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5.30 Novel Immunomodulatory Therapies for Respiratory Pathologies

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5.30.1 Introduction

The evolutionary role of the immune system is to defend the host against potential invaders (Kaufman, 2010). Several components of the mammalian immune system are indeed very ancient in evolutionary terms. Phagocytosis, for example, is a defense feature present in both invertebrates and vertebrates that was conserved during evolution (Buchmann, 2014). The ability of discriminating between self and non-self and neutralizing potential infections is crucial for the survival of every organism (Nicholson, 2016). In mammalians, the innate and adaptive immune responses are coordinated to ensure an effective defense against infections while...
contributes to other physiological events such as tissue repair and development (Buchmann, 2014). Therefore, malfunction of the immune system is related to several diseases.

Inflammation was one of the first “diseases” identified in ancient medicine and now has been considered a unifying component of several pathologies (Tavares et al., 2020b; Hunter, 2012; Parnes, 2008). In disease, inflammatory responses can be exaggerated, altered, or misplaced leading to tissue damage, symptoms and eventually death (Sousa et al., 2020b). However, inflammation is essential for health, protecting the host against infections and promoting tissue healing and adaptive responses (Iwasaki and Medzhitov, 2015). Several cellular and molecular controllers have been identified to finely tune inflammation in the body. Mucosal surfaces, such as the lungs, are in constant contact with a myriad of harmful particles and microorganisms from the environment. To keep up with the pulmonary physiological functioning, the immune system must encounter and clear infections while maintaining a high threshold of activation to prevent induction of unnecessary inflammation what can impair gas exchange, the primordial pulmonary function (Allard et al., 2018; Schleimer et al., 2007). Dysregulation of inflammatory responses in the lungs is associated with severity of chronic diseases such as asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis, and acute diseases such as pneumonia (Tavares et al., 2020b). Recognizing the endogenous circuits of inflammation and its regulation has paved the way for the development of therapeutics for respiratory pathologies.

The respiratory system is commonly divided into upper and lower respiratory tracts. The upper respiratory tract comprises the nasal cavity, mouth, pharynx, and larynx (voice box), whereas the lower respiratory tract includes the trachea, bronchi and the lungs, that are further subdivided into bronchioles and alveoli (Person and Mintz, 2005) (Fig. 1). The unique anatomy of the respiratory tract ensures that the inhaled air is warmed, filtered, and redirected to the alveoli to allow gas exchange (Person and Mintz, 2005). The dynamic process of respiration moves significant quantities of oxygen and carbon dioxide across the alveolar-capillary interface.
to allow the maintenance of cell metabolism. The adequate functioning of the respiratory tract is crucial to support the energetic requirements of the body and provides an effective barrier to invaders. Disorders of the respiratory tract are an enormous health burden worldwide, especially in developing countries (Forum of International Respiratory Societies, 2017a). Indeed, respiratory diseases are one of the leading causes of mortality, causing significant socioeconomic losses (WHO, 2018). Over the past years, a substantial amount of attention and investment has been given to understand the cellular and molecular components of these disorders, leading to a better appreciation of pathogenesis bases. Although the current therapies for respiratory diseases improved disease management considerably, limitations regarding the costs, indications and increases in pathogen resistance are challenges for the respiratory pharmacology (Chang and Rivera, 2013). New therapeutic approaches and improvement of existing treatments have been the focus of substantial research efforts to address this urgent clinical need. The immunopharmacology field employs features of the immune response as therapeutic targets to enhance protective pathways while reducing pathological ones. As such, the development of immunomodulatory therapies focuses on correcting and balancing the immune responses to reduce disease symptoms and severity. This article will present an overview of the immunological bases of respiratory pathologies and outline the current therapeutic strategies for the management of asthma, COPD, cystic fibrosis, and pneumonia focusing on novel immunomodulatory approaches. Targeting the dynamics of the immune system to push the response “back in track” is promising to control or ultimately cure respiratory pathologies.

5.30.2 Inflammation in the lungs: The good, the bad and the ugly

Inflammation is a response from the microvasculature against harmful stimuli, such as infections and injury (Medzhitov, 2010). The word inflammation is derived from the latin inflammare which means “to set on fire” and describes key features of the response including redness and increased temperature of the inflamed tissue (Rivas, 2010). Based on macroscopic findings, inflammation was described by five cardinal signs: redness (rubor), swelling (tumour), pain (dolor), heat (calor) and loss of function (functio laesa) (Medzhitov, 2010). In addition to its pathological role, inflammation is important for physiological responses including healing, adaptation to stress and, as mentioned before, defense against infections (Medzhitov, 2008). Inflammatory triggers include microorganism-derived bioparticles (pathogen associated molecular patterns—PAMPs) and host-derived molecules released upon damage (danger/damage associated molecular patterns—DAMPs). PAMPs and DAMPs can be sensed by pattern recognition receptors (PRRs) in cell surface and cytoplasm, such as toll-like receptors (TLRs), and NOD (nucleotide-binding oligomerization-domain protein)-like receptors (NLRs) (Medzhitov, 2008). In the lungs, innate receptors from resident macrophages, dendritic cells, mast cells and epithelial cells can detect and signal noxious stimuli leading to activation of intracellular signaling pathways that culminate with the production of chemokines, cytokines, vasoactive amines, and proteases. Almost immediately, the increased vascular permeability leads to edema and facilitate the recruitment of leukocytes into the tissue (Riches and Martin, 2018b).

The efficiency of the immune defenses in the respiratory tract is evidenced by the relatively small number of infectious diseases a healthy human has in a lifetime. The pulmonary mucosa is in contact with approximately 10,000 L of air per day carrying a multitude of harmful particles (Riches and Martin, 2018a). Therefore, without an efficient and coordinate system of defense, our respiratory tract would be an easy port of entry for a variety of airborne pathogens. The anatomy of the respiratory tract can be considered the first innate barrier to noxious stimuli (Nicod, 2005). The air turbulence created by the anatomical features of the branched respiratory tract ensures that particles from the inhaled air get trapped in the mucus layer in the epithelial surface (Riches and Martin, 2018b). The clearance of trapped microorganisms or particles is accomplished by the coordinated mucociliary clearance (Wanner et al., 1996). In addition, the respiratory epithelium presents structured cellular junctional complexes and produces antimicrobial molecules that form a physical and chemical barrier to environmental threats (Vareille et al., 2011). Further protection is conferred by the presence of immune resident cells that work on the surveillance of the respiratory tract, especially the lungs (Iwasaki et al., 2017). Importantly, immune cells that reside in the respiratory tract must differentiate between innocuous molecules and pathogens avoiding unwarranted inflammation that impair adequate organ functioning (Lloyd and Marsland, 2017). As such, leukocytes in the respiratory tract possess a higher threshold of activation and suppress immune responses leading to tolerance against harmless inhaled particles (Lloyd and Marsland, 2017; Curotto de Lafaille et al., 2010). In this regard, upon detection of harmless environment cues, airway macrophages secrete anti-inflammatory cytokines such as TGF-β and can physically interact with epithelial cells to prevent unnecessary inflammation (Curotto de Lafaille et al., 2010; Lloyd and Marsland, 2017; Thepen et al., 1994). In addition, dendritic cell antigen-presentation in the lymph nodes, without an inflammatory stimulus, leads to induction of regulatory T lymphocytes contributing to airway tolerance (Curotto de Lafaille et al., 2010) (Fig. 2A). On the other side, the immune cells in the respiratory tract must respond promptly to a given pathogen, activating an effective and self-restrained inflammatory response when needed (Fig. 2B). Activation of PRRs by PAMPs, triggers the activation of airway macrophages and epithelial cells to produce pro-inflammatory cytokines and chemokines, leading to the recruitment of granulocytes to the site of infection (Medzhitov, 2008). Mast cells can also be activated and release vasoactive amine and proteases increasing vascular permeability further contributing to the recruitment of cells (Moiseeva and Bradding, 2011). Neutrophils, eosinophils, NK cells and other innate lymphocytes are important coordinators of the antimicrobial responses in the respiratory tract. Neutrophils and eosinophils possess intracellular granules enriched in proteases, reactive oxygen species (ROS) and anti-microbial peptides that can kill microorganisms or parasites. NK cells and innate lymphocytes can target infected cells to avoid dissemination of infection while producing cytokines that coordinate the response (Iwasaki et al., 2017). The dynamics of the inflammation in the respiratory tract culminate with the induction of
adaptive immune responses that will aid to the overall defense and establish protective memory responses (Riches and Martin, 2018b).

Once the clearance of the potential invader is achieved, the inflammatory responses must be terminated. The resolution of inflammation is an active process that leads to termination of inflammation and restoration of tissue homeostasis (Levy and Serhan, 2014). As for inflammation itself, the five cardinal signals of resolution were described, and include: removal of pathogens and debris, restoration of vascular integrity, regeneration of tissue, remission of fever and relief of pain (Basil and Levy, 2016). The cellular and molecular events of resolution of inflammation involves the cessation of granulocyte recruitment in conjunction with the recruitment of macrophages that are important for the clearance of pathogens and debris, reduction of proinflammatory mediator’s secretion and induction of pro-repair pathways (Watanabe et al., 2019; Dalli and Serhan, 2017). The steps of resolution are governed by endogenous immunoioresolvents that can be lipids (collectively called specialized pro-resolving mediators—SPMs), proteins or gaseous molecules (Sugimoto et al., 2016). Pro-resolving mediators act mainly through binding to specific receptors in the cell surface triggering signaling pathways that lead to resolution cellular responses (Serhan and Levy, 2016). The production and action of pro-resolving mediators is tightly regulated by differential expression of cell receptors and biosynthetic enzymes, activation of signaling cascades and post-transcriptional control (e.g., miRNAs) (Recchiuti and Serhan, 2012).

Chronic respiratory diseases such as COPD, asthma and cystic fibrosis are characterized by the excessive production of pro-inflammatory cytokines and recruitment and activation of granulocytes in the airways that can cause increased pulmonary damage and impairment of lung function (Tavares et al., 2020b). In addition, the activation of immune cells leads to the secretion of vaso-active amines and lipid mediators (e.g., leukotrienes and prostaglandins) that cause bronchoconstriction reducing the air flow to the alveoli for gas exchange. Interestingly, one of the mechanisms that would potentially explain the uncontrolled inflammation and

Fig. 2 Immune tolerance versus activation in the conducting airways and alveoli of the lung. (A) Harmless particles are captured by the airway mucus and are transported out of the airways by the coordinate ciliary beating of the epithelium. Resident dendritic cells sense and capture these particles and, in the absence of other activation signals, migrate to the regional lymph nodes to promote tolerance to commonly inhaled particles. Alveolar macrophages present inhibitory receptors such as CD200R and TGFβR that mediate tolerance through binding to their respective ligands CD200 and TGFβ. In addition, TGFβ secreted by steady-state macrophages promote the differentiation of T regulatory cells that also contribute to the maintenance of tissue homeostasis. (B) Upon the detection of pathogen molecules (PAMPs), by pattern recognition receptors (PRRs) the innate immunity is activated. Epithelial cells and resident alveolar macrophages produce cytokines, chemokines and lipid mediators that mediate the recruitment of leukocytes, especially neutrophils, to the lungs. The recruited cells produce antimicrobial molecules including reactive oxygen species (ROS), enzymes and peptides and perform phagocytosis leading to pathogen clearance in the lungs. Airway dendritic cells are also activated by the PAMPs, leading to maturation and migration to regional lymph nodes stimulating lymphocyte proliferation and activation of adaptive immunity. Created with BioRender.com®.
chronification of these respiratory illnesses, is failure of resolution pathways including the production of pro-resolving mediators (Barrie et al., 2018; Duvall et al., 2017; Philippe and Urbach, 2018; Bozinovski et al., 2014). Furthermore, the loss of innate tolerance to innocuous particles in the respiratory tract initiates exacerbated immune responses, accumulation of fibrous exudates in the lungs and thickening of respiratory surface that ultimately impair gas exchange or lung adequate inance to innocuous particles in the respiratory tract initiates exacerbated immune responses, accumulation of enzymes (Rumzhum and Ammit, 2016; Haeggstrom, 2018; Nelson et al., 2007; Welliver et al., 2003). On the other hand, strategies for asthma and COPD, for example, can be reduced through the pharmacological inhibition of 5-lypoxygenase or cyclooxygenase-2 of the important pharmacological challenges for the use of small molecule antagonists (Roth-Walter et al., 2019).

Various pro-inflammatory cytokines are involved in disease pathogenesis for respiratory disorders. Much consideration has been given to the inhibition of specific cytokines to mitigate or prevent disease severity. Cytokine production can be targeted by therapies that block transcription factors that induce their expression in the cells. The prototypical pro-inflammatory transcription factor, nuclear factor kappa B (NF-κB), has received considerable attention as an inducer of cytokines that are pathogenic for asthma and COPD, for example (Schuliga, 2015). Inhibition of signaling messengers that culminate with activation of NF-κB or enhancing signaling pathways that prevent its translocation into the nucleus are examples of pharmacological approaches for reducing cytokine production. Cytokine-directed therapies also include strategies that block cytokines after their release from the cells. Humanized monoclonal antibodies (mAbs) can specifically neutralize cytokines or cytokine receptors (Kopf et al., 2010) reducing their pathogenic effects in specific diseases. However, there are some important risks to the use of mAbs including acute anaphylaxis, susceptibility to infections and cancer, serum sickness and the generation of antibodies against the treatment (Hansel et al., 2010). In addition, mAbs targeting cytokines, rather than their receptors, can enhance the secretion or signaling of the target molecule (Rudulier et al., 2016). Appropriate selection and monitoring of specific mAb-therapies should be implemented to minimize side effects during treatment of respiratory diseases. Besides antibodies, small molecule antagonists of cytokine receptors and soluble receptors are strategies that reduce cytokine activity during disease. Soluble cytokine receptors are employed to compete with the surface-bound receptors for the binding of target cytokines preventing, specifically, unwanted cytokine activity (Borish et al., 1999). Small-molecule antagonists can inhibit cytokine binding by allosteric changes in the receptor or by blocking the binding site (Roth-Walter et al., 2019; Guntur and Reinero, 2012; Knobloch et al., 2018). As for mAbs, small molecule antagonists also present limitations regarding treatment of respiratory diseases. Potential toxicity, short half-life and reduced specificity are some of the important pharmacological challenges for the use of small molecule antagonists (Roth-Walter et al., 2019).

Targeting key enzymes involved in the production of pro-inflammatory mediators represent a different strategy to modulate the immune responses in respiratory diseases. Leukotrienes and prostaglandins, known to play an important role in the pathogenesis of asthma and COPD, for example, can be reduced through the pharmacological inhibition of 5-lipoxygenase or cyclooxygenase-2 enzymes (Runzhum and Ammit, 2016; Haeggstrom, 2018; Nelson et al., 2007; Welliver et al., 2003). On the other hand, strategies that induce production of pro-resolving and anti-inflammatory mediators may counter-regulate the pathological aspects of inflammation in respiratory diseases. More recently, the identification of biosynthetic pathways and circuits of resolution set the ground for the development of the "resolution pharmacology" (Perretti et al., 2015). In the lungs, promotion of resolution by exogenous administration of SPMs or other immunoresolvents was shown to decrease important features of asthma, COPD, cystic fibrosis, and pneumonia, mostly in preclinical studies (Duvall et al., 2017; Wang et al., 2011; Basil and Levy, 2016).

In summary, the complex facets of inflammation can be pharmacologically modulated to preserve the protective aspects of the response while blunting pathological ones. Adequate selection, dosage and treatment scheduling are necessary to avoid the unwanted effects of immunomodulatory therapies. Identifying central aspects of pathogenesis in respiratory diseases is crucial for the advancement of respiratory immunopharmacology. The next sections will present a concise description of important respiratory diseases—asthma, COPD, cystic fibrosis, and pneumonia—and selected therapies focused on regulation of immune responses.

5.30.3 Asthma

Asthma is a chronic lung disease caused by the swelling and narrowing of the tubes (bronchi and bronchioles) that carry the air to and from the lungs. It is a multifactorial disease with combination of genetic and external factors. The most common symptoms are shortening of breath, coughing, and wheezing. Asthma normally starts in the childhood but can affect all ages being the most common chronic disease in the world. More than 300 million people around the world suffer from asthma but the majority of the mortality occurs in developing countries (WHO, 2020a,b). Despite the unavailability of a cure for asthma, different treatment
strategies were suggested to relieve the symptoms and prevent exacerbations. Occasionally, severe asthma attacks, or exacerbations, result in acute worsening of symptoms and significant loss of lung function, requiring intensification of treatment that may progressively involve systemic corticosteroid treatment, hospitalization, and ventilation support (Arron et al., 2013).

