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# Neutrophils in asthma: the good, the bad and the bacteria

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## ABSTRACT

Airway inflammation plays a key role in asthma pathogenesis but is heterogeneous in nature. There has been significant scientific discovery with regard to type 2-driven, eosinophil-dominated asthma, with effective therapies ranging from inhaled corticosteroids to novel biologics. However, studies suggest that approximately 1 in 5 adults with asthma have an increased proportion of neutrophils in their airways. These patients tend to be older, have potentially pathogenic airway bacteria and do not respond well to classical therapies. Currently, there are no specific therapeutic options for these patients, such as neutrophil-targeting biologics.

Neutrophils comprise 70% of the total circulatory white cells and play a critical defence role during inflammatory and infective challenges. This makes them a problematic target for therapeutics. Furthermore, neutrophil functions change with age, with reduced microbial killing, increased reactive oxygen species release and reduced production of extracellular traps with advancing age. Therefore, different therapeutic strategies may be required for different age groups of patients.

The pathogenesis of neutrophil-dominated airway inflammation in adults with asthma may reflect a counterproductive response to the defective neutrophil microbial killing seen with age, resulting in bystander damage to host airway cells and subsequent mucus hypersecretion and airway remodelling. However, in children with asthma, neutrophils are less associated with adverse features of disease, and it is possible that in children, neutrophils are less pathogenic.

In this review, we explore the mechanisms of neutrophil recruitment, changes in cellular function across the life course and the implications this may have for asthma management now and in the future. We also describe the prevalence of neutrophilic asthma globally, with a focus on First Nations people of Australia, New Zealand and North America.

## INTRODUCTION

Asthma is a common respiratory condition that affects an estimated 358 million people worldwide.<sup>1</sup> In 2015, the Global Burden of Disease Study by the WHO estimates that 26.2 million disability-adjusted life years were lost due to asthma, representing 1.1% of the total global disease burden.<sup>2</sup> Asthma accounts for an estimated 495 000 deaths<sup>3</sup> every year, with over 80% occurring in low and lower middle-income countries.<sup>4</sup>

Due to an absence of established gold standards,<sup>5</sup> the diagnosis of asthma is based predominantly on evidence of recurrent respiratory symptoms, such as cough, shortness of breath, wheeze, chest

tightness and presence of variable airway obstruction.<sup>5,6</sup> Many patients with asthma experience exacerbations (repetitive, subacute, flares of symptoms), which are a major cause of disease morbidity and increased healthcare costs. Some patients experience frequent exacerbations, and these repetitive insults are associated with a greater and progressive loss of lung function.<sup>7,8</sup>

The heterogeneity of asthma reflects symptomatic burden and the type and degree of airway inflammation and airway remodelling. This has led to the development of various phenotype models of asthma<sup>9</sup> in which clinical characteristics are clustered and, more recently, endotype models of asthma<sup>10</sup> in which distinct functional or pathobiological mechanisms are clustered.

There is a significant body of evidence supporting a distinct asthma endotype consisting of type 2 (T2)<sup>HIGH</sup> airway and predominantly eosinophilic inflammation, and high levels of interleukin (IL)-4, IL-5 and IL-13.<sup>11</sup> However, although many patients have evidence of T2<sup>HIGH</sup> airway inflammation, not all do. Cross-sectional cluster analyses have identified asthma groups based on cellular patterns as well as clinical features. Discriminant analysis has shown the two most influential variables for cluster assignment to be baseline FEV<sub>1</sub>% predicted and the proportion of sputum neutrophils.<sup>12</sup>

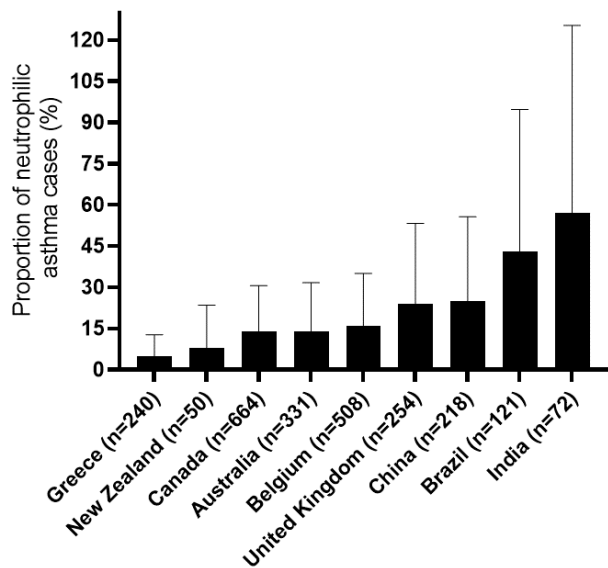
Adult patients with asthma with more than 60% (median) neutrophils in their sputum tended to be older, male, had late-onset asthma, more severe lung disease, were prescribed higher doses of inhaled corticosteroids and were more likely to be taking oral corticosteroids, and had a greater incidence of hospitalisation for asthma (65% vs 28%)<sup>12</sup> and more comorbidities such as hypertension, osteoporosis and gastro-oesophageal reflux disease.<sup>12,13</sup> Neutrophilic asthma is associated with less atopy and these patients usually have lower levels of exhaled nitric oxide (FeNO), often less than 30 parts per billion.<sup>14</sup> In children, airway neutrophilia is less common, but when it occurs it is associated with airway bacteria,<sup>15</sup> and unlike adult neutrophilic asthma, neutrophilia in paediatric severe asthma appears to have a different mediator profile without IL-17.<sup>16,17</sup> The studies in children are from the severe asthma cohorts and not from the mild to moderate disease.

