulmonary infections [145]. Moreover, human peripheral blood-derived mast cells have been shown to be activated to release type 1 interferons by several viruses including the influenza virus [214]. Conversely, it was also shown that activation of mast cells may be linked to the immunopathology observed during IAV infection [215]. Considering the limited information of mast cells involvement in LTRI, more studies that assess mast cells role in severe pulmonary infections are needed.

Although many studies attempted to describe strategies to modulate granulocytic activation and function during LRTI, there is an unmet medical need for the development of therapeutic approaches that would promote effective pathogens clearance while minimizing unwanted bystander lung injury. The timing of administration, dosage and possible side effects are also parts to be discussed and evaluated carefully through clinical studies. By focusing on the host immune responses, new strategies to treat pulmonary infections may prevent microbial resistance while targeting a broader spectrum of pathogens.

Perspectives and Conclusion

The early description of granulocytes dates back to the 18th century [216], and since then, there is much interest in understanding granulocytes functions and development. The different granule content of these immune cells is strongly related to their diverse role in host

699 defense processes, where proteases, histamine and antimicrobial agents are released after
700 activation of granulocytes; mediators that are important in killing the potential pathogens and
701 induce repair mechanisms at the site of injury. However, exaggerated recruitment or activation
702 of these granulocytes, and their consequent degranulation, leads to amplification of the
703 inflammatory response and bystander tissue injury (Figure 2). Therefore, the knowledge
704 gathered regarding the role of granulocytes in health and disease has led to the development of
705 therapeutic strategies that target specific features of granulocyte biology.

706 The enormous mucosal surface of the lungs allows an efficient gas exchange which is
707 impaired by the intense recruitment of granulocytes that fill the alveoli during disease [165].
708 Allergens, pollutants, or potential pathogens are recognized by the resident pulmonary cells
709 (e.g. epithelial cells and macrophages) and can initiate a significant inflammatory response
710 that, if uncontrolled, leads to tissue damage and respiratory failure. Therapeutics targeting
711 granulocyte recruitment and function are powerful strategies in controlling different respiratory
712 diseases (Table 1). It is important to account the unique aspects of the pathophysiology of each
713 respiratory disease during drug development. Of note, the immunosuppression of patients when
714 treated with CSs increases susceptibility to secondary infections and impair tissue repair [217,
715 218], but may be protective in some specific contexts [219, 220]. There are different
716 phenotypes of granulocytes that might be related to distinct pathological conditions or may not
717 even be associated to the induction of disease. Thus, the effectiveness of any drug might rely
718 on the responses of a given phenotype to that particular intervention and does not guarantee
719 the same results in other inflammatory diseases.

720 The future directions of granulocyte-targeted therapies for airway diseases lie in the
721 development of drugs that act on specific functions or selected phenotypes of granulocytes,
722 while not affecting the resident granulocytic populations responsible for host defenses.
723

724

Table 1- Granulocyte-targeted therapies for respiratory diseases

Disease	Treatment	Cell target	Evidence
Asthma	Corticosteroids	Eosinophils/ Neutrophils	Approved [62,96]
	Leukotriene receptor antagonist	Eosinophils	Approved [100,102]
	IgE-neutralizing humanized Abs	Eosinophils Basophils Mast cells	Approved [62,99]
	IL-5/IL-5R neutralizing humanized Abs	Eosinophils	Approved [104-108]
	IL-4R neutralizing humanized Abs	Eosinophils	Preclinical/ Clinical studies [113,114]
	IL-13 neutralizing humanized Abs	Eosinophils	Clinical studies [115-117]
	CXCR2 antagonist	Neutrophils	Clinical studies [91]
	Human anti-IL-17R monoclonal antibody	Neutrophils	Clinical studies [92]
	Macrolides	Neutrophils	Clinical studies [90]
COPD	Inhibitor of the ErbB family of receptor tyrosine kinases	Neutrophils	Preclinical studies [131]
	Matrine	Neutrophils	Preclinical studies [132]
	CXCR2 antagonist	Neutrophils	Clinical studies [134]
	Dipeptidyl Peptidase 1 Inhibitor	Neutrophils	Preclinical [137]
	IL-5R neutralizing humanized Abs	Eosinophils	Clinical studies [142]
	GATA3-specifc DNAzyme	Eosinophils	Clinical studies [143]
Acute lower respiratory tract infections	HMG-CoA reductase inhibitors	Neutrophils	Preclinical/ Clinical studies [172-180]
	Corticosteroids	Neutrophils	Preclinical/ Clinical studies [182-187]
	Macrolides	Neutrophils	Preclinical/ Clinical studies [169-170]
	CXCR2 antagonist	Neutrophils	Preclinical/ Clinical studies [188-192]
	Leukotriene receptor antagonist	Eosinophils	Clinical studies [209]

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