Neutrophilic inflammation in the pathogenesis of Chronic Obstructive Pulmonary Disease

Butler, Aidan; Walton, Georgia May; Sapey, Elizabeth

DOI:

[10.1080/15412555.2018.1476475](https://doi.org/10.1080/15412555.2018.1476475)

License:

None: All rights reserved

Document Version

Peer reviewed version

Citation for published version (Harvard):

Butler, A, Walton, GM & Sapey, E 2018, 'Neutrophilic inflammation in the pathogenesis of Chronic Obstructive Pulmonary Disease', *COPD: Journal of Chronic Obstructive Pulmonary Disease*, vol. 15, no. 4, pp. 392-404. <https://doi.org/10.1080/15412555.2018.1476475>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

Checked for eligibility 25/10/2018

This is an Accepted Manuscript of an article published by Taylor & Francis in *COPD: Journal of Chronic Obstructive Pulmonary Disease* on 31/07/2018, available online: <http://www.tandfonline.com/10.1080/15412555.2018.1476475>

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Neutrophilic Inflammation in the Pathogenesis of Chronic Obstructive Pulmonary Disease

Mr Aidan Butler, BSc; Miss Georgia M Walton, BSc; Dr Elizabeth Sapey BSc, MBBS, PhD

Mr Aidan Butler, Institute of Inflammation and Ageing, University of Birmingham, Birmingham, UK

Miss Georgia Walton, Institute of Inflammation and Ageing, University of Birmingham, Birmingham, UK

Dr. Elizabeth Sapey, Corresponding author.

Institute of Inflammation and Ageing, University of Birmingham, Birmingham, UK

Tel: +44 121 627 2000

Fax: +44 121 627 2000

Email: e.sapey@bham.ac.uk

Running title: Neutrophils and COPD

Key words: proteinases, emphysema, chronic bronchitis, chemotaxis, phagocytosis

Word count: 3215

2 Figures

1 table

Abstract

Chronic Obstructive Pulmonary Disease (COPD) is a common preventable disease characterized by an inflammatory infiltrate of the airways and progressive airflow obstruction. Whilst many inflammatory cells are implicated in COPD, the neutrophil is by far the most abundant and has been extensively associated with disease pathogenesis. Neutrophil products are thought to be key mediators of inflammatory changes in the airways of COPD patients, causing pathological changes such as emphysema and hypersecretion of mucus. Furthermore, both bacterial colonization and acute exacerbations of COPD are associated with increased neutrophil numbers and markers of activation, further highlighting their role in disease.

High rates of bacterial colonization and infective causes of acute exacerbations are observed in some patients with COPD despite this abundance of anti-bacterial neutrophils, raising the suggestion that neutrophil functions may be impaired in COPD. Exploring this hypothesis is complicated by the wide variety of functions exhibited by the neutrophil; impairment of any one of these could result in impaired bacterial clearance.

There is a need for new therapeutic strategies in COPD. Further explorations into neutrophil function in both health and COPD is allowing us to understand its role in disease pathogenesis and to elucidate whether this key inflammatory mediator represents a viable therapeutic target to prevent disease progression.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) remains a significant global health challenge. It has not seen the same improvements in morbidity and mortality as many other chronic inflammatory diseases and only one novel drug class has reached market in the past twenty years(1). After 25 years of smoking approximately 30 – 40% of adults will have developed COPD(2) but despite most patients having this shared risk factor, COPD is heterogeneous in presentation, age of onset and speed of decline. Whilst the disease is defined by the presence of airflow obstruction, patients can be divided into recognized clinical phenotypes. These include those with a predominance of emphysema(3), obstructive bronchiolitis (3, 4), the presence or absence of chronic bronchitis, evidence of bacterial colonisation of the lower airways with potentially pathogenic bacteria, those who experience frequent exacerbations(5) and those who experience a faster decline in lung function(6). Clinical phenotypes are stable within individual patients(6) and cluster within families(7) and thus are likely to reflect genetic traits. Indeed, genome wide association studies in COPD have identified a large number of signals for genes associated with lung development, lung parenchyma formation and repair and epigenetic regulation such as the inositol phosphate pathway(8, 9). These studies suggest there may be a wide range of therapeutic targets in different subsets of COPD patients, providing hope for personalised medicine. However drug development is costly and there are concerns about the affordability of targeting many different molecules for small groups of patients, especially in low income countries where the burden of COPD is rising fastest(10). This raises the question as to whether there might be a more common targetable mechanism across disease phenotypes which could provide a treatment for larger number of patients.

Inflammation in COPD

Most pro-inflammatory mediators and immune cells have been shown to be raised in lung secretions taken from patients with COPD(11) and this inflammation is heightened and self-sustaining in smokers who are susceptible to COPD in contrast to those smokers who are not (12, 13). However, while many cells and mediators have been implicated in COPD pathogenesis at some level, few have reliably demonstrated their importance as therapeutic targets in human studies. For example, despite the promising resistance to COPD-like lung damage shown by TNF α receptor (14) or IL-1 receptor 1 knock-out mice (15) studies targeting these individual mediators in unselected cohorts of patients with COPD have been disappointing with TNF α and IL-1 receptor 1 inhibition showed no improvements in disease endpoints(16, 17). This might suggest that, unlike murine models, only sub groups of patients will respond to individual mediator-based therapies. In support of this concept, there is variability in inflammatory patterns between patients, even when matched for age, smoking status and disease severity(18) which might reflect specific genetic traits as shown in some studies of TNF α (19) and IL-1 β (20) polymorphisms.

To complicate matters further, there is significant intra-patient variability in the concentration of plasma and sputum mediators and cells on a day to day basis. Some mediators increase while others decrease suggesting the variability not only reflects dilution but also fluctuations in the specific components of the inflammatory load (18, 21). This supports an alternative explanation to the negative trial results reported to date, where there is so much redundancy, compensation and overlap within the complex inflammatory storm that is established COPD(22) that end-cell effects

can be driven by an alternative cytokine, should one be abrogated. For example, toll-like receptors, TNF α and IL-1 signalling to NF- κ B all converge on a common I κ B kinase complex that phosphorylates the NF- κ B inhibitory protein I κ B α , despite the upstream signalling components being to a large part receptor-specific(23). The effects of these mediators are synergistic and inhibiting one has not proved efficacious enough to impact robustly on cellular inflammation or COPD disease progression. Potentially targeting the functions of the end-cell and not the intermediary cytokine might be more effective and there is a strong rationale for targeting the neutrophil in COPD.

Classical neutrophil functions in health

Neutrophils are the most abundant leukocyte, accounting for 70% of all circulating white blood cells. They are short-lived cells (with a half-life of around eight hours) with basal production of $1-2 \times 10^{11}$ neutrophils/day in health; though this can increase to 10^{12} during infection(24-26). Following myeloblastic differentiation in the bone marrow, the mobilisation of terminally differentiated neutrophils into the circulation is tightly controlled by bone marrow signals and circulating growth factors including those between neutrophil CXCR4 and bone marrow stromal cell CXCL12 causing cell retention and neutrophil CXCR2 resulting in neutrophil release(27, 28). Neutrophils are characterised by the presence of a multi-lobed nucleus and granular cytoplasm, due to the presence of Azurophilic (primary), Specific (secondary) and Gelatinase (tertiary) granules, as well as secretory vesicles. These granules and vesicles contain a complex and specialised arsenal of proteins which facilitate neutrophil migration from the systemic circulation through the dense extracellular matrix to areas of inflammation, and then permit microbial killing and tissue remodelling and degradation, as listed in Table 1. This is not an “all or nothing response” and neutrophils require different levels of activation (and subsequent calcium mobilisation) to release granules, with secretory vesicles requiring the least stimulation, mobilising to facilitate migration and adhesion, while azurophil granules (which are the most cytotoxic) requiring the most stimulation.