This raises important questions when considering new therapeutic strategies for asthma. It is clear that some patients with asthma have a high proportion of neutrophils in their pulmonary secretions. Evidence from other diseases with a similarly high burden of pulmonary neutrophils



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**Figure 1** Prevalence of neutrophilic asthma in different countries with size of cohort (n) studied. Error bars indicate CIs.<sup>23–25 35 126 130–133</sup>

suggests these cells are associated with tissue damage and disease progression (eg, COPD and bronchiectasis). In this review, the evidence for a distinct neutrophilic endotype will be summarised. Risk factors for neutrophilic asthma will be considered. The evidence that neutrophils can contribute mechanistically to the pathology and symptomatology of asthma will be reviewed, as will current progress in targeting these cells therapeutically.

## EVIDENCE FOR A NEUTROPHILIC ENDOTYPE

### The definition and epidemiology of a neutrophilic endotype

There is no agreed definition of neutrophilic asthma, but studies in healthy controls suggest the normal range of induced sputum neutrophil percentages is approximately 30%–50%.<sup>18 19</sup> If one uses age-corrected 95th percentile of neutrophil proportion in healthy controls,<sup>20</sup> the cut-off value identifying an increased presence of neutrophils can range from  $\geq 60\%$  to  $>76\%$  depending on respiratory sample type.<sup>21</sup> Nair *et al* have proposed that the term ‘neutrophilic asthma’ should be limited to those patients who consistently (on at least two occasions) have a sputum neutrophil count  $\geq 5 \times 10^9/L$ .<sup>22</sup>

There are several studies describing patients with asthma who display high sputum neutrophil concentrations. While neutrophilic asthma comprises approximately 20%–30% of all asthma cases,<sup>23</sup> its prevalence varies between regions (figure 1). For example, it has been estimated to be as low as 5% in Greece,<sup>24</sup> but as high as 57% in India.<sup>25</sup>

<sup>20</sup>The presence of sputum neutrophils is reproducible within individuals, with one study showing stability of sputum cell patterns at four weekly samplings over 5 months<sup>26</sup> and a further study demonstrating a similar pattern over 12 months.<sup>27</sup> A study by Jayaram *et al* found most exacerbations of asthma to be non-eosinophilic, and patients with non-eosinophilic exacerbations were predominantly neutrophilic in nature (68% neutrophils and 0.3% eosinophils) and remained so, irrespective of treatment strategy.<sup>28</sup>

### High pulmonary neutrophils in asthma cannot be fully explained by therapies

There is debate as to whether neutrophil-dominated inflammation in asthma represents a true endotype or is the result of treatment. Corticosteroids have been shown to increase the survival of neutrophils,<sup>29</sup> and corticosteroid treatment of airway eosinophils has been associated with a corresponding rise in sputum neutrophils. However, neutrophilic inflammation in patients with asthma can be observed regardless of corticosteroid therapy,<sup>30</sup> and data support the presence of a neutrophilic endotype even in steroid-naïve patients with asthma.<sup>31</sup>

In one large study, patients were divided into eosinophilic asthma (n=350), mixed granulocytic asthma (n=31), paucigranulocytic asthma (n=318) and neutrophilic asthma (n=134) groups. Across all groups, there were no differences in the percentage of sputum neutrophils comparing patients who were not taking inhaled corticosteroids to those who were, suggesting the predominance of neutrophils in some patients was not related to steroid exposure.<sup>32</sup>

While oral corticosteroids can induce airway neutrophilia, this has not been shown consistently with inhaled corticosteroids.<sup>33</sup> Furthermore, withdrawal of inhaled corticosteroids has been associated with an increase in sputum neutrophil counts and neutrophil-associated inflammatory mediators in some patients.<sup>34</sup> Optimisation of inhaled therapy (by stopping or reducing, or starting or increasing inhaled corticosteroid) has also been shown to have no effect on sputum neutrophil percentage or neutrophil-associated inflammatory markers.<sup>35</sup> These studies highlight that while in some patients, treatment strategies may increase sputum neutrophils, this is not ubiquitous and sputum neutrophilia can exist in the absence of steroid therapies.

Together, these data support airway neutrophilia being a feature of asthma, and one that is associated with worse outcomes (including worse lung function impairment and increased hospitalisations). There is also evidence to suggest that neutrophilic infiltration can lead to asthma symptomatology.