Asthma is a heterogeneous disease that can be classified by clinical, inflammatory, immunologic, and molecular aspects. Despite the description of these classifications, asthma phenotypes are highly overlapping in their clinical presentation and underlying inflammatory process. Asthma phenotypes have been defined based on characteristic disease features that are the result of a combination of hereditary and environmental factors. Understanding the specific features of the different phenotypes has enable the identification of the distinct disease causes and aetiological mechanisms while improves the development of more effective therapeutic measures for asthmatic patients. Common clinical factors that can stratify asthma patients includes the atopic status, age of onset, obesity, smoking history, and sputum eosinophil percentages. The inflammatory classification is based on sputum cells differentials and identification of four different asthma types: eosinophilic (counts more than 1.01%), neutrophilic (counts more than 61%), mixed granulocytic (high counts of eosinophil and neutrophil), and paucigranulocytic (normal to low counts of eosinophil and neutrophil) (Kaur and Chupp, 2019).

Based on immunological and molecular aspects there are 4 main asthma phenotypes: (1) early-onset mild allergic asthma, which begins in early childhood and presents high eosinophil counts and preserved lung function; (2) early-onset allergic moderate-to-severe remodeled asthma that starts in childhood and presents mixed granulocytic and lower lung function; (3) late-onset nonallergic eosinophilic asthma that normally occurs in adults which does not presented any previous persistent respiratory symptoms and presents high eosinophil counts with preserved lung function, and (4) late-onset noneosinophilic nonallergic asthma, which happen in adults with normal eosinophilic and high neutrophil counts with lower lung function (Kaur and Chupp, 2019).

In addition to the phenotypes, asthma can also be subclassified in endotypes that described mechanism related to the disease at a cellular and molecular level of the immune response. This endotypes are based on the cytokine secretion profile leading to two major different asthma endotypes: type 2 (T2-high) and non-type 2 (T2 low) asthma (Kuruvilla et al., 2019).

The T2-high endotype represents the classical form of asthma: a typical mild to moderate asthma, with increased eosinophil inflammation in the airway and inflammation. In this context, the inflammatory response begins when airways epithelial cells are activated after allergen exposure and release cytokines, named alarmins. Alarmins are epithelial cell derived mediators such as thymic stromal lymphopoietin (TSLP), interleukin (IL)-25 and IL-33 that are release in response to infection or allergen exposure. Once release, alarmins can activate type 2 innate lymphoid cells (ILC2). Mast cells and antigens-presenting cells (APCs). ILC2s are important producers of IL-5 and IL-13 whereas dendritic cells activate B and T cells and induce Th2 immune response through secretion of IL-4, IL-5, and IL-13. In response to these cytokines, T2-high asthmatic patients display increased IgG production sputum eosinophils and peripheral eosinophilia, increased mucus secretion in the airways and airway remodeling (Lloyd and Hessel, 2010).

The T2-low endotype represents the non-eosinophilic asthma which is normally characterized by increased Th1 and/or Th17 associated responses, neutrophilic or paucigranulocytic inflammation. The related inflammatory response is associated with steroid resistance therapy. The long-term and timely increase in the dose of corticosteroids leads to increased susceptibility to viral and/or bacterial infections of severe asthmatic patients (Juhn, 2014). T2-low asthma is associated with chronic lung infections and a strong innate immunity activation with involvement of NLRP3, toll-like receptor (TLR)-2 and TLR-4 activity (Zakari and Russo, 2018; Kim et al., 2017). The non-eosinophilic asthma, still need to be fully understood, but a remarkable feature is the increased release of pro-inflammatory cytokines such as TNF, IFN, IL-17, IL-6 and IL-1β that is associated with neutrophil activation (Kim et al., 2016; Cosmi et al., 2011; Simpson et al., 2016; Liu et al., 2017). Moreover, extra pulmonary factors, such as obesity, are also important drivers of the persistent symptoms in T2-low asthma. In summary, the T2-high endotype is typically associated with eosinophilic early-onset and late-onset and the T2-low with mixed or neutrophilic early-onset and late-onset (McDowell and Heaney, 2020).

In asthma, exposure to environment insults such as allergens, infections, and cigarette smoke leads to airway hyperresponsiveness and obstruction (Corren et al., 2017). Pathologically, infiltration of inflammatory cells contributes to release of inflammatory mediators, including histamine and leukotrienes from mast cells leading to increased vascular permeability, pulmonary oedema, smooth muscle hypertrophy and hyperplasia of mucus producing cells (mucus hypersecretion) (Fireman, 2003). The repeated acute attacks of asthma characterized by bronchoconstriction, presence of inflammatory exudate and mucus secretion on the airways culminate with lung injury and induction of airway remodeling with collagen deposition and epithelial membrane thickening (Carroll et al., 1996). In addition, sensory nerves from airways become sensitized due to chronic inflammation, contributing to enhancement of symptoms such as coughing and chest tightness (Trankner et al., 2014).

It is well established that activation of immune responses is responsible for most of the asthmatic symptoms and, the use of anti-inflammatory therapies, including corticosteroids, is the most common approach to control disease and avoid exacerbations. Depending on the patient medical history and adherence of treatment, different therapeutic strategies are recommended by the Global Initiative for Asthma (GINA). Based on GINA, pharmacological treatment options are divided in three main categories: Controller medications, that are used to control asthma symptoms and prevent exacerbations and include low dose of inhaled corticosteroid plus long-acting β agonist (LABA), i.e., formoterol, as needed; Relief or rescue medications used for controlling asthma attacks (e.g., post-exercising) and includes low doses of inhaled corticosteroid plus short-acting β agonist (SABA), i.e., salbutamol, or a leukotriene inhibitor as needed; and Add on therapies for patients with severe asthma, which is applicable for patients with persistent symptoms and exacerbations and consist in medium to high doses of inhaled and oral corticosteroid in combination with LABA and leukotriene inhibitor (Asthma GIF, 2020a,b). Importantly, uncontrolled asthma symptoms increase the risk of exacerbations. During exacerbations standard treatments are not effective and several alternative treatments have been suggested and/or
In asthma, inhaled corticosteroids (ICS) are the first-line treatment for adults and children targeting the disease symptoms. Low dose of ICS (i.e., budesonide) provides benefits for most of clinical manifestations of asthma and can be used as a maintenance treatment to prevent persistent asthma symptoms. Still, some patients may not respond to treatment, thus medium to high dose of ICS with or without the combination of a controller or relief medication is required. Increased doses of ICS must be balanced due to the risk of local and systemic side effects (Asthma GIF, 2020a,b). ICS act reducing mucosal edema, decreasing vascular permeability and the release of pro-inflammatory mediators, consequently reducing leukocyte infiltration into the airways (Townley and Suliaman, 1987). Moreover, ICS improve lung function and reduce the frequency of asthma exacerbations (O’Byrne et al., 2006). In some cases, mainly during exacerbations, oral corticosteroids can be used in short periods to better control the symptoms. At the cellular level, corticosteroids can pass through the cellular membrane and bind to its receptor on the cytoplasm, which after homodimerization, translocate to the nucleus to bind to specific sites on DNA named glucocorticoids responsive elements (GRE) (McGregor et al., 2019) leading to anti-inflammatory gene transcription (Barnes and Adcock, 2003).

The mechanism of action of corticosteroid in asthma (and also other pulmonary pathologies as will be discussed in the following sections) is based on the trans-activation of anti-inflammatory genes and trans-repression of pro-inflammatory genes. These actions are mediated by chromatin remodeling. Under resting conditions, the chromatin structure is closed, with DNA winding tightly around histones, thus suppressing transcription. Under activation, DNA is unwound from the histones enabling transcription of specific genes. Acetylation of histones allows changes on the chromatin conformation from close to open. In this regard, two enzymes regulate histone acetylation: histone acetyltransferase (HAT)—that act opening chromatin structure and activating gene transcription—and histone deacetylase (HDAC), which removes acetyl groups changing the conformation of

**5.30.3.1 Corticosteroids**

In asthma, some of the pharmacological approaches proposed for asthma management focusing on the control of the activation of the immune responses (Fig. 3).
and as a prophylactic protection against bronchospasm induced by exercise, due to its short duration of action (typically 4–6 h). SABAs are recommended to use as needed (Cazzola et al., 2012; Cullum et al., 1969). β2 agonists have been used to treat asthma due to their ability to reverse bronchoconstriction by relaxing the airway smooth muscle. There are two categories: short acting β agonist (SABA) and long acting β agonist (LABA) (Cazzola et al., 2013).

SABAs, which includes albuterol, fenoterol and terbutaline, are used in combination with ICS as relief medications for asthma and as a prophylactic protection against bronchospasm induced by exercise, due to its short duration of action (typically 4–6 h). SABAs are recommended to use as “as needed” medication. Similarly, LABAs, which includes salmeterol and formoterol, are used in combination with ICS as controller medications and display a duration of action around 12 h, offering a considerable clinical benefit and improvement of lung function (Cazzola et al., 2013). As noted, monotherapy of β2 agonists are contraindicated since the use of these medications is associated with increased adverse events and greater risk for severe exacerbations and mortality (Ortega and Peters, 2010).

The mechanism of action of β2 agonists is mediated by its receptor (β2AR), which is a member of the G-protein coupled receptor (GPCR) family. Once activated, β2AR catalyze the formation of cyclic AMP (cAMP) which activates the protein kinase A (PKA). Specifically in airway smooth muscle, PKA antagonizes pro-contractile signaling and directs inhibits mechanisms of contraction (Billington and Penn, 2003).

The combination of ICS and LABA have clearly shown clinical benefit. The combination of the ICS anti-inflammatory properties and LABA bronchodilation and bronchoprotection effects, synergistically enhance the overall anti-inflammatory protective response. Nevertheless, the mechanism underlying the additive effect between the two compounds still need to be fully investigated (Kips and Pauwels, 2001).

5.30.3.3 Leukotriene receptor antagonists

Leukotriene receptor antagonists (LTRA) are a proven effective treatment strategy to control asthma symptoms. Although less effective than ICS, LTRA are recommended for some patients who are unable to use ICS or have persistent symptoms even after ICS + SABA treatment. In addition, LTRA are also recommended as a prophylactic therapy to exercise induced bronchospasms in asthma patients. LTRAs, such as montelukast and zafirlukast, reduce asthma symptoms and decrease the need of rescue medication by reducing airway inflammation and improving lung function (Riccioni et al., 2007).

Leukotrienes are lipid mediators synthesized through the enzymatic conversion of arachidonic acid, a molecule release from the cell membrane after the activation of the cellular phospholipase A2. Arachidonic acid is metabolized by two main pathways, the cyclooxygenase pathway, leading to prostaglandin and thromboxane production, and the 5-lipoxygenase pathway generating leukotrienes. Cysteinyl leukotrienes (leukotrienes C₄, D₄ and E₄) act through the CysLT1 receptor binding inducing all the pathological features of asthma including airway eosinophilia, increase vascular permeability, bronchospasm, mucus production and airway remodeling (Holgate et al., 2003; Devillier et al., 1999). Treatment with LTRA was shown to reverse the hallmarks of asthma including symptoms triggered by allergens, exercise, cold air or aspirin challenges (Bisgaard, 2001). Although montelukast is recommended to prevent acute asthma attacks and can be used for long-term treatment of asthmatic adults and children, the use of this medication is associated with increased risk of mental health side effects that should be considered during treatment management (Aldean Perona et al., 2016).

5.30.3.4 Macrolides

Macrolides are class of antibiotics that exert antimicrobial and immunomodulatory effects on asthma. Given the antimicrobial actions of macrolides, asthma exacerbations are reduced through the impairment of bacterial colonization in the lungs. In addition, macrolides, specially the second generation of these drugs, possess important immunomodulatory effects in asthma. Clarithromycin and azithromycin, widely used to treat pneumonia and other pulmonary pathologies (Dinos, 2017), were shown to prevent hyperresponsiveness and granulocyte activation in asthma patients (Gibson et al., 2017). However, some studies have shown that the use of macrolides did not improve asthma control (Kew et al., 2015; Johnston et al., 2016). Cautious optimism is justified by different studies regarding the role of immunomodulatory actions of macrolides in asthma. According to GINA, Azithromycin...
can be used as an add-on treatment option for those patients that even using moderate to high ICS doses + LABA still display persistent symptoms. In this regard, treatment with Azithromycin three times a week reduces asthma exacerbations and improves patient’s quality of life (Asthma GIF, 2020b). The conflicting evidence in the literature can be explained by the different phenotypes and endotypes in asthma. Therefore, specific groups of asthma patients may benefit from the use of macrolides while others might not. Macrolides mechanism of action in asthma is related to the decreased activation of macrophages, neutrophils and production of pro-inflammatory cytokines, including TSLP (Zhu et al., 2013). Indeed, ex vivo studies using tracheal aspirates taken from premature infants developing bronchopulmonary dysplasia, showed that azithromycin decreased TNF-α-induced NF-κB activation and release of IL-6 and IL-8 (Aghai et al., 2007). Furthermore, macrolides potentially decrease some features of the T12 response that are related to asthma pathogenesis. Further mechanistic studies will clarify the immunomodulatory benefits of macrolides in asthma, uncovering possible synergistic effects with other treatment approaches.

5.30.3.5 Cytokine and antibody-targeted therapies

Currently, management of asthma symptoms relies on use of controller and relief medications. Yet, some patients are not able to control asthma, presenting persistent symptoms despite the treatment. To develop new alternative treatment strategies, biological therapies have stood out due to the beneficial effects observed. Biologic therapies are new approaches to control asthma symptoms targeting specific inflammatory pathways involved in asthma pathogenesis (McGregor et al., 2019).

Anti-IgE therapy was the first biologic approved for asthma treatment and consist in the administration of a humanized anti-IgE monoclonal antibody, called Omalizumab. The mechanism of action consists in the prevention of IgE binding to its receptor FceRI, present in mast cells and basophils. Preventing IgE binding leads to reduced activation of these leukocytes and release of pro-inflammatory mediators mitigating the downstream allergic responses (Holgate et al., 2005). Omalizumab, rather than binding to cell-bound IgE or its receptor, binds to free IgE decreasing the antibody serum levels. This therapeutic approach was design to target an epitope that specifically neutralizes IgE, without affecting other antibody classes (D’Amato, 2006). The treatment also result in reduced expression of FcεRI in basophils, mast cells and dendritic cells suggesting that the treatment not only reduce pro-inflammatory mediator release, but may decrease antigen processing and presentation (Lin et al., 2004; Prussin et al., 2003). Omalizumab can reduce asthma exacerbations, hospitalizations, and the dose of ICS required for disease management. It is indicated for patients aged ≥ 6 years with moderate to severe allergic asthma, uncontrolled by ICS, and with positive test for allergy and high serum levels of IgE (McGregor et al., 2019).

Another strategy to control asthma severity is to target eosinophil related cytokines. Anti-IL-5, was developed as a therapeutic option for eosinophilic asthmatic patients since IL-5 is strongly associated with eosinophil recruitment, differentiation, activation and survival (Pelaia et al., 2019). The humanized monoclonal antibody anti-IL-5, called Mepolizumab, was shown to reduce the number of eosinophils in blood and sputum of asthma patients, leading to reduced exacerbation rates and improved asthma control. The mechanism of action of Mepolizumab is based on blocking the connection between IL-5 and its receptor found on the surface of eosinophils and basophils. Treatment with Mepolizumab is indicated for patients aged ≥ 12 years with severe asthma associated to persistent eosinophilic inflammation despite corticosteroid treatment (Ortega et al., 2014). Reslizumab, another anti-IL-5 monoclonal antibody approved for asthma treatment, is indicated for patients over 18 years age. Differently from Mepolizumab, Reslizumab treatment requires weight adjusted dosage leading to better results on the management of late onset eosinophilic asthma patients (Brusselle et al., 2017). Noteworthy, targeting IL-5 response through its receptor is another strategy to prevent eosinophilic exacerbations in asthma. Benralizumab is an afucosylated monoclonal antibody that block the effects of IL-5 on eosinophils and basophils by targeting the IL-5 receptor and inducing antibody-mediated cell cytotoxicity (Kolbeck et al., 2010). Patients with severe uncontrolled asthma who received this treatment on clinical trials displayed reduced annual exacerbation rates and improved lung function (FitzGerald et al., 2016). Benralizumab was recently approved as add-on therapy for patients with severe asthma presenting eosinophilic phenotype (Markham, 2018).

