Meeting the Challenge of Identifying New Treatments for Type 2-Low Neutrophilic Asthma

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Asthma pathobiology can be broadly classified by endotypes of type 2-high and type 2-low disease, with a significant percentage of patients with asthma having a type 2-low endotype that is poorly responsive to the antiinflammatory actions of corticosteroids. Therapeutic approaches that target mediators of type 2-low asthma with neutrophilic airway inflammation have yet to enter clinical practice. Therefore, an unmet need exists for the development of new treatments for patients with a neutrophilic asthma, especially those with severe disease. A goal-oriented methodology may be used to address this problem, which focuses on identifying key mediators driving the type 2-low endotype, followed by the development of targeted treatments that can be administered to patients in whom the pathway is active and mediating disease in a precision medicine approach. Because multiple mediators can potentially promote neutrophilic airway inflammation, bioassays will be needed that identify specific factors that have a causal relationship with disease

pathobiology in individual patients. Furthermore, because neutrophils play a key role in host defense, rigorous consideration will need to be given to titrating the reduction in mediators that promote neutrophilic airway inflammation from pathologically elevated levels to concentrations that are typically found in the healthy lung, so that patients can retain normal host defense and immune surveillance functions. Fortunately, the future is bright for the type 2-low endotype as new pathways continue to be identified and targeted treatments are being advanced to clinical trials to establish their safety and efficacy for neutrophilic airway inflammation in patients with type 2-low asthma.

Asthma is a chronic disease that is characterized primarily by airway inflammation and a pathobiology that varies greatly among individuals. The distinction between eosinophilic and noneosinophilic asthmatic phenotypes has evolved into a

ABBREVIATIONS: APO = apolipoprotein; BALF = bronchoalveolar lavage fluid; BET = bromodomain and extraterminal; CXCL = C-X-C motif chemokine ligand; CXCR = C-X-C motif chemokine receptor; DNAzyme = deoxyribozyme; G-CSF = granulocyte-colony stimulating factor; GM-CSF = granulocyte/monocyte colony-stimulating factor; hMSC = human mesenchymal stem cell; IFN = interferon; IL = interleukin; ILC = innate lymphoid cell; LXA4 = lipoxin A4; NETosis = neutrophil extracellular trap cell death; NLRP = nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain-containing protein; ROR γ t = retinoic acid-related orphan receptor γ , thymus specific; Tbet = T box expressed in T cells; Th1, Th2 = helper T cell type 1, type 2; TNF = tumor necrosis factor; SAA = serum amyloid A

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classification scheme that dichotomizes airway inflammation into type 2-high and type 2-low endotypes.^{1,2} This pertains to the full range of asthma severity, as approximately one-half of patients with severe asthma treated with high doses of inhaled corticosteroids and/or systemic corticosteroids do not have ongoing type 2 airway inflammation.³ Mechanistically, type 2-high asthma is characterized by eosinophilic airway inflammation orchestrated by helper T cell type 2 (Th2) lymphocytes or group 2 innate lymphoid cells (ILCs). Conversely, patients with type 2-low asthma comprise a diverse group, whose disease is driven by less well-defined pathobiologic mechanisms and is characterized by neutrophilic or paucigranulocytic inflammation.

Neutrophils are polymorphonuclear granulocytes that mediate innate immunity in response to pathogen- and damage-associated molecular pattern signals. Chronic neutrophilic inflammation occurs when a cytokine microenvironment enhances cellular recruitment and survival. Activated neutrophils produce an oxidative burst, release multiple proteinases, and secrete cytokines that facilitate cross-talk with immune cells. Neutrophil activation can also induce NETosis (neutrophil extracellular trap cell death), a process that releases extracellular DNA traps, termed *neutrophil extracellular traps*, and generates enucleated neutrophils, termed *cytoplasts*. NETosis promotes neutrophilic airway inflammation in asthma. In an experimental murine model system, mice that had been sensitized with house dust mite plus lipopolysaccharide developed NETosis and neutrophilic airway inflammation on subsequent house dust mite challenge.⁴ In addition, bronchoalveolar lavage fluid neutrophil counts and lung cell expression of interferon (IFN)- γ and interleukin (IL)-17 were decreased in *Padi4* (peptidyl arginine deiminase 4) knockout mice with defective NETosis. Both extracellular DNA levels⁵ and cytoplasmic burden⁴ represent potential biomarkers of NETosis in the lungs of patients with severe asthma and are associated with increased IL-1 β and IL-17, respectively. Interestingly, eosinophils can also release DNA traps with resultant eosinophil extracellular trap cell death; however, eosinophil extracellular traps contain significantly fewer proteases than neutrophil extracellular traps and may therefore be more stable.⁶

