

# T2-“Low” Asthma: Overview and Management Strategies



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## Learning objectives:

1. To identify clinical features of type 2 (T2)-“low” asthma.
2. To identify molecular pathways of T2-“low” asthma.
3. To identify possible treatment approaches for patients with T2-“low” asthma.

**Recognition of Commercial Support:** This CME has not received external commercial support.

**Disclosure of Relevant Financial Relationships with Commercial Interests:** B. E. Chipps reports consultancy fees from AstraZeneca, Boehringer Ingelheim, Circassia, Genentech, Novartis, Sanofi, Regeneron Pharmaceuticals, Inc, and Teva, outside the submitted work; and serves on the speakers' bureaus for AstraZeneca, Boehringer Ingelheim, Circassia, Genentech, Novartis, and Teva. P. G. Woodruff reports personal fees for consulting from Glenmark Pharmaceuticals, Theravance, GlaxoSmithKline, NGM Pharmaceuticals, Genentech, Regeneron, Sanofi, Clarus ventures, 23andME, and Astra Zeneca, outside the submitted work. The rest of the authors declare that they have no relevant conflicts of interest. M. Schatz declares no relevant conflicts of interest.

Although the term “asthma” has been applied to all patients with airway lability and variable chest symptoms for centuries, phenotypes of asthma with distinct clinical and molecular features that may warrant different treatment approaches are well recognized. Patients with type 2 (T2)-“high” asthma are characterized by upregulation of T2 immune pathways (ie, IL-4 and IL-13 gene sets) and eosinophilic airway inflammation, whereas these features are absent in patients with T2-“low” asthma and may contribute to poor responsiveness to corticosteroid treatment. This review details definitions and

clinical features of T2-“low” asthma, potential mechanisms and metabolic aspects, pediatric considerations, and potential treatment approaches. Priority research questions for T2-“low” asthma are also discussed. © 2019 American Academy of Allergy, Asthma & Immunology (*J Allergy Clin Immunol Pract* 2020;8:452-63)

**Key words:** Asthma; Asthma in children; Asthma management; Biomarker; Endotype; Eosinophil; Neutrophil; Phenotype; Sensitization; Type 2 inflammation

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Conflicts of interest: B. E. Chipps reports consultancy fees from AstraZeneca, Boehringer Ingelheim, Circassia, Genentech, Novartis, Sanofi, Regeneron Pharmaceuticals, Inc, and Teva, outside the submitted work; and serves on the speakers' bureaus for AstraZeneca, Boehringer Ingelheim, Circassia, Genentech, Novartis, and Teva. P. G. Woodruff reports personal fees for consulting from Glenmark Pharmaceuticals, Theravance, GlaxoSmithKline, NGM

Pharmaceuticals, Genentech, Regeneron, Sanofi, Clarus ventures, 23andME, and Astra Zeneca, outside the submitted work. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication August 19, 2019; revised manuscript received and accepted for publication November 11, 2019.

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2213-2198

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<https://doi.org/10.1016/j.jaip.2019.11.006>

*Abbreviations used*

GINA- Global Initiative for Asthma

ILC- Innate lymphoid cell

NET- Neutrophil extracellular trap

type 1- T1

type 2- T2

## INTRODUCTION

Although the term “asthma” has been applied to all patients with airway lability and variable chest symptoms for centuries, heterogeneity is clearly evident among affected individuals. Since the early 1940s, “phenotypes” of asthma (ie, groups of patients with shared clinical characteristics) have been reported in the literature. The first phenotypes, described by Rackemann,<sup>1</sup> were those of “intrinsic” versus “extrinsic” asthma. In that report, Rackemann noted that patients with “extrinsic” asthma developed asthma in childhood in association with allergic sensitization, whereas patients with “intrinsic” asthma often developed asthma after the age of 40 years and tended to have less sensitization despite relatively low lung function.<sup>1</sup> Nearly 50 years later, Wenzel et al<sup>2</sup> described 2 groups of patients with severe, systemic corticosteroid-dependent asthma on the basis of presence and absence of airway eosinophils. Although the eosinophil-negative group had lesser evidence of airway remodeling, these patients also had marginally lower FEV<sub>1</sub> and significant symptomology despite significant anti-inflammatory treatment.<sup>2</sup> It was not until recently, in the era of widespread corticosteroid treatment for all patients with asthma, that it became evident that not all patients respond equally to this treatment approach.<sup>3</sup> This recognition of asthma heterogeneity fueled multiple attempts to describe clinical and molecular phenotypes of asthma in difficult-to-treat populations for the purpose of personalized, more effective treatment.<sup>4</sup>

In the resulting studies, consistent relationships were noted between upregulation of type 2 (T2) immune pathways (ie, IL-4 and IL-13 gene sets), eosinophilic airway inflammation, and airflow limitation in patients with asthma.<sup>5,6</sup> These patients have since been termed as having T2-“high” asthma. In the original studies that analyzed the expression of 3 bronchial epithelial genes after *ex vivo* IL-13 stimulation, these patients with T2-“high” asthma comprised about 50% of the study population and were more allergic, had more eosinophils, and had more bronchial hyperreactivity than did patients with low expression.<sup>7,8</sup> Moreover, patients with T2-“high” asthma also had robust improvement in FEV<sub>1</sub> after the initiation of inhaled corticosteroids, whereas patients with low gene expression did not.<sup>7</sup> This finding, coupled with the observation that T2-pathway-targeted biologics lacked efficacy in all-comers with asthma,<sup>9</sup> has resulted in broad recognition of a non-T2 or T2-“low” asthma phenotype that warrants alternative treatment.