Table 1. The contents of human neutrophil granules and secretory vesicles

Constituents	Azurophil	Specific	Gelatinase	Secretory
Matrix proteins	Elastase Proteinase 3 Cathepsin G Cathepsin D Defensins Lysozyme Myeloperoxidase Bacterial permeability increasing protein (BPI) Azuricidin α 1-antitrypsin β -glucuronidase Phospholipase A2	Collagenase Gelatinase hCAP-18 Histaminase Heprainase Lactoferrin Lysozyme NGAL β 2 microglobulin	Gelatinase Lysosyme β 2 microglobulin	Plasma proteins
Membrane proteins	CD63 CD68 Presenilin 1 V-type H ⁺ -ATPase	CD11b/CD18 Cytochrome b ₅₅₈ fMLP-R G-protein α -subunit Leukolysin VAMP-2	CD11b/CD18 Cytochrome b ₅₅₈ fMLP-R VAMP-2 V-type H ⁺ -ATPase	Alkaline phosphatase CD11b/CD18 CD16 CD10 CD13 CD14 fMLP-R

				C1q-R Cytochrome b ₅₅₈
--	--	--	--	--------------------------------------

Legend. The contents of the different sub-types of neutrophil granule, divided into membranous and matrix (cytosolic) proteins. R, receptor; hCAP-18, human cathelicidin protein; VAMP-2, vesicle-associated membrane protein 2. fMLP, N-formylmethionyl-leucyl-phenylalanine

Neutrophils are guided towards inflammation in a gradient-dependent manner by chemotactic signals (30). Circulating neutrophils cross the endothelium at sites of inflammation via the Leukocyte Adhesion Cascade (LAC), whereby neutrophils interact with endothelial cells at sites of high inflammatory signalling, facilitating passage across the endothelium and stimulating signalling cascades within the neutrophil associated with antimicrobial armament and activation. It is unclear whether the LAC occurs in the pulmonary circulation however, due to the small diameter of the capillaries found there, which may influence neutrophil function (31).

Neutrophil homeostasis is also regulated by signals from tissue resident macrophages. When neutrophils become activated they release IL23 which stimulates IL-17 production. IL-17 induces release of G-CSF, causing further mobilisation of neutrophils into the blood stream to assist with clearing an infection (19,20).

Changing perceptions of the neutrophil

Neutrophils in COPD

Neutrophils are considered central to the pathogenesis of COPD. Airway neutrophilia is a feature of COPD regardless of the clinical phenotype, severity of disease, rapidity of decline or age of onset. Their numbers and products in sputum and airway lavage fluid correlate with disease severity, evident in the degree of airway obstruction, decline in FEV₁ and severity of emphysema present (32-35). Within the airway wall, confirmation of a neutrophilic presence has been more enigmatic, with some studies confirming (36) and others refuting their presence (37). However, neutrophils are not tissue resident, and inconsistencies could reflect the short life span of these cells. To address this, studies have focused on tissue collection at times of significant neutrophil recruitment to the airways in COPD (during acute exacerbations), and here numbers have been shown to be consistently raised (38). Increased levels of human neutrophil lipocalin (HNL) and myeloperoxidase (MPO), products of neutrophils degranulation, in the sputum of COPD patients suggest increased neutrophil activity in COPD airways (39). Concordantly, 18-fluorodeoxyglucose positron emission tomography studies of neutrophilic inflammation in COPD show enhanced uptake in the emphysematous regions of the lungs and correlate with measures of disease severity (40). Elegant pathological studies by Hogg et al suggest small airways dysfunction and destruction may precede the development of emphysema and airflow obstruction, and may reflect the earliest pathological changes in the lungs of patients with COPD (41). Neutrophils have also been implicated here, showing a relationship with neutrophilic infiltration and tomographic measures of air trapping (42).

Neutrophils and co-morbidities of COPD

Potential targets and therapeutic challenges – CXCR2 data. From trial

Activated neutrophils degranulate during migration towards inflammation and infection, during frustrated phagocytosis and NETosis, releasing serine proteases and elastolytic enzymes (e.g. neutrophil elastase [NE], matrix metalloproteases [MMPs] 8 and 9, proteinase-3) into the airways of COPD patients (43, 44). Excessive activity of these enzymes in COPD airways is thought to degrade elastin and type III collagen, leading to destruction of alveolar tissue and consequently centrilobular emphysema, apparent in many cases of COPD (43, 44). Serine proteases are also potent stimulants of mucus secretion in the airways, and cigarette smoke is thought to have direct effects on cilia, shortening them and reducing mucociliary clearance (45-47). The resulting mucus accumulation further obstructs the airways and increasing the risk of bacterial colonisation, contributing to the inflammatory environment (45, 48). Figure 1 summarizes the inflammatory interactions thought to underlie COPD.

Alveolar macrophages also contribute to and may orchestrate this inflammatory milieu by the release of inflammatory mediators and macrophage-specific and scavenged proteinases, recruiting more neutrophils to the airways, which in turn promote monocyte recruitment, thus leading to a vicious cycle of inflammatory damage.

COPD is also associated with a number of chronic inflammatory co-morbidities, including cardiovascular disease, type 2 diabetes, osteoporosis and periodontitis; greater prevalence of these co-morbidities are seen in COPD patients even once contributory factors (such as age, smoking and sedentary lifestyle) have been taken into account. It has been hypothesized that the pulmonary inflammation present in COPD may “overspill” systemically, leading to the development of different co-morbidities (with the type most likely dependent on individual susceptibility and exposures). Interestingly, many of the co-morbidities shared with COPD are also characterized by neutrophilic inflammation. For example, neutrophil products are raised acutely following a myocardial infarction and predict outcomes(49), neutrophil receptor expression and function are altered in diabetes(50), neutrophil lymphocyte ratios are inversely related to bone density in elderly people(51) and neutrophilic inflammation appears central to the development of periodontitis(52).

As demonstrated in figure 1, macrophages(53), T cells(54), B cells and auto-immunity(54, 55), and eosinophils(56) are all implicated in COPD. The degree to which a certain cell is involved in individual patients may reflect varying genetic predispositions or environmental exposures which drive disease within that individual. However, just as with each clinical phenotype, and with the majority of co-morbidities associated with COPD, no matter what cell type has been implicated neutrophils remain at the heart of the disease. In the landmark paper by Brightling discussing eosinophilic signals in COPD(56), average airway cells constituted of 2.4% eosinophils, 21.5% macrophages, 0.4% T-lymphocytes and 67.9% neutrophils.