Neutrophilic asthma is characterized clinically by increased disease severity,⁷⁻¹¹ as well as more severe airflow obstruction.¹²⁻¹⁵ Furthermore, neutrophilic asthma can occur without concomitant infection, corticosteroid therapy, cigarette smoking, or air

pollution. The current definition of neutrophilic asthma is based on the presence of 40% to approximately 70% sputum neutrophils, which can result in an imprecise classification of patients. Therefore, the field currently lacks biomarkers for neutrophilic asthma that are specific, reproducible, and define druggable targets to facilitate optimal patient selection for clinical trials.

The goals of this article are to discuss the pathobiologic pathways that mediate neutrophilic airway inflammation in asthma, define therapeutic targets, and provide a glimpse into future venues in neutrophilic asthma research. It must be emphasized, that regardless of asthma endotype, lifestyle changes, such as smoking cessation and weight loss, are equally as important in reducing asthma symptoms and improving quality of life as pharmacologic interventions.

Druggable Pathways in Neutrophilic Asthma

General Considerations

An unmet need persists for the development of new treatments for neutrophilic asthma. A goal-oriented methodology to address this problem can focus, in a precision medicine approach, on the identification of key mediators that drive neutrophilic inflammation, followed by the development of targeted treatments that are administered to patients in whom that pathway is active and mediating disease. Because multiple mediators can potentially promote neutrophilic airway inflammation, bioassays will need to identify the specific factor that is causing disease in individual patients.

An important potential limiting factor that will need to be considered regarding the development of targeted therapies for neutrophilic airway inflammation is the risk of potential infectious and neoplastic complications, which may result from cytokine and/or cellular blockade. As a consequence, it will be important to titrate the reduction in mediators that promote neutrophilic airway inflammation from pathologically elevated levels to concentrations that are typically found in the healthy lung, so that patients can retain normal host defense and immune surveillance. Therefore, strategies that completely abrogate lung neutrophils and predispose patients to infectious or neoplastic complications need to be avoided. Furthermore, this concern may restrict therapies targeting mediators of neutrophilic airway inflammation to patients with severe and refractory asthma.

Potential mediators that can be targeted for the treatment of neutrophilic asthma are considered in the following sections (Fig 1).

Figure 1 – Selected pathways mediating neutrophilic airway inflammation in type 2-low asthma. (Figure created with BioRender.com.) APO = apolipoprotein; CXCL = C-X-C motif chemokine ligand; CXCR = C-X-C motif chemokine receptor; FPR = N-formyl peptide receptor; G-CSF = granulocyte colony-stimulating factor; G-CSFR = granulocyte colony-stimulating factor receptor; GM-CSF = granulocyte/monocyte colony-stimulating factor; GM-CSFR = granulocyte/monocyte colony-stimulating factor receptor; IFN = interferon; IL = interleukin; ILC = innate lymphoid cell; LXA = lipoxin; NLRP = nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain containing; SAA = serum amyloid A; SLPI = secretory leukocyte protease inhibitor; TGF = transforming growth factor; TNF = tumor necrosis factor.