In addition to neutrophil proportion, there is a possibility that other models such as gene signature of epithelial cells can also be used in the future for classification of inflammatory phenotypes in asthma. A recent study used gene signature of IL-17A response in bronchial epithelial cells of COPD to distinguish a distinct COPD phenotype that was exhibiting neutrophilic airway inflammation.<sup>36</sup>

### POTENTIAL REASONS FOR NEUTROPHIL INVOLVEMENT

Pulmonary neutrophil recruitment in asthma may reflect a ‘normal’ response to pulmonary inflammation caused, for example, by airway pollutants or the presence of potentially pathogenic bacteria. Alternatively, intrinsic factors related to patient demographics (including age-associated changes in neutrophil function) and comorbid disease, including obesity, body mass index (BMI) and insulin resistance, can influence too the airway neutrophilia.<sup>37 38</sup>

### Pollution and working environment

It has been known for some time that ozone and endotoxin exposure can exacerbate inflammation in the airway.<sup>39</sup> Traffic-related air pollution<sup>40</sup> and diesel exhaust<sup>41</sup> exposure enhances the ozone-induced airway neutrophilic inflammation in healthy humans and affects neutrophil function to favour more degranulation and local tissue damage.

Airway neutrophilia is also influenced by environmental exposures such as living in close proximity to a main road,<sup>42</sup> and a

neutrophilic endotype in asthma is more prevalent in countries with higher environmental pollutants.<sup>43</sup> This supports the low-prevalence studies in New Zealand, a country with some of the lowest particulate matter pollution levels globally.<sup>44</sup>

Pollution activates the airway epithelium and macrophages, leading to the production and release of proinflammatory mediators such as IL-8 and IL-6, recruiting neutrophils to the airways, enhancing inflammation and causing remodelling in the bronchi,<sup>45</sup> and potentially patients with asthma may be more prone to the deleterious responses, as suggested by studies of gene signatures.<sup>45</sup>

Injury to the airway epithelium is also likely to contribute to the pathogenesis of occupationally induced asthma, with oxidative stress associated with an influx of neutrophils to the airways.<sup>46</sup> Indeed, inhalation challenges of various work-based noxious stimuli are more commonly associated with neutrophilic inflammation than an eosinophilic signal, highlighting the importance of this endotype in occupational disease.<sup>47 48</sup>

### Smoking

There is a strong association between cigarette smoking and the severity of asthma including poor symptom control and an insensitivity to inhaled corticosteroids.<sup>49 50</sup> Smoking is also associated with a predominance of neutrophilic inflammation in asthma,<sup>51</sup> resulting from epithelial and macrophage activation<sup>52</sup> and heightened expression of IL-17A, IL-6 and IL-8, with IL-17A correlating with IL-8 and neutrophil numbers.<sup>53</sup>

### Obesity and insulin resistance

A number of host factors have been associated with a greater burden of neutrophilic inflammation in asthma. For example, there is a wealth of data to support the relationship between asthma and obesity.<sup>54</sup> Although there is still much to be understood about the pathogenesis of asthma in obese individuals, there is a signal of a more neutrophilic endotype in this group, as reviewed recently.<sup>55</sup> Previous studies have further shown an increased proportion of airway neutrophils in obese patients with asthma with high BMI compared with non-obese.<sup>37</sup> Resistance to insulin appears to amplify the negative association between asthma and obesity,<sup>38</sup> with activation of transforming growth factor beta 1 expression in the bronchial epithelium leading to airway remodelling,<sup>56</sup> which may affect the airway neutrophilia.<sup>38</sup>

### NEUTROPHIL FUNCTIONS WITH AGE AND ASTHMA

Neutrophils provide a crucial 'first-line' immune response and are among the first effector cells to be recruited to the lung during inflammation, irrespective of the cause, and are also the most abundant leucocyte, accounting for 70% of all circulating white cells.<sup>57</sup>

Once released from the bone marrow into the circulation, neutrophils patrol the blood vessels and navigate towards inflammation by sensing chemotactic gradients such as CXCL-8, which can be released due to interaction of microbial or damage-associated molecular patterns. During this process, neutrophils use their cellular polarity, regulated by Rho guanosine triphosphatases, to perform amoeboid movement to accurately reach the site of inflammation.<sup>58</sup> To enable migration through dense extracellular matrices, neutrophils release proteinases, such as neutrophil elastase and proteinase 3, to cleave components of the extracellular matrix such as elastin, fibronectin, vitronectin, laminin and collagen.<sup>59</sup>