## DEFINITION OF T2-“LOW” ASTHMA AND CLINICAL FEATURES

At the present time, definitions for T2-“low” asthma have not been established. For the purpose of this review, T2-“low” asthma is defined loosely as asthma in the absence of prominent T2-pathway signatures. This definition is admittedly flawed. Although the original studies of T2-“high” asthma relied on epithelial gene expression signatures to define immune

phenotypes,<sup>5,6</sup> bronchoscopy is not clinically indicated in all patients with asthma and epithelial-based definitions are therefore not practical in the real-world setting. Although some emphasis has also been placed on noninvasive biomarkers for the detection of T2-“high” asthma (ie, exhaled nitric oxide, blood eosinophils, or sputum eosinophils), the exact cutoff points for these markers are debatable.<sup>10</sup> These T2 markers may also have little concordance in the same patient<sup>11,12</sup> and may be subject to significant variability over time and in response to asthma treatments.<sup>13</sup> Indeed, T2-“low” asthma could result from suppression of T2-“high” asthma by corticosteroids. Therefore, it remains unclear whether T2-“low” asthma is a distinct disease entity necessitating unique treatment approaches.

Nonetheless, T2-“low” asthma is typically associated with a later age of onset, higher medication requirements, and significant symptomology (Table I).<sup>14-17</sup> Although lung function may be better than in patients with T2-“high” severe asthma, patients with T2-“low” asthma also tend to have a poorer response to short-acting bronchodilators, which may further contribute to symptom burden.<sup>18,19</sup> Obesity has also been associated with T2-“low” phenotypes of asthma,<sup>20</sup> which may worsen symptoms and lung function.<sup>21</sup> Uniformly, patients with T2-“low” asthma also have a poor response to corticosteroids<sup>7</sup> and may have secondary complications when these medications are used for long durations, such as worsening of obesity or metabolic dysfunction.

Airway neutrophilia may also be a feature of some patients with T2-“low” asthma, but this feature has not been consistently reproduced. Cutoff points for airway neutrophils have not been well established and largely depend on the method used and compartment measured (ie, biopsy tissue, bronchoalveolar lavage, or induced sputum). Indeed, various cutoff points for airway neutrophilia have been used in the literature, with a broad range from 40% up to 76%.<sup>22</sup> The consistency of airway neutrophils over time is also less than that of eosinophils,<sup>23</sup> and intensive corticosteroid treatment may further alter neutrophil survival.<sup>24,25</sup> Although airway neutrophil numbers do tend to increase in difficult-to-treat populations,<sup>26-29</sup> presumably in association with increased IL-17 levels<sup>30</sup> and other inflammatory cytokines and chemokines,<sup>31-33</sup> many patients with elevated airway neutrophils also have abundant airway eosinophils and other T2-“high” features. For example, in a recent study of adults with corticosteroid-refractory, moderate to severe persistent asthma, sputum neutrophil counts were negatively correlated with sputum eosinophil counts, but most patients with elevated neutrophil counts also had airway eosinophilia.<sup>34</sup> In that same study, there was also no correlation between neutrophil percentages in the mucosal, lower airway, and upper airway compartments.<sup>34</sup>

Airway neutrophil numbers (and IL-17) may also increase during acute exacerbations<sup>35</sup> and in patients with a history of smoking.<sup>36,37</sup> However, whether smoking is truly a T2-“low” phenotype is somewhat unclear. Although smoking can induce corticosteroid insensitivity through oxidative mechanisms that promote neutrophil and macrophage activation,<sup>38</sup> smoking may also increase susceptibility to allergen sensitization.<sup>39,40</sup> Indeed, many patients with asthma-chronic obstructive pulmonary disease overlap would have T2-“high” asthma as defined by the joint task force of the Global Initiative for Asthma (GINA) and the Global Initiative for Chronic Lung Disease, given that the minor criteria for diagnosis include a history of atopy, significant bronchodilator reversibility, and peripheral eosinophilia.<sup>41</sup>

Nonetheless, the concept of a neutrophilic T2-“low” asthma phenotype persists and has been supported by gene expression profiling studies.<sup>42</sup>

### MECHANISMS OF T2-“Low” Asthma

Potential mechanisms of T2 low asthma include the following: (1) non-T2 inflammation within the lung (airway neutrophilia, type 1 [T1] [IFN-mediated] or type 3 [IL-17-mediated] immune pathways), (2) systemic inflammation associated with IL-6, obesity, and metabolic dysfunction, and (3) noninflammatory (paucigranulocytic) mechanisms. In addition to explaining T2-low asthma, these mechanisms may co-occur with T2 inflammation, plausibly explaining the presence of more severe asthma in some patients.

### Neutrophils in asthma

Neutrophils are the most abundant innate immune effector cell whose primary functions include phagocytosis, degranulation, and formation of neutrophil extracellular traps (NETs), which trap and kill bacteria, viruses, and other organisms to prevent dissemination.<sup>43</sup> Airway neutrophilia has been reported in patients with more severe chronic asthma.<sup>44-46</sup> Although airway neutrophilia can be seen in the absence of airway eosinophilia, the combination of airway eosinophilia and neutrophilia (mixed granulocytic inflammation) is also associated with severe disease.<sup>26</sup> Airway neutrophilia has also been noted during asthma exacerbations<sup>47</sup> and may potentiate airway epithelial injury through the release of proteases, induction of oxidative stress, induction of goblet cell degranulation, and formation of NETs.<sup>30,48</sup> NETs also mediate inflammasome activation and IL-1 $\beta$  secretion from monocytes and contribute to epithelial cell injury.<sup>43</sup> Interestingly, NETs were recently described in response to rhinoviral infection and were shown to potentiate T2 T-cell responses and contribute to exacerbations of allergic asthma.<sup>49</sup> Increased NET formation has also been reported in a subset of patients with exacerbation-prone severe asthma who were more likely to be treated with systemic corticosteroids and who also had increased airway neutrophil counts (and mixed granulocytic inflammation)<sup>50</sup> and higher IL-17 concentrations.<sup>51</sup> Exposure of cultured airway epithelial cells to NETs further induced epithelial toxicity, reflected by upregulation of IL-6 and IL-8 mRNA and increased release of glucose-6-phosphate dehydrogenase.<sup>50</sup>