IL-4 and IL-13 are cytokines associated to the type 2 inflammation associated to certain phenotypes of asthma. Therapeutic strategies targeting both cytokines were developed since the inhibition of each cytokine independently did not reduce asthma exacerbations (Alijanpour and Aliakbarpour, 2017; Panettieri et al., 2018; Corren et al., 2010). Dupilumab is a human monoclonal antibody that targets the IL-4 receptor z and inhibits the action of IL-4 and IL-13. Both cytokines, are key mediators in the production of IgE, recruitment of inflammatory cell into the airways, hyperplasia of goblet cells and modulates airway hyperresponsiveness (Santini et al., 2017). The use of Dupilumab in patients with severe asthma reduced number of exacerbations, decreased blood eosinophil levels, improved lung function and asthma control (McGregor et al., 2019).

Anti-IL-17 therapies have been studied using two strategies based on blocking the IL-17 receptor (IL-17RA, Brodalumab) or the cytokine IL-17A (Secukinumab). IL-17A levels are elevated in the bronchoalveolar lavage of asthma patients and positively correlate with increased neutrophil inflammation and airway hyperresponsiveness. Brodalumab is a human monoclonal antibody that block the receptor preventing the activity of IL-17A, IL-17B and IL-25. Despite the potential therapeutic value of Brodalumab, the clinical trials did not demonstrated improvement on asthma symptoms (Busse et al., 2013) while its use was associated with increased risk of mental health problems leading to the discontinuation of the investigation (Lebowohl et al., 2018). Secukinumab is a human immunoglobulin G1 antibody that directly targets IL-17A. It was the first anti-IL-17 approved to treat psoriasis (Fargnoli, 2019). In patients with uncontrolled asthma, Secukinumab did not improved asthma control and the study was discontinued (ClinicalTrials.gov Identifier: NCT01478360).
**Anti-alarmins** have been identified as potential targets to prevent disease exacerbations (Al-Sajee et al., 2018). Due to complexity of the inflammatory pathway involved on asthma, current therapies are still not efficient to control all different asthma phenotypes and prevent exacerbations and therapies that target the initial stages of inflammation induction are of interest (Porsbjerg et al., 2020). Tezepelumab is an anti-TSLP monoclonal antibody that prevents TSLP to act on its receptor. The use of Tezepelumab in patients with moderate-to-severe asthma reduced annual exacerbation rates (Corren et al., 2017). In addition, the other alarmin IL-33 has also been tested in clinical studies for asthma management. Currently, there are two anti-IL-33 monoclonal antibodies being tested for asthma control but the results of these studies are not yet available (ClinicalTrials.gov Identifier: NCT03112577 and NCT03469934).

### 5.30.3.6 Pro-resolving mediators

A growing amount of evidence implicates a defective activation of pro-resolving pathways contributing to the persistent inflammation and disease severity in asthma. SPMs are produced by the enzymatic conversion of polyunsaturated essential fatty acids (PUFAs) including arachidonic acid (Smolen et al., 2020), eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) which yields potent bioactive autacoids inducers of resolution (Serhan and Levy, 2018). Noteworthy, the levels of SPMs including lipoxin A4 (LXA4) and 15-epi-LXA4 levels are reduced in bronchoalveolar lavage (Asma), exhale breath condensates, blood and sputum of severe asthma patients (Bhavsar et al., 2010; Levy et al., 2005; Fritscher et al., 2012; Kazani et al., 2013). These low levels were associated with increased number of neutrophils in the airways, high risk of exacerbations and lower lung function (Ricklefs et al., 2017). Decreased levels of lipoxins are at least partially related to the increased oxidative stress in severe asthma that dysregulate biosynthetic enzymes for lipoxins in the airways (Plana-guma et al., 2008; Ono et al., 2014). In addition to lipoxins, DHA levels in the lungs are decreased in asthma reducing the availability for the conversion of DHA-derived SPMs (Freedman et al., 2004). Consistent with that, biosynthesis of PD1 is also impaired in severe asthma patients suggesting that deficient generation of pro-resolving mediators lead to inadequate asthma control (Levy et al., 2007; Miyata et al., 2013). In contrast to anti-inflammatory therapies that lead to immunosuppression, pro-resolving mediators display a more protective and reparative response without compromising the host antimicrobial responses. In this regard, inhaled LXA4 was shown to prevent LTC4-induced airway obstruction in asthmatics patients (Christie et al., 1992). In addition, LXA4 acts on NK cells from asthmatic patients increasing its activation and mediating the NK-induction of eosinophil and neutrophil apoptosis (Barnig et al., 2013). Similarly, inhalation of LXA4 analogs have been shown to be effective, safe and well tolerated in asthmatic pediatric patients with acute moderate episodes (Kong et al., 2017). Moreover, the use of ACT-389949, an agonist of the LXA4 receptor, ALX/FPR2, was shown to be well tolerated in humans representing a potential pro-resolving drug for asthma. However, due to receptor desensitization, the chronic use of ACT-389949 might not be possible and new molecules with better pharmacologic characteristics are of interest (Stalder et al., 2017).

Lastly, the recently described SPMs Maresin Conjugates in Tissue Regeneration (MCTRs) were shown to counter regulate airway inflammation and hyperreactivity competing with cysteinyi leukotrienes for the cysteinyi leukotriene receptor 1 (CysLT1) in preclinical studies (Levy et al., 2020). Proof of concept studies in asthma patients will uncover the potential benefits of inducing resolution during this chronic lung inflammatory disease.

### 5.30.3.7 Challenges and perspectives

Despite the development of several novel immunomodulatory strategies to control asthma symptoms in the past years, the biological complexity of this airway disease and the distinct phenotypes and endotypes, contribute to treatment failure and high rates of hospitalizations. As mentioned before, asthma is a disease with several “flavors” and stratification of patients is helpful to identify specific therapeutic targets. The use of the guidelines (GINA) have improved the treatment of many patients, however, treatment options are still limited compared to the heterogeneous mix of pathobiological mechanisms involved in the disease. Further studies are needed to identify key pathways related to disease pathogenesis. In addition, targeting specific subtypes of cells, including eosinophils, might separate the protective from the detrimental responses of the immune system in asthma.

### 5.30.4 Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is currently the fourth leading cause of death worldwide and it is projected to be the third by the end of 2020 (Disease GIFICOL, 2020). It is estimated that 3.17 million of deaths were caused by COPD in 2017, with more than 90% occurring in low- and middle-income countries (WHO, 2017; Gershon et al., 2011; Beran et al., 2015). Tobacco smoking is by far the principal risk factor associated to the development of COPD; still, occupational, and indoor air pollution, caused by biomass fuel burning, are also potential causes of COPD. Because not all smokers develop COPD, pathogenesis is the result of a complex interplay between long-term cumulative exposure to noxious gasses and particles, combined with host factors that are not completely understood (Rennard and Vestbo, 2006). Dyspnea is the major clinical manifestation of COPD and is frequently associated with chronic airflow limitation and persistent respiratory symptoms. Two other classic manifestations are the destruction of lung alveoli (emphysema), with the consequent reduction in the surface for gas exchange, and/or Airways narrowing and inflammation that results in cough and increased sputum production (bronchitis).
In general, the characteristic chronic pulmonary inflammation triggered by the prolonged exposure to noxious gasses and particles is the main cause of COPD development, which is progressive and persists even when smoke ceases (Barnes, 2008). Increased oxidative stress is an important contributor to disease pathophysiology being smoke and air pollution the major exogenous sources of oxidative molecules. Curiously, increased oxidative stress persists even in ex-smokers, indicating that it also arises endogenously through the activation of inflammatory pathways (Barnes, 2020). The harmful environment created by continuous inhalation of noxious particles leads to the activation of several pattern recognition receptors (PRRs), especially Toll-like receptors (TLRs). The activation of TLRs, especially TLR2, TLR4, and TLR9, induce inflammatory cell recruitment and cytokine production (Zuo et al., 2015). The sustained exposure to inflammatory insults leads to persistent leukocyte recruitment to the lungs, especially neutrophils. These cells enhance tissue damage, induce oxidative stress by production of reactive oxygen species (ROS), provoke protease-mediated degradation of connective tissue elements, and induce apoptosis of type I pneumocytes and endothelial cells (Barnes, 2008). In addition, extracellular traps (NETs) released by neutrophils might also contribute to tissue inflammation and injury in COPD (Uddin et al., 2019). This inflammatory loop is further amplified by the release of several damage-associated molecular patterns (DAMPs) from the injured lung tissue (Huang et al., 2019; Pouwels et al., 2016). Moreover, COPD patients are particularly susceptible to respiratory infections caused by respiratory virus or bacteria, fuelling the ongoing inflammatory process in the lungs. In this regard, bacteria such as *Pseudomonas aeruginosa* and *Haemophilus influenzae* are often found colonizing lungs of COPD patients (Di Stefano et al., 2017). Altogether, cigarette smoke, oxidative stress, bacteria and viruses activates pro-inflammatory signaling cascades culminating with NF-kB and the p38 mitogen-activated protein kinase (MAPK) mediated transcription of pro-inflammatory mediators in airway epithelial cells. CXCL1, CXCL8, TNF-α, GM-CSF and leukotriene B4 (LTB4) secretion is significantly increased in COPD development leading to enhanced neutrophil recruitment into the lungs. Epithelium activation also induces TGF-β production and consequently fibrosis. Fibrosis around the small airways is thought to be the main factor contributing to the irreversible airway narrowing that characterizes COPD (Hogg et al., 2004). Besides, TGF-β production may be responsible for the loss of ciliated cells and goblet cell hyperplasia in COPD (Gohy et al., 2019).

In addition to neutrophils, increased numbers of macrophages are observed in the sputum of COPD patients. Monocytes from the circulation are recruited to the lungs in response to chemotactic mediators released from airway epithelial cells and resident macrophages (Costa et al., 2016; Traves et al., 2004). Activated macrophages secrete several elastolytic enzymes, including matrix metalloproteinases -2, 9 and 12, cathepsins K, L and S, and neutrophil elastase taken up from neutrophils. They also contribute to tissue oxidative damage by promoting ROS and peroxynitrite generation (Barnes, 2004). Another important feature of COPD inflammation is the infiltration of lymphocytes, specially TCD8+, into the lungs, defining the typical Th1 pro-inflammatory profile of COPD (Alter et al., 2020). Lymphocytes and monocytes can be recruited to the lung tissue via CXCR3 and CCR5 (Costa et al., 2016). The T111 lymphocytes are the main source of IFN-γ, which induces epithelial cell release of CXCL9, CXCL10 and CXCL11 further enhancing T11 cell recruitment. In addition, IL-23 production by macrophages promotes T117 cell differentiation additionally contributing to neutrophil recruitment through IL-17 production (Barnes, 2018). The role of cytotoxic T cells in COPD development is not yet clear, but they might be involved in the apoptosis and destruction of alveolar-wall epithelial cells (Barnes, 2000). Similarly, NK cells were also implicated in alveolar wall destruction and establishing emphysema due to their cytotoxicity actions (Freeman et al., 2014). Other innate lymphoid cells, particularly ILC1 and ILC3 cells, are also increased in the lungs of COPD patients. It is likely that these lymphocytes aid to the TH1 pro-inflammatory profile in COPD, enhancing neutrophilic inflammation and persistence of inflammation, even when the patients quit smoking (De Groe et al., 2016).

Despite the significant numbers of leukocytes recruited to the lungs; COPD patients show increased susceptibility to infections due to dysfunctional immune responses. Bacterial colonization in the lungs of COPD patients is a common finding (Traves et al., 2002). In addition, COPD patients present impaired anti-viral responses, despite significant numbers of cytotoxic T CD8 cells present in the patients’ lungs. Although the elevated production of IFN-γ leads to overactivation of these cells, they present reduced cytotoxic capacity in response to infection. This mechanism named T CD8 cell exhaustion is believed to heighten structural lung damage through an excessive and dysfunctional inflammatory process (Hsu et al., 2017; McKendry et al., 2016). Indeed, acute exacerbation of COPD (AECOPD) is the most problematic consequence of deficient control of infections, being the main cause of hospitalizations, morbidity and mortality (Viniol and Vogelmeier, 2018). During AECOPD, inflammatory markers in lung tissue and sputum increase and so does leukocyte recruitment. In this regard, sputum IL-1β was shown to be a good biomarker of bacterial exacerbations, whereas serum CXCL10 (IP10) is an indicator of virally induced exacerbations (Barnes, 2019).

The reason why inflammation persist to the point of chronic dyspnoea and lung parenchymal destruction in some smokers, and not others, remains to be completely understood. Certainly, there is no simple explanation as many different factors might be involved. For instance, two genetic-related endotypes had been identified so far. The best described is alpha-1 antitrypsin (AAT) deficiency. Smokers with defective production of AAT by the liver, present early onset of emphysema due to a protease/anti-protease imbalance but principally attributed to augmented neutrophil elastase and other serine proteases. However, it accounts for less than 1% of COPD patients. Telomerase polymorphisms are also found in approximately 1% of COPD patients and results in early-onset emphysema in smokers who are predominantly female. In these patients, telomere shortening leads to cellular senescence, which induces chronic inflammation (Barnes, 2019). In fact, induction of cellular senescence, defined as a permanent arrest of the cell cycle, is a possible mechanism associated with chronic inflammation in COPD. Cellular senescence represents an irreversible, stable, and long-term loss of proliferative capacity despite an active cell metabolism. Cellular stress (premature senescence) and accelerated aging may induce senescence in lung epithelial cells of COPD patients. Interestingly, the pattern of inflammatory proteins in the lungs and sputum of COPD patients is remarkably similar to the senescence-associated secretory phenotype (SASP) secreted by senescent cells. This includes IL-1β, TNF-α, IL-6, CXCL8, CXCL10, MMP-2, MMP-9 and TGFβ (Kumar et al., 2014).
Moreover, plasminogen activator inhibitor-1 (PAI-1) is a characteristic mediator of SASP and is increased in the sputum and lungs of COPD patients (To et al., 2013). Finally, reduced levels of endogenous antiaging molecules such as sirtuin-1, sirtuin-6 and Klotho were reported in COPD patients (Gao et al., 2015; Rajendrasozhan et al., 2008).

In any case, the sustainment of the inflammatory process in the lung is the principal factor of pathogenesis in COPD, for that reason, pharmacological approaches that seek the control of the inflammatory process are of special interest. To date, there is no evidence that any existing treatments for COPD modify the long-term decline in lung function or avoid disease progression. Thus, by the moment, pharmacological therapy for COPD is used to reduce symptoms, the frequency and severity of exacerbations, and improve health status (Disease GIFCOL, 2020). Novel immunotherapies are urgent needed.

### 5.30.4.1 Corticosteroids

Corticosteroids are the most effective anti-inflammatory drug available. Unfortunately, most studies have shown that treatment with inhaled or oral corticosteroids does not avoid FEV1 decline or disease progression, neither reduce mortality of COPD patients (Disease GIFCOL, 2020). Corticosteroid resistance is a major barrier to the effective management of COPD patients. Several molecular mechanisms have been proposed to explain the reduced anti-inflammatory effects of corticosteroids in those patients (Fig. 4).

The ability of corticosteroids to repress pro-inflammatory gene expression depends on the reduction of acetylation on histones located in the activated inflammatory gene complex. This is enzymatically achieved by histone deacetylase-2 (HDAC2), which is recruited to the gene complex once glucocorticoid receptor α (GRα) gets activated. Noteworthy, HDAC2 expression is markedly reduced in airways and peripheral lung tissue of patients with COPD (Ito et al., 2005). The increased oxidative stress, that is consistently associated with corticosteroid resistance, induces activation of phosphoinositide-3-kinase (PI3K)-δ, which leads to phosphorylation, ubiquitination, and degradation of HDAC2. In addition, the formation of peroxynitrite inactivates HDAC2 by nitration of tyrosine residue, which impairs HDAC2 activation and promotes its degradation (Barnes, 2020). In this line, it has been suggested that activation of PI3Kδ/akt and MAPK p38 pathways lead to phosphorylation of GR, reducing or impairing its translocation to the nucleus of peripheral blood mononuclear cells (PBMCs) from COPD patients. Furthermore, increased levels of IFN-γ may contribute to steroid resistance in COPD via JAK/STAT1 activation (Mei et al., 2019).