AGE  
→

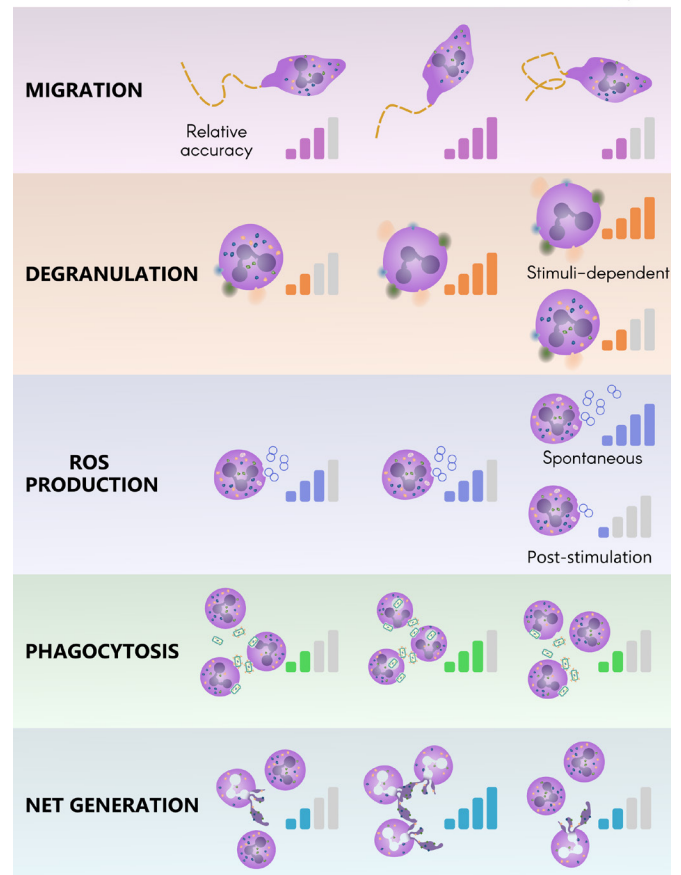
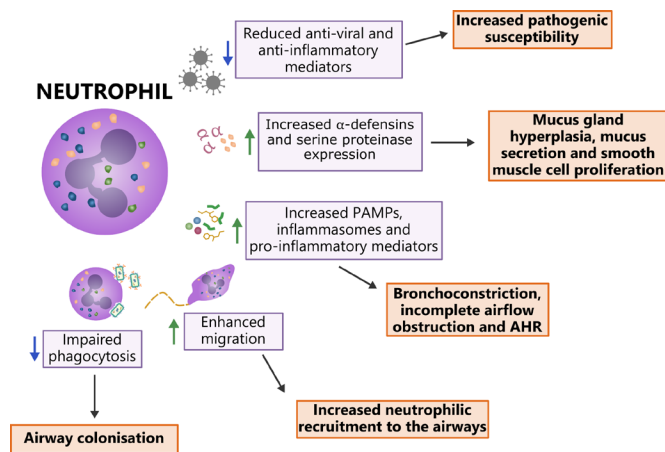


Figure 2 Change in neutrophil functions with increasing age.<sup>32–48</sup>

At the site of inflammation, neutrophils engulf smaller target material by phagocytosis, such as *Haemophilus influenzae* or *Streptococcus pneumoniae*. Engulfed microbes are then destroyed in the neutrophil phagosome through pH change, and exposures to reactive oxygen species (ROS) and cytotoxic enzymes.<sup>60</sup> Besides phagocytosis, neutrophils can also perform microbial killing by degranulation, a process in which neutrophils extracellularly release cytotoxic ROS and proteolytic enzymes such as neutrophil elastase and proteinase 3 at the site of infection.<sup>59 61</sup> Neutrophils can also release neutrophil extracellular traps (NETs), web-like structures consisting of cytosolic granule proteins embedded on a scaffold of decondensed chromatin—to capture and destroy pathogens.<sup>62</sup>

Studies suggest that neutrophil functions change as the host ages (figure 2). In young children, isolated neutrophils display reduced migratory accuracy,<sup>63</sup> degranulation,<sup>64</sup> phagocytosis<sup>65</sup> and NET generation following stimulation,<sup>66</sup> but preserved ROS generation,<sup>67</sup> compared with young adults. Together, these differences would support a less effective response to infection, but also a reduced ability to harm host tissue. In old age, there is a decline in cellular function, including reduced migratory accuracy,<sup>68</sup> NET generation,<sup>69</sup> phagocytosis,<sup>70</sup> but increased spontaneous ROS production,<sup>71</sup> with decreased ROS production following stimulation.<sup>72</sup> In contrast to cells from young children, those from older adults could predispose towards less effective pathogen clearance but *increased*



**Figure 3** Visualisation of the changes in neutrophil functions observed in asthma and how they lead to asthma pathophysiology.<sup>38 49–56</sup> AHR, airway hyperresponsiveness; NET, neutrophil extracellular trap; PAMPs, pathogen associated molecular patterns; ROS, reactive oxygen species.

bystander tissue damage, and this may be further exaggerated during severe infections<sup>73</sup> to which the old and young are more susceptible.