### Type 3 (IL-17-mediated) immunity

The IL-17 cytokine family members are designated IL-17A through IL-17F, and most studies of IL-17 cytokines in asthma focus on IL-17A. IL-17A is secreted by a subset of T-helper cells (ie, T<sub>H</sub>17 cells), natural killer T cells, CD8<sup>+</sup> T cells, and gamma delta T cells<sup>52-55</sup> and a subset of innate lymphoid cells (ILCs) including T1 ILCs and type 3 ILCs<sup>56</sup> (Figure 1). Possible pathogenic roles for IL-17A in the asthmatic airway include promotion of airway neutrophilia via upregulation of colony-stimulating factor 3 and chemokine ligand chemokines,<sup>52,57</sup> mediation of airway hyper-responsiveness,<sup>58,59</sup> and corticosteroid resistance.<sup>57</sup> In mice, allergic sensitization through the airway or the skin promotes bronchial T<sub>H</sub>17 responses and can induce airway hyper-responsiveness.<sup>60,61</sup> High levels of IL-17 or IL-17-producing cells have been reported in blood, sputum, and bronchial biopsies in some patients with severe asthma.<sup>62-71</sup> Unfortunately, despite this relatively large body of observational study evidence, a randomized

clinical trial of IL-17 blockade with brodalumab in inadequately controlled moderate to severe asthma did not show clinical benefit in all-comers.<sup>72</sup> Whether better targeting of this therapeutic approach based on biological markers would be beneficial is uncertain. For example, early work examining anti-IL-5 (mepolizumab) was disappointing in all-comers because the patients were not recruited on the presence of an eosinophilic phenotype.<sup>9</sup> A more recent translational study suggested that T2 cytokine suppression itself may promote T<sub>H</sub>17 responses and neutrophilic inflammation in the lung in humans with asthma (a cross-sectional analysis) and in mice treated with IL-13 and IL-17 blockade, suggesting that combination therapies targeting both T2 and IL-17-related pathways could be beneficial.<sup>73</sup>

### T1 (IFN-mediated) immunity

T1 cell-mediated effector immunity protects against intracellular microbes and is mediated by IFN- $\gamma$ -producing helper cells (ie, CD4<sup>+</sup> T<sub>H</sub>1 cells and T1 ILCs), as well as a subset of CD8<sup>+</sup> T cells and natural killer T cells.<sup>74</sup> In addition, airway epithelial cells contribute to mucosal immunity by releasing IFN- $\alpha$ , IFN- $\beta$ , and IFN- $\lambda$  as part of a stereotypic response to viruses and some bacteria.<sup>75,76</sup> Two bodies of literature suggest potentially conflicting pathogenic roles of T1 immunity in asthma. On the one hand, studies suggest that IFN signaling in the airway epithelium can be deficient in asthma<sup>77</sup> and that this deficiency renders patients with asthma more susceptible to viral airway infection and asthma exacerbations.<sup>78</sup> Unfortunately, randomized trials of inhaled IFN therapy have not succeeded to date in improving asthma symptoms or reducing exacerbations caused by respiratory viruses,<sup>79,80</sup> in part due to a relatively low number of exacerbations and small change in symptoms after perceived colds in these controlled studies. On the other hand, other studies suggest that increased IFN signaling in the airways in a subset of patients with asthma may be pathogenic.<sup>81-85</sup> For example, patients with severe asthma have more IFN- $\gamma$ <sup>+</sup> (and IL-17A<sup>+</sup>) CD4<sup>+</sup> T cells in bronchoalveolar lavage than do patients with mild to moderate asthma despite high-dose corticosteroid treatment.<sup>82</sup> These high IFN- $\gamma$  levels were associated with higher CXCL10 gene expression, a mast cell signature,<sup>84</sup> and higher expression of IFN regulatory factor 5 in bronchoalveolar lavage cells.<sup>83</sup> Each of these findings was recapitulated in accompanying mouse models of corticosteroid-resistant airway inflammation, which supported the pathogenic potential of IFN- $\gamma$ <sup>+</sup>, and IFN regulatory factor 5 through the use of genetically deleted mice.<sup>82,83</sup> Even in mild to moderate asthma, analysis of airway epithelial brushings shows induction of IFN-related gene expression signatures that are associated with lower lung function,<sup>85</sup> and, when elevated in blood cells, are associated with poor response to omalizumab (an anti-IgE therapy that is more effective in T2-“high” asthma).<sup>86</sup>

### Systemic inflammation

A subset of patients with asthma manifests obesity, metabolic dysfunction, and low-grade systemic inflammation.<sup>87</sup> This clinical scenario will be familiar to clinicians who care for patients with severe asthma. This low-grade systemic inflammation originates with adipocytes and inflammatory macrophages in adipose tissue that secrete various proinflammatory cytokines (including IL-6).<sup>88</sup> Plasma IL-6 concentrations are elevated in some patients with asthma,<sup>89</sup> particularly in patients with a higher body mass index and

**TABLE I.** Common features of T2-“high” vs T2-“low” asthma

| Feature                              | T2-“high”                          | T2-“low”                           |
|--------------------------------------|------------------------------------|------------------------------------|
| Age of onset                         | Earlier onset                      | Later onset                        |
| Symptoms                             | May be significant                 | May be significant                 |
| Life-threatening exacerbations       | More exacerbations                 | Fewer exacerbations                |
| Obesity/metabolic dysfunction        | May be present                     | Often present                      |
| Lung function                        | More obstruction                   | Less obstruction                   |
| Short-acting bronchodilator response | More responsive                    | Less responsive                    |
| Allergic sensitization               | Present                            | Absent                             |
| Exhaled nitric oxide                 | Normal to elevated                 | Low to normal                      |
| Airway eosinophilia                  | Present                            | Absent                             |
| Airway neutrophilia                  | May be present                     | May be present                     |
| Medication requirements              | More responsive to corticosteroids | Less responsive to corticosteroids |