The increased expression of GRδ has also been linked to decreased corticosteroid responsiveness. GRδ is induced by pro-inflammatory cytokines and compete with GRα for binding to glucocorticoid response element (GRE) in the DNA acting as a dominant-negative inhibitor. The expression of GRδ is considerably lower than GRα in most cells, except for neutrophils, one of the most enriched cells in COPD lungs. Also, IL-17, a cytokine that is not suppressed by corticosteroids, significantly increases the expression of GRδ in airway epithelial cells (Vazquez-Tello et al., 2010). Another mechanism that might be related to corticosteroid resistance, is the activation of c-Jun N-terminal kinase (JNK) by pro-inflammatory cytokines leading to phosphorylation of GR at Ser226 and prevention of binding to GRE. Although JNK is activated in patients with COPD, whether this directly contributes to corticosteroid resistance is yet to be clarified (Barnes, 2013). Altogether, different mechanisms prevent corticosteroid from switching off activated inflammatory genes in COPD, resulting in amplified inflammation and corticosteroid resistance. For that reason, several studies seek to develop new treatment strategies that target oxidative stress or specific kinases activation to regain corticosteroid anti-inflammatory effects in COPD.

#### 5.30.4.1.1 Corticosteroid combined therapies

Some beneficial effects had been found when combining long-acting β2-adrenergic receptor agonists (LABA) with inhaled corticosteroids (ICS) compared to each treatment alone. It was reported that this therapeutic combination improves lung function and patient’s health and leads to a modest reduction in the rates of exacerbations in moderate and severe COPD patients. The evidence supporting the step-up to triple inhaled treatment with long-acting muscarinic antagonist (LAMA)/LABA/ICS is weak. However, triple therapy was shown to reduce by approximately 10% the rate of moderate and severe exacerbations when compared to LABA/ICS treatment (Lipson et al., 2018).

There is no evidence confirming that combined LABA/ICS therapy improves survival in patients with COPD. Yet, post-hoc analyses of data suggest that certain subtypes of COPD patients may benefit from it, particularly those with severe airflow limitation and/or frequent exacerbations (Agusti et al., 2018). The advantages in combining corticosteroid treatment to regular bronchodilator treatments in COPD might relate to evidences that show that LABA or LAMA can increase the anti-inflammatory effects of corticosteroids. For instance, LABAs were shown to increase nuclear translocation of GR by the reduction in PI3Kδ activity (Barnes, 2013). Similarly, a study using neutrophils isolated from COPD patients showed that Azelastine bromide, a LAMA bronchodilator for COPD treatment, inhibited PI3Kδ activity through muscarinic M2 receptor blockade and enhanced fluticasone propionate-mediated transactivation of glucocorticoid response element (GRE) (Milara et al., 2016).

Given their modest effectiveness and concerns about safety, particularly the increased risk for pneumonia, ICS treatment protocols should be personalized and carefully considered. In this regard, some COPD patients that present eosinophilic infiltration in airways may benefit from ICS, as eosinophilic inflammation is more susceptible to this treatment. In fact, administration of ICS in COPD patients who have increased sputum eosinophils did reduce exacerbations (Siva et al., 2007). It is uncertain whether blood eosinophil count correlates with sputum or lung tissue eosinophilia. However, increased numbers of blood eosinophils corresponded to the better effect of ICS in reducing exacerbations and disease progression. Still, ICS could not ameliorate lung function or symptoms in this specific group of patients (Pascoe et al., 2015).
Novel Immunomodulatory Therapies for Respiratory Pathologies

- N-acetylcysteine (NAC)
- Carbocisteine
- Erdosteine
- Ebselen

Aminoguanidine

ROS Peroxynitrite

- NOX
- IC8714
- PI3Kδ
- Akt
- Ub
- HDAC2

JAK
- JAK
- IL-17
- IFN-γ

Jakinibs

iNOS

mTORC

- Rapalogs
- X-1125

SHIP

Degradation

HDAC2 + Nitro-Tyrosine

- Losmapimod
- PH-797804
- Acumapimod
- RV-568

PI3Kδ

p38

ROS

mTORC

GRα

GRβ

Nfr2 activators:
- Bardoxolone methyl
- Dimethyl fumarate

Nrf2

Antioxidants

Pro-inflammatory genes

Anti-inflammatory genes

GC

Ac

Ac
In summary, ICS should not be used as a single therapy for COPD. Most patients that benefit from the addition of ICS to LABA therapy, are those with: (i) history of multiple or severe exacerbations despite appropriate use of bronchodilator, (ii) history of concomitant asthma, and (iii) those with blood eosinophil counts above 300 cells/μL. (Agustí et al., 2018). In patients with less than 100 circulating eosinophils/μ and recurrent bronchial infections, ICS treatment increases the risk of pneumonia (Martínez-García et al., 2020; Barnes, 2019).

### 5.30.4.2 Antioxidants

Given the primordial role of oxidative stress in COPD pathogenesis and corticosteroid resistance, several strategies using antioxidants with potential therapeutic effects are currently being studied. For instance, treatments with thiol based mucolytic drugs with recognized antioxidant effects like N-acetylcysteine (NAC), carbocisteine and erdosteine have been tested. Overall, treatment with these drugs have shown to reduce disease exacerbations but have no significant effect enhancing lung function or life quality. Likewise, there is no evidence that these antioxidants increase corticosteroid sensibility in patients (Rogliani et al., 2019; Poole et al., 2019; Zheng et al., 2014). A possible explanation is that the high level of oxidative stress in COPD lungs may provoke a rapid inactivation of this class of molecules. Consequently, this increased the research efforts to find more effective antioxidant molecules for COPD management.

NADPH (nicotinamide adenine dinucleotide phosphate) oxidase (NOX) is a multi-component enzyme bound to the cytoplasmatic membrane, that mediates electron transfer from nicotinamide adenine dinucleotide phosphate (NADP) to molecular oxygen, resulting in the production of superoxide anions. This protein complex is the major source of endogenous ROS in COPD. Several NOX inhibitors have been developed to dampen oxidative stress-induced tissue damage (Cifuentes-Pagano et al., 2012). Apocynin is a non-selective NOX inhibitor that reduced lung inflammation in a mouse model of AECOPD triggered by the combination of cigarette smoke and viral infection (Oostwoud et al., 2016). Nebulized apocynin given to COPD patients reduced H2O2 and nitrite concentrations in exhaled breath condensate, but no clinical parameters were reported (Stefanska et al., 2012). Despite NOX represents an interesting drug target for lung inflammatory diseases, it has been proved difficult to develop selective inhibitors. Recently, some potent and specific inhibitors were developed but most of these compounds were only validated in vitro or mice models (Chocy and Leloup, 2020).

Myeloperoxidases (MPO) are other important sources of oxidative radicals. In addition to hydrogen peroxide (H2O2), these enzymes produce large amounts of chloride anion (Cl−) and hypochlorous acid (HOCl), both of which are significantly aggressive oxidants. Neutrophil granules contains high amounts of MPO, therefore MPO activity is markedly increased in COPD (Keatings and Barnes, 1997). Treatment with a selective irreversible MPO inhibitor, 2-thioxanthine AZD5904, in guinea pigs exposed to cigarette smoke for 6 months reduced airway remodeling and emphysema. The treatment also inhibited oxidative pulmonary damage and improved lung function (Churg et al., 2012). However, although well tolerated in human volunteers, the clinical studies for this drug were discontinued for unknown reasons (Barnes 2020).

Other antioxidant strategies include antioxidant mimetics, which are designed to restore antioxidants activity, thus, lowering the impact of oxidative stress in tissue. Ebselen, a GPx mimic, was shown to reduce airway inflammation in a model of AECOPD triggered by a combination of cigarette smoke and viral infection (Oostwoud et al., 2016). Like Ebselen, other drugs are effective in animal models of oxidative stress, albeit, no clinical studies using antioxidant mimetics in COPD have been reported (Smith et al., 2002; Barnes, 2020). Nitric oxide contributes to oxidative stress in COPD through the formation of the highly reactive peroxynitrite ions (Osoata et al., 2009; Smith et al., 2002). Nebulized aminoguanidine, a relatively selective inhibitor of inducible nitric oxide synthase (iNOS), may reverse corticosteroid resistance. Likewise, Nrf2 activators including bardoxolone methyl or dimethyl fumarate increase the transcription of antioxidant genes. Inhibitors of PI3k/Akt/mTORC pathway (IC8714), Rapalogs (Src homology 2 domain-containing inositol 5-phosphatase-1. Created with BioRender.com).
though Nrf2 activators are an attractive therapeutic approach, it to the development of potent and specific Nrf2 activators is difficult. Sulforaphane, a natural activator of Nrf2, over 4 weeks failed to increase antioxidant gene targets of Nrf2 or reduce oxidative stress. Bardoxolone methyl, or dimethyl fumarate, showed several side effects in clinical trials (Gold et al., 2012; Rossing, 2013). Even though Nrf2 activators are an attractive therapeutic approach, it to the development of potent and specific Nrf2 activators is difficult.

5.30.4.3 Kinases inhibitors

Activation of protein kinases has been implicated in the transduction of signals triggered by inflammatory mediators. Several kinases were shown to be overactivated in COPD, thus promoting hyper-contractility of airways, mucus hypersecretion, immune cell infiltration, and airway remodeling (Defnet et al., 2020; Barnes, 2016; Pirozzi et al., 2019). Kinase inhibitors, mostly developed for cancer treatment, might be particularly interesting in the context of COPD to cease the inflammatory process.

5.30.4.3.1 p38 Inhibitors

The major MAPK pathways involved in inflammatory diseases are ERK (extracellular regulating kinase), p38 MAPK, and JNK (c-Jun NH2-terminal kinase). Nonetheless, most attention was focused on inhibition of the p38 MAPK pathway because there is enough evidence showing that p38 MAPK is activated in COPD and plays an important role driving chronic inflammation as well as corticosteroid resistance (Chung, 2011). Different extracellular pro-inflammatory stimuli that are relevant to COPD, such as cigarette smoke, cytokines, toll-like receptor agonists and oxidative stress, increase p38 phosphorylation and activity. Indeed, increased activation of p38α was shown in epithelial cells, alveolar macrophages, and lymphocytes in the lungs from COPD patients (Renda et al., 2008; Gaffey et al., 2013). In this regard, a p38α inhibitor, SD-282, was shown to suppress the neutrophilic inflammatory response in mice exposed to cigarette smoke and to ozone, even when corticosteroids were ineffective (Williams et al., 2008; Mclnicherla et al., 2008). Noteworthy, in PBMC from COPD patients, the reduced responsiveness to corticosteroids was reversed by treatment with a p38α inhibitor (Khorasani et al., 2015). P38γ could also promote corticosteroid insensitivity since it induces GRζ phosphorylation reducing its nuclear translocation (Mercado et al., 2011).

Activation of p38 is directly associated to the production of inflammatory cytokines from airway epithelial cells infected with respiratory viruses which can contribute to COPD exacerbations (Grego et al., 2000). In this regard, several orally administered p38 inhibitors have been developed to target inflammation in COPD. Losmapimod, an oral dual p38α/b inhibitor, was evaluated in a phase II study with 300 COPD patients over 12 weeks. Even though it reduced phosphorylation of hsp-27 and plasma fibrinogen by approximately 11%, it did not affect sputum neutrophils (Lomas et al., 2012). During a larger clinical trial studying 600 COPD patients over 24 weeks, three doses of losmapimod were ineffective to improve exercise tolerance (6-minute walking distance), although there was a trend for reduction in inflammatory exacerbations. Nevertheless, there were considerable side effects at the highest dose tested (Watz et al., 2014). Of interest, further studies discard any effect of losmapimod on exacerbations (Pascoe et al., 2017).

PH-797804, an oral p38α-selective inhibitor, studied in 230 COPD patients for 6 weeks, increased trough FEV1 values and improve dyspnea at some doses (MacNee et al., 2013). Unfortunately, most of these oral p38 inhibitors in high doses have caused unacceptable side effects including liver toxicity and skin reactions (Barnes, 2016). Yet, an exploratory study using Acumpimod, a highly selective p38α/β MAPK inhibitor, orally active, for the treatment of COPD exacerbations showed beneficial effects over FEV1 values in a post-hoc analysis. As exacerbations are acute events, patients just received either 1 or 2 doses of Acumpimod which prevented the occurrence of adverse events (Strambu et al., 2019).

Aiming to diminish systemic treatment toxicity, inhaled p38 inhibitors were developed. For instance, RV-568 is a potent inhaled p38 inhibitor that inhibits all four isoforms of p38 and a member of Src family kinase “Hck”, thus, is referred to as a narrow spectrum kinase inhibitor. RV-568 presented significant anti-inflammatory activity in animal models of COPD and increased FEV1 and reduced sputum malondialdehyde, a product of oxidative damaged lipids, after 2 weeks of administration in COPD patients. Although RV-568 was well-tolerated by COPD patients, it only demonstrated a modest clinical benefit (Charron et al., 2017). Another pharmaceutical approach to avoid the occurrence of adverse effects of p38 inhibitors is to target the p38 substrate MAP-activated protein kinase 2 (MK2), which mediates many inflammatory actions of p38 (Singh and Najmi, 2019). No clinical studies were yet performed to assess the effectiveness of these inhibitors for COPD.

5.30.4.3.2 PI3K-inhibitors

PIK3s are a family of lipid kinases that phosphorylate phosphatidylinositol in the cell membrane to generate phosphatidylinositol-(3,4,5)-trisphosphate (PIP3), a lipid second messenger that regulate many cellular processes, including those related to immune responses (Hawkins and Stephens, 2015; Vanhaesebroeck et al., 2012). Class I PI3Ks, are heterodimers comprising a catalytic subunit with four isoforms (designated p110α, β, γ and δ) and an adaptor subunit of either p85 (class IA) or p110/p84 (class IB). While PI3Kα and PI3Kβ are ubiquitously expressed, PI3Kδ and PI3Kγ are localized predominantly in leukocytes. Several receptor tyrosine kinases (RTKs) activate PI3Kδ, while PI3Kγ activation is triggered by G-protein-coupled receptors including chemokine receptors (Hawkins and Stephens, 2015). Both isoforms promote PIP3 accumulation and PI3K/Akt/mTORC pathway.
activation. Noteworthy, PI3Kγ-induced activation of Akt is the pivotal signaling pathway mediating neutrophil recruitment to the lung after the instillation of chemokines (Thomas et al., 2005).

Inhibition of PI3Kγ with IC87114 reverses corticosteroid-resistance in mice exposed to cigarette smoke. Moreover, the deletion of PI3Kγ restored histone deacteylase 2 activity and the anti-inflammatory effects of glucocorticoids, whereas PI3Kγ deletion did not affect glucocorticoids sensitivity (Marwick et al., 2009). In addition, the silencing of PI3Kγ in a monocytic cell line avoided corticosteroid-resistance, whereas the silencing of PI3Kγ did not (To et al., 2010). This suggests that PI3Kγ might be directly involved in corticosteroid resistance induced by oxidative stress. Hence, several clinical trials are ongoing to test the safety and efficacy of inhaled PI3Kγ inhibitors in COPD (Erra et al., 2018; Wilson et al., 2019). Nevertheless, there is a good rationale for the development of dual PI3Kγ/δ inhibitors for lung inflammatory diseases with principal emphasis on COPD. Besides the weak improvement of the anti-inflammatory profile with PI3Kγ inhibition, the blockage of both isoforms may avoid compensatory mechanisms that are often observed, at least in cancer treatment, when specific kinases are inhibited (Winkler et al., 2013).

mTOR is highly activated in PBMC derived from COPD patients compared to PBMC from healthy smokers. Therefore, rapamycin, a macrolide that functions as an allosteric inhibitor of mTORC1, was suggested as another therapeutic approach for COPD. Interestingly, rapamycin treatment in COPD PBMCs reduced mTOR activation, IL-8 production and corticosteroid resistance (Mitani et al., 2016). Given the increased spectrum of adverse effects, there is a particular interest in finding less toxic analogs of rapamycin with improved pharmacokinetics (rapalogs). Phosphatases are also interesting targets since their activation switches off the pro-inflammatory intracellular signaling in the context of COPD. For instance, activation of Src homology 2 domain-containing inositol 5-phosphatase-1 (SHIP), might be useful since it dephosphorylates Akt preventing the activation of the PI3K/Akt/mTOR signaling pathway. The safety and efficacy of AQX-1125, a SHIP activator, has been tested in clinical trials with COPD patients, showing a favorable safety profile (ClinicalTrials.gov Identifier: NCT01954628).