In asthma, neutrophil functions can be altered further (figure 3). Patients with neutrophilic asthma display upregulation of neutrophil  $\alpha$ -defensins and serine proteases<sup>74</sup> and increased NET formation.<sup>75</sup> In addition, neutrophils isolated from patients with neutrophilic asthma display enhanced migration but reduced speed of phagocytosis compared with healthy controls in vitro.<sup>76</sup> These changes are reminiscent of the dysfunction observed in both early and late life. Furthermore, adults with neutrophilic asthma display altered innate immune responses, such as reduced antiviral interferon (IFN) production,<sup>77</sup> anti-inflammatory deficiencies associated with reduced galectin-3 and IL-1RA/IL-1 $\beta$ ,<sup>78</sup> increased expression of pathogen-associated molecular pattern receptors, such as TLR-2 and TLR-4,<sup>79</sup> elevated expressions of inflammasomes such as NLRP3<sup>80</sup> and elevated release of proinflammatory mediators such as CXCL-8 and IL-1 $\beta$ .<sup>79</sup>

Although not well studied, airway neutrophils have been associated with respiratory symptoms that are typical of neutrophilic asthma. Airway neutrophils are associated with breathlessness, a faster decline in FEV<sub>1</sub> and incomplete airflow obstruction reversibility.<sup>81</sup> Neutrophil elastase has been shown to be causally associated with induced airway mucus gland hyperplasia, mucus secretion and airway smooth muscle cell proliferation.<sup>74</sup> In keeping with this, inhibiting neutrophil elastase significantly reduced airway hyper-responsiveness, goblet cell metaplasia and inflammatory cell infiltration in a murine model of allergic airway inflammation.<sup>82</sup> In this model, levels of IL-4, IL-5 and IL-13, and eotaxin were reduced in alveolar in the bronchoalveolar lavage (BAL) fluid following treatment.<sup>82</sup>

These studies suggest neutrophils are present in higher percentages than expected in a proportion of patients with asthma where they have reduced function and appear capable of causing some of the features of asthma. The next question to consider is why neutrophils are recruited to the airways. Neutrophilic asthma is more common in adults where potentially pathogenic bacteria can be detected in the lower airways,<sup>83–85</sup> suggesting an association between neutrophilic asthma and airway microbiology.

## INFLAMMATION OR INFECTION: THE ROLE OF AIRWAY MICROBIOLOGY IN ASTHMA

The characteristics of airway microbiology differ between asthma endotypes. T2-eosinophilic asthma is associated with increased susceptibility to acute respiratory infection by bacterial pathogens, including *S. pneumoniae*, *S. pyogenes*, *Bordetella pertussis*, *Legionella pneumophila*, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* (reviewed elsewhere<sup>86</sup>). However, in the absence of acute infection, levels of bacteria in the airways of individuals with eosinophilic asthma are typically low, and detectable bacteria largely represent common members of the upper respiratory microbiota.<sup>8</sup> Positive associations between eosinophilia and bacterial genera, including *Gemella*, *Streptococcus* and *Neisseria*,<sup>83 87</sup> have been identified, with either no association,<sup>83 87</sup> or a positive association,<sup>88 89</sup> between eosinophilia and airway bacterial diversity.

In contrast, the airway microbiology of individuals with neutrophilic asthma is characterised by substantially increased levels of bacteria within the airways<sup>89</sup> and markedly reduced bacterial diversity.<sup>83 89 90</sup> These traits are consistent with other chronic respiratory conditions characterised by airway neutrophilia, including bronchiectasis,<sup>91</sup> COPD<sup>92</sup> and cystic fibrosis.<sup>93</sup>

Similarities in the types of bacteria present are also evident, with the apparent reduction in bacterial diversity associated with proliferation of a small group of bacterial taxa that can exploit the growth environment of the lower airways and withstand host immune response. Most commonly, members of Gammaproteobacteria, particularly *H. influenzae* and *Moraxella catarrhalis*,<sup>83 85 89 90</sup> are prevalent within neutrophilic airway secretions,<sup>85</sup> with clear parallels to airway microbiology typical of patients with bronchiectasis<sup>94</sup> and COPD.<sup>95</sup> Relationships between airway neutrophilia, increased bacterial abundance, reduced diversity and the prevalence of Gammaproteobacteria appear to be continuous, becoming more pronounced where neutrophil levels are greatest.<sup>83 85 89</sup>

The characteristics of airway microbiology in neutrophilic asthma are in keeping with a model of respiratory microbiota developed in other chronic respiratory contexts.<sup>96</sup> Rather than representing acute infective events, bacteria within this scheme accumulate within airway secretions as a result of impaired airway clearance and mucus hypersecretion. Common respiratory opportunist pathogens, such as *H. influenzae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, can proliferate within these secretions, leading to an increase in bacterial load, and a reduction in detectable bacterial diversity. Increased airway inflammation that is associated with bacterial proliferation within the airways acts to further compound poor airway clearance and reduced microbiota diversity, contributing to a persistent cycle of chronic bacterial colonisation and airway neutrophilia.