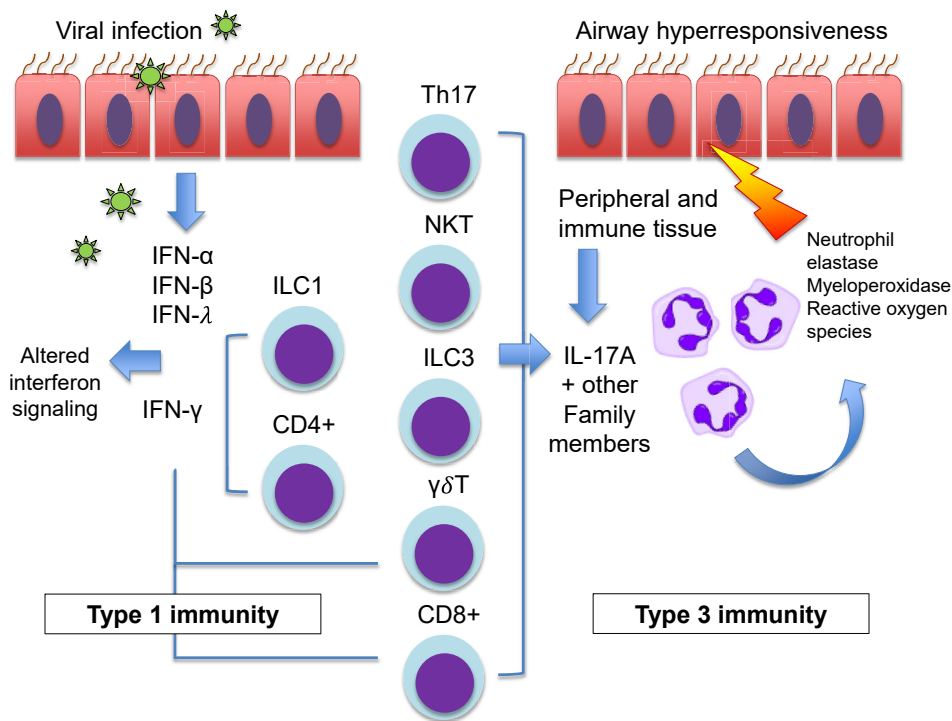


**FIGURE 1.** T1 and type 3 immune pathways in T2-“low” asthma. *NKT*, Natural killer T;  $\gamma\delta T$ , gamma delta T cell.

metabolic disease. These IL-6 “high” obese patients with asthma also have diminished lung function and increased exacerbation frequency compared with IL-6 “low” obese patients with asthma.<sup>87</sup> Plasma IL-6 levels in obese patients with asthma are also associated with increased blood neutrophils and plasma C-reactive protein concentrations, suggesting a more general systemic inflammatory state.<sup>87</sup> Genetic studies have also shown that polymorphisms in genes relevant to IL-6 signaling are associated with asthma.<sup>90</sup> Interestingly, elevated plasma IL-6 levels and systemic inflammation can be seen in both non-T2 asthma and T2-“high” asthma and are not associated with upstream T2 inflammation.<sup>87</sup> Plasma IL-6 levels also do not correlate with airway IL-6 concentrations<sup>87</sup> and are instead likely derived from inflammatory macrophages in white adipose tissue.<sup>91</sup> Whether systemic IL-6 is a causal factor for asthma or mediator of asthma is still uncertain.

**Noninflammatory (paucigranulocytic) mechanisms**

Studies of cellular airway inflammation using induced sputum show that some patients with asthma are “paucigranulocytic” in that they have neither sputum eosinophilia nor sputum neutrophilia.<sup>92</sup> Although one must be cautious about interpreting this as a lack of inflammation when a patient is actively treated with anti-inflammatory medications, this intriguing observation is relatively common and begs a mechanistic explanation. Possible mechanisms include “innate” airway smooth muscle dysfunction,<sup>93</sup> remodeling of airway smooth muscle or other structural cells,<sup>94</sup> and neurogenic mechanisms referable to parasympathetic nerves<sup>95</sup> or taste and olfactory receptors on resident lung cells.<sup>96</sup>

**METABOLIC ASPECTS OF T2-“LOW” ASTHMA**

Asthma encompasses a heterogeneous group that includes not only those with asthma with varying degrees of airway

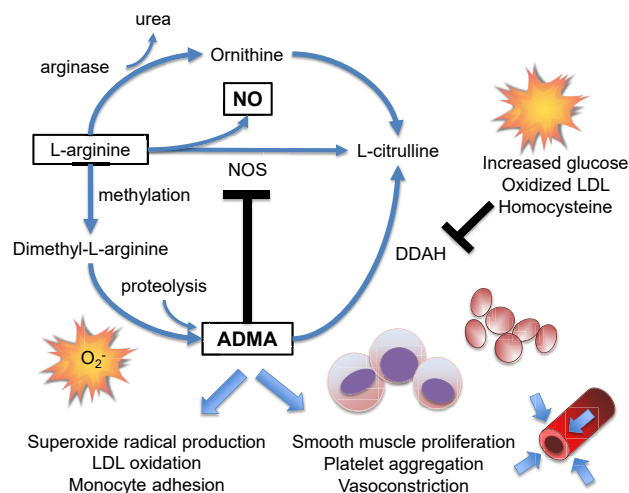


**FIGURE 2.** Metabolic alterations in T2-“low” asthma. ADMA, asymmetric dimethyl arginine; DDAH, dimethylarginine dimethylaminohydrolase; LDL, low-density lipoprotein; NO, nitric oxide; NOS, nitric oxide synthase.

**TABLE II.** Priority research questions for T2-“low” asthma

Basic/translational research

- What are the underlying molecular pathways associated with T2-“low” asthma?
- What is the role (and specificity) of neutrophils in patients with T2-“low” asthma?
- Are there specific biomarkers associated with T2-“low” asthma?