Other kinases like Janus Activated Kinases (JAK), which principally mediates the transduction of signals in response to cytokines, are also truly relevant in chronic inflammation on COPD. Several oral JAK inhibitors (jakinibs) are now approved or in advanced stages of clinical trials for the treatment of other inflammatory diseases such as rheumatoid arthritis (Schwartz et al., 2016). Inhaled jakinibs have been developed for lung diseases, however, there are still no randomized clinical trials to evaluate the effectiveness of jakinibs in COPD (Menet et al., 2013; Schwartz et al., 2016; Barnes, 2016).

Much consideration should be given to the development of treatment protocols for COPD that include kinase inhibitors. Once a particular kinase becomes activated it can phosphorylate several different proteins that share a consensus sequence, activating signaling pathways that regulate other metabolic processes leading to off-target effects. For that reason, kinase inhibitors should ideally have increased selectivity and be extensively screened for toxicity. Moreover, identifying the specific signaling pathway related to disease development and the ultimate route of administration for the inhibitor is challenging.

### 5.30.4.4 Phosphodiesterase 4 inhibitors

Second messengers control cellular processes in time, length, intensity, and space allowing the compartmentalization of cellular processes. Cyclic AMP (cAMP) is an important intracellular second messenger controlling several anti-inflammatory and pro-resolving effects. Increased levels of cAMP suppress NF-κB-mediated transcription of inflammatory proteins through the activation of protein kinase A (PKA), or the exchange proteins directly activated by cAMP (EPAC1 and 2) (Tavares et al., 2020a). cAMP can also reduce TNF-α, CXCL8 and IL-6 production by inhibition of TNF-α-converting enzyme (TACE) independently of NF-κB in alveolar epithelial cells (Wyatt et al., 2014). In addition, cAMP promotes granulocyte apoptosis and phagocytosis and induces macrophage polarization towards an anti-inflammatory phenotype, two pivotal steps of resolution of inflammation (Tavares et al., 2020a). Moreover, it promotes the biosynthesis of pro-resolving molecules such as Annexin A1 (AnxA1) (Tavares et al., 2017). cAMP production is mediated by adenylyl and guanylyl cyclases, while the degradation is mediated by a superfamily of phosphatases containing inositol 5-phosphatase-1 (SHIP), might be useful since it dephosphorylates Akt preventing the activation of the PI3K/Akt/mTOR signaling pathway. The safety and efficacy of AQX-1125, a SHIP activator, has been tested in clinical trials with COPD patients, showing a favorable safety profile (ClinicalTrials.gov Identifier: NCT01954628).

PDE4 is the predominant isoenzyme in inflammatory cells. It is also expressed in the airways smooth muscle, brain, and cardiovascular tissues, being the most characterized PDE isoenzyme (Boswell-Smith et al., 2006). The first generation of PDE4 inhibitors showed effective reduction of a wide range of inflammatory cell functions in vitro observed in eosinophils, lymphocytes, basophils, and neutrophils (Nielson et al., 1990; Weston et al., 1997; Giembycz et al., 1996; Dent et al., 1991). Furthermore, they were highly effective at suppressing inflammation in animal models of respiratory disease (Torphy and Undem, 1991). Additionally, PDE4 inhibitors were shown to induce relaxation of isolated human bronchus (Cortijo et al., 1993), suggesting that one single molecule could condensate the two most desirable effects in COPD treatment: inhibition of inflammation and bronchoconstriction. Nevertheless, gastrointestinal disturbances such as emesis, associated with noradrenergic pathway interference in the brain, and nausea demanded the development of second generations of PDE4 inhibitors to be safe enough for COPD treatment (Boswell-Smith et al., 2006).

Currently, Cilomilast and Rolflumilast, two second generation PDE4 inhibitors, are approved as an add-on therapy for a subgroup of people with persistent symptoms or exacerbations despite optimal COPD management (e.g., people whose condition is not controlled by fixed-dose long-acting beta2-agonist (LABA) and inhaled corticosteroid (ICS) combinations). Despite no observable effect over mortality, PDE4 inhibitors provokes a small improvement in forced vital capacity and quality of life, although it is uncertain if they are capable to increase FEV₁ (Disease GIFCOL, 2020). In addition, treatment roflumilast or reduced by
approximately 20% the odds of experiencing a COPD exacerbation over a year in COPD patients. Despite the improvements on drug safety, some patients still suffer from gastrointestinal adverse events when treated with second generation PDE4 inhibitors (Janjua et al., 2020). The scientific community is optimistic about the recent development of dual PDE3 and PDE4 inhibitors that present potent bronchodilation and anti-inflammatory effects (Beute et al., 2018; Venkatasamy and Spina, 2016). On the other hand, the development of inhaled PDE4 inhibitors might limit their distribution to the brain and reduce side effects (Ti et al., 2019).

5.30.4.5 Chemokine-targeted therapies

The pivotal role of neutrophils in COPD pathogenesis raises significant interest in possible ways to selectively inhibit cell migration to the lungs. Chemokines are a specific subset of cytokines, which principal function relies on driving cell migration. Although many chemokines bind to more than one chemokine receptor, CXCL8 induces neutrophil chemotaxis through the activation of CXCR1 and CXCR2 receptors with enough accuracy to be considered a potential therapeutic target. Therefore, several pharmacological approaches were suggested to inhibit the neutrophil recruitment via CXCL8-CXCR1/2 axis blockage in COPD.

Neutralizing antibodies against CXCL8 were shown to decreased neutrophil counts in the sputum of COPD patients but no significant clinical benefits were observed (Henrot et al., 2019). Therefore, strategies focused on the receptor for neutrophil chemokines were proposed. Navarixin, a CXCR2 antagonist, reduced sputum neutrophils in COPD patients and improved FEV₁ efficiently in current smokers but not in former smokers in phase II clinical study. It also increased the time-to-first exacerbation and reduced overall inflammation (Rennard et al., 2015). Although the treatment did not increase infection rates, the clinical trial was terminated probably due to the significantly low neutrophil blood counts observed in treated patients (NCIT01006616). Another CXCR2 antagonist, Danirixin, was shown to inhibit neutrophil migration and activation in the lung in preclinical models of neutrophilic airway inflammation. A clinical trial with 93 patients showed that Danirixin treatment tended to improve respiratory symptoms and health status in COPD patients (Lazaar et al., 2018). However, a later study involving 614 participants for 26 weeks, showed no significant clinical benefit associated with Danirixin treatment compared to the placebo group. Furthermore, the treatment with the highest dose (50 mg) increased the rate of exacerbations and the number of pneumonia cases (Lazaar et al., 2020).

Despite the lack of relevant clinical improvements of CXCR2 inhibition in COPD, recent studies have been evaluating the use of superior antagonists, with dual inhibition of CXCR1 and CXCR2, that may present higher potency. For instance, Ladarixin is an allosteric non-competitive CXCR1/2 antagonist that has shown satisfactory reduction in neutrophilic inflammation in several models of lung diseases. Currently, clinical trials are ongoing to test Ladarixin in patients with new-onset of type-1 diabetes (Tavares et al., 2017a). Recently, preclinical studies identified a protective effect of this drug in a model of COPD in mice (Mattos et al., 2020).

5.30.4.6 Challenges and perspectives

This section enumerates, some of the immunomodulatory strategies tried so far to prevent disease progression and improve lung function in COPD patients. Pro-resolving lipid mediators (Hsiao et al., 2015), PDE2 antagonists (Snell et al., 2013), antibodies directed against inflammatory cytokines (Barnes, 2018), are among the other several approaches which, at the moment, did not demonstrate a relevant clinical benefit when tested in clinical trials despite their success in animal models. Among all the immunomodulatory strategies mentioned along the section, just two of them, both showing limited clinical benefit, were approved for clinical use (corticosteroids and PDE4 inhibitors). This reinforce the enormous pharmacological challenge that chronic inflammation in COPD represents. Of note, the optimization of low molecular weight drugs (LMWDs) is a major field of drug discovery and management of inflammatory diseases. The improvement of selectivity and pharmacokinetics will, hopefully, bring up more potent and efficient drugs to promote anti-inflammatory pathways or inhibit inflammatory ones. This will result in reduced costs, side effects, and why not, allow sophisticated and personalized drug combinations. In addition, epigenetic modulation of inflammatory genes may offer a novel strategy for the treatment of lung diseases.

5.30.5 Cystic fibrosis

Cystic fibrosis is an autosomal recessive genetic disease characterized by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (Elborn, 2016). CFTR gene codifies a chloride-conducting transmembrane channel that regulates anion transport in the airways, intestines, pancreas, kidneys, sweat gland and male reproductive tract (Elborn, 2016). The CFTR protein have additional functions, including bicarbonate secretion, with consequent regulation of airway pH, and inhibition of the epithelial sodium channel (ENaC), that controls the hydration of secretions and mucus (Gentzsch and Mall, 2018). Numerous variants of CFTR gene were described in cystic fibrosis patients, with different CFTR functional consequences that define disease severity (Bell et al., 2020). Patients presenting CFTR gene variants that lead to little or no protein function usually have a more severe phenotype of disease, while other variants lead to residual CFTR function and less severe disease (Boyle and De Boeck, 2013). Cystic fibrosis is an important cause of morbidity and mortality affecting more than 80,000 patients worldwide (De Boeck and Amaral, 2016). Indeed, cystic fibrosis has been considered the most common lethal genetic disease in the Caucasian population (O'Sullivan and Freedman, 2009).
Cystic fibrosis patients can experience exocrine pancreatic insufficiency, hepatobiliary manifestations, nutrient malabsorption, male infertility, and pulmonary disease (Elborn, 2016). The lung pathology is characterized by bronchiectasis, mucus accumulation due to impaired mucociliary clearance, increased susceptibility to infections and hyper-inflammation (Stoltz et al., 2015). The sustained neutrophilic inflammatory response in the lungs of cystic fibrosis patients is associated with progressive pulmonary damage and function impairment (Jacquot et al., 2008). Importantly, respiratory failure is the leading cause of death among cystic fibrosis patients that do not receive lung transplant (Elborn, 2016). Pulmonary inflammation in cystic fibrosis is self-perpetuating as the intense recruitment of neutrophils, increased oxidative stress and chronic infections cause airway destruction amplifying the response through liberation of DAMPs and PAMPs (Cohen-Cymberknoh et al., 2013). It is not yet clear whether the hyper-inflammatory state in the lungs of cystic fibrosis patients is caused by chronic airway infections or is directly related to the CFTR gene mutation itself. Defects in the CFTR impairs chloride movement into the airway lumen, leading to increased absorption of sodium and water that result in dehydration of the airway surface liquid (Matsui et al., 1998). This culminates with mucus concentration and accumulation (given the reduced mucociliary clearance) forming a fertile environment for bacterial growth that can trigger intense airway inflammation (Armstrong et al., 2005). Furthermore, equivalent increased levels of pro-inflammatory cytokines were observed in cystic fibrosis patients with detectable or undetectable infections (Muhlebach et al., 2004) and spontaneous inflammatory responses were observed within uninfected lung xenografts from cystic fibrosis patients in mice (Tirouvanziam et al., 2002). Further studies will help to elucidate the origins of inflammation during cystic fibrosis; however, it is common ground that pulmonary inflammation is a determinant of disease severity, associates with the impaired immune responses and is amplified by the presence of chronic infections (Roesch et al., 2018).

In addition to bacterial infections, fungi and viruses are other causes of inflammation exacerbation in cystic fibrosis. Among the bacterial pathogens, Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenzae and Burkholderia cepacia complex, are the most common (Bhagirath et al., 2016). Of importance, P. aeruginosa is one of the most prevalent bacterial infection in cystic fibrosis patients causing chronic and antibiotic-resistant infections (Parkins et al., 2018). Respiratory virus such as influenza, rhinovirus, respiratory syncytial virus, and adenovirus are the most common viral causes of inflammatory exacerbations leading to lung function impairment in cystic fibrosis patients (Shale, 1992). Fungi infections, although often underdiagnosed, contribute to increased hypersensitivity pulmonary responses and inflammation in cystic fibrosis patients. In this regard, allergic bronchopulmonary aspergillosis can affect up to 15% of patients and is characterized by increased type 2 inflammatory responses and deterioration of lung function (Janahli et al., 2017).

The inflammatory responses in the lungs of cystic fibrosis patients start very early in life even before the first clinical manifestations of disease (Armstrong et al., 1995; Khan et al., 1995). Pulmonary inflammation in cystic fibrosis patients are characterized by elevated concentrations of pro-inflammatory cytokines including IL-6, CXCL8, IL-17, IL-33, GM-CSF, G-CSF, and HMG-1 (Bongfeldt et al., 1995; Moser et al., 2005; Koller et al., 1997) and other mediators such as leukotriene B4 (LTB4) (Konstan et al., 1993; Lawrence and Sorrell, 1994). The increased secretion of pro-inflammatory cytokines is related to important disease symptoms. For instance, increased TNF-α secretion is associated with increased frequency of asthma, higher neutrophil activation, and decreased bone density in cystic fibrosis (Shmarina et al., 2013). In addition, pro-inflammatory cytokines induce leukocyte recruitment and activation leading to secretion of reactive oxygen species and proteases. As such, excessive amounts of IL-17 and CXCL8 in the airways of cystic fibrosis patients are associated with the increased recruitment and activation of neutrophils in the lungs (Oglesby et al., 2015; Taylor et al., 2016). Despite its protective role against P. aeruginosa and other pathogens in the respiratory tract of patients, neutrophils are the main players in the immunopathogenesis of cystic fibrosis (Tirouvanziam, 2006). Neutrophil activation leads to degranulation and release of proteases such as elastase and metalloproteinases. Of importance, levels of neutrophil elastase correlate with severity of disease in cystic fibrosis (Sly et al., 2013; Sagel et al., 2012). Neutrophil elastase (NE) can promote alveoli damage, mucus hypersecretion and reduction of CFTR function (Le Gars et al., 2013; Lee and Downey, 2001; Voynow et al., 2004). As such, NE levels in bronchoalveolar lavage or sputum has often been considered a biomarker for cystic fibrosis lung disease (Mayer-Hamblett et al., 2007; Sly et al., 2013; Sagel et al., 2012; Meyer and Zimmerman, 1993). Metalloproteinases, in addition to serine proteases (such as elastase), are also upregulated in cystic fibrosis patients and correlates with airway inflammation and impaired pulmonary function (Roderfeld et al., 2009; Delacourt et al., 1995; Sagel et al., 2005). The increased release of neutrophil proteases has been implicated in the cleavage of immune receptors and consequent reduced function of lymphocytes (Doring et al., 1995), macrophages (Vandivier et al., 2002), dendritic cells (Roghanian et al., 2006) and neutrophils themselves (Hartl et al., 2007). The reduced effector function of neutrophils may also be attributed to the absence of a functional CFTR. Defective CFTR in the phagolysosome membrane of neutrophils reduces the levels of chloride anion in the phagosome compartment, the production hypochlorous acid and killing of phagocytosed pathogens (Painter et al., 2008). In addition, impaired phagocytosis ability was also described for neutrophils of cystic fibrosis patients (Morris et al., 2005). Despite that, neutrophils of cystic fibrosis patients are alive and maintain an active ROS production and granule release (McKeon et al., 2010). Phenotypically, neutrophils from cystic fibrosis present her levels of activation markers including CD80 and major histocompatibility complex type II, and lower levels of CD16 and CD14, important receptors for phagocytosis (Tirouvanziam et al., 2008).