Th1/ILC1 Cytokines

IFN- γ is produced by Th1 CD4⁺ T cells and group 1 ILCs to promote innate immune responses to infection. Dysregulated IFN- γ production may promote severe asthma, as individuals with mixed neutrophilic and eosinophilic airway inflammation have increased IFN- γ -producing airway CD4⁺ T cells.¹⁶ IFN- γ induces C-X-C motif chemokine ligand (CXCL) 10 expression, which is a chemokine with chemoattractant activity toward Th1 CD4⁺ T cells and neutrophils, and also decreases airway epithelial cell production of secretory leukocyte protease inhibitor, which may promote increases in airway hyperresponsiveness, exhaled nitric oxide production, and airway remodeling.^{16,17} Therefore, an IFN- γ /

CXCL10-mediated type 1 bias may exist in patients with severe, steroid-unresponsive asthma that might be targeted to reduce neutrophilic airway inflammation. Given the key role of this pathway in host defense, neutralization strategies may potentially be associated with infectious complications. Therefore, therapies would need to be titrated so that host defense is preserved.

Tumor necrosis factor (TNF)- α is produced by multiple cell types, including macrophages and Th1 CD4⁺ T cells, and promotes neutrophilic inflammation.² Neutrophilic inflammation has been correlated with sputum TNF- α levels in patients with severe asthma,¹⁸

whereas inhalation of TNF- α by nonasthmatic patients increases sputum neutrophils and causes airway hyperreactivity.² The largest clinical trial exploring the effect of anti-TNF- α antibody, golimumab, on lung function and asthma control in patients with severe asthma failed to show a beneficial effect and was associated with increased adverse events, including serious infections and cancer, that resulted in early termination of the study.¹⁹ A limitation of this study was that it did not restrict enrollment to patients with TNF- α -high asthma. Although the unfavorable risk-to-benefit profile related to TNF- α inhibition suggested that this approach may not be suitable for all patients with asthma, it is not known whether anti-TNF- α therapies with fewer side effects might be used for the targeted treatment of patients with asthma with an endotype of TNF- α -mediated neutrophilic airway inflammation.

Deoxyribozymes (DNAzymes) are single-stranded DNA antisense molecules with catalytic activity that specifically bind and cleave target mRNAs. This approach has already been implemented in clinical trials to target GATA3 in Th2 cells.²⁰ Inhibition of the Th1-specific transcription factor Tbet (T-box transcription factor 21, T-box expressed in T cells) may therefore represent a target for Th1-predominant asthma, as *Tbet*-deficient mice are resistant to double-stranded RNA-induced neutrophilic airway inflammation.²¹ Although a Tbet-specific DNAzyme has been studied in murine skin inflammation models, this approach has not been applied to asthma.²² Tbet inhibition will also attenuate Th1-mediated antimicrobial immunity and increase host susceptibility to infection. As a consequence, the effect of depleting Th1 cells on airway host defense will need to be rigorously considered.

Janus kinase 1 mediates signaling by both type 1 and 2 interferons, type 2 cytokines, and IL-6. An inhaled small-molecule inhibitor of Janus kinase 1 has been developed that attenuated neutrophil inflammation in a murine model of allergic asthma.¹ Finally, human mesenchymal stem cells (hMSCs) are thought to have reparative and immunomodulatory properties. Intravenous administration of hMSCs in an experimental murine model of allergen-mediated asthma attenuated neutrophilic airway inflammation.²³ hMSCs also suppressed IFN- γ production by human peripheral blood mononuclear cells.

Th17/ILC3 Cytokines

IL-17 cytokines promote neutrophilic inflammation and are secreted by Th17 cells and group 3 ILCs.^{1,2} Although

IL-17A and IL-17F are increased in the airways of patients with severe asthma, the role of IL-17 in mediating neutrophilic airway inflammation remains incompletely defined, which in part reflects conflicting results regarding the association between IL-17 expression and neutrophilic inflammation in airway biopsy specimens.^{1,2} Our understanding of the cause-and-effect relationship between Th17 response and airway neutrophilia is evolving, as a recent murine study showed that neutrophil-derived cytoplasts activate lung dendritic cells to induce a Th17 response, which suggests that neutrophilic inflammation may promote IL-17 production.¹