Clinical application

- How should T2-“low” asthma be defined in patients?
- Is a single biomarker or multiple biomarkers of T2-“low” asthma more useful? If multiple biomarkers are used, what is the best course of action in the setting of mixed results?
- Are there clinical features, apart from molecular features, that are unique to patients with T2-“low” asthma?
- Is T2-“low” asthma a distinct molecular phenotype/endotype, or T2-“high” asthma in remission?
- What is the temporal stability of the T2-“low” phenotype?
- What therapies have the greatest efficacy in patients with T2-“low” asthma?
- Is T2-“low” asthma present in children, and if so, do clinical features and therapeutic approaches differ from those in adults?

eosinophilia but also those in which airway function is directly impaired or underlying inflammation worsened by changes in metabolic pathways. Obese patients, particularly females, who develop asthma after childhood are an example of a frequent T2-“low” phenotype in which metabolic dysregulations have been associated with increased asthma morbidity.<sup>27,97-99</sup> Clinically, these patients have low or normal exhaled nitric oxide levels, less atopy, and reduced numbers of eosinophils in the airway lumen.<sup>100</sup> As such, these patients are less likely to achieve symptom control or improve FEV<sub>1</sub> in response to inhaled corticosteroids.<sup>101</sup>

Metabolic alterations potentially affecting airway function in obese patients include changes in L-arginine/nitric oxide

metabolism, mitochondrial dysfunction, insulin resistance, and immune dysregulation associated with diet and lipid metabolism.<sup>102</sup> Obesity and asthma are both chronic diseases that are characterized by having increased arginase activity and reduced L-arginine levels.<sup>103,104</sup> In addition, these conditions have greater asymmetric dimethyl arginine levels,<sup>105,106</sup> which is a protein methylation product that accumulates with oxidative stress. (For an in-depth review of this metabolic pathway, please see Teerlink<sup>107</sup>). In the airway epithelium of obese patients with asthma, a lower L-arginine/asymmetric dimethyl arginine ratio uncouples nitric oxide synthase 2, preferentially producing anion superoxide at the expense of nitric oxide.<sup>108</sup> Ultimately, this reduces nitric oxide airway bioavailability, which could impair bronchodilation and cause airway oxidative stress, which reduces inhaled corticosteroid responsiveness (Figure 2).<sup>109,110</sup> Moreover, because nitric oxide normally inhibits mitochondrial respiration, reduced nitric oxide bioavailability consequently increases mitochondrial respiration, which in turn can produce mitochondrial reactive oxygen species and activate additional inflammatory pathways.<sup>104</sup> Indeed, compared with the lean counterparts, the airway epithelium in obese patients with asthma has greater maximal mitochondrial respiration and glycolytic rates.<sup>104</sup> Whether or not obesity-mediated mitochondrial dysfunction translates into increased asthma morbidity is yet to be defined.

Other dysregulations in metabolism frequently associated with obesity that can also adversely impact asthma include metabolic syndrome factors such as hyperinsulinemia and dyslipidemias. Insulin resistance, for example, has been associated with T1 polarization, an increased risk for asthma exacerbations,<sup>111</sup> and reduced lung function.<sup>112,113</sup> Insulin has also been shown to induce airway smooth muscle proliferation and increase contractility in murine models,<sup>114</sup> yet the relevance of these findings to human airway smooth muscle cells is less certain, because airway smooth muscle from obese patients with asthma, particularly females, have increased agonist-mediated contractility that is independent from insulin.<sup>115</sup> Dyslipidemias (ie, low high-density lipoprotein and high triglycerides), however, have been associated with reduced lung function, wheezing, and monocyte activation.<sup>116,117</sup>

## T2-“LOW” ASTHMA IN SCHOOL-AGE CHILDREN

Compared with adults, school-age and prepubertal children with asthma tend to have more episodic disease, namely, severe exacerbations triggered by viral infections and/or allergen exposures, with asymptomatic periods between these episodes.<sup>118</sup> Most children with asthma (>80%) also have aeroallergen sensitization<sup>119-122</sup> and thus the predominant asthma phenotypes in children are most aligned with the early-onset, T2-“high” phenotype in adults.<sup>27</sup> Indeed, the most “severe” pediatric asthma phenotypes identified by cluster analyses of children in the National Heart, Lung, and Blood Institute’s Severe Asthma Research Program<sup>123</sup> and Childhood Asthma Management Research Program<sup>124</sup> were characterized by a high degree of atopy and eosinophilia and the lowest lung function. Although the eosinophilic/T2-“high” phenotype in adults is generally accepted to be more corticosteroid-responsive,<sup>7</sup> these children had little clinical improvement with daily inhaled corticosteroid monotherapy,<sup>124</sup> but did improve with the addition of salmeterol to the inhaled corticosteroid.<sup>125</sup> However, there were also other atopic phenotypes identified, namely, those with comorbidities,

that essentially had no response to guideline-based asthma treatment.<sup>123,125</sup>

Other important differences in children do exist, even among those with atopy and T2-“high” disease. For example, significant obstruction is relatively uncommon in children with asthma.<sup>122</sup> Instead, lung function (ie, FEV<sub>1</sub>) is often normal in children despite symptom burden,<sup>126-128</sup> but distal lung resistance may be increased in the absence of large airway involvement.<sup>129</sup> Therefore, others have argued that alternative measures such as the ratio of FEV<sub>1</sub> to forced vital capacity, forced expiratory flow rates, or airway hyperresponsiveness to bronchodilators may better discriminate asthma severity or related outcomes in this age group.<sup>126</sup> Furthermore, although there are subsets of children with hyperinflation,<sup>119</sup> these children tend to have less airway resistance than do adults<sup>130</sup> and are generally reversible with bronchodilation.<sup>131</sup> However, these features may worsen during adolescence,<sup>132</sup> particularly in adolescents with worsening symptoms,<sup>133,134</sup> and may increase the risk of more severe asthma, fixed airflow obstruction, or even chronic obstructive pulmonary disease in later adulthood.<sup>135,136</sup> Indeed, in the Melbourne asthma cohort, 14% of all participants developed fixed airflow obstruction by the age of 50 years, defined as a postbronchodilator FEV<sub>1</sub>/forced vital capacity ratio of less than 0.7. In children with severe asthma, 44% of children with severe asthma developed fixed airflow obstruction at age 50 years, which was largely attributed to a lack of increase in FEV<sub>1</sub> %predicted value during the ages of 14 to 21 years.<sup>136</sup> The mechanisms underlying this transition are poorly understood. Sex hormones may be involved, because girls tend to maintain hyperresponsiveness and symptoms longer than do males.<sup>137-139</sup>

