HHS Public Access

Author manuscript

Curr Opin Immunol. Author manuscript; available in PMC 2021 November 04.

Published in final edited form as:

Curr Opin Immunol. 2020 October ; 66: 123–128. doi:10.1016/j.coi.2020.10.002.

Mechanisms of non-type 2 asthma

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Abstract

Non-type 2 inflammation (Non-T2)-mediated asthma is difficult to define due to lack of signature biomarkers. It exists in the absence of T2-high or eosinophilic inflammation and includes neutrophilic and paucigranulocytic subtypes. Several cell types and cytokines, including Th1, Th17, IL-6, and IL-17, contribute to mechanisms of non-T2 asthma. Neutrophil extracellular traps (NETs) and inflammasome activation likely play a role in severe neutrophilic asthma. Several mechanisms lead to uncoupling of airway hyperresponsiveness and remodeling from airway inflammation in paucigranulocytic asthma. Recent research on transcriptomics and proteomics in non-T2 asthma is discussed in this review. Investigations of specific drug therapies for non-T2 asthma have been disappointing, and remain an important area for future clinical studies.

Introduction

Asthma is a heterogeneous chronic obstructive airway disease characterized by multiple distinct endotypes. Asthma is commonly categorized by the type of inflammation associated with its pathobiology. The majority of asthma shows evidence of cytokines associated with T-helper 2 cell (T2)-mediated inflammation and is termed T2-high. The pathogenesis of T2-high asthma is chiefly orchestrated by interleukins (IL)-4, IL-5 and IL-13 and is usually accompanied by eosinophil infiltration. T2-high disease is clinically determined by elevated peripheral blood or sputum eosinophil levels using consensus-derived, numerical cutoffs. Conversely, there is currently no agreed upon definition or signature biomarker for T2-low or non-T2 asthma other than absence of T2-high inflammation. In this review, we will discuss several proposed mechanisms underlying non-T2 asthma and potential research directions (Table 1).

Clinical identification of non-T2 asthma

Sputum cytology can be used to categorize airway inflammation as eosinophilic, neutrophilic, mixed granulocytic, or paucigranulocytic. Non-T2 asthma encompasses the neutrophilic and paucigranulocytic categories; whether similar mechanisms drive neutrophilic inflammation in the mixed granulocytic and neutrophilic-only categories is unknown, as is the longitudinal stability of these categories. Although there is no agreed upon numerical criterion, neutrophilic asthma (NA) has been defined as ≥50% sputum

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JCC and SH have nothing to disclose.

neutrophils [1]. Paucigranulocytic asthma is characterized by absence of increased sputum neutrophil or eosinophil levels, coupled with stereotypical asthmatic features, principally airway smooth muscle (ASM) hypertrophy, reversible airway obstruction and airway hyperresponsiveness (AHR).

Non-T2 asthma currently lacks confirmatory biomarkers. A study with >500 asthmatic participants identified the combination of three endogenously generated, exhaled volatile organic compounds — hexane, nonanal, and 1-propanol — as associated with NA [2]. This finding requires confirmation in other cohorts.

Non-T2 asthma is heterogeneous, reflecting the combination of neutrophilic and paucigranulocytic categories. It is more common in those with adult-onset disease, corticosteroid resistance, and comorbidities such as obesity and gastroesophageal reflux disease [3]. Triggers for neutrophilic airway infiltration are diverse and include smoking, air pollution, and bacterial infections.

Induction of neutrophilic airway inflammation in asthma

Multiple cytokines are implicated in the development of neutrophilic airway inflammation in asthma. IL-17 levels in bronchial biopsies correlate with airway neutrophil infiltration and are increased in patients with severe and exacerbation-prone asthma relative to those with milder disease [4]. Th17 cells secrete IL-17 cytokines, including IL-17A, which promote neutrophil recruitment in the airways by acting on airway epithelial cells (AEC) to secrete neutrophil chemokines such as CXCL1 and CXCL8. Murine studies suggest that lung dendritic cell expression of TNF-α-induced protein 3 (TNFAIP3) stimulates Th2 expression whereas its absence results in Th17 expression and neutrophilic airway infiltration following intranasal dust mite extract administration [5]. A gene polymorphism in the IL4-receptor-alpha chain (R576) promotes inducible T-regulatory to Th17 cell conversion and associates with severe asthma [6]. Treatment with an anti-IL6-R-antibody decreased exacerbation rates and improved lung function in a severe asthmatic child homozygous for the IL4-R-alpha(R576) allele while decreasing Th17 expression in peripheral blood [7]. However, the pathogenic role for IL-17 cytokines, such as IL-17A and IL-17F, in asthma is questioned by a failed clinical trial of an anti-IL-17-receptor antibody to improve asthma control [8]; but these participants were not selected for NA, which may explain the negative results. IL-17 also induces IL-6 secretion from AECs, which may stimulate neutrophilic inflammation by driving naïve T-helper cells into Th17 differentiation. IL-6 is implicated in severe NA, as documented in a study by the Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes (U-BIOPRED) Group, which identified a subgroup of severe asthmatic patients with elevations in sputum of both IL-6 levels and neutrophil counts [9]. In contrast, a recent study from the National Heart Lung and Blood Institute (NHLBI)-sponsored Severe Asthma Research Program 3 (SARP3) reported that serum IL-6 associates with asthma severity, although IL-6 correlated with elevated blood but not sputum neutrophil counts [10•]. This observation suggests that IL-6 may be important in asthma pathogenesis through mechanisms other than neutrophil infiltration in the airways, such as mucus hypersecretion [11].

