Provisional chapter

New Frontiers in the Diagnosis and Treatment of Chronic Neutrophilic Lung Diseases

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Additional information is available at the end of the chapter

1. Introduction

Neutrophils, or polymorphonuclear leukocytes (PMNs), are a key component in the innate immune system and a powerful player in host defense. Because of this, PMNs have been studied for over a century, although current understanding of their primary function, trafficking to sites of infection and catabolyzing microbial pathogens, is unchanged. PMNs are viewed by some as mere blunt immune instruments, utilized by the host against a broad array of pathogens. However, a careful review of both neutrophil function and dysfunction reveals a cell of discrete coordination in both normal homeostasis and disease. Herein, we provide a review of neutrophil biology focusing on PMNs role in chronic inflammatory lung disease. We provide a summary of the current knowledge of these cellular first responders and detail novel therapeutics related to combating their dysfunction in chronic disease.

2. Evolutionary origin of neutrophils

The evolutionary origins of the human neutrophil lie in phagocytic cells found in simple organisms. These evolutionary precursors to the human PMNs, originally studied in starfish, were first observed migrating to a site of injury over a century ago. Since the seminal immunological discovery of cells that attack invading pathogens, various phagocytic immune cells along the evolutionary continuum have been described. Phagocytic cells with functions and signaling mechanisms similar to mammalian neutrophils have been described in organisms as simple as the slime mold *Dictyostelium discoideum*. [1] Phagocytes containing bactericidal granules analogous to those in the human neutrophil are

found in insects. Although functionally similar, these immune cells differ from their human counterparts significantly in lifespan and nuclear morphology, suggesting that a short-lived, multi-lobed phagocyte is a more recent evolutionary development. [2] This trend continues with non-mammalian vertebrates. Both amphibians and bony fish have granulocytic phagocytes with multi-lobed nuclei that are genetically and morphologically similar to the human PMN. [3] Although the structure, morphology, function, and genetic make-up of neutrophils is highly conserved within mammals, the percentage of total immune cells represented by neutrophils varies significantly. Even within primates neutrophil counts vary a great deal; neutrophils represent approximately 50% of chimpanzee's circulating immune cells, whereas the human neutrophil accounts for almost 70% of white blood cells. [4],[5] The commonality of PMNs and PMN-like cells make it clear that the neutrophil is an ancient player on the immunological stage.

3. Hematopoietic origin, differentiation/maturation of neutrophils

Neutrophil biogenesis occurs in the bone marrow from an undifferentiated hematopoietic stem cell. Regulation of transcription factors through cytokine and growth factor signaling dictates neutrophil differentiation, a process called granulopoiesis. Granulopoiesis is the successive differentiation of a pluripotent hematopoietic stem cell, to a multipotent committed myeloid progenitor cell (myeloblast), to a bipotent granulocyte-macrophage progenitor cell (metamyelocyte) and finally to a unipotent committed granulocyte. The final stage of PMN maturation, or terminal granulopoiesis, is characterized morphologically by the appearance of a multi-lobed granulated nucleus. On a molecular level, granule protein synthesis and granule packaging mark neutrophil maturation. These granules and their cargo proteins are among the primary weapons in neutrophils' antimicrobial arsenal. [6]-[8]Synthesis of granules and granule proteins progresses concurrently with granulopoiesis. Granules are traditionally classified as primary, secondary and tertiary according to the stage of differentiation during which they are formed. This is important because the granules formed at different stages of differentiation exhibit drastically different protein cargo and thus play different roles in the immune and inflammatory response. [9]The array of neutrophilic granule cargo might include myeloperoxidase, lactoferrin, haptoglobin and alpha-1-antitrypsin. [10] Specific granule proteins and their respective roles in neutrophilic lung disease will be addressed below.

4. Release and homeostasis

Once mature, neutrophils are released from the bone marrow. Locally, release of neutrophils into circulation is governed by cytokine signaling. Toll like receptors (TLRs) and granulocyte colony stimulating factor (G-CSF) receptors are crucial in neutrophil production, but CXCR2 and CXCR4 appear to be the primary receptors involved in neutrophil release into the circulation. [11],[12] Whereas activation of CXCR4 favors retention of mature PMNs in

the bone marrow, activation of CXCR2 promotes their release into circulation. Under homeostatic conditions a normal human adult produces 1 - 2 X 10[11] neutrophils per day. The rate of neutrophil production and release is dictated largely by G-CSF in a negative feedback mechanism whereby an increasing number of apoptotic neutrophils decreases the amount of G-CSF. The apoptotic neutrophils are phagocytosed by tissue macrophages, which decrease their release of interleukin-23 (IL-23). IL-23 stimulates the release of IL-17 by helper T (T_H) cells. [13] IL-17 is in turn the primary stimulus for the release of G-CSF. Thus, increased controlled destruction of neutrophils leads to decreased levels of macrophage derived IL-23 released by macrophages, IL-17 released by T_H17 cells, G-CSF released by osteoblasts, and thus a decrease in neutrophil synthesis and release. Conversely, IL-17 has been shown to act through p38 MAPK to augment IL-8 release from pulmonary epithelial cells. This mechanism, ideally, allows the body to rapidly speed neutrophil production and release during infection in a regulated fashion to minimize potential damage to the host. [10],[14]Another method by which the host regulates circulating neutrophil numbers is through the phenomenon of margination and demargination. Margination occurs when resting neutrophils travel at a significantly slower pace along the endothelium of the blood vessels. The expression of previously mentioned adhesion molecules creates distinct organ-specific (marginated) pools of cells. Exercise induced stress, infection, or other sources of systemic stress leads to an increase in blood flow, a release of epinephrine and demargination of the neutrophil into the general circulation.[15]