Although T2-“high” phenotypes predominate in children, plausible T2-“low” phenotypes characterized by few or no eosinophils have also been reported.<sup>140-145</sup> In a relatively large bronchoscopic study of 126 children with severe asthma, 52% were paucigranulocytic, with less postbronchodilator airflow limitation and less blood eosinophilia, whereas 15% had isolated airway neutrophilia.<sup>145</sup> Among children with isolated airway neutrophilia, 65% had detectable microbes (either respiratory virus or bacteria), whereas the remainder had no clinically recognized fungal or bacterial infection.<sup>145</sup> Other recent studies likewise suggest that airway neutrophil elevations may be present in up to 30% of children with severe asthma.<sup>144,146</sup> In 1 study, increased neutrophils were noted in a subpopulation of children with severe asthma and were associated with upregulation of proteins responsible for neutrophil activation and chemotaxis.<sup>147</sup> Others have also noted increased expression of IL-17 receptor A, which promotes neutrophil maturation,<sup>142</sup> and largely undetectable IL-4, IL-5, and IL-13 concentrations in the airways of most children with severe asthma,<sup>148</sup> suggesting that the inflammation in these children is not isolated to eosinophils alone. Indeed, a recent study of children and adolescents with severe asthma and elevated airway neutrophils also noted that the neutrophils from these patients were highly proinflammatory, with greater phagocytic capability and neutrophil extracellular trap formation, but more impaired respiratory burst.<sup>143</sup> Airway macrophages from children with neutrophil “high” severe asthma were also highly proinflammatory with disrupted innate immune defenses, which may render those children more susceptible to lower respiratory tract infections.<sup>143</sup> However, it should be noted that most patients in that study did not have isolated airway neutrophilia but instead had a mixed granulocytic pattern with

concordant elevations in lung lavage eosinophils. Unfortunately, these studies have been too small to identify immune phenotypes. Large molecular “omic” studies in children are needed.

## TREATMENT OF T2-“LOW” ASTHMA

Although progress has been made in the identification of T2-“high” asthma, to date, well-validated noninvasive biomarkers for T2-“high” or T2-“low” asthma are not available. Airway neutrophilia is also challenging to ascertain and cannot be detected on the basis of currently available biomarkers. Indeed, 1 study attempted to validate a greater than or equal to 3% sputum eosinophil cutoff point in patients with asthma with a combination of exhaled nitric oxide, blood eosinophils, and total IgE and found that the combination of these biomarkers could accurately predict sputum eosinophilia in only 58% of the population.<sup>149</sup> Likewise, although increased blood eosinophils have also been associated with increased odds and shorter time to exacerbation in children and adolescents, the addition of other markers of T2 inflammation did not markedly improve exacerbation prediction.<sup>10</sup> It may be that more novel methods are needed. In a recent study of 521 patients with asthma, volatile organic compounds in the exhaled breath were more likely than existing biomarkers to discriminate between neutrophilic and eosinophilic asthma.<sup>150</sup> In that study, hexane and 2-hexanone had the highest classification performance in eosinophilic asthma, whereas nonanal, 1-propanol, and hexane had the highest classification performance for neutrophilic asthma.<sup>150</sup>

Given current inability to accurately define either T2-“high” or T2-“low” asthma, the persistence of these immune phenotypes is unclear. Therefore, T2-“low” asthma remains poorly understood, and there are currently very limited options for treatment. Furthermore, because the concept of T2-“low” asthma is relatively new, there are few studies that stratify treatment on the basis of immune phenotype. The most commonly used therapies for T2-“low” asthma, tiotropium and macrolides, are prescribed on the basis of presumed efficacy and are not explicitly approved (or even studied) for this indication. Other possible therapies, namely, weight reduction, smoking cessation, and bronchial thermoplasty, are also discussed briefly below.

## Tiotropium

Tiotropium has now been studied in several populations of asthma. A landmark study in 2010 involving 210 patients with mild to moderate asthma uncontrolled with low-dose inhaled corticosteroid compared the addition of tiotropium (18 µg daily by HandiHaler) to doubling of the inhaled corticosteroid dose or the addition of salmeterol with a 3-way, double-blind, triple-dummy, crossover study design. Compared with doubling the inhaled corticosteroid dose, tiotropium resulted in the superior primary outcome, with a morning peak flow mean difference of 25.8 L/min. Superiority in secondary outcomes was also noted, with an evening peak flow mean difference of 35.3 L/min, prebronchodilator FEV<sub>1</sub> mean difference of 100 mL, and greater proportion of asthma control days. The addition of tiotropium was not inferior to the addition of salmeterol for all the assessed outcomes, but prebronchodilator FEV<sub>1</sub> increased more than it did with salmeterol with a difference of 110 mL. It should be noted that patients who had a better response to tiotropium were younger patients who had a greater beta-agonist response and a lower resting heart rate.<sup>151</sup>

Tiotropium has also been studied in patients with more severe poorly controlled asthma as an add-on to combination therapy with inhaled corticosteroids and long-acting beta-agonists. Adults with poorly controlled asthma despite inhaled corticosteroids plus long-acting beta-agonists with a mean baseline FEV<sub>1</sub> of 60% predicted and a mean age of 53 years received tiotropium (5 µg daily through the Respimat Soft Mist inhaler) or placebo for 24 weeks, after which the mean difference in peak FEV<sub>1</sub> from baseline was measured.<sup>152</sup> In the trials reported in that publication, the mean difference in FEV<sub>1</sub> was 86 ± 34 mL in trial 1 and 154 ± 32 mL in trial 2. The predose FEV<sub>1</sub> also improved compared with placebo, as well as the time to first exacerbation, which increased to 282 days (tiotropium) versus 226 days (placebo), with an overall reduction of 21% in the risk for severe exacerbations.<sup>152</sup> In 2 subsequent meta-analyses of tiotropium (5 µg or 2.5 µg) in patients with symptomatic asthma despite low-to-medium dosages of inhaled corticosteroid,<sup>153,154</sup> both doses of tiotropium were similarly associated with significant improvements in peak flow and FEV<sub>1</sub>. Tiotropium also decreased the risk of severe exacerbations and improved asthma control indices.