The association between COPD and neutrophils is further evidenced by Alpha-1 Antitrypsin Deficiency (AATD). Alpha-1 Anti-trypsin is an anti-protease that directly antagonizes the proteolytic activities of NE on a one to one molar basis, and is thought to provide greater than 90% of the protective mechanisms against NE in the lungs (44, 57, 58). AATD is the most extensively documented genetic risk factor for COPD, where the inhibition of NE proteolysis predisposes to increased NE-mediated degradation of extracellular matrix and development of emphysema (58). Emphysema occurs even in the absence of smoking in some individuals and rates of decline in lung function are faster in those who smoke. Furthermore, there is evidence that emphysema progression is slowed by augmentation with replacement Alpha-1 Anti-trypsin(59) although the effect of augmentation on FEV₁ decline is less clear(60).

Of note, the persistently progressive and self-perpetuating inflammatory pattern observed in ex-smoking patients who develop COPD is not observed in ex-smokers who do not develop COPD(61, 62), suggesting a differential inflammatory response in individuals who are susceptible to disease in comparison to those who are not. Furthermore, only 25-50% of smokers develop COPD(63) and COPD also occurs in non-smokers(64), hence chronic exposure to cigarette smoke is neither sufficient alone to cause disease nor necessary for disease development, and other factors must play a role in disease development. In keeping with this, familial and genome association studies support the presence of a number of inherited traits that either promote or protect the host from COPD (65). Our current understanding of these traits is limited because genetic studies have not identified polymorphisms that are common in a large proportion of COPD patients. Furthermore, there is evidence that lung development (with early childhood and potentially in utero stimuli) contributes to disease burden (66). This has led to the orphan disease hypothesis of COPD(67); many potential genetic drivers and developmental factors, combined with the appropriate environment trigger, lead to the clinical symptoms and airflow obstruction that defines disease. Such a diverse array of potential susceptibility factors is challenging, both in terms of understanding disease pathogenesis and developing treatment strategies, and there is great interest in finding a unifying therapeutic target for the highest number of patients. The neutrophil may represent such a potential candidate target for therapeutic modulation.

Bacterial Colonisation and Acute Exacerbations

Bacterial colonisation of the lower respiratory tract is common in COPD; approximately 20-30% of stable COPD patients have a positive bacterial sputum culture and 60% show evidence of lower respiratory tract bacteria using culture independent assays. Bacterial colonisation correlates with more severe airflow obstruction, a more pronounced decline in lung function, worse health status and increased incidence of acute exacerbations (68-73). Bacterial colonisation is also associated with worsened pathological changes: increases in inflammatory cytokines are associated with neutrophil recruitment (such as IL-8 and LTB₄) and activation (TNF α), increased mucus secretion(74), reduced ciliary beat frequency(75), and epithelial damage(76, 77).

Many sufferers of COPD experience acute periods of exacerbation of respiratory symptoms, thought to be partly caused by an increase in the inflammatory infiltrate of the airways. For the majority of exacerbations (as high as 78%), this is induced by bacterial or viral infection, although many patients suffer exacerbations in the absence of identified infection (78-80). Acute exacerbations of COPD are significant medical events, often resulting in hospitalisation and occasionally mortality (81).

Irrespective of aetiology, airway neutrophilia shows a clear increase during exacerbations of COPD (38, 82). There is also evidence of increased matrix metalloproteinase-9 (MMP-9) activity, the neutrophil proteolytic enzyme (44). Furthermore, increased airway neutrophilia appears to be associated with critical expiratory flow limitation, dynamic lung hyperinflation, and consequently increased respiratory distress (68, 78, 83).

Neutrophil Functions in Health and COPD

Not all smokers develop COPD even when matched for exposure and there are differences in neutrophilic inflammatory burden in ex-smokers with and without COPD, therefore differential neutrophil functions are implicated in COPD pathogenesis.

Once within inflamed tissue, the neutrophil utilizes chemotactic gradients created by host-derived inflammatory cytokines (e.g. IL-8) and bacterial products (e.g. Lipopolysaccharide [LPS], N-formyl-methionyl-leucyl-phenylalanine [fMLP]) released from the site of infection, to migrate towards invading pathogens (30). These chemoattractant molecules bind their cognate receptors inducing functional changes in the neutrophil (30) such as the assembly of oxidative burst machinery (84). External gradients are amplified within the cell, allowing accurate mobilisation of internal structures (pseudopods for migration, granules for phagolysis) towards the inflammatory or infectious insult and facilitating full use of functions such as phagocytosis, production of toxic oxidative products, release of bactericidal granular proteins and production of neutrophil extracellular traps (NETs) (30).

The neutrophil granule system is designed to store highly toxic substances within the cell, facilitating fast release in response to invading pathogens (29). Granules are traditionally divided into four sub-types: azurophilic (primary)(29, 85) and specific (secondary)(29, 86, 87) granules contain predominantly bactericidal products (e.g. myeloperoxidase, α -defensins, lactoferrin), whereas gelatinase (tertiary)(30, 88) granules and secretory vesicles(89-91) contain products that aid neutrophil migration and endothelial transmigration (e.g. metalloproteases [MMPs], β_2 -integrins, Fc γ III [CD16]). Different granule sub-types are mobilized and released dependent on cytosolic free calcium levels, which allows coordination of granule release to match cellular requirement, for example mobilisation of azurophilic granules occurs only once the neutrophil comes into contact with a pathogen (92). Details of the contents of neutrophil granules are provided in table 1 (29).

Reactive Oxygen Species (ROS) are released both into phagosomes and externally during migration, degranulation and NETosis (see later). NADPH oxidase acts as a channel for electrons from the cytosol into phagosomal vacuoles, stimulating reduction of oxygen (O_2) to the superoxide anion O_2^- (93). Superoxide can then dismutate, a process accelerated by the enzyme superoxide dismutase (SOD), to form the highly oxidative hydrogen peroxide (H_2O_2), which can react further (the MPO-halide-hydrogen peroxide antibacterial system) to form strongly bactericidal hypohalous acids (e.g. HOCl) (85, 94-98). The role of ROS and the NADPH oxidase in elimination of pathogens is long-believed to be a dominant killing mechanism in neutrophils, however the exact role of these processes in neutrophil bacterial killing has come under recent scrutiny with some research supporting their role as facilitatory rather than obligatory (96).

Phagocytosis is an active, receptor-dependent process through which a phagocyte internalizes material into membrane-bound vacuoles (99). Direct interactions between neutrophilic pattern recognition receptors (PRRs) and surface-expressed pathogen-associated molecular patterns

(PAMPs) can generate signalling cascades that lead to ingestion of the target pathogen, a process termed 'unopsonized' phagocytosis (30, 100, 101). Phagocytosis of opsonized particles is a much faster process; ingestion of an IgG-opsonized particles by a neutrophil can take less than 20 seconds (102). The most extensively studied opsonin receptors of neutrophils, the Fc receptors (e.g. FcγRIIA [CD32], FcγRIIb [CD16]) bind IgG-bound particles triggering a signalling cascade involving the tyrosine kinase Syk and phosphatidylinositol 3-kinase (PI3K). Complement molecules also opsonize pathogens to phagocytic killing by interacting with neutrophil complement receptors (e.g. CR1 [CD35], CR3 [CD11b/CD18]) (103-106). Binding activates a signalling cascade involving phospholipase D (PLD), diacylglycerol (DAG) and the GTPase Rho. Both IgG and complement mediated phagocytosis result in cytoskeletal rearrangements that facilitate particle ingestion (107, 108).

