



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



## 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.<sup>1</sup> Neutrophils have often been overlooked, typically 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.<sup>2</sup> 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.<sup>3,4</sup> 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.<sup>1</sup> 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<sup>5</sup>; when applied in bronchiectasis, patients experienced more frequent exacerbations and lung function decline.<sup>6</sup> One speculative explanation for this may be en-masse release of active proteases previously inhibited by bound DNA.<sup>7</sup>

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,<sup>8</sup> bronchiectasis<sup>9</sup> and COPD.<sup>10</sup> Whilst seemingly well tolerated in healthy human participants,<sup>11</sup> antagonism successfully reduced airway neutrophil numbers, but either resulted in no observable patient benefit, or potentially increased exacerbation frequency and pneumonia incidence,<sup>10</sup> raising concerns regarding uncontrolled infection. Other studies targeting similar mechanisms, like leukotriene B<sub>4</sub> 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.<sup>12</sup> Furthermore, treatment in COPD with CXCR2 antagonist did not reduce airway neutrophil or NETs levels, although in vitro neutrophil activation was lessened donor-dependently.<sup>13</sup> 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,<sup>14</sup> 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,<sup>15</sup> 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

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.<sup>16,17</sup>

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.<sup>16</sup> 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.<sup>18</sup> However, protease inhibitor therapies trialed have included the neutrophil elastase inhibitors Alvestat which failed to reduce neutrophilic inflammation in bronchiectasis<sup>19</sup> and showed no clinical benefits in COPD,<sup>20</sup> and Sivelestat which did not show clear benefit in ARDS.<sup>21</sup> 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.<sup>22</sup> 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,<sup>23</sup> including a randomised-trial of an inhaled PI3K $\delta$  inhibitor in acute exacerbations of COPD which improved lung function and recovery<sup>24</sup> but also resulted in adverse effects of cough. Previous investigations suggest PI3K $\delta$  inhibition can rescue defective neutrophil migration in aged individuals,<sup>25</sup> as did high-dose simvastatin in community-acquired pneumonia patients with sepsis, where treatment improved chemotaxis and reduced NETosis.<sup>26</sup> Neutrophil metabolic reprogramming has also been suggested to promote a more activated neutrophil type capable of more damage in airway disease,<sup>27</sup> 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.<sup>16,28</sup>

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.

## Funding

British Lung Foundation.