Unfortunately, very few studies have compared the efficacy of tiotropium by T2 phenotype. One exploratory analysis of patients with moderate to severe symptomatic asthma in 2 separate trials receiving tiotropium as an add-on therapy to either inhaled corticosteroid therapy or inhaled corticosteroid plus long-acting beta-agonist therapy found no differences in treatment responses between T2-“high” and T2-“low” patients.<sup>155</sup> In that study, T2-“high” asthma was defined by a physician determination of allergic asthma (yes vs no), serum IgE cutoff point of 430 µg/L (equivalent to 179.2 IU/L), and a blood eosinophil cutoff point of 600 cells/µL, although other cutoff points were also modeled and did not change interpretation of the results.<sup>155</sup> In other published studies, it is very difficult to determine what percentage of patients had T2-“low” asthma as opposed to T2-“high” asthma.

A recent article by Lazarus et al<sup>156</sup> provides some insight into the efficacy of tiotropium for patients with low sputum eosinophils (and presumed T2-“low” asthma). In this 42-week, double-blind, crossover trial, 295 adolescents and adults with mild persistent asthma received either mometasone 200 µg daily, tiotropium 18 µg capsule inhaled once a day, or placebo in a 42-week, crossover design with a 2-week washout between periods. In this study, 73% of the patients had low sputum eosinophil levels. Among these patients with low sputum eosinophils, there was no significant difference in the response to mometasone or tiotropium when compared with placebo. In contrast, among patients with higher eosinophil levels (and presumed T2-“high” asthma), the response to mometasone was significantly better than to placebo.<sup>156</sup> Similarly, studies of preschool children with recurrent wheezing and persistent asthma have also noted a significant improvement in symptoms and exacerbations with daily inhaled corticosteroid treatment, but only in those children with elevated blood eosinophils and aeroallergen sensitization.<sup>157,158</sup>

Tiotropium effectiveness was also analyzed in a pilot study of patients with severe noneosinophilic asthma. In that study, 17 patients classified as having noneosinophilic asthma were treated with tiotropium added to conventional therapy for 4 weeks, which was well tolerated.<sup>159</sup> In addition, FEV<sub>1</sub> improved significantly. However, additional studies of the effectiveness of tiotropium in T2-“low” asthma are still needed.

## Macrolides

Macrolides, namely, azithromycin, have been widely used in patients with other chronic obstructive lung diseases, including panbronchiolitis in elder Japanese males, cystic fibrosis, bronchiectasis, and chronic obstructive pulmonary disease. Although current guidelines from the European Respiratory Society and American Thoracic Society recommend against routine use of macrolide therapy for all patients with severe asthma, these agents may be useful in patients with airway neutrophilia with recurrent infections or when allergic bronchopulmonary aspergillosis is suspected.<sup>160</sup> Indeed, the 2019 GINA guidelines recommend the addition of low-dose macrolide for patients who do not respond to standard treatment, but emphasize that this is an off-label use that should be weighed against the potential for antibiotic resistance.<sup>161</sup>

Azithromycin has been shown to inhibit IL-6 and IL-8 protein production<sup>162,163</sup> and reduce neutrophil attraction to the site of infection,<sup>164</sup> likely through inhibition of nuclear factor kappa-B. Studies have also reported antiviral effects with azithromycin,<sup>163</sup> although azithromycin does not directly impact viral load.<sup>165</sup> Instead, the benefit of azithromycin for respiratory infection may be related to alterations in the airway microbiome, namely, bacterial composition and abundance.<sup>166</sup>

The long-term benefit of treating patients with asthma with azithromycin was reported in a meta-analysis in 2003.<sup>167</sup> In that analysis, macrolide administration for asthma over 3 or more weeks was not associated with improvement in FEV<sub>1</sub> but did result in significant improvement in peak flow, symptoms, quality of life, and airway hyperreactivity.<sup>167</sup> More recently, Gibson et al<sup>168</sup> performed a large, randomized, double-blind, placebo-controlled trial of azithromycin given at 500 mg Monday, Wednesday, Friday in 420 adults older than 18 years with symptomatic asthma despite the use of inhaled corticosteroid and long-acting bronchodilator. Azithromycin significantly reduced asthma exacerbations, which were 1.07 per patient-year (95% CI, 0.85-1.29) compared with 1.86 (95% CI, 1.54-2.18) in the placebo arm. The proportion of patients who experienced at least 1 asthma exacerbation was also reduced to 94 patients (44%) in the azithromycin group, compared with 127 patients (61%) in the placebo group. Azithromycin also significantly improved asthma-related quality of life. In prespecified subgroup analyses, there were no differences in azithromycin treatment effects between patients with eosinophilic and noneosinophilic asthma, with eosinophilic asthma defined as either greater than or equal to 3% sputum eosinophils or greater than or equal to 300 blood eosinophils/mL. However, efficacy in T2-“low” versus T2-“high” patients with asthma was not specifically examined. The side effects of azithromycin are also somewhat concerning regarding QT prolongation.<sup>169</sup> However, if a predosing electrocardiogram suggests that the QT interval is not prolonged, the chance of ventricular tachycardia or ventricular fibrillation is dramatically decreased. If the QT interval is within the normal range, macrolide antibiotic can likely be used without significant predictable adverse events.<sup>169</sup> Hearing should also be monitored because there are rare reports of sensorineural hearing loss with azithromycin, even after uncomplicated infections.<sup>170</sup>