Phagosome maturation refers to the equipping of the nascent phagosome with the antimicrobial potential required to eliminate a pathogen (103). Unlike the unquestionable acidification observed in the macrophage phagosome(103, 109), the neutrophil phagosome undergoes a transient alkalinisation, despite evidence of V-ATPase proton-pumping activity, and antimicrobial activity is thought to be predominantly acquired through delivery of neutrophil granules and the NADPH oxidase machinery (30, 110, 111). Membrane trafficking supports the controlled delivery of bactericidal granular proteins into the phagosome (92, 112). These processes are depicted in Figure 2.

Neutrophils are also able to produce extracellular traps (NETs), consisting of a backbone of uncondensed chromatin to which bactericidal products such as cathepsins, MPO and nuclear histones are bound (113). Microscopic studies suggest NETs are responsible for the killing of a wide range of pathogens, including Gram-negative(113) and Gram-positive(113, 114) bacteria as well as fungi(115), however the process of NETosis occurs later in neutrophil activation than other killing processes such as phagocytosis or generation of ROS (116). NETs may also have detrimental effects on the host by exposing host molecules (i.e. DNA) within regions of active inflammation, introducing risk of autoimmunity. NETs have been implicated in cases of systemic lupus erythematosus (SLE), sepsis-induced hepatotoxicity and deep vein thrombosis (117-120).

There is a paradox that airway secretions from patients with COPD are neutrophil replete, correlating closely with disease severity, but that airway colonisation and infections are common (43, 121-123). Hence, it is likely that neutrophils present in COPD airways are impaired, weakening antimicrobial function and contributing to lung damage, and there is evidence supporting this hypothesis. COPD neutrophils demonstrate migratory inaccuracy, able to migrate towards chemotactic signals with greater speed, but decreased accuracy and velocity than neutrophils from age-matched healthy controls (124, 125). *In vitro* modelling suggests this results in a longer and more convoluted migratory path, increased secretion of damaging digestive enzymes and a delay in bacterial killing processes, potentially responsible for the increased rates of bacterial colonisation and infective exacerbations observed in COPD patients (124). Flow cytometric studies demonstrate enhanced respiratory burst in neutrophils of COPD sufferers as compared to healthy smokers(126), and furthermore increased markers of oxidative activity both in COPD airways and systemically suggest increased ROS-producing capacity in stable disease (127). Evidence in acute exacerbations is less clear, with some studies(128) suggesting the 'frequent exacerbator' phenotype of COPD demonstrates decreased oxidative burst in comparison to both healthy controls and stable COPD patients, and others demonstrating increased oxidative burst in active exacerbations (129, 130).

Similarly, there is conflicting evidence of NETosis; increased quantities of NETs and NET-producing neutrophils are observed in the sputum of both stable and exacerbating COPD patients(131, 132), however when isolated from blood, neutrophils of exacerbating patients demonstrate attenuated NET-producing ability (133). A potential explanation for this is an impaired clearance of NETs by DNases(119) rather than increased production, but this is currently unexplored. The efferocytosis function of alveolar macrophages however, which mediates clearance of neutrophils(134), is thought to be impaired in COPD (135). The resulting accumulation of dead neutrophils in the airways may lead to secondary necrosis and excessive release of neutrophil granules and pro-inflammatory cytokines, contributing to an increasing neutrophilic infiltration and promoting an inflammatory environment (135, 136). There is limited data about the phagocytic functions of COPD neutrophils. Ingestion of opsonized *E. coli* and *Candida* species have been found to be reduced in COPD neutrophils compared to age-matched healthy controls (137, 138). However, neither Venge et al. nor Muns et al. observed any difference between the phagocytic abilities of COPD neutrophils and controls(139, 140), suggesting any defect may be both stimulant and assay dependent.

If neutrophil functions are dysregulated in COPD, favouring inflammation but impeding bacterial clearance, understanding the reasons for this is crucial to allow treatment. Genome wide studies have not consistently shown polymorphisms in pathways implicated in neutrophil functions, suggesting there are no common neutrophil-based genetic disorders causing COPD. However, transcriptional changes in neutrophil genotype have recently been described that appear to effect function in lupus patients(141), and it may be that the combination of multiple genetic factors, in themselves insufficient to cause disease, lead to epigenetic changes in neutrophils following environmental exposures which lead to long-term alterations in gene expression, perpetuating the inflammatory damage seen in COPD. Understanding these changes may unlock new strategies for COPD treatment.

Conclusion

Neutrophils are innate immune cells that have been widely implicated in the pathogenesis of COPD. They are a feature of all disease phenotypes and neutrophilic inflammation is described in many of the shared co-morbidities. Their ability to damage lung parenchyma as part of the inflammatory pathology of COPD has been well-described and there is mounting evidence of neutrophil dysfunction in COPD, which might explain the continued inflammatory response. All these data suggest the neutrophil may represent a unifying therapeutic target in COPD, but the necessary functions of these cells make this target a challenging one. Normalizing their activity whilst maintaining their ability to participate in host defence may be a crucial step in preventing COPD progression.

Declarations

AB, GMW have nothing to declare. ES has received funding from the Medical Research Council, British Lung Foundation , Wellcome Trust and has received non-commercial funding from GSK and Pfizer.

