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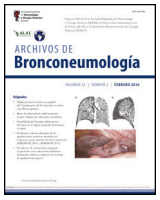

Editorial

Treating Neutrophilic Inflammation in Airways Diseases

Initially thought to be homogenous and short-lived with limited function, neutrophils are now recognised as critical and dynamic immune cells with longer life spans than previously conceived. Acting as Jekyll and Hyde, these abundant granulocytic leukocytes are typically described as first-responders to infection, crucial to host defence against invading pathogens, with an equal propensity for host damage, contributing to the pathogenesis of acute and chronic inflammatory disease.[1] Neutrophils have often been overlooked, topically epitomised in emerging research on severe COVID-19. Research efforts first largely disregarded these cells for a role in viral infection, but neutrophils have been identified as key to processes like cytokine storm and development of acute respiratory distress syndrome (ARDS), as well as modulation and dysregulation of the adaptive immune response, leading to a call for neutrophil-targeting therapies for COVID-19.[2] Furthermore, in line with a paradigm shift for the established roles of innate and adaptive immune cells, cancer and HIV studies have recently indicated important neutrophil anti-tumour or anti-viral activity, respectively, with parallel contributions to tumour growth and progression or viral transmission and immunosuppression.[3,4] These three distinct diseases typify the complex role of neutrophils, progressing from host defenders to disease drivers and therapeutic targets—herein lies the challenge of targeting neutrophil activity without compromising appropriate immune responses. Indeed, several attempts to block neutrophil activity in airway disease have resulted in harmful immunosuppression and careful consideration of inflammatory mechanisms in individual patients is required in the selection of therapies.

Better established is the role of neutrophils in inflammatory lung diseases considered to be neutrophil-driven, including chronic obstructive pulmonary disease (COPD), bronchiectasis and cystic fibrosis (CF), as well as neutrophilic asthma. Dysfunctional neutrophil activity in these disease processes involves release of neutrophil extracellular traps (NETs), which are less efficient at bacterial killing with greater host damage, defective phagocytic mechanisms resulting in reduced pathogen clearance, impaired migration causing greater tissue destruction, persistent or inappropriate neutrophil recruitment to the lung tissue or prolonged neutrophil survival perpetuating the inflammatory response, and also increased activation, degranulation and reactive oxygen species generation.[1] Neutrophils may also contribute to their own persistence by impairing efferocytosis of apoptotic neutrophils by alveolar macrophages, resulting in necrosis and release of harmful cellular contents. Importantly, despite apparently common neutrophil-driven mechanisms across several airway diseases, neutrophil-targeting approaches may not necessarily be applied ubiquitously. For example, aiming to degrade NETs and extracellular DNA, recombinant human deoxyribonuclease (DNAse) has been utilised in CF management for over three decades[5]; when applied in bronchiectasis, patients experienced more frequent exacerbations and lung function decline.[6] One speculative explanation for this may be en-masse release of active proteases previously inhibited by bound DNA.[7]

Further attempts to curb neutrophil activity with limited success have included antagonism of CXCR2, a chemokine receptor with ligands including CXCL8 and CXCL1, key in neutrophil recruitment and also NETosis, to prevent neutrophil accumulation in the airways in asthma,[8] bronchiectasis[9] and COPD.[10] Whilst seemingly well tolerated in healthy human participants,[11] antagonism successfully reduced airway neutrophil numbers, but either resulted in no observable patient benefit, or potentially increased exacerbation frequency and pneumonia incidence,[10] raising concerns regarding uncontrolled infection. Other studies targeting similar mechanisms, like leukotriene B_4 receptor antagonism, showed similar risk of adverse events in CF. Mechanistic studies revealed decreased airway neutrophils were associated with increased inflammation and *Pseudomonas aeruginosa* counts in the lungs and blood following treatment and infection, demonstrating the importance of neutrophil-mediated bacterial clearance even in chronic neutrophil-driven disease.[12] Furthermore, treatment in COPD with CXCR2 antagonist did not reduce airway neutrophil or NETs levels, although in vitro neutrophil activation was lessened donor-dependently.[13] Other investigations demonstrated in vitro CXCR2 antagonism in COPD patient blood and sputum neutrophils had inhibitory effects on NETosis; whilst the authors proposed this was via blocking NET-inducing effects of airway CXCL8,[14] this mechanism was not proven in the study and comparison with a stimulated airway neutrophil condition was lacking. It was previously noted that in vitro CXCL8 effects on NET production could be highly condition and donor dependent,[15] underlining a need for further in-depth mechanistic studies of NETosis and for caution in application of in vitro findings. In general, it has been noted that