The stability of airway neutrophilia in asthma, and the associated microbiological traits, is not well understood, however may contribute to progressive airway damage and remodelling. The contribution of chronic neutrophilic inflammation and airway infection is well described in, for example, bronchiectasis,<sup>97</sup> a condition that is also common in patients with severe uncontrolled asthma.<sup>98</sup>

Whether this association between age and neutrophilia contributes to reduced airway microbiota diversity, and the increased prevalence of opportunistic pathogens within the airways, is unclear. Both age and sputum neutrophil levels have been identified as independent predictors of microbiota diversity,<sup>83</sup> while disease duration is also positively correlated with

the total relative abundance of the *Haemophilus*, *Moraxella* and *Streptococcus* genera.<sup>85</sup>

Differentiating cause from effect in relationships between neutrophilia and airway microbiology in asthma is challenging, and not unexpected, given the central role played by the neutrophilic response in combating bacterial airway infection; experiments using mouse models suggest that the presence of neutrophilia-associated bacteria within the airways contributes to the neutrophilic endotype. Airway infection with *M. catarrhalis* in mice during allergen sensitisation, for example, is associated with neutrophilic infiltrates, and high levels of IL-6, IL-17, IFN- $\gamma$  and tumour necrosis factor alpha.<sup>99</sup> In mice infected with *H. influenzae*, steroid-sensitive allergic airway disease (Th2 cells and eosinophils) converts into steroid-resistant disease (Th1 cells and neutrophils), dominated by IL-17 responses.<sup>100</sup> In keeping with the presence of bacteria precipitating neutrophilia in asthma, initial airway infection may arise as a consequence of high doses of inhaled corticosteroids, suppressing both innate and adaptive immune response. Indeed, the relation between airway microbiology and neutrophilia seems dependent on inhaled corticosteroids, with no association between these observed in a study of steroid-free patients with asthma.<sup>101</sup>

At the same time, the association between neutrophilic inflammation and a relatively small group of bacterial taxa, which is seen consistently across chronic respiratory conditions, suggests that neutrophilic inflammation also presents a considerable selective pressure on the composition of the airway microbiota.<sup>15</sup> Of note, most of these respiratory conditions are more prevalent with increasing age, which suggests that the vicious cycle between airway bacteria, inflammation and neutrophil recruitment requires significant time to establish. Were this the case in neutrophilic asthma, one might expect differences in childhood disease.

### NEUTROPHILS IN CHILDHOOD ASTHMA

Due to paucity of diagnostic tests and clinically useful biomarkers, clinicians classify young children with recurrent episodes of wheeze into episodic wheeze (wheeze only with viruses) who are usually treated with intermittent bronchodilators, and multitrigger wheeze (wheeze with viruses, activity, exposure to allergy) who are prescribed inhaled steroids and bronchodilators, such clinical phenotypes overlap.<sup>102</sup> When clinically stable, preschoolers with severe wheeze show increased luminal neutrophils in the presence of bacteria. Unbiased cluster analysis shows airway neutrophilia is associated with altered airway microbiology.<sup>15</sup>

A number of studies have demonstrated neutrophilic childhood asthma. For example, in a study of 67 children with severe asthma, 38 had high BAL neutrophil count (>5%).<sup>16</sup> In these patients, high neutrophil count was associated with increased proinflammatory cytokine/chemokine release, higher markers of neutrophil activation and greater neutrophil trap formation, suggestive of heightened proinflammatory endotype. However, no difference in the clinical characteristics was noted between children with high or low neutrophil counts, in keeping with early adult studies. In another study of induced sputum in 40 children with asthma, 13 were classified as severe therapy-resistant asthma (STRA) and 27 were classified as controlled severe asthma. Although both the groups had eosinophilic inflammation and similar clinical characteristics, STRA group had elevated neutrophils, IL-10 and IFN- $\gamma$  in comparison with controlled severe asthma group.<sup>103</sup> However, in three other studies in children with STRA, no evidence of increased BAL<sup>104</sup>

or sputum neutrophil counts<sup>105 106</sup> was reported. The above studies highlight the lack of clarity around the role of airway luminal neutrophils in promoting disease severity in children with severe asthma. In contrast, increased intraepithelial neutrophils identified in bronchial biopsies of children with STRA are associated with better lung function, less frequent asthma attacks and lower effective dose of inhaled steroids.<sup>107</sup>

Children with evidence of neutrophilic asthma appear to display inflammatory mediator patterns that differ from adults with neutrophilic asthma. For example, sputum IL-17A, a proinflammatory cytokine involved in T cell-mediated neutrophil recruitment, correlates with disease severity in adults.<sup>17</sup> However, while elevated in children with severe asthma, there is no correlation with disease severity.<sup>108</sup> It is unclear whether IL-17 mediates disease severity by neutrophil recruitment in children or if its elevated levels reflect the use of steroids.<sup>109</sup> Emerging data suggest the importance of interactions between neutrophils and innate lymphoid cells (ILCs). In mouse models of house dust mite allergic inflammation, depletion of neutrophils results in worsening airway inflammation through the granulocyte colony-stimulating factor and ILC2 interactions.<sup>110</sup> Recently, IL-5 receptor- $\alpha$  has been identified in the BAL neutrophils of children with severe asthma.<sup>111</sup>

In summary, there is probably insufficient evidence to link airway neutrophilia with positive or adverse outcomes in childhood asthma, but intriguing early studies highlight the presence of these cells and a potential relationship with inflammation and bacteria.