References

1. Calverley PMA, Rabe KF, Goehring U-M, Kristiansen S, Fabbri LM, Martinez FJ. Roflumilast in symptomatic chronic obstructive pulmonary disease: two randomised clinical trials. *The Lancet*. 2009;374(9691):685-94.
2. Løkke A, Lange P, Scharling H, Fabricius P, Vestbo J. Developing COPD: a 25 year follow up study of the general population. *Thorax*. 2006;61(11):935.
3. Jeffery PK. Comparison of the structural and inflammatory features of COPD and asthma. Giles F. Filley Lecture. *Chest*. 2000;117(5 Suppl 1):251S-60S.
4. Bignon J, Khoury F, Even P, Andre J, Brouet G. Morphometric study in chronic obstructive bronchopulmonary disease. Pathologic, clinical, and physiologic correlations. *The American review of respiratory disease*. 1969;99(5):669-95.
5. Wedzicha JA, Brill SE, Allinson JP, Donaldson GC. Mechanisms and impact of the frequent exacerbator phenotype in chronic obstructive pulmonary disease. *BMC medicine*. 2013;11:181.
6. Vestbo J, Edwards LD, Scanlon PD, Yates JC, Agusti A, Bakke P, et al. Changes in forced expiratory volume in 1 second over time in COPD. *N Engl J Med*. 2011;365(13):1184-92.
7. Patel BD, Coxson HO, Pillai SG, Agustí AGN, Calverley PMA, Donner CF, et al. Airway Wall Thickening and Emphysema Show Independent Familial Aggregation in Chronic Obstructive Pulmonary Disease. *American Journal of Respiratory and Critical Care Medicine*. 2008;178(5):500-5.
8. Probert K, Miller S, Kheirallah AK, Hall IP. Developmental genetics of the COPD lung. *COPD Research and Practice*. 2015;1(1):10.
9. Wain LV, Shrine N, Artigas MS, Erzurumluoglu AM, Noyvert B, Bossini-Castillo L, et al. Genome-wide association analyses for lung function and chronic obstructive pulmonary disease identify new loci and potential druggable targets. *Nature Genetics*. 2017;49:416.
10. Ferkol T, Schraufnagel D. The Global Burden of Respiratory Disease. *Annals of the American Thoracic Society*. 2014;11(3):404-6.
11. Barnes PJ, Chowdhury B, Kharitonov SA, Magnussen H, Page CP, Postma D, et al. Pulmonary biomarkers in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2006;174(1):6-14.
12. Hoonhorst SJM, Timens W, Koenderman L, Lo Tam Loi AT, Lammers J-WJ, Boezen HM, et al. Increased activation of blood neutrophils after cigarette smoking in young individuals susceptible to COPD. *Respiratory Research*. 2014;15(1):121.
13. Rutgers SR, Postma DS, H. thN, Kauffman HF, W. vDMT, Koeter GH, et al. Ongoing airway inflammation in patients with COPD who Do not currently smoke. *Chest*. 2000;117(1931-3543 (Electronic)):262S.
14. Churg A., Wang RD, Tai H, Wang X, Xie C, Wright JL. Tumour necrosis factor alpha drives 70% of cigarette smoke-induced emphysema in the mouse. *Am J Respir Crit Care Med*. 2004;170:492 - 8.
15. Pauwels NS, Bracke KR, Dupont LL, Van Pottelberge GR, Provoost S, Vanden Berghe T, et al. Role of IL-1 α and the Nlrp3/caspase-1/IL-1 β axis in cigarette smoke-induced pulmonary inflammation and COPD. *European Respiratory Journal*. 2011;38(5):1019.
16. Rennard SI, Fogarty C, Kelson S, Long W, Ramsdell J, Allison J, et al. The safety and efficacy of infliximab in moderate to severe chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2007;175:926 - 34.
17. Calverley PMA, Sethi S, Dawson M, Ward CK, Finch DK, Penney M, et al. A randomised, placebo-controlled trial of anti-interleukin-1 receptor 1 monoclonal antibody MEDI8968 in chronic obstructive pulmonary disease. *Respiratory Research*. 2017;18:153.
18. Stone H, McNab G, Wood AM, Stockley RA, Sapey E. Variability of sputum inflammatory mediators in COPD and alpha1-antitrypsin deficiency. *Eur Respir J*. 2012;40(3):561-9.
19. Sapey E, Wood AM, Ahmad A, Stockley RA. Tumor necrosis factor- α rs361525 polymorphism is associated with increased local production and downstream inflammation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2010;182(2):192-9.
20. Xie Z-K, Huang Q-P, Huang J, Xie Z-F. Association between the IL1B, IL1RN polymorphisms and COPD risk: A meta-analysis. *Scientific Reports*. 2014;4:6202.

21. Sapey E, Bayley D, Ahmad A, Newbold P, Snell N, Stockley RA. Inter-relationships between inflammatory markers in patients with stable COPD with bronchitis: intra-patient and inter-patient variability. *Thorax*. 2008;63(6):493-9.
22. Nish S, Medzhitov R. Host Defense Pathways: role of redundancy and compensation in infectious disease phenotypes. *Immunity*. 2011;34(5):629-36.
23. Verstrepen L, Bekaert T, Chau TL, Tavernier J, Chariot A, Beyaert R. TLR-4, IL-1R and TNF-R signaling to NF- κ B: variations on a common theme. *Cellular and Molecular Life Sciences*. 2008;65(19):2964-78.
24. Borregaard N. Neutrophils, from Marrow to Microbes. *Immunity*. 2010;33(5):657-70.
25. Mayadas TN, Cullere X, Lowell CA. The multifaceted functions of neutrophils. *Annu Rev Pathol*. 2014;9:181-218.
26. Summers C, Rankin SM, Condliffe AM, Singh N, Peters AM, Chilvers ER. Neutrophil kinetics in health and disease. *Trends in immunology*. 2010;31(8):318-24.
27. Kolaczowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nature Reviews Immunology*. 2013;13:159.
28. Eash KJ, Greenbaum AM, Gopalan PK, Link DC. CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. *The Journal of Clinical Investigation*. 2010;120(7):2423-31.
29. Faurschou M, Borregaard N. Neutrophil granules and secretory vesicles in inflammation. *Microbes and infection*. 2003;5(14):1317-27.
30. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: from mechanisms to disease. *Annual review of immunology*. 2012;30:459-89.
31. Borregaard N. Neutrophils, from marrow to microbes. *Immunity*. 2010;33(5):657-70.
32. Thompson AB, Daughton D, Robbins RA, Ghafouri MA, Oehlerking M, Rennard SI. Intraluminal airway inflammation in chronic bronchitis. Characterization and correlation with clinical parameters. *The American review of respiratory disease*. 1989;140(6):1527-37.
33. Pilette C, Colinet B, Kiss R, Andre S, Kaltner H, Gabius HJ, et al. Increased galectin-3 expression and intra-epithelial neutrophils in small airways in severe COPD. *Eur Respir J*. 2007;29(5):914-22.
34. Donaldson GC, Seemungal TA, Patel IS, Bhowmik A, Wilkinson TM, Hurst JR, et al. Airway and systemic inflammation and decline in lung function in patients with COPD. *Chest*. 2005;128(4):1995-2004.
35. Parr DG, White AJ, Bayley DL, Guest PJ, Stockley RA. Inflammation in sputum relates to progression of disease in subjects with COPD: a prospective descriptive study. *Respir Res*. 2006;7:136.
36. Di Stefano A, Capelli A, Lusuardi M, Balbo P, Vecchio C, Maestrelli P, et al. Severity of airflow limitation is associated with severity of airway inflammation in smokers. *Am J Respir Crit Care Med*. 1998;158(4):1277-85.
37. O'Shaughnessy TC, Ansari TW, Barnes NC, Jeffery PK. Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of CD8+ T lymphocytes with FEV1. *Am J Respir Crit Care Med*. 1997;155(3):852-7.
38. Qiu Y, Zhu J, Bandi V, Atmar RL, Hattotuwa K, Guntupalli KK, et al. Biopsy neutrophilia, neutrophil chemokine and receptor gene expression in severe exacerbations of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2003;168(8):968-75.
39. Keatings VM, Barnes PJ. Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. *Am J Respir Crit Care Med*. 1997;155(2):449-53.
40. Subramanian DR, Jenkins L, Edgar R, Quraishi N, Stockley RA, Parr DG. Assessment of pulmonary neutrophilic inflammation in emphysema by quantitative positron emission tomography. *Am J Respir Crit Care Med*. 2012;186(11):1125-32.