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the success of previous clinical trials in airway disease may have been negatively impacted by lack of understanding and appropriate selection of those who may be most likely to benefit from a therapy as well as selecting appropriate endpoints. Adoption of patient phenotyping and endotyping approaches has helped to identify the specific mechanisms active in individual patients, highlighting a crucial strategy to direct personalised medicine and future clinical trials, including for neutrophil-targeting therapies.[16,17]

Therapies directly targeting and inhibiting neutrophil activity may need to modulate functions not necessarily vital for the normal immune response. NETs facilitate the explosive release of neutrophil proteases and are associated with worse disease, tissue damage and unfavourable changes in the lung microbiome.[16] Study of Papillon-Lefèvre syndrome, a rare autosomal recessive disease in which individuals lack active dipeptidyl peptidase-1 (DPP1)—implicated in NETosis and a master regulator of neutrophil serine protease activation in the bone marrow—demonstrated that absence of protease activity did not seem to result in marked immunodeficiency.[18] However, protease inhibitor therapies trialed have included the neutrophil elastase inhibitors Alvestat which failed to reduce neutrophilic inflammation in bronchiectasis[19] and showed no clinical benefits in COPD,[20] and Sivelestat which did not show clear benefit in ARDS.[21] It was therefore a significant breakthrough when a phase-2 trial in bronchiectasis of the reversible DPP1 inhibitor, Brensocatib, demonstrated large reductions in sputum neutrophil elastase activity, prolonged time to first exacerbation and reduced exacerbation frequency.[22] No increase in infections were seen and treatment was well tolerated. Inhibiting neutrophil protease activity before cell release into the circulation rather than at the inflammatory site may be a key proponent of success in this case, as well as blockade of multiple inflammatory targets including elastase but also cathepsin G and proteinase-3. A phase-3 trial of the drug has recently been initiated (NCT04594369). Since NETosis and neutrophil serine proteases are major contributors to other neutrophilic diseases, DPP1 inhibition has potential across airway diseases and a trial in CF has also recently been announced.

Direct approaches which attempt to block activity intertwined with the normal immune response can come with higher risk. Therapies which promote normal neutrophil function to restore homeostasis, modulate the immune response via anti-inflammatory mechanisms, modulate immunometabolism, or reduce immunosenescence, may be safer solutions. Other approaches to rescue neutrophil function have had some success,[23] including a randomised-trial of an inhaled PI3Kδ inhibitor in acute exacerbations of COPD which improved lung function and recovery[24] but also resulted in adverse effects of cough. Previous investigations suggest PI3Kδ inhibition can rescue defective neutrophil migration in aged individuals,[25] as did high-dose simvastatin in community-acquired pneumonia patients with sepsis, where treatment improved chemotaxis and reduced NETosis.[26] Neutrophil metabolic reprogramming has also been suggested to promote a more activated neutrophil type capable of more damage in airway disease,[27] and proposed approaches include treatment with the AMPK activator metformin, typically used in the treatment of type 2 diabetes.

For now, low dose macrolides remain the only immunomodulatory therapy with activity on neutrophilic inflammation in widespread use. The mechanism of action of azithromycin and other macrolides is controversial but recent data adds to the view that they have direct effects on airway neutrophils and NETs and that this links to their efficacy in reducing exacerbation rates in several chronic airway diseases.[16,28]

Targeting the neutrophil may be the most important and simultaneously the most difficult therapeutic challenge in airways diseases today. The role of neutrophils in inflammation is highly complex and further research is required to fully understand the intricacies of neutrophilic disease processes. There may be no silver bullet or one-size-fits-all approach in the treatment of neutrophilic inflammation, but instead there is a growing armoury of therapies in development, with increasingly accessible tools to characterise patients with airway disease to select the best possible treatment course, and select appropriate outcome measurements, guiding implementation and development of therapeutic strategies.

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