### ASTHMA IN FIRST NATIONS PEOPLE

Asthma has a disproportionate impact on indigenous communities. In adult Indigenous Australians, the prevalence of asthma is 17.5% compared with 10.1% in non-Indigenous adults, and asthma is the second most common cause of hospitalisation for Indigenous Australians after renal failure.<sup>112 113</sup> High rates of asthma in First Nations people are also evident in other indigenous populations. For example, asthma prevalence in Māori (22.1%) and Pacific Islander populations (20.6%) in New Zealand is substantially above the overall prevalence (15.2%).<sup>114</sup> Similarly, prevalence of asthma in First Nations people in Canada is around 12.9% compared with 7.8% in non-Aboriginal Canadians, and in the USA the prevalence in Native Americans is around 13.1% compared with 8.2% in non-Hispanic whites.<sup>115</sup>

A common factor across these communities is considerable social disadvantage. Despite living in affluent countries with modern healthcare sectors, indigenous peoples are typically subject to substantial health inequality, arising as result of poverty, social exclusion and often remote location with limited access to primary healthcare.<sup>116</sup>

Rates of respiratory infection and smoking are both more common in Indigenous Australians, especially in those living in very remote areas. Both of these factors are associated with the development of neutrophil-dominated inflammation<sup>52 117</sup> and potentially contribute to higher rates of late onset of asthma,<sup>113 118</sup> which may not respond well to classical therapies like inhaled corticosteroids.<sup>84 119</sup> Elevated blood neutrophils in combination with reduced lung function have also been demonstrated in Aboriginal adults.<sup>120</sup>

Aside from this, increased prevalence of bronchiectasis, a neutrophil-dominated airway disease, is found in Indigenous Australian children.<sup>121</sup> This highlights the possibility of a neutrophil-dominated airway inflammation in Aboriginal adults with asthma. Therefore, a better understanding of patterns of

airway inflammation in the Australian Aboriginal and Torres Strait Islander communities, and in indigenous peoples and those subject to healthcare inequity more widely, is essential if clinical care is to be delivered effectively. Better understanding of airway inflammation in these groups can contribute substantially to the development of precision models of care that specifically address the disease characteristics most common in these populations.

### POTENTIAL TARGETS AND STRATEGIES FOR TREATMENT

Targeting neutrophils to improve patient outcomes is challenging, as the consequences of blunting the innate immune response could be considerable. However, there are a number of therapeutic approaches under investigation (table 1).

Therapies based on inhibiting the recruitment of neutrophils in airways such as CXCR2 inhibitors have shown effectiveness in reducing sputum and blood neutrophils, and mild exacerbations in patients with severe asthma, but have no effect on severe exacerbation in patients with uncontrolled asthma. These trials of CXCR2 inhibitors were also met with safety challenges such as neutropenia.<sup>122</sup> That said, recent developments in the understanding of the CXCR2 structure and its implication on cellular signalling and activation may provide grounds for further pharmacological investment in CXCR2 inhibitors.<sup>123</sup>

Nemiralisib, an inhaled phosphoinositide 3-kinase inhibitor, developed to reduce activation and recruitment of neutrophils

in the airways, has shown promising results in COPD participants by improving lung function and reducing exacerbation when used as an adjunct treatment.<sup>124</sup> However, when tried in persistent uncontrolled asthma as a monotherapy, it failed to deliver any meaningful outcome from a clinical perspective.<sup>125</sup>

Failure of both treatments in asthma could be a result of not targeting those with neutrophilic airway disease, a lesson previously learnt in eosinophilic asthma.

In contrast, therapies like macrolides have shown more promising results in patients with asthma as they were successful in reducing neutrophil-dominated inflammation biomarkers and exacerbations in patients with severe asthma.<sup>126</sup> Further studies are now warranted, especially to determine the mechanism of action and assess if bacterial resistance is a likely side effect.