5. Response to infection/smoking

Although the rate of neutrophil production and release may increase during an immunological challenge, as the most populous circulating white blood cells, neutrophils serve as first line responders to injury and infection. During the course of their 6 to 8 hour life span in circulation, neutrophils tend to remain near the vascular endothelium. PMNs constitutively express two glycoprotein ligands, PSGL-1 and L-selectin, allowing neutrophils to detect inflamed or injured endothelium. At sites of inflammation, bacterial peptides such as lipopolysaccharide (LPS), and f-Met-Leu-Phe (fMLP), along with host pro-inflammatory cytokines (i.e., tumor necrosis factor-α [TNF-α]) stimulate the vascular endothelium to produce to adhesion molecules such as lymphocyte function antigen (LFA) and the immunoglobulin-derived intercellular adhesion molecule (ICAM). [4],[15]

The adhesive force between the endothelial adhesion molecules and neutrophil selectins produces a Velcro®-like action that slows the neutrophil down, a process known as rolling. Rolling also prompts the neutrophil to express surface molecules known as β-integrins, which further slow the neutrophil. It is at this early stage that PMNs have already begun to become activated and are preparing the intracellular machinery necessary to combat the invading pathogens. Slow rolling is followed by arrest and firm adhesion via clustering of β2-integrins. Arrest initiates actin polymerization vital to migration across the endothelial surface via a G-protein coupled receptor (GPCR) signaling cascade. [16],[17] Transendothelial migration, or exocytosis, begins as the adhesive force between the neutrophil and endo-

thelium increases and the neutrophil "crawls" in search of a suitable route to cross the vessel wall, either paracellular or transcellular. At this point the neutrophil extends pseudopod-like surface projections that penetrate the endothelium. Upon penetration the neutrophil increases expression of surface integrins and releases proteases that function to break through the vascular basement membrane and into the inflamed tissue. [16]

Once in the interstitium, PMNs must target the specific site of infection amidst large number of healthy cells. This is accomplished by a two pronged method of sensing inflammatory chemoattractant gradients. PMNs sense a chemoattractant gradient of IL-8 produced by damaged host cells and resident monocyte/macrophages through CXCR1 and CXCR2 (both GPCRs), and also detect fMLP (FPR1 receptor) LPS (TLR4), flagellin (TLR5), through pattern recognition receptors. [18] Although PMNs have been traditionally thought to promulgate an active innate immunity with little regulation, more recent evidence suggests that PMNs carefully coordinate a well-tailored immune response. A classic example of this regulated coordination is the elegant response of PMNs to IL-8. [19] As PMNs travel along the IL-8 gradient activation and release of microbicidal molecules occurs in a step-wise manner. With increasing concentrations of IL-8, neutrophils first produce more β-integrins, subsequently begin the oxidative burst, and finally degranulate potent proteases into the intracellular space. [20] The trafficking of neutrophils to sites of inflammation is with dual purpose: 1) to release their antimicrobial arsenal, and 2) to recruit more neutrophils and other innate immune cells to the site of inflammation.

The neutrophil's arsenal includes the following weapons with which the neutrophil attacks pathogens: release of aforementioned granules with their anti-microbial contents, synthesis and release of anti-microbial peptides, production of reactive oxygen species (ROS) during the respiratory/oxidative burst, phagocytosis (mainly utilized to remove debris) and the release of neutrophil extracellular traps (NETs), composed of DNA material that entraps invading pathogens. The second objective is accomplished through the release soluble mediators such as IL-12 and IFN-gamma that form a complex network of recruitment of other neutrophils, dendritic cells, natural killer cells and macrophages. [21] Neutrophils can also act as antigen presenting cells in communication with CD8+ T cells, thus forming a link between innate and adaptive immunity. Such a potent response to injury and inflammation depends on negative feedback mechanisms, the short life span of neutrophils and the clearance of apoptotic neutrophils by macrophages. If left unchecked, the inflammatory response mediated by neutrophils can be a major contributor to chronic disease. [4] (Fig. 1)

Neutrophils are capable of responding to a number of inflammatory stimuli other than infection. Cigarette smoking has been shown to be a primary stimulus for the activation and migration of neutrophils into the tissues. Neutrophil treatment with cigarette smoke induces β_2-integrin activation and firm adhesion to fibrinogen. Increased levels of neutrophil elastase, and matrix metalloproteases has been demonstrated with exposure to cigarette smoke. Furthermore, there is a decrease in superoxide production in the presence of cigarette smoke, indicating that smoking may lead to an impaired response to bacterial challenge.

It is with this potential for destructive dysregulation that we provide the following review of selected neutrophil mediated, inflammatory lung diseases. The definition, etiology, epi-

demiology, cellular pathophysiology, diagnosis and current treatment of each condition will be discussed briefly. Following each disease will be a discussion of recent advancements in the understanding of the disease and advancements in therapeutics directed toward each condition.