The increased recruitment of neutrophils contributes greatly for the increased pulmonary oxidative stress of cystic fibrosis patients (Van Der Vliet et al., 1997; Kettle et al., 2004). Activated neutrophils release increased levels of superoxide anion (O2·−), hydrogen peroxide (H2O2) and hydroxyl free radicals (OH·), adding up to the production of ROS by airway epithelial cells (Galli et al., 2012). Abnormal levels of ROS contribute to epithelial damage, and lead to oxidation of pulmonary surfactant which alters its
biophysical properties aiding to reduced lung function in cystic fibrosis (Pulfer and Murphy, 2004; Hull et al., 1997; Galli et al., 2012). In addition, ROS molecules work as second messengers activating pro-inflammatory transcription factors such as NF-κB (Rahman et al., 2006). In cystic fibrosis, ROS-induced inflammatory signaling boosts the production of cytokines which amplifies the already increased lung inflammation (Chen et al., 2008). Moreover, the neutrophil-induced oxidative stress can trigger neutrophil extracellular traps (NETs) formation (Fuchs et al., 2007). Cystic fibrosis patients present increased levels of extracellular DNA in the airways which have been associated with increased release of NETs (Dwyer et al., 2014). The primary function of NETs is to act as an anti-bacterial defense, promoting the entrapment of bacteria and release of anti-microbial molecules (Brinkmann and Zychlinsky, 2012). In cystic fibrosis, the released extracellular DNA increases the mucus viscosity in the airways contributing to airway obstruction (Marcos et al., 2015; Martinez-Aleman et al., 2017). In addition, NET release may also contribute to pulmonary damage inducing airway epithelial cytotoxicity (Saffarzadeh et al., 2012). While NETs seems to have little effect preventing the proliferation of bacteria in the airways of cystic fibrosis patients, NET release may be more harmful than beneficial in the context of disease (Martinez-Aleman et al., 2017).

Macrophages and monocyte chemokines are also enriched in the airways of cystic fibrosis patients (Brennan et al., 2009). Alveolar macrophages are important sentinels in the airways, responding to infections through phagocytosis and killing of invaders and by the production of pro-inflammatory mediators. In addition, macrophages are also important to coordinate termination of inflammation (Watanabe et al., 2019). Efferocytosis and production of pro-resolving mediators are important macrophage tasks. In this regard, different phenotypes of macrophages coordinate these contrasting responses (Hussell and Bell, 2014). In cystic fibrosis, macrophages present a hyper-inflammatory phenotype and present impaired efferocytosis and anti-bacterial abilities (Bruscia and Bonfield, 2016). Macrophages derived from cystic fibrosis patients secrete high amounts of pro-inflammatory cytokines upon stimulation and have lower expression of surface receptors including MARCO, CD206 and CD11b, molecules involved in phagocytosis of bacteria and efferocytosis of apoptotic neutrophils (Wright et al., 2009; Simonin-Le Jeune et al., 2013). The functional defect of macrophages is both intrinsic, as it relates to impaired CFTR function (Zaman et al., 2004; Di et al., 2006), and extrinsic, depending on the neutrophil inflammation and release of cytokines proteases (Vandivier et al., 2002).

Macrophages are bridges between the innate and adaptive immunity. Functioning as antigen presenting cells, macrophages can influence lymphocyte responses (Bruscia and Bonfield, 2016). Cystic fibrosis patients present increased numbers of pulmonary Th17, Th2 and γδ T cells (Tan et al., 2011; Raga et al., 2003; Tiringer et al., 2013; Mueller et al., 2011). Of importance, Th17 cells are important producers of IL-17 and CXCL8 amplifying the neutrophilic inflammation in the lungs (Tan et al., 2011). In addition, γδ T cells also contribute to inflammation through the production of TNF-α, IFN-γ and IL-17 when the airways are infected with P. aeruginosa (Raga et al., 2003). On the other hand, regulatory T cells (Tregs), important controllers of inflammation, are reduced in blood and airways of cystic fibrosis patients (Hector et al., 2015). Altogether the overall response in cystic fibrosis favors a pro-inflammatory environment, at the expense of the anti-inflammatory counterpart.

In the past years, the molecular and cellular components of cystic fibrosis pathogenesis were uncovered and allowed the development of different therapies to mitigate symptoms and manage disease. As such, it was observed a significant increase in the life expectancy in cystic fibrosis to more than 40 years, in developed countries (O’Sullivan and Freedman, 2009). Normalization of the host response and ultimately cure of cystic fibrosis depends on the molecular correction of the CFTR defect. Nevertheless, there is an urgent need for novel therapeutics focused on modulating pulmonary inflammation, the main cause of disease morbidity (Jacquot et al., 2008).

Anti-inflammatory drugs used in association with aggressive antibiotic protocols and mucolytic therapies are valuable for cystic fibrosis. Different pharmacological strategies have been proposed to modulate inflammatory exacerbations therefore lowering the progression of disease. Given the early onset of pulmonary inflammation (Armstrong et al., 1995, Khan et al., 1995), immunomodulatory therapies for cystic fibrosis should, in theory, be prescribed during early stages of disease (Torphy et al., 2015). Although several clinical studies were carried out to uncover the potential benefits of anti-inflammatory drugs in cystic fibrosis, only few drugs were translated to clinical use. The length of this section precludes a detailed description of all immunomodulatory strategies proposed to treat cystic fibrosis. Here, we summarize important pharmacological approaches proposed to control exacerbated inflammation in cystic fibrosis (Fig. 5).

### 5.30.5.1 Corticosteroids

Corticosteroids are non-specific anti-inflammatory molecules that have been used to treat several chronic inflammatory disorders, including respiratory pathologies. The mechanism of action of corticosteroids involve the inhibition of pro-inflammatory signaling pathways (e.g., NF-κB), the secretion of several proinflammatory mediators and chemotaxis, all of which are features of the inflammatory responses in cystic fibrosis (Roesch et al., 2018). The first study evaluating the systemic and prolonged use of corticosteroids for cystic fibrosis started over 30 years ago (Auerbach et al., 1985). The 4-years clinical trial evaluated a small group of cystic fibrosis pediatric patients with mild to moderate disease that received alternate-day prednisone or placebo. The prednisone group presented improved lung function, superior weight gain and vital capacity, and fewer hospital admissions compared to placebo (Auerbach et al., 1985). Later, a multicenter study evaluated the same treatment schedule in a larger number of cystic fibrosis patients. Similarly, the second study observed beneficial effects of prednisone in lung function analysis of patients, but they also reported high incidence of corticoid-related side effects (Eigen et al., 1995). Impaired glucose metabolism, obesity, acne, muscle wasting, growth impairment in children and thinning of the skin are some of the adverse events associated with high doses of systemic corticosteroids (Balfour-Lynn et al., 2019). To undermine the adverse events related to systemic corticosteroids, drug administration by...
Inhalation was proposed. In the United States, a significant proportion of cystic fibrosis patients are prescribed inhaled corticosteroids (ICS) (Cystic Fibrosis Foundation, 2019). The efficacy of ICS to treat cystic fibrosis symptoms was evaluated in different studies in the past years. The randomized trials that were conducted to test the potential beneficial of ICS to treat pulmonary exacerbations presented contrasting results. Recently, an updated Cochrane systematic review of randomized trials concluded that evidence compiled by the randomized trials is insufficient to confirm whether ICS are valuable for cystic fibrosis treatment (Balfour-Lynn et al., 2019). Among the studies, small increases in lung function and decreases in inflammation and bronchial hyperresponsiveness to histamine were reported (Wojtczak et al., 2001, Ren et al., 2008, van Haren et al., 1995; Bisgaard et al., 1997), while others did not find any significant improvements (Balfour-Lynn et al., 1997, 2006; De Boeck et al., 2007; Schiotz et al., 1983). Different schedule of treatment (short-term versus long-term) and doses of corticosteroid were evaluated in the studies (Balfour-Lynn et al., 2019). No consistency regarding duration or dosage was associated to the reported beneficial effects of ICS. In addition, important adverse events were described including impairment in growth in patients with high dose ICS (de Boeck et al., 2007; Ren et al., 2008). Therefore, given the inconsistency in the trials and unwanted effects observed, the Cystic Fibrosis Foundation recommends against the regular use of ICS or systemic corticosteroid therapies to improve lung function in cystic fibrosis patients without asthma or allergic bronchopulmonary aspergillosis (Mogayzel et al., 2013).

### 5.30.5.2 Ibuprofen

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) that inhibits the activity of cyclooxygenases (COX-1 and COX-2), leading to decreased synthesis of prostaglandins. In addition to this classic mechanism of action, the anti-inflammatory effects of ibuprofen also relies on the suppression of NF-κB and AP-1 activity (Tegeder et al., 2001). Moreover, high doses of ibuprofen were shown to stimulate the Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) (Lehmann et al., 1997), a negative regulator of inflammation that is downregulated in CFTR-deficient cells (Ollero et al., 2004).

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**Fig. 5** Immunomodulatory strategies for cystic fibrosis. Schematic representation of a cystic fibrosis lung and the underlying exaggerated inflammatory that contribute to the worsening of disease: excessive recruitment and activation of leukocytes with consequent production of pro-inflammatory mediators, reactive oxygen species (ROS), proteases and neutrophil extracellular traps (NETs). In addition, the dysfunctional immune responses in the lungs and the increase accumulation of mucus lead to overgrowth of bacteria in the pulmonary mucosa. Different immunomodulatory therapies were proposed to overcome this problem and are shown in the figure: antagonists of receptors for leukotrienes and pro-inflammatory cytokines, anti-proteases, CFTR modulators, monoclonal antibodies targeting cytokines, DNAse to target NETs, antioxidants that decrease the levels of ROS and immunomodulatory antibiotics that reduce both inflammation and bacterial colonization in the lungs of cystic fibrosis patients. Created with BioRender.com.
In cystic fibrosis patients, high-dose ibuprofen was shown to significantly reduce the rate of decline in lung function when compared to placebo groups (Konstan et al., 1995; Konstan et al., 2007). Recently, the decreased lung function impairment associated with 2-year high-dose ibuprofen treatment, was correlated with improved survival among children (Konstan et al., 2018). Of note, the slower decline in lung function seems to be more pronounced in younger children than individuals aged 13 years and older (Lands and Stanojevic, 2019). Based on pharmacokinetics analysis, patients receive an adjusted dose of ibuprofen (generally 20–30 mg/kg, given twice a day) to allow a peak plasma concentration of 50–100 µg per milliliter (Konstan et al., 1993). The beneficial effects of ibuprofen can be related to the drug-induce reduction in neutrophil chemotaxis (Konstan et al., 2003; Brown and Collins, 1977; Venezio et al., 1985), probably due to inhibition of LTB4 synthesis (Konstan et al., 1990). In addition, the suppression of NF-kB by high doses of ibuprofen could theoretically decrease CXCL8, an important chemoattractant for neutrophils. However, an in vitro study reported no differences in CXCL8 production by respiratory epithelial cells of cystic fibrosis patients treated with ibuprofen (Daal et al., 2010). In agreement with that, the levels of pro-inflammatory mediators including CXCL8, TNF-α and IL-1β were not reduced in the sputum of patients receiving this drug (Chmiel et al., 2015). Therefore, the ibuprofen mechanisms for reduction in neutrophil infiltration are not completely understood.

Despite reducing the inflammatory response, preclinical studies have suggested that high doses of ibuprofen do not impair bacterial clearance in cystic fibrosis patients (Oermann et al., 1999). On the contrary, the antimicrobial activity of ibuprofen against different bacteria, including cystic fibrosis-associated pathogens, was determined in vitro and in vivo (Elvers and Wright, 1995; Shah et al., 2018; Shirin et al., 2006), aiding to the activity of other antimicrobial drugs (Byrne et al., 2007; del Prado et al., 2006; Chan et al., 2017). Nevertheless, the use of ibuprofen during intravenous administration of aminoglycosides is associated with increased risk of acute renal failure and must be avoided during administration of this class of antimicrobial drugs (Kavesi et al., 1998; Scott et al., 2001; Bertenshaw et al., 2007). Another important concern regarding the routine use of NSAIDs is the potential adverse events. Abdominal pain is listed as the most common side effect, and few cases of gastrointestinal bleeding are also described (Fennell et al., 2007). The prophylactic use of a H2-blocker or proton ion pump inhibitor might be valuable to protect cystic fibrosis patients from gastrointestinal unwanted effects (Lands et al., 2007). Given the amount of evidence supporting the use of ibuprofen for cystic fibrosis, the Cystic Fibrosis Foundation Guideline recommends the chronic oral treatment with high doses of the drug in patients with 6–17 years of age (Kapnadak et al., 2020).

### 5.30.5.3 Cytokine-targeted therapies

Few pre-clinical studies have been carried to evaluate the specific inhibition of pro-inflammatory cytokines to prevent lung neutrophilic inflammation in cystic fibrosis. IL-17 neutralization using monoclonal antibodies was shown to decrease inflammation-induced lung injury in a murine model of cystic fibrosis (Hsu et al., 2016). In addition, approaches that block signaling of CXCL8 were attempted. Exposure of bronchoalveolar lavage fluid (BALF) from cystic fibrosis patients to PA401, an CXCL8 decoy, reduced its chemoattractant activity to neutrophils (Elvers et al., 2015). Moreover, the antagonism of the CXCL8 receptor CXCR2 by SB-656933, an oral and well-tolerated small molecule, showed minor trends for reduction of inflammatory markers in sputum from cystic fibrosis patients (Moss et al., 2013). While blockage of cytokine binding and signaling is an interesting strategy to control inflammation, none of these approaches presented significant evidence for protection of lung damage in cystic fibrosis. More recently, the potential involvement of IL1-β inducing inflammation and mucus secretion in cystic fibrosis was proposed and might represent a novel therapeutic target for young cystic fibrosis patients (Chen et al., 2019).

Besides cytokines, pro-inflammatory lipid mediators are targets for immunomodulatory therapies in cystic fibrosis. The synthesis of leukotrienes initiates with the conversion of arachidonic acid (AA) by the sequential activity of enzymes including 5-lipoxygenase (5-LOX) and LTA4 hydrolase (LTA4H) (Wan et al., 2017). LTB4 is a potent neutrophil chemoattractant that binds to the high-affinity receptor BLT1 and the low-affinity receptor BLT2 (Saeki and Yokomizo, 2017). The antagonism of BLT1 to treat cystic fibrosis was evaluated in a phase 2 clinical trial of the drug amelutin (BII. 284 BS) (Konstan et al., 2014). The study was terminated prematurely due to an unexpected increase in pulmonary exacerbations, possibly related to overly decreased inflammation and increased susceptibility to infections (Konstan et al., 2014). This example serves as a cautionary tale for the development of novel immunomodulatory drugs to treat cystic fibrosis and other chronic inflammatory diseases in the lung. More recently, a different strategy to decrease LTB4 activity was formulated. Acebilustat (CTX-4430) is an oral inhibitor of LTA4H that was found to be safe and well tolerated (Elborn et al., 2017). During the phase 1 trials, Acebilustat diminished inflammatory markers in the sputum of patients with cystic fibrosis (Elborn et al., 2017). Therefore, the second phase was launched evaluating a larger population to access the potential benefits of Acebilustat in cystic fibrosis patients (Elborn et al., 2018). Recently, the clinical trial was completed, and the results will clarify the therapeutic potential of this drug (ClinicalTrials.gov Identifier: NCT02443688). Another approach to decrease leukotriene-mediated inflammation, is the use of antagonists for the cysteinyi leukotriene receptor-1 (CysLTR1), a receptor for LTC4, LTD4 and LTE4 (Kanaoka and Boyce, 2004). Montelukast, a competitive antagonist of CysLTR1, given once daily was shown to reduce inflammatory markers in a small group of young cystic fibrosis patients (5–18 years) taking the drug for 21 days (Schmitt-Grohe et al., 2002). The long-term effects of Montelukast in the same population were observed in a follow-up study and showed sustained reductions in inflammatory markers and a significant decrease in P. aeruginosa colonization after treatment in combination with antibiotics (Schmitt-Grohe et al., 2007). In addition, a longer treatment schedule (8 weeks) was evaluated in moderate cystic fibrosis patients and showed improvements in lung function measurements, inflammatory markers and decreases in cough and wheezing scale scores (Stelmach et al., 2005). Further studies with larger populations will uncover the extent of the beneficial effects of antagonists of CysLTRs in cystic fibrosis patients.
5.30.5.4 Anti-proteases

Serine proteases such as NE are important targets for treating the unbalanced proteolytic activity in cystic fibrosis. Inhaled administration of the anti-protease z-anti-trypsin (AAT) was proposed as a safe therapeutic approach that decreased NE levels in the respiratory epithelial lining fluid (ELF) of a small group of cystic fibrosis patients and restored neutrophil defenses against bacteria (McElvaney et al., 1991; Martin et al., 2006). In agreement with that, a subsequent study showed that inhaled AAT given for 4 weeks significantly decreased inflammatory markers in the sputum of cystic fibrosis patients in comparison with baseline levels (Griese et al., 2007). Nevertheless, an important limitation of this study is the absence of placebo controls to assess the potential benefits of inhaled AAT therapy (Griese et al., 2007). A randomized, placebo-controlled trial showed reduced levels of myeloperoxidase in sputum of cystic fibrosis patients after inhaled AAT therapy, but no reduction in NE levels was observed (Martin et al., 2006). Similarly, inhaled prostatin, a therapeutic formulation of alpha-1 proteinase inhibitor, decreased taurine (an indirect measurement of neutrophils), but not NE levels in sputum of cystic fibrosis patients (Cantin et al., 2006). The contrasting results may be related to the formulation and devices for administration of the anti-protease therapy and to the difficult sampling of the airway environment in cystic fibrosis patients.