41. McDonough JE, Yuan R, Suzuki M, Seyednejad N, Elliott WM, Sanchez PG, et al. Small-airway obstruction and emphysema in chronic obstructive pulmonary disease. *N Engl J Med*. 2011;365(17):1567-75.
42. Berger P, Laurent F, Begueret H, Perot V, Rouiller R, Raheison C, et al. Structure and function of small airways in smokers: relationship between air trapping at CT and airway inflammation. *Radiology*. 2003;228(1):85-94.
43. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol*. 2016;138(1):16-27.
44. Abboud RT, Vimalanathan S. Pathogenesis of COPD. Part I. The role of protease-antiprotease imbalance in emphysema. *Int J Tuberc Lung Dis*. 2008;12(4):361-7.
45. Fahy JV, Dickey BF. Airway mucus function and dysfunction. *N Engl J Med*. 2010;363(23):2233-47.
46. Leopold PL, O'Mahony MJ, Lian XJ, Tilley AE, Harvey BG, Crystal RG. Smoking is associated with shortened airway cilia. *PloS one*. 2009;4(12):e8157.
47. Tamashiro E, Xiong G, Anselmo-Lima WT, Kreindler JL, Palmer JN, Cohen NA. Cigarette smoke exposure impairs respiratory epithelial ciliogenesis. *Am J Rhinol Allergy*. 2009;23(2):117-22.
48. Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. *N Engl J Med*. 2008;359(22):2355-65.
49. Mangold A, Alias S, Scherz T, Hofbauer T, Jakowitsch J, Panzenbock A, et al. Coronary neutrophil extracellular trap burden and deoxyribonuclease activity in ST-elevation acute coronary syndrome are predictors of ST-segment resolution and infarct size. *Circulation research*. 2015;116(7):1182-92.
50. Freire MO, Dalli J, Serhan CN, Van Dyke TE. Neutrophil Resolvin E1 Receptor Expression and Function in Type 2 Diabetes. *Journal of immunology (Baltimore, Md : 1950)*. 2017;198(2):718-28.
51. Ozturk ZA, Yesil Y, Kuyumcu ME, Bilici M, Ozturk N, Yesil NK, et al. Inverse relationship between neutrophil lymphocyte ratio (NLR) and bone mineral density (BMD) in elderly people. *Archives of gerontology and geriatrics*. 2013;57(1):81-5.
52. Hobbins S, Chapple ILC, Sapey E, Stockley RA. Is periodontitis a comorbidity of COPD or can associations be explained by shared risk factors/behaviors? *International Journal of Chronic Obstructive Pulmonary Disease*. 2017;12:1339-49.
53. Barnes PJ. Alveolar macrophages as orchestrators of COPD. *Copd*. 2004;1(1):59-70.
54. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med*. 2004;350(26):2645-53.
55. Kirkham PA, Caramori G, Casolari P, Papi AA, Edwards M, Shamji B, et al. Oxidative stress-induced antibodies to carbonyl-modified protein correlate with severity of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2011;184(7):796-802.
56. Brightling CE, Monteiro W, Ward R, Parker D, Morgan MD, Wardlaw AJ, et al. Sputum eosinophilia and short-term response to prednisolone in chronic obstructive pulmonary disease: a randomised controlled trial. *Lancet*. 2000;356(9240):1480-5.
57. Gadek JE CR. Alpha-1 antitrypsin deficiency. In: WStanbury JB WJ, Frederickson DS, editor. *The metabolic basis of inherited disease*. 5th ed. New York: McGraw-Hill; 1983. p. 1450-67.
58. Stoller JK, Aboussouan LS. A review of alpha1-antitrypsin deficiency. *Am J Respir Crit Care Med*. 2012;185(3):246-59.
59. Balbi B, Ferrarotti I, Miravittles M. Efficacy of augmentation therapy for emphysema associated with alpha1-antitrypsin deficiency: enough is enough. *Eur Respir J*. 2016;47(1):35-8.
60. Stockley RA, Miravittles M, Vogelmeier C. Augmentation therapy for alpha-1 antitrypsin deficiency: towards a personalised approach. *Orphanet journal of rare diseases*. 2013;8:149.
61. Louhelainen N, Ryttilä P, Haahtela T, Kinnula VL, Djukanovic R. Persistence of oxidant and protease burden in the airways after smoking cessation. *BMC pulmonary medicine*. 2009;9:25.

62. Willemse BW, ten Hacken NH, Rutgers B, Lesman-Leegte IG, Postma DS, Timens W. Effect of 1-year smoking cessation on airway inflammation in COPD and asymptomatic smokers. *Eur Respir J*. 2005;26(5):835-45.
63. Rennard SI, Vestbo J. COPD: the dangerous underestimate of 15%. *Lancet*. 2006;367(9518):1216-9.
64. Salvi SS, Barnes PJ. Chronic obstructive pulmonary disease in non-smokers. *Lancet*. 2009;374(9691):733-43.
65. Wain LV, Shrine N, Artigas MS, Erzurumluoglu AM, Noyvert B, Bossini-Castillo L, et al. Genome-wide association analyses for lung function and chronic obstructive pulmonary disease identify new loci and potential druggable targets. *Nature genetics*. 2017;49(3):416-25.
66. Stocks J, Sonnappa S. Early life influences on the development of chronic obstructive pulmonary disease. *Therapeutic advances in respiratory disease*. 2013;7(3):161-73.
67. Rennard SI, Vestbo J. The many "small COPDs": COPD should be an orphan disease. *Chest*. 2008;134(3):623-7.
68. Sapey E, Stockley RA. COPD exacerbations . 2: aetiology. *Thorax*. 2006;61(3):250-8.
69. McHardy VU, Inglis JM, Calder MA, Crofton JW, Gregg I, Ryland DA, et al. A study of infective and other factors in exacerbations of chronic bronchitis. *British journal of diseases of the chest*. 1980;74(3):228-38.
70. Mobbs KJ, van Saene HK, Sunderland D, Davies PD. Oropharyngeal gram-negative bacillary carriage in chronic obstructive pulmonary disease: relation to severity of disease. *Respir Med*. 1999;93(8):540-5.
71. Sethi S. Infectious etiology of acute exacerbations of chronic bronchitis. *Chest*. 2000;117(5 Suppl 2):380s-5s.
72. Zalacain R, Sobradillo V, Amilibia J, Barron J, Achotegui V, Pijoan JI, et al. Predisposing factors to bacterial colonization in chronic obstructive pulmonary disease. *Eur Respir J*. 1999;13(2):343-8.
73. Wilkinson TM, Patel IS, Wilks M, Donaldson GC, Wedzicha JA. Airway bacterial load and FEV1 decline in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2003;167(8):1090-5.
74. Adler KB, Hendley DD, Davis GS. Bacteria associated with obstructive pulmonary disease elaborate extracellular products that stimulate mucin secretion by explants of guinea pig airways. *Am J Pathol*. 1986;125(3):501-14.
75. Wilson R, Roberts D, Cole P. Effect of bacterial products on human ciliary function in vitro. *Thorax*. 1985;40(2):125-31.
76. Read RC, Wilson R, Rutman A, Lund V, Todd HC, Brain AP, et al. Interaction of nontypable *Haemophilus influenzae* with human respiratory mucosa in vitro. *The Journal of infectious diseases*. 1991;163(3):549-58.
77. Khair OA, Devalia JL, Abdelaziz MM, Sapsford RJ, Tarraf H, Davies RJ. Effect of *Haemophilus influenzae* endotoxin on the synthesis of IL-6, IL-8, TNF-alpha and expression of ICAM-1 in cultured human bronchial epithelial cells. *Eur Respir J*. 1994;7(12):2109-16.
78. Celli BR, Barnes PJ. Exacerbations of chronic obstructive pulmonary disease. *Eur Respir J*. 2007;29(6):1224-38.
79. Papi A, Bellettato CM, Braccioni F, Romagnoli M, Casolari P, Caramori G, et al. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. *Am J Respir Crit Care Med*. 2006;173(10):1114-21.
80. Papi A, Luppi F, Franco F, Fabbri LM. Pathophysiology of exacerbations of chronic obstructive pulmonary disease. *Proc Am Thorac Soc*. 2006;3(3):245-51.
81. Parker CM, Voduc N, Aaron SD, Webb KA, O'Donnell DE. Physiological changes during symptom recovery from moderate exacerbations of COPD. *Eur Respir J*. 2005;26(3):420-8.
82. Mercer PF, Shute JK, Bhowmik A, Donaldson GC, Wedzicha JA, Warner JA. MMP-9, TIMP-1 and inflammatory cells in sputum from COPD patients during exacerbation. *Respir Res*. 2005;6:151.