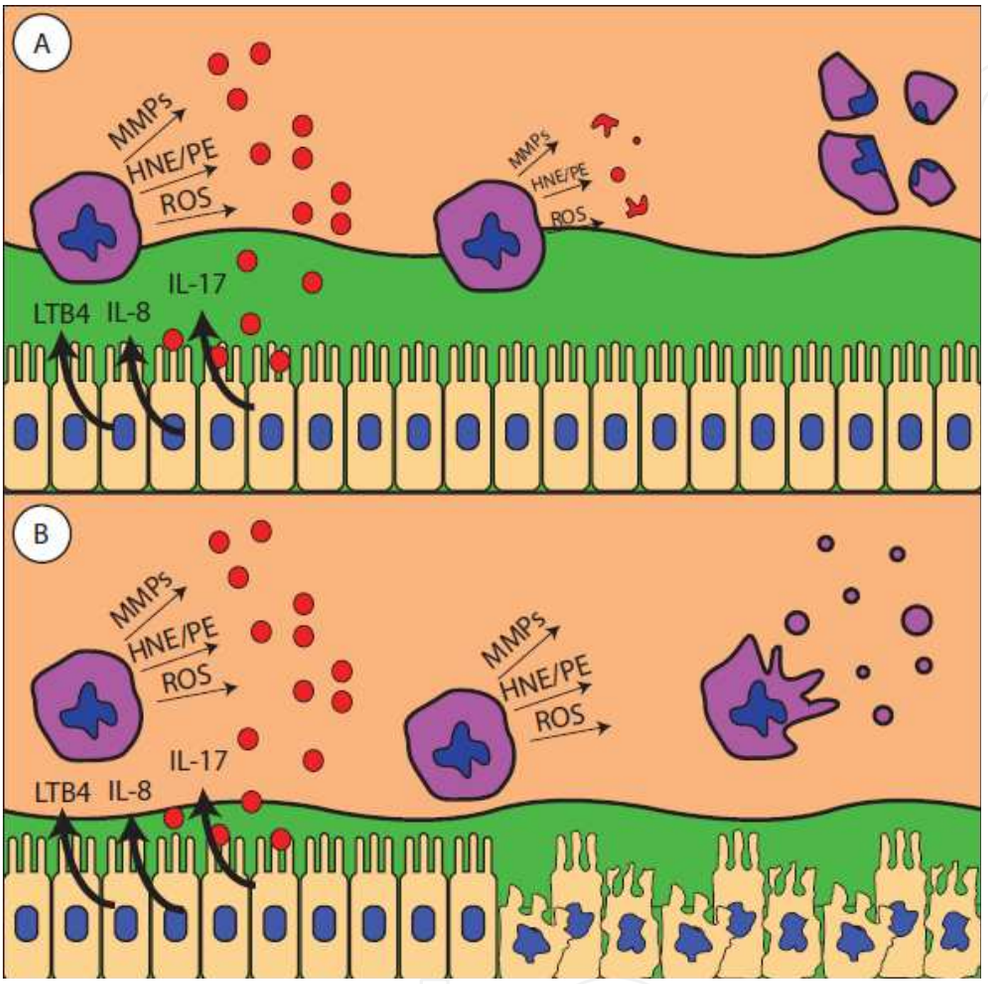

Figure 1. Neutrophils in both normal and chronic inflammatory responses. Neutrophils are recruited into the airway in normal acute information through the release of chemokines such as IL-8, IL-17 and LTB4. Once in the airway they release proteases and reactive oxygen species (ROS) to combat bacteria (shown in red). After the infection in resolved, the neutrophils undergo apoptosis and prevent destructive release of their proteases and ROS into the interstitium. (A) In chronic inflammation, neutrophils continue to release harmful proteases with no pathogen presence. Eventually the neutrophils undergo necrosis which futher damages the epithelium and creates a feed-forward process of disease. (B)

6. COPD

6.1. Overview and epidemiology

Chronic obstructive pulmonary disease (COPD) is marked by progressive and irreversible airway limitation, chronic bronchitis, pulmonary hypertension and emphysema. These tis-

sue changes are secondary to persistent, chronic pulmonary inflammation in response to persistent exposure to toxic gases or particles, primarily tobacco smoke. In addition to a baseline inflammatory state, the disease is associated with frequent exacerbations due to constant inflammation. [22],[23] Although preventable and treatable, COPD is a significant cause of morbidity and mortality worldwide. As the leading cause of pulmonary-related death in the world, COPD was the fifth leading cause of death worldwide in 2001 and is expected to move to third by 2020. [24]

6.2. Etiology

An overwhelming proportion of COPD is related directly to cigarette smoking. Other environmental irritants that have been implicated in the development of COPD include coal and metal mining dust, urban pollution, and indoor cooking with biofuels. [25] Irrespective of secondary irritants, greater than 90% of individuals diagnosed with COPD are current or former smokers. However, only a minority of current and former smokers develop symptomatic COPD (15-20%), suggesting COPD is a confluence of environmental factors and genetic susceptibility. [26] The complex nature of the inflammatory process and its response to environmental factors in COPD confounds the search for individual susceptibility genes. [27] To that end, several genome-wide association studies (GWAS) have been performed with the goal of elucidating the genetic factors related COPD pathogenesis. Unfortunately, even in this current age of rapid whole genome sequencing, multiple GWAS studies have only postulated loose corollaries of genes and association with disease. No genes associated with COPD have displayed a Mendelian mode of inheritance with respect to causation of COPD. [28]

6.3. Pathophysiology

On a cellular level, the pathophysiology of COPD is essentially a heightened, perpetually active inflammatory process. Inflammation is typically localized in the small airways and parenchyma of the lungs, where irritant molecules become trapped. The difference between the normal inflammatory cascade and that seen in COPD is the damage immune cells and their mediators inflict upon the lung tissues due to persistent activation. Lung function decline, characteristic of COPD, is linked to three distinct but synergistic mechanisms: destruction of alveolar walls (emphysema), narrowing of the small airways, and hypersecretion of mucus. [29]

The inflammatory cascade leading to COPD is a complex interaction of immune cells and molecular mediators. The process begins with the inhalation of cigarette smoke or other chemical irritants, which damages the airway epithelium leading to release of chemoattractant molecules. Cigarette smoke was also shown by Braber et al. to induce β2 integrin-dependant migration of neutrophils across endothelial cells. [30] These ligands bind and activate chemokine receptors on circulating neutrophils, helper T cells, cytotoxic T cells and monocytes and recruit them to the lungs. Monocytes migrate across the epithelium and differentiate, joining the resident macrophages. As the congregation of immune cells grows they release proteases, such as matrix metalloproteinase-9 (MMP-9) and human neutrophil

elastase (HNE), which degrade connective tissue, particularly elastin, of the alveolar wall, leading to emphysema. [31]-[33] Airway narrowing results from fibroblast proliferation and collagen deposition around the bronchioles in response to TGF B (transforming growth factor) released by macrophages and irritated epithelial cells.