## References

- Liew PX, Kubes P. The neutrophil's role during health and disease. *Physiol Rev.* 2019;99:1223–48.
- Ackermann M, Anders HJ, Bilyy R, Bowlin GL, Daniel C, De Lorenzo R, et al. Patients with COVID-19: in the dark-NETs of neutrophils. *Cell Death Differ.* 2021;1–15.
- Moses K, Brandau S. Human neutrophils: their role in cancer and relation to myeloid-derived suppressor cells. *Semin Immunol.* 2016;28:187–96.
- Hensley-McBain T, Klatt NR. The dual role of neutrophils in HIV infection. *Curr HIV/AIDS Rep.* 2018;15:1–10.
- Shak S, Capon DJ, Hellmiss R, Marsters SA, Baker CL. Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum. *Proc Natl Acad Sci U S A.* 1990;87:9188–92.
- O'Donnell AE, Barker AF, Ilowite JS, Fick RB, Treatment of idiopathic bronchiectasis with aerosolized recombinant human DNase I. rDNase study group. *Chest.* 1998;113:1329–34.
- Duranton J, Belorgey D, Carrère J, Donato L, Moritz T, Bieth JG. Effect of DNase on the activity of neutrophil elastase, cathepsin G and proteinase 3 in the presence of DNA. *FEBS Lett.* 2000;473:154–6.
- O'Byrne PM, Metev H, Puu M, Richter K, Keen C, Uddin M, et al. Efficacy and safety of a CXCR2 antagonist, AZD5069, in patients with uncontrolled persistent asthma: a randomised, double-blind, placebo-controlled trial. *Lancet Respir Med.* 2016;4:797–806.
- De Soysa A, McDonnell MJ, Goeminne PC, Aliberti S, Lonni S, Davison J, et al. Bronchiectasis rheumatoid overlap syndrome is an independent risk factor for mortality in patients with bronchiectasis: a multicenter cohort study. *Chest.* 2017;151:1247–54.
- Lazaar AL, Miller BE, Donald AC, Keeley T, Ambery C, Russell J, et al. CXCR2 antagonist for patients with chronic obstructive pulmonary disease with chronic mucus hypersecretion: a phase 2b trial. *Respir Res.* 2020;21:149.
- Leaker BR, Barnes PJ, O'Connor B. Inhibition of LPS-induced airway neutrophilic inflammation in healthy volunteers with an oral CXCR2 antagonist. *Respir Res.* 2013;14:137.
- Döring G, Bragonzi A, Paroni M, Aktürk FF, Cigana C, Schmidt A, et al. BIIL 284 reduces neutrophil numbers but increases *P. aeruginosa* bacteremia and inflammation in mouse lungs. *J Cyst Fibros.* 2014;13:156–63.
- Keir HR, Richardson H, Fillmore C, Shoemark A, Lazaar AL, Miller BE, et al. CXCL-8-dependent and -independent neutrophil activation in COPD: experiences from a pilot study of the CXCR2 antagonist danirixin. *ERJ Open Res.* 2020;6.
- Pedersen F, Waschki B, Marwitz S, Goldmann T, Kirsten A, Malmgren A, et al. Neutrophil extracellular trap formation is regulated by CXCR2 in COPD neutrophils. *Eur Respir J.* 2018;51.
- Marcos V, Zhou Z, Yildirim AO, Bohla A, Hector A, Vitkov L, et al. Retraction Note: CXCR2 mediates NADPH oxidase – independent neutrophil extracellular trap formation in cystic fibrosis airway inflammation. *Nature Med.* 2011;17:899.
- Keir HR, Shoemark A, Dicker AJ, Perea L, Pollock J, Giam YH, et al. Neutrophil extracellular traps, disease severity, and antibiotic response in bronchiectasis: an international, observational, multicohort study. *Lancet Respir Med.* 2021;9:873–84.
- Shaw DE, Sousa AR, Fowler SJ, Fleming LJ, Roberts G, Corfield J, et al. Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. *Eur Respir J.* 2015;46:1308–21.
- Pham CT, Ivanovich JL, Raptis SZ, Zehnauer B, Ley TJ. Papillon-Lefèvre syndrome: correlating the molecular, cellular, and clinical consequences of cathepsin C/dipeptidyl peptidase I deficiency in humans. *J Immunol.* 2004;173:7277–81.
- Stockley R, De Soysa A, Gunawardena K, Perrett J, Forsman-Semb K, Entwistle N, et al. Phase II study of a neutrophil elastase inhibitor (AZD9668) in patients with bronchiectasis. *Respir Med.* 2013;107:524–33.
- Kuna P, Jenkins M, O'Brien CD, Fahy WA. AZD9668, a neutrophil elastase inhibitor, plus ongoing budesonide/formoterol in patients with COPD. *Respir Med.* 2012;106:531–9.

21. Pu S, Wang D, Liu D, Zhao Y, Qi D, He J, et al. Effect of sivelestat sodium in patients with acute lung injury or acute respiratory distress syndrome: a meta-analysis of randomized controlled trials. *BMC Pulm Med.* 2017;17:148.
22. Chalmers JD, Haworth CS, Metersky ML, Loebinger MR, Blasi F, Sibila O, et al. Phase 2 trial of the DPP-1 inhibitor brensocatib in bronchiectasis. *N Engl J Med.* 2020;383:2127–37.
23. Belchamber KBR, Hughes MJ, Spittle DA, Walker EM, Sapey E. New pharmacological tools to target leukocyte trafficking in lung disease. *Front Immunol.* 2021;12:704173.
24. Cahn A, Hamblin JN, Robertson J, Begg M, Jarvis E, Wilson R, et al. An inhaled PI3K $\delta$  inhibitor improves recovery in acutely exacerbating COPD Patients: a randomized trial. *Int J Chron Obstruct Pulmon Dis.* 2021;16:1607–19.
25. Sapey E, Greenwood H, Walton G, Mann E, Love A, Aaronson N, et al. Phosphoinositide 3-kinase inhibition restores neutrophil accuracy in the elderly: toward targeted treatments for immunosenescence. *Blood.* 2014;123:239–48.
26. Sapey E, Patel JM, Greenwood H, Walton GM, Grudzinska F, Parekh D, et al. Simvastatin improves neutrophil function and clinical outcomes in pneumonia. A pilot randomized controlled clinical trial. *Am J Respir Crit Care Med.* 2019;200:1282–93.
27. Giam YH, Shoemark A, Chalmers JD. Neutrophil dysfunction in bronchiectasis: an emerging role for immunometabolism. *Eur Respir J.* 2021;58(2.).
28. Baines KJ, Wright TK, Gibson PG, Powell H, Hansbro PM, Simpson JL. Azithromycin treatment modifies airway and blood gene expression networks in neutrophilic COPD. *ERJ Open Res.* 2018;4(4.).

Merete B. Long, James D. Chalmers\*  
*Division of Molecular and Clinical Medicine, University of Dundee,  
Dundee, UK*

Corresponding author.  
*E-mail address: jchalmers@dundee.ac.uk (J.D. Chalmers).*