The largest clinical trial of the anti-IL-17 receptor A monoclonal antibody, brodalumab, in patients with severe asthma did not improve asthma control, which may reflect that subjects were not selected on the basis of increased neutrophilic airway inflammation or increased IL-17 levels.²⁴ Therefore, it remains unknown whether administration of anti-IL-17 therapies to patients with neutrophilic asthma or patients with IL-17-high asthma can improve disease control. Two phase 2 clinical trials investigating the efficacy and safety of an anti-IL-17A monoclonal antibody in patients with moderate-to-severe type 2-low asthma,²⁵ and an anti-IL-23 antibody, risankizumab, which blocks Th17 cell differentiation in patients with severe asthma,²⁶ will provide additional data regarding the possible role of anti-IL-17 strategies for neutrophilic asthma.

ROR γ t (retinoic acid-related orphan receptor γ , thymus specific) is the major transcription factor for Th17 cells, and ROR γ t-overexpressing mice develop steroid-resistant neutrophilic airway inflammation.²⁷ Therefore, a ROR γ t-targeting DNAzyme might be developed for Th17-mediated neutrophilic asthma. Finally, histone acetylation is an epigenetic mechanism that regulates gene transcription. Bromodomain and extraterminal (BET) proteins can bind histone-acetylated lysine residues, whereas BET inhibitors displace BET proteins from histones and attenuate gene expression. A highly selective BET small-molecule inhibitor, CPI-203, was effective in attenuating Th17-mediated airway inflammation in a murine model of allergic asthma.²⁸

IL-1 β

IL-1 β is a proinflammatory cytokine generated by the NLRP3 (nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain-containing protein 3) inflammasome, mainly in monocytes and macrophages, and plays a central role in mediating

innate immunity.²⁹ Both the NLRP3 inflammasome and IL-1 β have been linked to neutrophilic airway inflammation and increased disease severity in severe, steroid-resistant asthma. The concept of targeting IL-1 β for neutrophilic asthma has advanced to early-stage clinical trials. The IL-1 receptor antagonist anakinra attenuated lipopolysaccharide-induced neutrophilic airway inflammation in healthy volunteers with reductions in sputum levels of IL-1 β , IL-6, and IL-8.³⁰ The effect of anakinra on airflow obstruction in mild allergic asthma is currently being evaluated in a phase 1/2 clinical trial.³¹ The anti-IL-1 β monoclonal antibody canakinumab has also been shown to be safe and well tolerated in a phase 1/2 clinical trial of patients with mild asthma.³² Additional studies will be needed to ascertain the safety and effectiveness of anti-IL-1 β strategies for neutrophilic asthma.

C-X-C Motif Chemokine Receptor 2

IL-8 (CXCL8), a major neutrophil chemoattractant and activator, is secreted mainly by epithelial and immune cells and interacts with low- and high-affinity C-X-C motif chemokine receptors (CXCRs) 1 and 2, respectively, on neutrophils. Airway IL-8 levels positively correlate with neutrophilic inflammation, asthma severity, and impaired lung function.² In a small study, the CXCR1/2 inhibitor SCH527123 significantly attenuated the primary end point of sputum neutrophil counts, but led to a modest improvement in asthma control.³³ In a larger multicenter study of patients with uncontrolled asthma and high blood neutrophil counts, the CXCR2 antagonist AZD5069 did not reduce severe asthma exacerbations.³⁴ Although this suggests that targeting CXCR2-mediated neutrophil recruitment may not be effective for severe asthma, the identification of more specific biomarkers of the CXCR2 pathway could potentially justify revisiting this approach. Other chemokines with neutrophil chemoattractant activity, such as CXCL1, CXCL3, CXCL5, CXCL10, and CCL20,^{16,35} have been shown to correlate with neutrophilic airway inflammation in asthma and may represent potential targets for future drug development.