6.4. Role of neutrophils in COPD

Neutrophils themselves are a primary factor in the continuation of the pro-inflammatory state seen in COPD. The hypersecretion of mucus is linked to the accumulation of PMNs. Neutrophil elastase stimulates mucin gene expression; hence goblet cells and mucus glands produce excess mucus, leaving the bronchioles further obstructed. [34],[35] HNE is a one of a family of neutrophil serine proteases that have pluripotent effects in COPD. Not only can HNE degrade the basement membrane but it also directly affects ciliary beat frequency and cleaves CD2, CD4, and CD8 on T-cells, affecting their function. Additionally, HNE cleaves CXCR1 on PMNs, creating an impotent neutrophil that travels to the site of infection but is incapable of acting once it arrives. [36] Furthermore, other neutrophil proteins such as protienase-3, cathepsin G, and myeloperoxidase are all pro-inflammatory molecules released by the neutrophil upon activation or necrosis. [37]

6.5. Diagnosis of COPD

The current standard for COPD diagnosis is spirometry. Lung spirometry measures the volume of exhaled air, thus providing a functional assessment of airway obstruction. Two key spirometric values are FEV1 (forced expiratory volume), the volume of exhaled air over the first second of forced expiration, and FVC (forced vital capacity) or the total volume of air exhaled during forced expiration. These values are interpreted as a ratio (FEV1/FVC) whereby a decreasing value indicates increasing airway obstruction. A ratio less than 0.70 after bronchodilator treatment is diagnostic for COPD. [38],[39] Clinical indications for spirometric evaluation include age greater than 40 years, family history of COPD, past exposure to inhaled irritants, chronic cough and sputum production and dyspnea. [22]

6.6. Traditional COPD therapeutics

Pharmacological therapy of COPD is rooted in combating the symptoms that present secondary to the tissue damage described above. Currently drug therapy is limited to a small cadre of drug classes. Therapeutic agents include bronchodilators, glucocorticosteroids and phosphodiesterase inhibitors. Bronchodilators are the mainstay of COPD therapy. B 2 receptor agonists act on bronchial smooth muscle, promoting relaxation and airway dilation. Both long acting (daily therapy) and short acting (acute exacerbation) formulations are used. [40] Anticholinergics, or acetylcholine antagonists complement the airway dilating mechanism of β-agonists by blocking parasympathetic muscarinic receptors that otherwise cause bronchial smooth muscle contraction. [41] Inhaled glucocorticosteroids aid in controlling inflammation, but are typically only used in conjunction with other drug classes. Oral, or systemic, glucocorticoid therapy is reserved for acute

exacerbations because of chronic immunosuppression and undesirable side-effect profiles from long-term daily use. [42]

7. Cystic fibrosis

7.1. Disease overview

Cystic fibrosis (CF) results from a genetic defect in the cystic fibrosis transmembrane conductance regulator (CFTR), an epithelial cell ion transporter. Although the resultant lung pathology is the main source of morbidity and mortality, there is multi-system dysfunction due to the prevalence of the channel in several cell types. The myriad manifestations of CF include lung disease, pancreatic insufficiency (both endocrine and exocrine), male infertility, liver disease, meconium ileus, and distal intestinal obstruction. [43][,][44] Among these, the primary cause of morbidity and mortality in cystic fibrosis is pulmonary disease. Pulmonary complications stem from impaired ion transport in the airways, which results in thick mucus, reduced ciliary beat frequency and pathogen clearance from the respiratory tract. These pathogens constantly bombard and eventually colonize the CF patient's airways, which leads to a state of persistent inflammation marked by recurrent infections and exacerbations. [45]

7.2. Epidemiology/prevalence/survival

CF predominately affects Caucasians, and has an estimated prevalence of roughly 80,000 people worldwide. When it was first described in 1938, CF virtually guaranteed death shortly after diagnosis. However, advances in knowledge of the disease process and clinical management of CF have led to improved life expectancy of 25 years in 1985 and currently approaching 40 years. Individuals diagnosed with CF today are expected to survive beyond 50 years of age. [46]

7.3. Etiology

The cystic fibrosis gene is situated on the long arm of chromosome 7. CF mutations are transmitted in an autosomal recessive pattern [47] and over 1500 unique mutations of the CF gene have been identified. [48] Such a large number of different mutations, and the demonstrated influence of other genes (i.e. TGFB1) suggests the probability of considerable variability in genotype, phenotype and disease severity. [49] Indeed, this is the case; symptoms, onset and severity vary widely across the CF population. CF patients are classified I-VI according to the type of defect the mutation causes in the resultant protein. Class I mutations are nonsense or stopgain mutations that cause the protein to be truncated. Class II are typically missense mutations that affect the tertiary structure of the protein and prevent it from trafficking to the cell membrane (this is by far the most common type of mutation seen in CF). The most common of all CFTR mutations is termed ΔF508, a deletion of three nucleotides resulting in a deletion of phenylalanine at position 508, and is a class II mutation. In

class III mutations, the CFTR is fully formed and traffics correctly to the cell membrane but does not function properly upon reaching it. Class IV mutations are similar to that of class III but they are solely malfunctions in the opening of the channel. Class V mutations result in less than normal amounts of CFTR, although what is made functions correctly. Finally, class VI mutations are similar to that of class V but they are unique in that what CFTR protein is made is degraded too quickly and there is a functional deficit in the necessary amount of CFTR present on the apical membrane. [50]Typically genotypes in classes I-III have worse phenotypic presentations and higher mortality. Like genotype, sex is also a mortality predictor; males have a higher survival rate than females until the age of 20. [51],[52]