Recently, the publication of the WILLOW trial in non-cystic fibrosis bronchiectasis has highlighted clinical benefits in targeting neutrophils in neutrophil-predominant disease. This study was of brensocatib (INS1007), an oral reversible inhibitor of dipeptidyl peptidase 1, an enzyme responsible for the activation of neutrophil serine proteases. A 24-week treatment with this agent was associated with a reduction in exacerbations of disease as well as a reduction in neutrophil proteinase activity with an acceptable side effect profile.<sup>127</sup>

Further studies are now warranted in this asthma endotype, especially to determine the mechanism of action and assess

**Table 1** Summary of trial outcomes from therapies targeting specific molecular targets in asthma

Therapy	Target	Patient population	Outcome	Reference
SCH527123	CXCR2	Severe asthma	Reduction in sputum neutrophil percentage and mean absolute neutrophil blood count (at week 4, recovered at week 5). Fewer mild exacerbations and lower ACQ score.	134
AZD5069	CXCR2	Persistent asthma Moderate persistent neutrophilic asthma	No reduction in severe exacerbations. Reduction in neutrophil counts in bronchial mucosal tissue, induced sputum and blood.	135 136
Golimumab	TNF	Uncontrolled asthma	No meaningful clinical outcome.	137
Etanercept	TNF	Severe asthma Refractory, mild/moderate asthma	Reduction in ACQ and AQLQ, and improvement in lung function. Reduction of histamine in sputum.	138 139
Nemiralisib	PI3K	COPD Uncontrolled asthma	Reduction in activation and recruitment of neutrophils in COPD. No meaningful clinical outcome in uncontrolled asthma.	124 125
Losmapimod	P38MAPK	COPD Severe asthma	No meaningful clinical outcome in COPD. Corticosteroid sensitivity was recovered in PBMCs of severe asthma.	140 141
Roflumilast	PDE4	Asthma	Improvement in lung function. Reduction in eosinophil and neutrophil counts and airway inflammation.	142
GSK2190915	5-Lipoxygenase-activating protein (FLAP)	Persistent asthma	Reduction in symptom scores, SABA use and urinary leukotriene E <sub>4</sub> .	143
Macrolides: azithromycin		Severe asthma	Improved AQLQ score in non-eosinophilic asthma. Reduced exacerbations in asthma. Reduced Hi, IL-6, IL-1b.	126 144 145
Brensocatib	DPP-1	Non-cystic fibrosis bronchiectasis	Reduction of neutrophil serine protease activity. Improvements in clinical outcomes.	127
Brodalumab	IL-17 receptor	Inadequately controlled moderate to severe asthma	No meaningful clinical benefit observed.	146
Tocilizumab	IL-6 receptor	Mild asthma	Reduction in level of C-reactive protein, IL-6 and soluble IL-6R. But treatment reported no effect on allergen-induced bronchoconstriction.	147

ACQ, asthma control questionnaire; AQLQ, asthma quality of life questionnaire; DPP-1, dipeptidyl peptidase 1; IL, interleukin; PMBC, peripheral blood mononuclear cell; PI3K, phosphoinositide 3-kinase; TNF, tumour necrosis factor.

if bacterial resistance is a likely side effect with macrolides, whether longer term brensocatib therapy is associated with an increase in infections and whether more careful patient selection could improve patient outcomes with the other potential therapies; however, a clear precedent has been set.

There are increasing collaborative efforts to undertake molecular phenotyping in asthma, such as the Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes study which will inform this field further.<sup>128</sup>

## CURRENT UNCERTAINTIES

There remains much to learn about neutrophils and asthma. The differences in patient characteristics, inflammatory patterns and airway microbiology suggest a separate phenotype and endotype that warrants a specific therapeutic approach. However, these patients have often had recurrent exacerbations, corticosteroids and antibiotics, and this may suggest the endotype is a consequence and not a cause of asthma management. Only carefully conducted, longitudinal studies that include steroid-naïve patients will provide the needed clarification. Understanding why neutrophils are present may facilitate new treatment pathways that prevent this cellular infiltration and subsequent tissue damage. However, until this is achieved, treatment options for this manifestation of disease are needed. The role of neutrophils in childhood asthma/wheeze is even less clear. Here, obtaining adequate airway samples will continue to be a barrier to research in children, but there are opportunities to learn from the basic science experience in neutrophilic paediatric airway diseases like cystic fibrosis. In vitro models using peripheral blood from children to study neutrophil function should be explored. Grunwell *et al* have reported use of a novel in vitro model to study transepithelial neutrophil migration in various lung environments in children.<sup>129</sup> Such novel in vitro methodologies should be explored to understand the role of neutrophils in childhood acute and chronic wheeze/asthma.

## CONCLUSION

There is an emerging body of evidence suggesting that neutrophilic asthma may form a distinct asthma endotype. The presence of neutrophils in adult asthma is associated with worse disease outcomes including narrowing of the airway microbiota. However, there is less clarity about these cells in childhood disease, with some studies supporting a more benign or even protective role.

Neutrophil populations demonstrate heterogeneity with proinflammatory and anti-inflammatory subsets as well as altered neutrophil functions at the extremes of age. Characterisation of these subsets in the airways of participants may provide more information about the nature of neutrophilic inflammation across the life course, as well as identifying potential therapeutic targets.

Trials of new therapies targeting recruitment of neutrophils to the airways have met with safety issues or lacked an efficacy signal in the small studies conducted to date. Targeting neutrophils' cytotoxic weaponry like serine proteases may help in containing host tissue damage and thus the vicious cycle of continuing neutrophilia-associated damage to airways in asthma.

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