## Lifestyle modifications

Given the high prevalence of obesity in patients with T2-“low” asthma, weight reduction strategies should be included in the treatment plan for obese patients. Numerous clinical

studies have shown that weight reductions in obese patients not only improve asthma symptoms and medication use but also reduce the risk for severe asthma exacerbations and improve asthma-related quality of life, perhaps through improvements in lung function.<sup>171-173</sup> Although bariatric surgery dramatically improves asthma symptoms and lung function, even modest weight losses of 5% to 10% can result in clinically important improvements in asthma symptom control, quality of life, and inflammation. However, the 2019 GINA guidelines emphasize that a weight reduction program plus twice-weekly aerobic and strength exercises may be more effective than weight reduction alone.<sup>161</sup> In a small clinical trial of weight reduction plus aerobic exercise versus weight reduction alone, the addition of aerobic exercise resulted in fewer asthma symptoms, improvements in lung function and inflammation, and greater weight loss.<sup>174</sup> Similar studies of exercise plus weight reduction also noted improvement in sleep and depressive symptoms as well as an increase in asthma symptom-free days.<sup>174,175</sup>

Weight reduction, along with exercise and dietary modification, is also beneficial for patients with metabolic syndrome. In a recent meta-analysis of exercise training interventions of 12 weeks or greater for patients with metabolic syndrome in the general population, aerobic exercises led to reductions in body mass index and waist circumference as well as reductions in systolic and diastolic blood pressure, fasting blood glucose, triglycerides, and low-density lipoprotein and increases in high-density lipoprotein.<sup>176</sup> In a recent study of obese patients with moderate to severe asthma, weight reduction plus exercise was similarly associated with changes in body composition and improvements in lung function that were accompanied by a significant reduction in IL-6 and other inflammatory cytokines and chemokines (ie, CCL2, IL-4, TNF- $\alpha$ ) and an increase in anti-inflammatory IL-10.<sup>174</sup> Unfortunately, to date, there have been few published interventional studies for patients with asthma and metabolic syndrome. One retrospective analysis of patients with asthma undergoing bariatric surgery found that metabolic syndrome was strongly predictive of a loss of asthma control by 12 months after the procedure ( $\sim 92\%$  higher risk compared with patients without metabolic syndrome),<sup>177</sup> further suggesting a causal link between metabolic syndrome and asthma. A secondary analysis of administrative claims from 23,920 individuals with asthma and diabetes also found that initiation of metformin improved insulin resistance and metabolic function and also lowered the hazard of asthma exacerbation.<sup>178</sup>

Smoking cessation (for patients who smoke) and avoidance of second-hand smoke may also dramatically improve asthma control. In addition to increasing the risk for exacerbations and chronic obstructive pulmonary disease, smoking is an established risk factor for cardiovascular disease and may increase the risk of sudden cardiac death.<sup>179</sup> Cessation of smoking for at least 6 weeks improves neutrophilic airway inflammation and lung function.<sup>36</sup> Likewise, avoidance of second-hand smoke reduces hospital visits for acute exacerbations in both children and adults.<sup>180</sup> Patients who smoke should be strongly encouraged to quit and provided with access to counseling and smoking cessation programs.<sup>161</sup> In 1 study, smokers with chronic lower respiratory disease including asthma were more likely than smokers without respiratory disorders to respond to a proactive outreach intervention for smoking cessation.<sup>181</sup>

## Bronchial thermoplasty

Bronchial thermoplasty involves direct application of radio-frequency pulse to an airway segment to reduce smooth muscle mass. This is a potential treatment option for patients with severe, treatment-refractory asthma who remain uncontrolled despite specialty care. However, the number of studies remains small. A recent meta-analysis of bronchial thermoplasty in adults with moderate to severe asthma found that thermoplasty resulted in lower rates of asthma exacerbations and improved quality of life, with no clear benefit in day-to-day asthma control.<sup>182</sup> However, in pooled analyses, the impact on asthma control did not meet the threshold of clinical significance.<sup>182</sup> Moreover, there is a significant placebo effect and risk of bias in several studies that lacked sham treatment arms. The procedure is also associated with more adverse events during treatment.<sup>182</sup> Long-term safety is not fully established, but 5-year follow-up studies demonstrate a reasonable safety profile and suggest that the effects are durable in many patients.<sup>183</sup> Nonetheless, European Respiratory Society/American Thoracic Society severe asthma treatment guidelines<sup>160</sup> and current GINA treatment guidelines<sup>161</sup> both recommended that the procedure only be performed in adults with severe asthma in the context of an institutional review board–approved systematic registry or clinical study, so that further safety and effectiveness data can be gathered. The role of bronchial thermoplasty in phenotype-directed asthma therapy is also not clear, because there are no current predictors of treatment response.

## CONCLUSIONS AND FUTURE DIRECTIONS

Although significant advances have been made for patients with T2-“high” asthma driven by IL-4–, IL-5–, and IL-13–mediated eosinophilic inflammation, T2-“low” asthma remains poorly understood. There are currently no proven pathways for T2-“low” asthma, although IL-8 and IL-17, obesity/metabolic derangements, and other exposures may play a role. As such there are no established biomarkers for T2-“low” asthma and multiple priority research questions remain (Table II). Current treatment approaches are therefore relatively nonspecific and are based on presumed efficacy, because true effectiveness studies in the population are lacking. Further studies of T2-“low” mechanisms are greatly needed to identify potential biomarkers and develop more effective therapies. Although no single treatment approach will be effective for all patients, careful clinical phenotyping and immune stratification in future clinical studies may hasten progress for these patients.

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