7.4. Pathophysiology

As noted above, CF presents with numerous extra-pulmonary symptoms, but only pulmonary complications will be addressed herein. Pulmonary manifestations of CF can be understood as a stepwise melding of the following pathologic processes:

1. Defective CFTR
2. Reduced ASL height
3. Disrupted mucociliary clearance
4. Colonization/chronic infection/exacerbation
5. Neutrophil dominated inflammation

(1) The underlying genetic defect in CF results in either a dysfunctional or absent CFTR channel. The submucosal glands in the distal airways express CFTR, a protein that spans the membrane of epithelial cells. It employs a cAMP-mediated, PKA activated mechanism to conduct chloride ions across the lipid bilayer. Other functions of CFTR that have been described are affected with varying degrees based on the type of mutation (congenital bilateral absence of the vas deferens). How those secondary functions are altered may explain the phenotypic severity in CF, but dysregulation and dysfunction in chloride conductance is the primary pathology of the CFTR in CF.

(2) Ineffective secretion of chloride anions (and unregulated absorption of sodium ions) leads to a reduced volume of airway surface liquid (ASL) due to the diminished electrolyte content in the airway and very little osmotic pull. In turn, this alters the consistency of airway mucus to a thick, desiccated, hyper-viscous layer that adheres to the airway epithelium. [53]

(3) The adherent mucus creates plaques that obstruct the airways and disrupt the mucociliary clearance mechanism. The detrimental effect of poor mucus clearance is two-fold. First, lung function, as measured by spirometry, declines due to the physical obstruction of the airways. Clogged with mucus plugs, the small airways conduct air less efficiently. Second, the adherent mucus becomes a nidus for infection. [54]

(4) The airways are thus persistently colonized by multiple species of bacteria, namely Pseudamonas aeruginosa, Burkholderia cepacia, Hemophila influezae and Staphylococcus aur-

eus. These organisms are difficult to eradicate in the CF patient, even with continuous prophylactic antibiotic treatment. Colonization is a doorway for infection, and CF is marked by periods of infection and significant decline in lung function known as exacerbations. The etiology of CF exacerbations is closely linked to fluctuations in the balance of bacterial flora in the airway. [55]

(5) Airway obstruction, colonization and episodic exacerbations promote a state of chronic inflammation in CF. In fact, the clinical status of the CF patient, especially during an exacerbation, is tied more closely to the inflammatory response than the quantity and types of organisms responsible for the infection. That inflammatory response is dominated by neutrophils (PMNs) and is responsible for the bulk of tissue damage in the CF airways. Bronchoalveolar lavage and sputum specimens from patients during exacerbation reveal high concentrations of both PMNs and their effectors and signaling molecules, such as neutrophil elastase and IL-8, respectively. [56] IL-8 is a powerful recruiter of PMNs, and excess levels of this signaling molecule likely explains the PMN dominated inflammation in CF. Excess PMN recruitment to the lungs results in the discharge of their destructive weapons (described above) and subsequent killing of pathogens, apoptosis and damage to lung and airway tissue. More often the extreme inflammatory state leads to an aggregation of dying PMNs that result in dysregulated cell lysis, or necrosis, instead of a controlled destruction that mitigates tissue damage. When PMNs undergo necrosis instead of apoptosis they release all of the activated enzymes and molecules designed to destroy pathogen into the interstitial space, further damaging an already taxed pulmonary environment. This damage results in a collection of mucopurulent debris that further clogs airways and provides a breeding ground for further infection. [57]

7.5. Diagnosis of cystic fibrosis

The accepted method for diagnosing CF is by quantitative analysis of the chloride ion content of the sweat. This is based on the premise that the CFTR protein is expressed in sweat glands as well, leading to excessive chloride ion concentration in the sweat. DNA immunoreactive trypsinogen screening techniques are available that detect the presence of many of the common CF mutations. [52],[58] Although not standard, newer diagnostic and screening techniques include genotyping and measurement of the nasal potential difference. No matter what the sweat chloride results, diagnosis of CF remains incomplete without molecular analysis of CFTR gene. Identification of the mutations, and confirmation of their trans state is necessary to provide the patient with an accurate prognosis and clear counseling to parents as to their future reproductive options. Nasal potential difference assesses ion conductance in vivo by observing changes in the voltage potential difference across the nasal epithelium. [59]

7.6. Current cystic fibrosis therapeutics

Advancements in therapeutics in CF in recent decades are the foundation for the prolonged survival of individuals noted above. Unfortunately, almost all current therapies the clinician has at his or her disposal only address the symptoms of CF without correcting the underly-

ing channelopathy. Current CF therapeutics are best understood in relation to how they address the five pathological processes described above. Efforts to address the underlying cause of CF, mutations of the CFTR gene, are underway in the form of gene therapy. The central problem surrounding gene therapy is the search for a suitable delivery mechanism. Viral vectors have been studied, but immune provocation remains to be an obstacle. [60] Reduced ASL height has been successfully addressed with inhaled hypertonic saline and enzymes, such as recombinant human DNAase (dornase alfa). In conjunction with the inhalation therapies noted above, techniques such as breathing exercises, positive expiratory pressure masks, and chest compression (both manual and automated) seek to disrupt the mucus plaques that line the CF airways. Aggressive antibiotic therapy is employed to combat both chronic colonization and acute infections. Although inhaled tobramycin and ciprofloxacin therapy have been effective, the wide array of bacteria in the CF airway precludes development of antibiotic therapy protocol. Finally, high dose ibuprofen and macrolide (typically Azithromycin) treatments stifle the persistent, PMN-dominated inflammation seen in CF. [46],[61],[62] There have been recent exciting discoveries regarding treatment of CF and these will be discussed in a later section.