Granulocyte and Granulocyte/Monocyte Colony-Stimulating Factors

Granulocyte colony-stimulating factor (G-CSF) and granulocyte/monocyte colony-stimulating factor (GM-CSF) are potent chemoattractants, activators, and antiapoptotic signals for neutrophils that may promote neutrophilic airway inflammation in asthma. The effectiveness of an anti-GM-CSF antibody, KB003, has

been investigated in a phase 2 trial of patients with inadequately controlled asthma, but showed no effect on the primary end point of improvement in mean FEV₁.³⁶ A limitation of this study was that it did not endotype participants regarding GM-CSF levels in blood or sputum. Therefore, the possibility exists that future studies that identify subjects with a GM-CSF-mediated endotype of neutrophilic asthma might demonstrate a beneficial effect of anti-GM-CSF therapy.

IL-6

A new asthma endotype, IL-6-high asthma, has been identified that is characterized by elevated plasma IL-6 levels, increased markers of systemic inflammation, metabolic dysfunction, and obesity.³⁷ In addition, patients with IL-6-high asthma had worse lung function and asthma control. Although sputum neutrophils did not differ between IL-6-high and IL-6-low patients, blood neutrophils were increased in the IL-6-high group, which suggests a role for systemic IL-6-mediated neutrophilic inflammation in mediating an “outside-in mechanism of lung dysfunction.”³⁷ Another study, however, showed that asthmatics with increased sputum neutrophils had a greater percentage of subjects with high plasma IL-6 levels as compared to nonneutrophilic asthmatics.³⁸ The relevance of plasma IL-6 as a biomarker of systemic inflammation that mediates an endotype of severe asthma will need to be confirmed by studies that investigate whether administration of IL-6-neutralizing therapies to patients with IL-6-high asthma reduces disease severity. This concept can promptly be advanced to clinical trials as anti-IL-6 receptor humanized monoclonal antibodies have already entered clinical practice for other inflammatory diseases. These studies can also assess whether plasma IL-6 levels mediate increased airway neutrophils in asthma.

Lipoxin 4 and Serum Amyloid A

A biochemical endotype of severe asthma has been identified with low bronchoalveolar lavage fluid (BALF) levels of the antiinflammatory, proresolving lipid mediators lipoxin A4 (LXA4) and 15-epi-LXA4, and high BALF levels of proinflammatory serum amyloid A (SAA).³⁹ BALF levels of LXA4 and 15-epi-LXA4 were inversely correlated with BALF neutrophils and were lower in patients with severe asthma, whereas BALF levels of SAA were positively correlated with BALF neutrophils and were higher in patients with severe asthma, which defines a new mechanism of neutrophilic airway inflammation.³⁹ Furthermore, patients with LXA4-low/SAA-high asthma were more likely to have

TABLE 1] Potential Druggable Targets to Attenuate Neutrophilic Airway Inflammation in Patients With Asthma

Pathway	Potential Biomarkers	Potential Therapeutic Interventions to Attenuate Neutrophilic Airway Inflammation in Patients With Asthma
Th1/ILC1	IFN- γ , CXCL10, SLPI, TNF- α , Tbet	Small-molecule inhibitor (JAK1) Neutralizing antibody (TNF- α) Soluble receptor fusion protein (TNF- α) DNAzyme (Tbet)
Th17/ILC3	IL-17A, IL-17F, IL-23A, ROR γ t	Neutralizing antibody (IL-17A, IL-17 receptor A, IL-23A) DNAzyme (ROR γ t) Small-molecule inhibitor (BET protein)
Inflammasome/IL-1 β	IL-1 β	IL-1 receptor antagonist Neutralizing antibody
CXCR1/CXCR2	IL-8 (CXCL8)	Small-molecule inhibitor
CXCR3	CXCL10	Small-molecule inhibitor
Colony-stimulating factor	G-CSF, GM-CSF	Neutralizing antibody APOA1 mimetic peptide
IL-6	IL-6	Neutralizing antibody (IL-6, IL-6 receptor)
LXA4/SAA	LXA4-low/SAA-high	LXA4