83. O'Donnell DE, Parker CM. COPD exacerbations . 3: Pathophysiology. *Thorax*. 2006;61(4):354-61.
84. Zarbock A, Ley K. Mechanisms and consequences of neutrophil interaction with the endothelium. *Am J Pathol*. 2008;172(1):1-7.
85. Klebanoff SJ. Myeloperoxidase. *Proceedings of the Association of American Physicians*. 1999;111(5):383-9.
86. Cramer E, Pryzwansky KB, Villeval JL, Testa U, Breton-Gorius J. Ultrastructural localization of lactoferrin and myeloperoxidase in human neutrophils by immunogold. *Blood*. 1985;65(2):423-32.
87. Oram JD, Reiter B. Inhibition of bacteria by lactoferrin and other iron-chelating agents. *Biochim Biophys Acta*. 1968;170(2):351-65.
88. Borregaard N, Cowland JB. Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood*. 1997;89(10):3503-21.
89. Borregaard N, Sorensen OE, Theilgaard-Monch K. Neutrophil granules: a library of innate immunity proteins. *Trends in immunology*. 2007;28(8):340-5.
90. Sengelov H, Kjeldsen L, Diamond MS, Springer TA, Borregaard N. Subcellular localization and dynamics of Mac-1 (alpha m beta 2) in human neutrophils. *The Journal of clinical investigation*. 1993;92(3):1467-76.
91. Detmers PA, Zhou D, Powell D, Lichenstein H, Kelley M, Pironkova R. Endotoxin receptors (CD14) are found with CD16 (Fc gamma RIII) in an intracellular compartment of neutrophils that contains alkaline phosphatase. *Journal of immunology (Baltimore, Md : 1950)*. 1995;155(4):2085-95.
92. Sengelov H, Kjeldsen L, Borregaard N. Control of exocytosis in early neutrophil activation. *Journal of immunology (Baltimore, Md : 1950)*. 1993;150(4):1535-43.
93. Segal AW. How neutrophils kill microbes. *Annual review of immunology*. 2005;23:197-223.
94. Winterbourn CC, Hampton MB, Livesey JH, Kettle AJ. Modeling the reactions of superoxide and myeloperoxidase in the neutrophil phagosome: implications for microbial killing. *J Biol Chem*. 2006;281(52):39860-9.
95. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem*. 1969;244(22):6049-55.
96. Levine AP, Segal AW. The NADPH Oxidase and Microbial Killing by Neutrophils, With a Particular Emphasis on the Proposed Antimicrobial Role of Myeloperoxidase within the Phagocytic Vacuole. *Microbiology spectrum*. 2016;4(4).
97. Klebanoff SJ. Iodination of bacteria: a bactericidal mechanism. *The Journal of experimental medicine*. 1967;126(6):1063-78.
98. Klebanoff SJ, Clark RA. Iodination by human polymorphonuclear leukocytes: a re-evaluation. *The Journal of laboratory and clinical medicine*. 1977;89(3):675-86.
99. Greenberg S, Grinstein S. Phagocytosis and innate immunity. *Current opinion in immunology*. 2002;14(1):136-45.
100. Hayashi F, Means TK, Luster AD. Toll-like receptors stimulate human neutrophil function. *Blood*. 2003;102(7):2660-9.
101. Ekman AK, Cardell LO. The expression and function of Nod-like receptors in neutrophils. *Immunology*. 2010;130(1):55-63.
102. Segal AW, Dorling J, Coade S. Kinetics of fusion of the cytoplasmic granules with phagocytic vacuoles in human polymorphonuclear leukocytes. *Biochemical and morphological studies. The Journal of cell biology*. 1980;85(1):42-59.
103. Lee WL, Harrison RE, Grinstein S. Phagocytosis by neutrophils. *Microbes and infection*. 2003;5(14):1299-306.
104. Silverstein SC, S. Greenberg, F. Di Virgilio, T.H. Steinberg. Phagocytosis. In: William PE, editor. *Fundamental Immunology*. New York: Raven Press; 1989. p. 703.
105. van Kessel KP, Bestebroer J, van Strijp JA. Neutrophil-Mediated Phagocytosis of *Staphylococcus aureus*. *Front Immunol*. 2014;5:467.

106. Ehlers MR. CR3: a general purpose adhesion-recognition receptor essential for innate immunity. *Microbes Infect.* 2000;2(3):289-94.
107. Fallman M, Andersson R, Andersson T. Signaling properties of CR3 (CD11b/CD18) and CR1 (CD35) in relation to phagocytosis of complement-opsonized particles. *Journal of immunology* (Baltimore, Md : 1950). 1993;151(1):330-8.
108. Caron E, Hall A. Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. *Science.* 1998;282(5394):1717-21.
109. Lukacs GL, Rotstein OD, Grinstein S. Phagosomal acidification is mediated by a vacuolar-type H(+)-ATPase in murine macrophages. *J Biol Chem.* 1990;265(34):21099-107.
110. Jankowski A, Scott CC, Grinstein S. Determinants of the phagosomal pH in neutrophils. *J Biol Chem.* 2002;277(8):6059-66.
111. Segal AW, Geisow M, Garcia R, Harper A, Miller R. The respiratory burst of phagocytic cells is associated with a rise in vacuolar pH. *Nature.* 1981;290(5805):406-9.
112. Nordenfelt P, Tapper H. Phagosome dynamics during phagocytosis by neutrophils. *Journal of leukocyte biology.* 2011;90(2):271-84.
113. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science.* 2004;303(5663):1532-5.
114. Buchanan JT, Simpson AJ, Aziz RK, Liu GY, Kristian SA, Kotb M, et al. DNase expression allows the pathogen group A *Streptococcus* to escape killing in neutrophil extracellular traps. *Current biology : CB.* 2006;16(4):396-400.
115. Urban CF, Reichard U, Brinkmann V, Zychlinsky A. Neutrophil extracellular traps capture and kill *Candida albicans* yeast and hyphal forms. *Cellular microbiology.* 2006;8(4):668-76.
116. Brinkmann V, Zychlinsky A. Beneficial suicide: why neutrophils die to make NETs. *Nature reviews Microbiology.* 2007;5(8):577-82.
117. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nature medicine.* 2007;13(4):463-9.
118. Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD, Jr., et al. Extracellular DNA traps promote thrombosis. *Proc Natl Acad Sci U S A.* 2010;107(36):15880-5.
119. Hakkim A, Furnrohr BG, Amann K, Laube B, Abed UA, Brinkmann V, et al. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proc Natl Acad Sci U S A.* 2010;107(21):9813-8.
120. Lande R, Ganguly D, Facchinetti V, Frasca L, Conrad C, Gregorio J, et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Science translational medicine.* 2011;3(73):73ra19.
121. Stanescu D, Sanna A, Veriter C, Kostianev S, Calcagni PG, Fabbri LM, et al. Airways obstruction, chronic expectoration, and rapid decline of FEV1 in smokers are associated with increased levels of sputum neutrophils. *Thorax.* 1996;51(3):267-71.
122. Donaldson GC, Wedzicha JA. COPD exacerbations .1: Epidemiology. *Thorax.* 2006;61(2):164-8.
123. Fagon JY, Chastre J, Trouillet JL, Domart Y, Dombret MC, Bornet M, et al. Characterization of distal bronchial microflora during acute exacerbation of chronic bronchitis. Use of the protected specimen brush technique in 54 mechanically ventilated patients. *The American review of respiratory disease.* 1990;142(5):1004-8.
124. Sapey E, Stockley JA, Greenwood H, Ahmad A, Bayley D, Lord JM, et al. Behavioral and structural differences in migrating peripheral neutrophils from patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2011;183(9):1176-86.
125. Burnett D, Chamba A, Hill SL, Stockley RA. Neutrophils from subjects with chronic obstructive lung disease show enhanced chemotaxis and extracellular proteolysis. *Lancet.* 1987;2(8567):1043-6.