8. Alpha 1 AT

8.1. Overview/epidemiology

α1-Antitrypsin (A1AT) deficiency (A1AD) is a form of COPD that is an extreme form of the condition, with the potential to develop COPD without excessive smoking. Patients with A1AT deficiency comprise approximately 5% of the global COPD population and have a predicted life span more than 10 years less than those of a life-long smoke with COPD. A1AT is believed by many to be an underreported condition affecting up to 1:2000 people. [63] Although this may seem extremely prevalent, those with the condition only begin to appear symptomatic with cigarette smoking. The underreporting of A1AD may also be due to clinicians giving a diagnosis of idiopathic COPD, or COPD without a known underlying cause. [64]

8.2. Etiology

A1AD is a genetic disorder inherited in an autosomal recessive manner. Patients with A1AT have at least two mutations in a *trans* configuration in the A1AT gene. A1AD patients most often become symptomatic after cigarette smoking, although it is possible to present with emphysema in the third decade of life with no prior tobacco use. Patients present with wheezing, shortness of breath, rales, rhonci, and in some cases, liver failure. [65]

8.3. Pathophysiology

A1AT, an acute phase protein, is member of the serpin family of protease inhibitors. It is produced in the liver and serves to prevent activation and function of the neutrophil serine

proteases HNE and proteinase-3. It is normally present at relatively high concentrations (1.5g/L) in the blood and is believed to play a prominent role in resolving inflammation under normal homeostasis. A1AD derived COPD is believed to be due to a protease/antiprotease imbalance in which normal levels of HNE and proteinase-3 (P3) are uninhibited at sites of minor infection or inflammation. [66] The constitutively active HNE and P3 are left unencumbered to degrade extra-cellular matrix and begin a pro-inflammatory cascade of molecules that only further exacerbate the inflammation. Cigarette smoking is so destructive to those with A1AD because cigarette smoke directly inactivates A1AT, wreaking even further havoc on an already taxed system. The pathophysiology of A1AD is similar to that of COPD and thus the underdiagnosis of this condition.

8.4. Role of neutrophils in A1AD

Very similar to the role they play in traditional COPD, PMNs are both effect and maintain inflammation seen in COPD. As producers of HNE they are responsible for the initial pathology seen in the condition. [67]Under normal circumstances, A1AT is loosely bound to HNE, among other serine proteases. However, in the chronic inflammatory condition associated with A1AD, HNE is constantly active and degrades the basement matrix. Furthermore, HNE has been shown to be capable of cleaving the inactive form of MMP-9, pro-MMP-9 to the active form, creating more protease stress on the system. MMP-9 and HNE are capable of degrading multiple matrix proteins present in the lung. This destruction of the basement collagen, elastin, etc. creates a "leaky" vasculature, only making it easier for other immune cells to move into the lung interstitium. [68] This movement of cells and proteins into the intracellular space brings with it fluid from the circulation and edema results. As the producers of HNE, neutrophils are integral to the pathogenesis and continuation of A1AD associated COPD.

8.5. Diagnosis of A1AD

Diagnosis of A1AD is only made in those cases of COPD where there is an unexplained cause of the condition. A1AT serum levels are measured using enzyme linked adsorbent assays (ELISA), or more recently mass spectrometry. Like CF, there is a spectrum of phenotypes that are observed in the condition and they are categorized based upon the circulating levels of A1AT. Patients with the most severe phenotype are those individuals with concentrations less than 15% of normal in their serum. [69]

8.6. Treatment of A1AD

Because of the nature of the disease treatment of A1AD is very similar to that of traditional COPD, with one exception. Patients with a severe lung phenotype are treated with intravenous infusion of A1AT isolated from human serum. [70] Additionally, liver transplant has been utilized to address the absence of circulating A1AT. [71] In addition to these therapies, the common treatments for COPD mentioned previously are employed to address the specific symptoms of A1AD.

9. Neutrophilic/steroid resistant asthma

9.1. Overview/epidemiology

Asthma was first defined in 1860 by Salter, a British clinician who ascertained that attacks were related to smooth muscle contraction. Asthma, at its core, is a chronic airway disease characterized by wheezing, coughing, and breathlessness with variable airway obstruction on pulmonary function testing. Asthma is a relatively common disease; recent reports by the Center for Disease Control (CDC) place its prevalence at approximately 12% (children) and 10% (adults) in the US. (www.cdc.gov) There appears to be a predominance of childhood asthma in non-Hispanic blacks, whereas non-Hispanic Whites, Hispanics, Asians, and Native Americans all have similar frequencies of asthma. Additionally, the condition is significantly more common among females than males.

9.2. Etiology

The development of asthma is thought to be associated with three major risk factors: genetic predisposition, and occupational and environmental factors. Although a precise list of genes associated with the atopic response in human has yet to be collated, GWAS studies in human, and canines have revealed multiple loci related to the IgE response known to be important in the etiology of asthma. [72],[73]

9.3. Pathophysiology

Asthma begins in the airways with host contact of an allergen, following this, specific IGE antibodies are upregulated and initiate mast cell activation. Mast cell activation, in turn begins the early and late phase response. The early phase response is mediated by histamine, leukotriene C4, D4, and E4, and prostoglandin D2. After the early phase/hypersensitivity response, that late phase response begins. Eosinophils, basophils, neutrophils, and T cells are all recruited to the airway and produce inflammatory cytokines that propogate the allergic response that is a hallmark of asthma.