Currently, a phase II randomized, placebo-controlled multicenter study is evaluating the safety and pharmacodynamics of POL6014 a potent and selective inhibitor of human NE (ClinicalTrials.gov Identifier: NCT03748199) (Barth et al., 2020). In addition, the NE inhibitor CHF6333 has been investigated in a phase I, randomized, placebo-controlled clinical trial (ClinicalTrials.gov Identifier: NCT03056326).

5.30.5.5 CFTR modulators

The development of CFTR modulators has significantly changed the landscape of cystic fibrosis clinical practice. Targeting the origin defect of CFTR instead of treating the secondary effects of CFTR impairment has been an area of extensive research (Wainwright et al., 2015; Taylor-Cousar et al., 2017; Ramsey et al., 2011; Middleton et al., 2019; Heijerman et al., 2019). Of interest, CFTR modulators not only improve lung function and mucus accumulation but were also shown to regulate features of the inflammatory response in cystic fibrosis. CFTR modulators are drugs that improve or even restore the functional alterations of CFTR caused by specific mutations. Depending on their effects, CFTRs can be classified in: potentiators, correctors, stabilizers, read-through agents, and amplifiers (Lopes-Pacheco, 2019).

Ivacaftor, the first CFTR modulator approved, significantly enhances CFTR activity in cystic fibrosis patients with G551D-CFTR mutations (Ramsey et al., 2011). Contrasting studies have evaluated inflammatory markers following treatment with ivacaftor. No differences in inflammatory markers were found in a small group of patients treated orally with ivacaftor for 6 months (Rowe et al., 2014). On the other hand, a different evaluation of patients treated with ivacaftor showed significant reductions in CXCL8, IL-1β, NE and other inflammatory markers (Hisert et al., 2017). In agreement with that, other studies reported that the number of pulmonary exacerbations, related to increased inflammation, was reduced in patients treated with ivacaftor (Ramsey et al., 2011; Middleton et al., 2019). Moreover, ivacaftor treatment of monocyte-derived macrophages from cystic fibrosis patients increased the expression of CFTR, partially restore macrophage polarization and bacteria killing, and reduced the secretion of pro-inflammatory cytokines by these cells (Barnaby et al., 2018). According to the Cystic Fibrosis Foundation, ivacaftor is recommended to treat patients aged 6 years or older to improve lung function, quality of life and reduce exacerbations (Kapnadak et al., 2020). Regardless of the beneficial actions of CFTR modulators on cystic fibrosis pulmonary disease, these drugs are unlikely to fully restore CFTR function and revert an established disease. In this regard, CFTR therapy might be administered in combination with other anti-inflammatory drugs and antibiotics.

5.30.5.6 Pro-resolving mediators

An innovative strategy to control chronic inflammatory diseases as cystic fibrosis, is to harness the pathways of resolution of inflammation. SPMs are produced by the enzymatic conversion of polyunsaturated essential fatty acids (PUFAs) including arachidonic acid (AA), eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) which yields potent bioactive autacoids inducers of resolution (Serhan and Levy, 2018).

Cystic fibrosis patients present significantly reduced levels of the AA-derived SPM Lipoxin A4 (LXA4) (Karp et al., 2004; Yang et al., 2012) and the EPA-derived RvE1 (Yang et al., 2012) in sputum and BAL. The levels of DHA-derived SPMs might also be reduced in cystic fibrosis given the lower DHA concentrations in the airways of these patients (Freedman et al., 2004). Of note, decreased expression of biosynthetic enzymes for SPMs was observed in BAL of pediatric cystic fibrosis patients (Ringholz et al., 2014) and nasal epithelial cells of adult patients (Janssen et al., 2014). Moreover, P. aeruginosa was shown to secrete an epoxide hydrolase that disrupts the biosynthesis of the SPM 15-epi lipoxin A4 (15-epi LXA4) (Flitter et al., 2017). Adding to the impaired lipid mediator production, the expression of protein pro-resolving mediators such as Annexin A1 is diminished in human nasal epithelial cells obtained from cystic fibrosis patients (Bensalem et al., 2005). Altogether, cystic fibrosis pathogenesis also relies in the impairment of important pathways of resolution of inflammation. Based on that, pharmacological strategies to increase the levels of pro-resolving mediators were proposed.

Lenabasum (JBT-101) is an oral agonist for the cannabinoid CB2 receptor (CBR2) that enhances the biosynthesis of LXA4 leading to resolution of inflammation in experimental peritonitis in mice (Zurier et al., 2009). Indeed, lenabasum treated
monocyte-derived macrophages from cystic fibrosis patients expressed decreased markers of activation, secreted lower amounts of pro-inflammatory cytokines and had higher bacterial killing activity when compared to untreated cells (Tarique et al., 2020). Currently, a multicenter, double-blind, randomized, placebo-controlled phase 2b study is evaluating the efficacy and safety of lenabasum for cystic fibrosis and will clarify potential positive effects of CBR2 agonists (ClinicalTrials.gov Identifier: NCT03451045).

Another strategy to treat the unresolved inflammation in cystic fibrosis is elevating the levels of SPM precursors through dietary supplementation. The effects of diets enriched with omega-3 essential fatty acids in cystic fibrosis were recently summarized in an updated Cochrane systematic review of randomized trials (Watson and Stackhouse, 2020). The duration of supplementation and concentrations of DHA or EPA is variable among the existing studies (Lawrence and Sorrell, 1993; Keen et al., 2010; Hanssens et al., 2016; Watson and Stackhouse, 2020). Despite the overall lack of evidence for improvements in lung function, omega-3 supplementation was shown to reduce pulmonary exacerbations (Hanssens et al., 2016) and inflammatory markers (Keen et al., 2010), and improved neutrophil function (Lawrence and Sorrell, 1993). The potential benefits of omega-3 supplementation are to be confirmed in larger, long-term, randomized, controlled trials. Nevertheless, data from preclinical studies have shown that pro-resolving mediators are protective in experimental models of cystic fibrosis. For instance, LXA₄ was shown to increase secretion of chloride and increase epithelial repair in cultures of cystic fibrosis epithelial cells (Higgins et al., 2014; Verriere et al., 2012). In addition, resolvin D1 (RvD1), a DHA-derived SPM, regulates the secretion of pro-inflammatory mediators and enhances the phagocytic and bacterial killing ability of macrophages from cystic fibrosis patients (Ringholz et al., 2018; Isopi et al., 2020). In agreement with that, a positive correlation between the ratio of RvD1/CXCL8 levels in sputum and lung function was observed in cystic fibrosis patients (Eickmeier et al., 2017). Moreover, Annexin A1 treatment was shown to reduce the pulmonary inflammation in a murine model of cystic fibrosis (Dalli et al., 2010).

Promoting resolution is especially interesting in the context of cystic fibrosis, in which the uncontrolled inflammation is associated to chronic infections. Anti-inflammatory therapies might cause immunosuppression, whereas pro-resolving therapeutics reduce inflammation while enhance the patient anti-microbial responses.

### 5.30.5.7 Immunomodulatory antibiotics

Macrolides, such as azithromycin, are drugs with both antimicrobial and immunomodulatory actions (Cigana et al., 2006). The chronic use of azithromycin is recommended for cystic fibrosis patients of 6 years of age or older with or without chronic *P. aeruginosa* infections (Mogayzel et al., 2013). The mechanism of action behind the immunomodulatory effects of azithromycin can be related to both inhibition of the activation of NF-κB and other pro-inflammatory signaling pathways (Cigana et al., 2006) and its antibacterial properties (Imperi et al., 2014). In cystic fibrosis, azithromycin reduces the number of pulmonary exacerbations, inflammatory markers and improves lung function (Clement et al., 2006; Saiman et al., 2003, 2010; Southern et al., 2012). An important concern regarding the continuous use of azithromycin is the possible development of resistance in individuals with occult or active nontuberculous mycobacteria (NTM) infection, impairing NTM treatment (Kapnadak et al., 2020). In addition, azithromycin might inhibit the antibiotic activity of tobramycin (Nichols et al., 2017).

Another antimicrobial drug that possesses anti-inflammatory actions is doxycycline, a tetracycline antibiotic against Gram-positive bacteria. Doxycycline reduces neutrophil chemotaxis, nitric oxide production and harbors anti-metalloproteinase activity (Golub et al., 1995; Hanemaaijer et al., 1998; Hoyt et al., 2006; Elewski et al., 1983). In cystic fibrosis, doxycycline attenuates the proteolytic imbalance in the airways of patients, improved lung function and the time to next exacerbation (Xu et al., 2017).

### 5.30.5.8 Challenges and perspectives

The development of novel immunomodulatory therapies for cystic fibrosis faces different and important challenges. First, the large number of CFTR mutations and associated chronic bacterial infections result in different disease phenotypes for cystic fibrosis. Therefore, personalized biomarkers might be useful to predict treatment efficacy in specific group of patients. In addition, the clinical trials for immunomodulatory drugs for cystic fibrosis often require a significant number of study subjects and take 2–4 years for its conclusion. Moreover, the lack of an experimental model that recapitulates all the features of cystic fibrosis renders the preclinical studies of anti-inflammatory drugs of limited value to rationalize clinical trials.

Another important consideration should be given regarding potential immunosuppression caused by drugs that counter-regulate inflammation in cystic fibrosis. As such, the antagonist of LTB₄ receptor, BIL284BS, was shown to increase exacerbations in the lungs, potentially due to bacteria overgrowth. In addition, anti-inflammatory drugs might interact with other therapies for cystic fibrosis leading to unwanted events.

As the survival of cystic fibrosis patients increases overtime, the development of pharmacological strategies long-term management is urgent needed. The novel immunomodulatory approaches should target specific elements of inflammation associated to disease pathogenesis, while sparing the protective responses.

### 5.30.6 Pneumonia

Lower respiratory tract infections (LRTI—bronchitis, bronchiolitis, and pneumonia) are a major health threat comprising the deadliest communicable disease in the world (Forum of International Respiratory Societies, 2017b). Pneumonia is responsible for
approximately 15% of all deaths of children under 5 years old, being the most common cause of child mortality. Pneumonia can be caused by bacteria, viruses, or fungi and be acquired in the community (Community acquired pneumonia—CAP) or in hospitals (nosocomial pneumonia) during the treatment of other health conditions. The emergence of new respiratory viruses and the growing levels of pathogen resistance to antimicrobials are important infectious disease challenges of the 21st century. Yearly epidemics of respiratory infections cause increased morbidity and mortality while pandemics can spread rapidly worldwide leading to even higher mortality rates and economic losses. Influenza A virus infects up to 5 million people every year, leading to 250,000–650,000 deaths. Moreover, influenza pandemics are devastating marks during human history. The most extreme example is the 1918 pandemic, that caused 50–100 million deaths within a short period of time. Recently, the causative pathogen of the new pandemic COVID19 was identified and designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). By the end of August 2020, nearly 25 million cases and 800,000 deaths due to COVID19 have been reported since the start of the outbreak (WHO, 2020c). Bacterial causes of pneumonia, including Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Pseudomonas aeruginosa, among many others, have high prevalence among the elderly and children, and clinical management is challenged by the increasing rates of antibiotic resistance. Similarly, the treatment of fungal pneumonia, a very serious form of pneumonia, is often complicated. Therefore, despite the improvements in disease management and the efforts for vaccine development, novel therapeutic strategies for pneumonia are urgently needed.

In the lungs, the inflammatory responses triggered by an infection are essential to control pathogen proliferation and dissemination. Resident immune and parenchymal cells respond promptly to the presence of a microorganism through the activation of PRRs and secretion of pro-inflammatory and antimicrobial cytokines, such as type I interferons. Resident alveolar macrophages perform phagocytosis and pathogen killing, while secrete cytokines and chemokines that lead to the activation of endothelial cells and recruitment of neutrophils to the lungs. Neutrophils are one of the first cell responders to respiratory infections (Aulakh, 2018) and are classically recruited by chemokines such as CXCL1, CXCL2 and CXCL8 (Aulakh, 2018). The granular content of mature neutrophils include an extensive arsenal of antimicrobial peptides and proteases that will mediate pathogen clearance upon cell activation and degranulation (Cowland and Borregaard, 2016). In addition, the production of ROS and subsequent release of NETs entrap and kill potential pathogens in the lungs. Indeed, the vital role of neutrophils controlling infections in the lungs is evidenced by the higher susceptibility of pneumonia patients with deficits in neutrophil quality (neutropenia) or defects in function (chronic granulomatous disease) (Rawat et al., 2016; Turner and Nasir, 2015). Moreover, neutrophils are important for the development of adaptive immune responses following viral lung infections (Lim et al., 2015).

Inflammatory recruited macrophages are also important players in pulmonary pathogen control. These cells are derived from circulating monocytes and possess increased phagocytic activity and production of reactive nitrogen and oxygen species aiding to the innate defenses in the lung. In addition, eosinophils were shown to be protective against viral infections (Samarasinghe et al., 2017; Percopo et al., 2014; Drake et al., 2016; Handzel et al., 1998), including with SARS-CoV-2 (Liu et al., 2020). Therefore, the different players of the innate immune system, coordinate the effective defense against the invading pathogen in the lungs. On the other hand, exacerbation of inflammatory responses is associated with increased lung damage and pneumonia severity.

Inflammatory responses in the lungs must be regulated to guarantee proper pathogen clearance avoiding bystander lung injury. The pulmonary mucosa comprises a huge surface for gas exchange ensuring effective delivery of oxygen to the cells. Exaggerated pulmonary inflammation is characterized by increased edema and infiltration of neutrophils that fill up the alveoli decreasing gas diffusion while alveoli damage reduces the respiratory surface. The higher levels of pro-inflammatory cytokines aggravate inflammation in the lungs by activating immune cells, increasing vascular permeability, and are associated with systemic symptoms such as fever, myalgia, and anorexia. The collateral damage in the lungs can perpetuate inflammation by the release of DAMPs (damage associated molecular patterns) that stimulate immune and parenchyma cells to produce even more pro-inflammatory mediators fueling the response culminating with acute respiratory distress syndrome (ARDS). Of interest, mechanical ventilation in ARDS patients may further increase the inflammatory response (Verbrugge et al., 2007). Currently, there is limited information regarding the immune responses and physiopathology of COVID19. The present evidence suggests that a hyperinflammatory status is associated with the most severe cases of disease and, as for severe influenza infections, anti-inflammatory therapies could mitigate some of the symptoms and improve disease outcome. Other respiratory infections caused by bacteria pathogens such as Streptococcus pneumoniae are also associated to increased inflammatory responses that can contribute to disease progression and severity. Noteworthy, different pathogens have evasion mechanisms to overcome the host immune defenses. In those cases, enhancing inflammation may cause more harm than good. Therapeutic strategies that focus on the host immune responses are interesting as they are less prompt to induce resistance and can be used to treat disease caused by different pathogens. On the other hand, immunomodulatory therapies should be carefully considered to avoid immunosuppression increasing disease severity due to pathogen overgrowth. Here we are going to discuss novel immunomodulatory strategies to control pneumonia burden pushing the inflammatory responses back in the rails. Importantly, immunomodulatory therapies for pneumonia do not replace antimicrobial drugs, such as antibiotics or antivirals, yet the adjunctive immunomodulatory treatment aid to reduce mortality and morbidity of severe pneumonia patients.