126. Noguera A, Batle S, Miralles C, Iglesias J, Busquets X, MacNee W, et al. Enhanced neutrophil response in chronic obstructive pulmonary disease. *Thorax*. 2001;56(6):432-7.
127. Rahman I. The role of oxidative stress in the pathogenesis of COPD: implications for therapy. *Treatments in respiratory medicine*. 2005;4(3):175-200.
128. Jones AW, Robinson R, Mohamed P, Davison G, Izzat HJ, Lewis KE. Impaired Blood Neutrophil Function in the Frequent Exacerbator of Chronic Obstructive Pulmonary Disease: A Proof-of-Concept Study. *Lung*. 2016.
129. Vaitkus M, Lavinskiene S, Barkauskiene D, Bieksiene K, Jeroch J, Sakalauskas R. Reactive oxygen species in peripheral blood and sputum neutrophils during bacterial and nonbacterial acute exacerbation of chronic obstructive pulmonary disease. *Inflammation*. 2013;36(6):1485-93.
130. Rahman I, Morrison D, Donaldson K, MacNee W. Systemic oxidative stress in asthma, COPD, and smokers. *Am J Respir Crit Care Med*. 1996;154(4 Pt 1):1055-60.
131. Grabcanovic-Musija F, Obermayer A, Stoiber W, Krautgartner WD, Steinbacher P, Winterberg N, et al. Neutrophil extracellular trap (NET) formation characterises stable and exacerbated COPD and correlates with airflow limitation. *Respir Res*. 2015;16:59.
132. Obermayer A, Stoiber W, Krautgartner WD, Klappacher M, Kofler B, Steinbacher P, et al. New aspects on the structure of neutrophil extracellular traps from chronic obstructive pulmonary disease and in vitro generation. *PloS one*. 2014;9(5):e97784.
133. Pullan J, Greenwood H, Walton GM, Stockley RA, Sapey E. Neutrophil extracellular traps (NETs) in COPD: A potential novel mechanism for host damage in acute exacerbations. *European Respiratory Journal*. 2015;46(suppl 59).
134. McCubbrey AL, Curtis JL. Efferocytosis and lung disease. *Chest*. 2013;143(6):1750-7.
135. Hodge S, Hodge G, Scicchitano R, Reynolds PN, Holmes M. Alveolar macrophages from subjects with chronic obstructive pulmonary disease are deficient in their ability to phagocytose apoptotic airway epithelial cells. *Immunology and cell biology*. 2003;81(4):289-96.
136. Quint JK, Wedzicha JA. The neutrophil in chronic obstructive pulmonary disease. *J Allergy Clin Immunol*. 2007;119(5):1065-71.
137. Prieto A, Reyes E, Bernstein ED, Martinez B, Monserrat J, Izquierdo JL, et al. Defective natural killer and phagocytic activities in chronic obstructive pulmonary disease are restored by glycoprophosphopeptical (immunoferon). *Am J Respir Crit Care Med*. 2001;163(7):1578-83.
138. Shanmugam L, Ravinder SS, Johnson P, Padmavathi R, Rajagopalan B, Kindo AJ. Assessment of phagocytic activity of neutrophils in chronic obstructive pulmonary disease. *Lung India : official organ of Indian Chest Society*. 2015;32(5):437-40.
139. Venge P, Rak S, Steinholtz L, Hakansson L, Lindblad G. Neutrophil function in chronic bronchitis. *Eur Respir J*. 1991;4(5):536-43.
140. Muns G, Rubinstein I, Bergmann KC. Phagocytosis and oxidative burst of blood phagocytes in chronic obstructive airway disease. *Scandinavian journal of infectious diseases*. 1995;27(4):369-73.
141. Coit P, Yalavarthi S, Ognenovski M, Zhao W, Hasni S, Wren JD, et al. Epigenome profiling reveals significant DNA demethylation of interferon signature genes in lupus neutrophils. *Journal of autoimmunity*. 2015;58:59-66.

Figure Legends

Figure 1 Inflammatory Airways of COPD. Inhaled irritants such as cigarette smoke induce inflammation in the airways, the degree of which is affected by lung development and epigenetic factors (A). Cigarette smoke stimulates release of cytokines from bronchial epithelial cells (B), including TGF- β which induces excessive collagen production from fibroblasts and consequently fibrosis of the small airways (C). Bronchial epithelial cells are also thought to recruit eosinophils to the airways via release of IL-33, although the exact role of the eosinophil in COPD is unclear (D). Alveolar macrophages are activated both by direct interactions with cigarette smoke and through cytokine signalling from bronchial epithelial cells (E). As well as further

recruitment of circulating monocytes (F), alveolar macrophages work with bronchial epithelial cells to recruit neutrophils in vast quantities (G). Neutrophils, aided by alveolar macrophages, secrete proteolytic and elastolytic enzymes (H) that mediate destruction of alveolar parenchyma (I) and induce hypersecretion of mucus within the airways (J), both contributing to airway obstruction. It has also been hypothesized that this pulmonary inflammation ‘overspills’ systemically (K), resulting in associated inflammatory co-morbidities, for example diabetes, myocardial infarction (MI) and reduction in bone density.

Figure 2 Phagosome Maturation. The neutrophil NADPH oxidase machinery, activated by delivery of its membrane-bound components to the phagosome, pumps electrons into the phagosomal space to generate toxic ROS. Membrane trafficking allows delivery of primary and secondary granules to the phagosomal membrane, which release a variety of microbicidal proteins into the phagosomal space. MPO released from primary granules reacts with ROS to further produce highly toxic substances . Adapted from(112). All abbreviations given in text if appropriate.