9.4. Role of neutrophils in neutrophilic and steroid-resistant asthma

Asthma is typically thought of as an eosinophilic disease, yet there have been numerous studies reporting an increase in neutrophil number and activation in sputum collected from steroid-resistant asthma patients. There are reports of up to 50% of asthma cases that have an increase in IL-8 and neutrophil burden, separate from eosinophilic inflammation. [74], [75] Because of the variability seen in primary immune cell burden in asthma neutrophilic asthma has recently begun to be viewed as a specific sub-type of the condition. [76] Patients with neutrophilic asthma have a more severe progression of disease, respond poorly to therapy, and are burdened with much high health care costs than typical asthma patients. Unfortunately, there is little, if any, established dogma regarding neutrophilic asthma. Studies have only been able to describe correlative relationships between neutrophil burden and the phenotypic profile observed in neutrophilic asthma patients. [77] There has been extensive

work performed investigating the role of MMP-9 in the pathogenesis of asthma. In a report by Cundall et al the authors state that MMP-9 concentrations in BAL fluid correlate with eosinophils but not neutrophil or monocyte/macrophage counts. [78] They hypothesize that PMNs and macrophages release MMP-9 which breaks down the basement membrane, making it easier for the eosinophils to migrate into the airways. In another study, MMP-9 levels in BAL fluid were correlated significantly with decreases in FEV1 seen in asthma patients.

HNE, another potent neutrophil derived protease, has also been correlated with symptoms of asthma. [79] Patients with allergic rhinitis has significantly elevated levels of HNE in their nasal lavage compared to control patients in which no rhinitis was observed. To add to the myriad of evidence that neutrophils are at the very least, associated with asthma, a study by Norzila et al demonstrated that myeloperoxidase (MPO), a neutrophil mediator of the oxidative burst, is elevated in induced sputum collected from certain asthma patients compared to control patients. [80] Because MPO, HNE, and MMP-9 are all contained in intracellular granules of the neutrophil it is evidence that neutrophils present in/around the lung in asthma patients are activated and degranulate.

9.5. Diagnosis of asthma

Diagnosis of asthma is made through evaluation of symptoms and pulmonary function testing (PFT) via spirometry. An increase in FEV of ≥15% in conjunction with reported wheezing, chest tightness, and coughing is diagnostic for asthma. A difficulty arises when patients present with normal spirometry results. To address this, home PFT devices are available to record lung function data over a period of time to encapsulate more data points. Additionally, controlled exacerbation of asthma attacks with methacholine in the clinician's office is a reliable method of eliciting the necessary response to confirm a diagnosis of asthma. [81]

9.6. Traditional asthma therapeutics

Similar to the other lung diseases discussed in this chapter, treatment of asthma is relegated to management of symptoms. Monitoring of frequency and severity of attacks is vital to administering correct dosages of medication. Patients are encouraged to keep records of attacks with information regarding date/time, location, duration, and triggers. The standard treatment of asthma is glucocorticoid (GC) inhaler with a long-acting β-agonist. [82] The GC treatment is directed at reducing the constant inflammatory state, whereas the β-agonist is a bronchodilator intended to ameliorate airway obstruction. So physicians will also prescribe the use of IgE inhibitors or neutralizing antibodies such as omaluzimab to combat the high levels of the pro-inflammatory molecule. [83] In neutrophil associated and steroid-resistant asthma, clinicians have fewer options with which to treat this potentially deadly condition. A patient's response to a two week trial of traditional asthma therapy will indicate whether or not they are a candidate for alternative asthma therapy. Because certain forms of asthma are refractory to GC therapy, the focus of treatment in such patients shifts to a more aggressive immunosuppressive approach. Treatment with cyclosporine, tacrolimus, and methotrexate have been associated with some benefit, although the risk of side effects is significantly higher in these classes of medicines. Finally, IV immunoglobulin therapy is uti-

lized in extreme cases, but due to its expense and limited evidence of efficacy, its use is not widespread. Because of the lack of knowledge about the cellular and molecular etiology of neutrophilic asthma, current therapies are limited to those already employed in traditional asthma. As might be expected, these have limited efficacy in patients diagnosed with neutrophilic asthma.