APOA1 = apolipoprotein A1; BET = bromodomain and extraterminal; CXCL = C-X-C motif chemokine ligand; CXCR = C-X-C motif chemokine receptor; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte/monocyte colony-stimulating factor; IFN = interferon; IL = interleukin; ILC = innate lymphoid cell; JAK = Janus kinase; LXA = lipoxin A; ROR γ t = retinoic acid-related orphan receptor γ , thymus specific; SAA = serum amyloid A; SLPI = secretory leukocyte protease inhibitor; Tbet = T-box transcription factor TBX21; Th1, Th17 = helper T-cell type 1 and type 17; TNF = tumor necrosis factor.

severe asthma and worse lung function. Mechanistically, BALF macrophages from patients with severe asthma had increased expression of ALX/FPR2 (lipoxin A4/N-formyl peptide receptor 2), whereas BALF fluid from patients with LXA4-low/SAA-high asthma increased IL-8 production by an epithelial cell line that expressed ALX/FPR2. This suggests that low levels of LXA4 and high levels of SAA might be developed as a biomarker to identify patients with asthma with this endotype of neutrophilic disease.

Macrolides

The macrolide antibiotic azithromycin has been shown in a clinical trial in adults with persistent uncontrolled asthma (Asthma and Macrolides: the Azithromycin Efficacy and Safety Study [AMAZES]) to reduce asthma exacerbations and improve quality of life, including a subgroup of noneosinophilic subjects.⁴⁰ The mechanism by which azithromycin reduces asthma exacerbations has not been definitively established. Although a previous trial suggested that the beneficial effect of macrolides on neutrophilic inflammation in asthma might be related to reductions in IL-8, the modulation of other pathways, such as calcineurin and mammalian target of rapamycin, has been proposed.⁴⁰ Although prolonged macrolide therapy is frequently used in clinical practice, the potential of inducing antibiotic-resistant bacteria should be considered with long-term

macrolide therapy for asthma, as an analysis of AMAZES showed that azithromycin increased the carriage of macrolide and tetracycline resistance genes by the airway microbiome.⁴¹

Apolipoproteins

A therapeutic effect of apolipoprotein A-I (APOA1) on neutrophilic asthma has been identified in a murine model of ovalbumin-induced neutrophilic airway inflammation.⁴² APOA1 transports cholesterol and lipids out of cells via the ABCA1 transporter. Experiments using *Apoa1* and *Abca1* knockout mice identified an APOA1/ABCA1 pathway in the lung, where APOA1 is expressed by alveolar epithelial cells and interacts with ABCA1 transporters expressed by pulmonary vascular endothelial cells and alveolar macrophages, which suppresses neutrophilic airway inflammation via a G-CSF-dependent mechanism. Furthermore, a positive association exists between serum APOA1 levels and less severe airflow obstruction in patients with allergic asthma. On the basis of these results, an inhaled formulation of an APOA1 mimetic peptide, termed 5A, is being developed for clinical trials.

In contrast, apolipoprotein E (APOE) may promote neutrophilic airway inflammation in asthma. APOE activated the NLRP3 inflammasome and IL-1 β secretion from ex vivo cultures of asthmatic alveolar

macrophages.²⁹ APOE also induced the secretion of both CXCL5 and IL-8, which have chemoattractant activity toward neutrophils, by ex vivo cultures of asthmatic airway epithelial cells via a TLR4/TAK1/IKK β /NF- κ B/TL2/2/JNK signaling pathway.³⁵

In conclusion, the identification of biomarkers to distinguish endotypes that mediate neutrophilic asthma can guide the development of targeted interventions that are delivered in a precision medicine approach to those subjects in whom the pathway is active (Table 1). Because neutrophils also play a key role in host defense, the benefits of suppressing exaggerated neutrophilic inflammation in asthma will need to be carefully balanced against the potential risk of infection.

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