5.30.6.1 Corticosteroids

The clinical use of corticosteroids as an adjunctive therapy for pneumonia is highly controversial, especially for viral infections. During influenza outbreaks, the use of corticosteroids to treat severe patients has been shown to be either harmful, especially when given early during infection, or lack any beneficial effect (Lee et al., 2015; Martin-Loeches et al., 2011; Cao et al., 2016;
Patients were shown to have higher mortality rates, nosocomial infections, and present longer duration of viral shedding in the lungs. However, most of the data on corticosteroids and influenza pneumonia comes from observational studies that might present other confounders. Also, most of the studies evaluated the effect of corticosteroids without an association with antiviral drugs. Given the amount of studies presenting evidence opposing the use of corticosteroids during influenza pneumonia, the Infectious Diseases Society of America recommends against its use in this context (Uyeki et al., 2019). Randomized clinical trials will confirm this conclusions. In pre-clinical studies, adjunctive corticosteroid treatment during influenza and bacteria coinfection was shown to protect mice from lung injury and mortality (Ghoneim and McCullers, 2014). For other respiratory viral infections, such as RSV, systemic corticosteroids also did not present substantial beneficial effects for disease management in both clinical (Lee et al., 2011) and animal studies (Domachowske et al., 2001). Recently, researchers on the RECOVERY COVID19 trial release a preliminary report that shows 8–26% lower mortality in participants with severe COVID19 given 6 mg dexamethasone once daily (Horby et al., 2020). Although the study is neither peer reviewed or published yet, the results were received in the scientific community with both enthusiasm and concern. Similarly, a previous study of a small cohort of COVID19 patients who developed ARDS showed a that treatment with methylprednisolone decreased the risk of death (Wu et al., 2020). Of interest, the severity of COVID-19 is correlated to an aggressive uncontrolled inflammatory response in the lungs and overproduction of cytokines systemically ("cytokine storm"). Therefore, the anti-inflammatory actions of low-to-moderate dose of corticosteroids are potentially protective to critically ill COVID-19 patients (Shang et al., 2020).

In general, corticosteroids may be helpful in managing hyperinflammation during severe pneumonia in conjunction with appropriate antimicrobial therapy. A Cochrane review identified 17 randomized clinical trials that evaluated the use of systemic corticosteroids in a total of 2264 participants who had acquired pneumonia in the community (community-acquired pneumonia (CAP)) being treated in the hospital. The population of patients was diverse as well as the pathogens causing pneumonia. The overall conclusion was that corticosteroid treatment lead to lower clinical failure rates (death, worsening of imaging studies, or no clinical improvement), a reduced hospital stay, and fewer complications (Stern et al., 2017). Indeed, the use of corticosteroids in combination with antibiotics is a cost effective strategy leading to significant reduced health care costs (Pliakos et al., 2019). Among the adverse effects relate to corticosteroid use in pneumonia, hyperglycemia was shown to be more frequent in treated patients (Blum et al., 2015). However, the reported side effects do not seem to outweigh the benefits of corticosteroids in severe pneumonia management.

The mechanisms of action of corticosteroids include the reduction in the levels of TNF-α, IL-6, CXCL8, and other inflammatory mediators (Remmelts et al., 2012) and recruitment of neutrophils into the lungs (Rhen and Cidlowski, 2005). Therefore, the overall diminished inflammatory response prevents lung edema, injury, and the inflammation-related impaired pulmonary function. On the other side, the timing and dosage for corticosteroid administration are extremely important as it can lead to severe immunosuppression favoring pathogen overgrowth in the lungs. Noteworthy, based on the clinical studies, corticosteroids cannot be generally recommended in pneumonia but might be beneficial specifically to patients presenting a hyperinflammatory disease phenotype.

Currently, clinical trials aiming to assess the therapeutic potential of corticosteroids in pneumonia, specially caused by SARS-Cov-2, are ongoing (ClinicalTrials.gov Identifier: NCT02517489, NCT04344288, NCT04451174).

5.3.0.6.2 Statins

Hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors, also known as statins, are drugs used as lipid-lowering agents to prevent and treat cardiovascular disease (Piepoli et al., 2016). In addition, statins have important pleiotropic anti-inflammatory and anti-thrombotic actions (Blum and Shamburek, 2009). The immunomodulatory effects of statins rely on the inhibition of the mevalonate pathway, leading to reduced levels of isoprenoids and inhibition of signaling molecules like Rho, Ras, and Rac that are implicated in several features of inflammation (Viasus et al., 2010). Additionally, statins reduce the activation of NFκ-B (Hilgendorff et al., 2003) while increases the levels of anti-inflammatory actions for disease management in both experimental and clinical studies that evaluate the role of statins in pneumonia management. In preclinical studies, statins were shown to reduce inflammation-related damage and enhance host defenses in mice infected with Streptococcus pneumoniae or Staphylococcus aureus (Boyd et al., 2012; McDowell et al., 2011). Nevertheless, a different study reported increased pulmonary inflammation and reduce survival of Escherichia coli infected mice that was treated with statins in comparison with the vehicle group (Hussein et al., 2019). In the clinical settings, observational studies also presented conflicting results. While different retrospective studies showed that pneumonia patients in use of statins presented better disease outcomes in comparison with patients that were not (Doshi et al., 2013; Mortensen et al., 2012; Mortensen et al., 2005), others reported lack of beneficial effect (Dublin et al., 2009; Yende et al., 2011). Although less frequent than the observational studies, randomized clinical trials were performed and, once again, diverging results were observed. No significant improvements in pneumonia severity or inflammation features were observed in the community-acquired pneumonia patients that were treated for 4 days with statins in comparison to placebo (Viasus et al., 2015). On the other side, a randomized controlled study with 62 septic pneumonia patients...
treated with high dose statins (80 mg per day) or placebo showed a reduction the 6-month mortality and re-admissions of patients and improvement in patient's neutrophil function (Sapey et al., 2019).

The discrepancies in the studies can be mostly explained by differences in risk factors in the populations analyzed and that pneumonia can be caused by a multitude of pathogens leading to distinct severity levels of disease. Therefore, controlling for infection etiology and comorbidities in the studied population is necessary to determine the groups of patients that will benefit from the use of statins in combination with antimicrobial drugs during pneumonia. Currently, clinical studies evaluating the therapeutic value of statins reducing the inflammatory exacerbations observed in severe COVID19 patients are ongoing (ClinicalTrials.gov Identifier: NCT04407273, NCT04472611, NCT04348695, NCT04380402).

5.30.6.3 Pro-resolving mediators

Impaired resolution of inflammation might contribute to pneumonia pathogenesis (Sousa et al., 2020a). Indeed, the severity of infection and clinical symptoms of influenza patients is inversely correlated with the levels of 12-lipoxygenase-derived pro-resolving mediators (Tam et al., 2013). In addition, infection with highly pathogenic influenza A virus, H5N1, is associated with decreased lipoxin signaling leading to increased pulmonary inflammation and dissemination of virus in mice (Cilloniz et al., 2010). Moreover, in a preclinical model of bacterial pneumonia, increased inactivation of AnxA1 was shown to be associated to the peak of inflammatory response while restoration of AnxA1 levels was observed at the resolution of infection (Tavares et al., 2016). Inducing resolution, rather than blocking inflammation, prevents the harmful activation of inflammatory responses in the lungs while enhances host antimicrobial responses. The possibility to exogenously give mediators of resolution to treat infectious inflammatory diseases has uncovered a new window of therapeutic opportunities for severe pneumonia (Basil and Levy, 2016).

Agonists of resolution of inflammation signal the cessation of granulocyte recruitment while increase the recruitment of macrophages favoring the clearance of pathogens and inflammatory cells leading to restoration of tissue homeostasis (Buckley et al., 2014). In addition, these molecules reduce pro-inflammatory mediator production by airway epithelial cells and macrophages and induce granulocyte apoptosis and repair of damaged epithelial cells (Levy and Serhan, 2014). All of those features, which can potentially be controlled by specialized pro-resolving mediators, are important steps to control pneumonia severity (Fig. 6).

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**Fig. 6** Protective actions of specialized pro-resolving mediators (SPMs) in pneumonia. The left panel illustrates the alveolar environment in the context of severe pneumonia, which is characterized by increased edema, epithelial damage, secretion of pro-inflammatory mediators and consequent activation and recruitment of leukocytes. Activated neutrophils perform NETosis and accumulate in the alveoli fueling the response. In contrast, the right panel shows the alveoli in the setting of active inflammation resolution promoted by SPMs. SPMs enhance the antimicrobial actions of macrophages increasing phagocytosis and killing of pathogens, decrease the pro-inflammatory cytokine production, stimulate apoptosis of granulocytes and efferocytosis of dead cells and debris. In addition, SPMs can active epithelial repair programs and enhance the tight junction integrity leading to restoration of the epithelial barrier in the lungs. Created with BioRender.com®.
Currently, there are no pro-resolving mediators approved for the treatment of pneumonia. However, a recent clinical trial was launched to evaluate the therapeutic potential of Angiotensin 1–7, a pro-resolving heptapeptide, to treat inflammatory exacerbations in COVID19 patients (NCT04332666).

The therapeutic potential of SPMs has been explored in pre-clinical models of pneumonia. For instance, exogenous administration of 15-epi-LXA₄ enhances lung anti-bacterial responses while modulates inflammation through NFkB inhibition (Sham et al., 2018). Other lipid mediators of resolution, including RvD1 and AT-RvD1, were shown to decreased pathogen burden, neutrophil infiltration, and secretion of pro-inflammatory cytokines while enhanced efferocytosis in murine models of bacterial pneumonia (Abdulnour et al., 2016; Codagnone et al., 2018; Croasdell et al., 2016; Wang et al., 2017). Similarly, treatment with protein mediators of resolution, such as Annexin A1, was shown to protect mice to pneumonia caused by Streptococcus pneumoniae (Machado et al., 2020). Mediators of resolution also possess antiviral properties. The SPM protectin D1 (PD1) was shown to prevent mice lethality and impairment of lung function by blunting influenza virus proliferation (Morita et al., 2013). Proof of concept studies in humans will illuminate the therapeutic effectiveness of this approach for pneumonia management.

### 5.30.6.4 Cytokine-targeted therapies

Severe pneumonia are often characterized by an intense secretion of pro-inflammatory cytokines and chemokines that lead to leukocyte recruitment and activation, to control pathogen replication (Fernandez-Serrano et al., 2003). Yet, the uncontrolled production of cytokines systemically and in the lungs is associated to increased pulmonary damage and multiorgan failure. Therefore, therapeutic strategies that target pro-inflammatory cytokines or its receptors were suggested to control inflammatory exacerbations during severe pneumonia.

Cytokines such as IL-17, IL-6 and IL-1β are associated to severity of viral pneumonia including influenza and SARS-CoV2 infections. In preclinical studies of acute lung injury or ARDS, deficiency in IL-17 or administration of specific antibodies targeting this cytokine resulted in improved survival, less pulmonary infiltration of leukocytes and better pathology scores (Li et al., 2016). Indeed, given that IL-17 mediates the recruitment of neutrophils, experimental models of pneumonia characterized by a neutrophilic pathological response shows that targeting IL-17 might be of interest to prevent the inflammatory damage (Ritchie et al., 2018; Crowe et al., 2009). Recently, IL-17 was suggested as an interesting target for COVID19 (Pacha et al., 2020). Noteworthy, IL-17 is crucial for the host response against bacteria and fungi challenging the clinical use of anti-IL-17 in the context of pneumonia.

Tocilizumab and Anakinra, a recombinant humanized monoclonal antibody targeting IL-6 and a recombinant IL-1 receptor antagonist, both approved to treat autoimmune diseases, are currently being evaluated as therapeutics for COVID19 inflammatory exacerbations. The correlation between increased levels of IL-6 and IL-1 in critical ill COVID19 patients set the basis for these trials (Mehta et al., 2020). The results of the COVACTA trial (NCT04320615) that evaluated the efficacy of tocilizumab for severe COVID19 showed negative results. In contrast, an observational study in Italy showed that high dose high-dose anakinra in patients with COVID-19 led to a significant improvement of patients. Observational studies and randomized clinical trials have important differences and further controlled randomized clinical trials will clarify whether inhibiting these cytokines is helpful to control the exaggerated pulmonary inflammation in severe COVID19 patients (Cavalli et al., 2020).

Chemokine receptors are also suitable targets for immunomodulation in the context of severe pneumonia. Antagonists of CXCR1 and 2, binding sites for neutrophil-related chemokines, have been tested as treatment strategies for influenza, S. pneumoniae and Klebsiella pneumoniae pulmonary infections (Wei et al., 2013; Tavares et al., 2017a; Nair et al., 2012; Miller et al., 2015). Experimental studies showed that the antagonism of CXCR1/2 during influenza, decreased neutrophil numbers, pro-inflammatory cytokine production and prevented lung injury and mortality, with additive effects when combined to antiviral drugs (Washburn et al., 2019; Tavares et al., 2017a). The results from the phase II clinical study of the antagonist of CXCR2 Danrixin (GSK1325756) showed the well-tolerability of the drug but the efficacy assessment was limited due to the small number of participants in the study (NCT02927431) (Madan et al., 2019). Antagonists of CXCR2 may also be useful to block neutrophil recruitment and activation in COVID19 patients (Koenig et al., 2020). In the context of bacterial pneumonia, CXCR2 antagonism was shown to prevent lung injury and inflammation in mice, without compromising the host ability to deal with infection (Wei et al., 2013; Tavares et al., 2017a).

The immune responses triggered by different pathogens in the lungs can be very distinct. In this regard, a better understanding of the immunopathology associated to different types of pneumonia will guide the development of drugs focused on harmful pathways without compromising the protective ones. While targeting cytokines can be interesting in the context of inflammatory diseases, it might lead to increased susceptibility to secondary infections and should be considered carefully for patient management.

### 5.30.6.5 Immunomodulatory antibiotics

Macrolides are a class of broad-spectrum bacteriostatic antibiotics against many gram-positive and some gram-negative bacteria. Beyond their direct antimicrobial effects, macrolides are an interesting immunomodulatory treatment for severe pneumonia patients. The immunomodulatory actions of macrolides rely on the inhibition of pro-inflammatory transcription factors including NFκB and AP-1 (Desaki et al., 2000). In addition, macrolides promote efferocytosis apoptotic cells by alveolar macrophages, thus...
immunomodulatory drugs in the context of pneumonia, most of them were not yet translated to the clinical settings. Pneumonia

The world market for respiratory disease drugs is anticipated to expand significantly in the following years given the increased prevalence of these diseases in the population worldwide, especially due to the COVID19 pandemic (The Business Research Company, 2020; Barnes et al., 2015). In this regard, immunomodulatory therapies are promising strategies to cope with disease burden in the respiratory tract. Dysregulated immune responses are a common feature of respiratory diseases and are targets for novel therapeutic approaches. Drug discovery is a long expensive process that include tests for safety, pharmacokinetics, bioavailability, and efficacy (Wehling, 2009). Therefore, the screening of numerous compounds with positive results in preclinical studies will result and a handful of new drugs that will be translated to clinical trials. Drug repurposing is an attractive strategy to overcome some of the challenges of drug development. As such, the antibiotics macrolides, firstly designed to treat bacterial infections, are now been suggested as immunomodulatory drugs for inflammatory diseases (Kwiatkowska and Maslinska, 2012; Kobayashi et al., 2013). Another example is the use of small doses of the bronchodilator theophylline to treat the increased oxidative stress in COPD (To et al., 2010). Anti-inflammatory drugs for respiratory diseases should be considered with caution. The available immunomodulatory strategies mostly rely on drugs that present anti-inflammatory effects but lack specificity in their mechanism of action. Immunosuppressive effects are not desirable in the context of pneumonia and cystic fibrosis, for example, in which uncontrolled proliferation of pathogens can worsen disease. Importantly, the unique pathophysiology of each respiratory disease must be considered for drug development. Specific pathways or features of inflammation might relate to disease severity, while others are associated to protective responses. Therapies that target certain phenotypes of leukocytes, rather than others, can improve disease outcome and prevent untoward effects such as susceptibility to infections. Thus, the efficacy of a given immunomodulatory drug might depend on the different inflammatory components of each respiratory disease. More recently, the pharmacological potential of agonist of resolution of inflammation has shed light on new strategies to modulate inflammatory diseases in the respiratory tract (Krishnamoorthy et al., 2018). Leveraging the potent actions of pro-resolving mediators is promising to treat infectious and non-infectious pulmonary diseases, enhancing host anti-microbial responses while timely regulating the extent of inflammation (Duvall et al., 2017; Krishnamoorthy et al., 2018).

Respiratory pathologies remain a significant health burden worldwide with considerable unmet clinical challenges. Important research efforts are being made to address the limitations of the respiratory tract pharmacology. Improvements in drug development and testing, especially regarding animal models and in silico studies, facilitate the advancement of therapy development and respiratory medicine.
Reference text extracted from the image provided.
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**Relevant Websites**

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https://www.who.int/ — World Health Organization.

https://clinicaltrials.gov/ — Clinical Trials.gov.

https://www.cf.org/ — Cystic Fibrosis Foundation.