10. Novel therapeutics in neutrophilic lung diseases

With better understanding of neutrophilic lung disease has come more advanced and targeted therapeutics. Towards that end, recent work by the Blalock and Gaggar groups at the University of Alabama at Birmingham (UAB) has expanded the role of PMNs in multiple chronic inflammatory lung diseases, including COPD, CF, and BOS. They described a novel concept of neutrophils proteases producing a neutrophil chemokine from extra-cellular collagen that acted in a feed-forward mechanism of disease. Seminal papers by Weathington et al and Gaggar et al detail the step-wise manner in which IL-8 draws PMNs into the interstitium, upon activation they release MMP-8 and MMP-9 which perform an initial digestion of collagen from macromolecule size. Subsequently, neutrophils release prolyl endopeptidase (PE), a serine protease previously only known to be a processor of neuropeptides. PE performs the final digestion of collagen to the tri-peptide proline-glycine-proline (PGP) from the PPGP amino acid motif that is repeated over 40 times throughout a single collagen molecule. [84],[85] PGP binds to the same receptors as IL-8, CXCR1 and CXCR2 acting a neutrophil chemoattractant and activator. [86] The authors showed that not only are the proteases responsible for PGP production present and elevated in BAL fluid collected from COPD and CF patients, both stable and in exacerbations, but PGP is also measurably elevated by mass spectrometry in the BAL fluid of such patients and correlates with PMN burden in disease. [39],[87],[88] These data indicate that not only is PGP a potential biomarker for chronic inflammatory neutrophilic lung disease, but the system of proteases responsible for PGP's production, and the receptors upon which it acts are potential targets for the development of novel precise therapeutics. Furthermore, work by Hardison et al, and Braber et al have demonstrated that cigarette smoke and its constituents are capable of acetylating PGP into the more potent and stable n-terminal acetylated form, AcPGP. [89],[90] AcPGP has proven to be resistant to degradation by leukotriene A4 hydrolase (LTA4H), a hydrolase/amino-peptidase also produced a number of cells, including neutrophils. In a 2010 Science paper, Snelgrove et al described a novel function for the dual purpose enzyme in resolving acute neutrophilic inflammation in a mouse model of influenza. [91] It would be extremely useful to have pharmaco-interventions able to modulate the PGP system of neutrophil inflammation, either at the genesis (MMP, PE) or terminus (CXCR, LTA4H).

Although any therapeutics derived from such work may be years away from fruition, there are other recent advancements that are already making an impact on patient morbidity and mortality. Kalydeco, a drug produced by Vertex Pharmaceuticals is the first drug developed that addresses the underlying genetic cause of CF. First released on the market in January of 2012, it is effective in patients that carry the G115D amino acid change. [92] This is a class III

mutation in which the protein traffics the cell surface but the channel does not function properly. Kalydeco interacts with the channel and increases the open probability of the channel. Another Vertex product, currently titled VX-809, is designed to act in patients with class II mutations (i.e., ΔF508). VX-809 acts in the endoplasmic reticulum, allowing improperly folded CF protein to pool and undergo corrected folding which results in trafficking to the cell membrane. [93] Both Vertex products are the result of so-called high throughput small molecule screening in which hundreds of thousands of small molecules are screened in a recombinant cell-based assay for an effect on cell function. The discovery of drugs that address the underlying genetics cause is an exciting advancement in any genetic disease, but made even more so by the fact that CF is one of the more common, and fatal diseases caused by a genetic malformation. Whether any of these drugs change the number or activation state of PMNs in the airway is currently unknown

Patients with COPD, an even larger cohort than those with CF may also soon benefit from new therapies targeted at resolving the underlying cause rather than merely treating symptoms. There is currently only a single phosphodiesterase 4 (PDE4) inhibitor, Daliresp that is approved for treatment of COPD in the United States. However, there are clinical trials currently underway researching the effects of multiple other PDE4 inhibitors. [94] PD4 is a cAMP specific phosphodiesterase present, primarily, in inflammatory cells and also in epithelial cells. Treatment with Daliresp has been shown to reduce the release of pro-inflammatory cytokines by neutrophils and resident monocyte/macrophages. Unfortunately, there are several side effects associated with Daliresp and thus the need for better, more targeted PDE4 inhibitors is apparent. Additionally, there have been recent advancements made in traditional COPD therapies. The development of ultra-long acting β2 agonists has proved beneficial in a number of lung diseases, including COPD, A1AT, and asthma. [95] Research is also underway into the identification of biomarkers for smokers who will develop COPD, allowing treatment or prevention to possibly begin earlier. Investigators at Weill Cornell College of Medicine are using a metabolomics approach in a cohort of smokers to establish a thorough catalogue of abnormal cell changes in airway epithelium after cigarette smoking. (weill.cornell.edu) Utilizing serum, epithelial lining fluid, and airway epithelial samples, Dr. Crystal's group aims to identify the early changes in airway epithelium that indicate if a patient will develop COPD later.

McNab et al recently published work detailing their investigation of "compound cg," a small molecule that assists in reducing aggregates of abnormal A1AT protein. [96] GC was effective in an *in vitro* model of A1AT deficiency and showed significant reduction in At1AT aggregates by both immunohistochemistry and Western blot analysis. Gene therapy is another approach, also applicable to CF that is being investigated as a potential source of curing the disease in A1AT deficiency. A group at UMass has pioneered a dual gene therapy approach that addressed both the lung malfunction and liver disease so often associated with aggregation of mutant protein. In utilizing an adeno-associated virus (AAV) to introduce corrected protein product in the lung, and microRNAs (miRNA) in the liver to reduce production of dysfunctional protein, the investigators have presented the possibility of curative therapy for patients with A1AT deficiency. [97]

Many of the therapies previously mentioned are also in use in the treatment of PMN-related or glucocorticoid resistant asthma. The PDE4 inhibitors, along with ultra-long acting β2 agonists have begun to be used in combating the airway dysfunction associated with asthma. [98] There is work being done to abrogate the ability of inflammatory cells such as neutrophils to bind adhesion molecules such as the integrin VLA-4. [99] Furthermore, kinase inhibitors being investigated that target p38 MAPK and PI3K would also effect neutrophil recruitment and activation in asthma. [100]

11. Conclusion

Chronic neutrophilic airway inflammation is a clinically similar, but foundationally heterogeneous cohort of disease. Although neutrophils are necessary and effective components of the innate immune system in resolving infection, when dysregulated, they can be potent mediators of devastating inflammation. Current therapeutics in a variety of neutrophilic lung diseases fail to address the underlying causes of the conditions and yield questionable benefit to patients. Fortunately, advances in identifications of biomarkers such as PGP and others afford the opportunity to develop targeted therapeutics aimed at resolving and preventing the progressive destruction that is a hallmark of chronic neutrophilic lung disease.

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