Th1 inflammatory cytokines are also implicated in NA. In one report, bronchoalveolar lavage fluid (BALF) from severe asthmatic patients showed greater Th1 cells and neutrophil numbers accompanied by higher interferon-gamma levels, a signature Th1 cytokine [12]. These investigators linked interferon-gamma to AHR through suppression of secretory leukocyte protease inhibitor, a protein expressed by AEC and associated with normal ASM tone.

Finally, the nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain containing 3 (NLRP3) inflammasome and IL1-beta are intracellular sensors of microbial and other danger signals important for innate immunity. NLRP3 and IL1-beta are both upregulated in sputum from patients with NA [13]. NLRP3 inflammasome-related genes were upregulated in patients with NA and correlated with sputum IL1-beta levels in a U-BIOPRED study [14]. In murine models of NA, bacterial infection with *Haemophilus a*nd *Chlamydia* upregulated the inflammasome, IL1-beta levels and neutrophilic airway inflammation. Pharmacological inhibition of the inflammasome and neutrophil depletion both attenuated AHR. In this study, airway inflammation reoccurred following intranasal IL1-beta administration [15]. Understanding the contribution of neutrophil regulation to asthma pathobiology requires additional research.

Pathologic role of NETs in asthma

The major roles of neutrophils include phagocytosis of pathogens, antimicrobial enzyme degranulation, and generation of neutrophil extracellular traps (NETs) from ejected nuclear contents [16]. NET dysregulation may result in asthmatic pathobiology. A recent SARP3 study found that patients with more severe asthma exhibit higher airway neutrophil-derived sputum extracellular DNA levels which correlated with NET formation and inflammasome activation [17•]. These processes may result in asthma through AEC injury. Interestingly, a subset of NETs does not involve rupture of but rather resealing of the neutrophil plasma membrane, which creates an enucleated cytoplast. In murine models of NA, neutrophil cytoplasts correlated with Th17-mediated asthmatic responses independent of NETs [18]. In this study, neutrophil cytoplasts, identified by flow cytometry in BALF, also correlated with asthma exacerbation frequency among SARP3 severe asthmatic patients.

Airway dysbiosis in neutrophilic asthma

Airway dysbiosis refers to alterations in normal airway microbiome composition. Multiple investigators have documented dysbiosis in association with NA. In a study including >150 asthmatic participants, the sputum microbiome of those with NA had less bacterial species diversity compared with those with eosinophilic disease [19•]. Two groups identified sputum *Proteobacteria* as overrepresented in NA [20,21]. Recently, a U-BIOPRED study showed the relative stability of individual subject sputum microbiome after >12 months of follow up for NA. *Haemophilus, Moraxella*, and *Streptococcus* were noted more frequently [22•]. Whether these microbiome signatures are causative of NA or a confounder to another underlying mechanism remains to be determined.

Paucigranulocytic asthma

As the name implies, this phenotype involves asthma without an increased granulocytic presence in the airways, described as an ‘uncoupling’ of airway obstruction from airway inflammation [23]. There are several proposed mechanisms for paucigranulocytic asthma, including modulation of neural mediators, sphingolipid synthesis, and regulators of bronchoconstrictive signaling.

Efferent nerves in the airways are controlled by postganglionic, parasympathetic cholinergic neurons and elicit ASM contraction. Changes in their regulation may provoke AHR. A murine model demonstrated that nerve growth factor (NGF) administration by either nasal instillation or genetic engineering elicits similar degrees of AHR as allergen sensitization and challenge, but without attendant airway inflammation [24,25]. In a study using human bronchial biopsies, asthmatic patients exhibited greater cholinergic nerve density relative to healthy controls. Greater tropomyosin receptor kinase B expression in asthmatic airways may drive this difference, which is independent from airway eosinophil levels [26]. Conversely, SARP3 study participants with severe T2-high asthma exhibited greater sputum and AEC levels of brain-derived neurotrophic factor (BDNF; a factor important in neuronal survival) compared to T2-low asthma [27], suggesting that neural dysregulation in asthma may involve multiple pathways, not all specific to paucigranulocytic asthma.

Other mechanisms may explain the uncoupling of AHR and airway inflammation.

Genome-wide association studies have identified chromosome 17q21 as an asthma susceptibility locus. Within this locus, gene polymorphisms associated with overexpression of oromucoid-like 3 (ORMDL3) may underlie paucigranulocytic asthma. ORMDL3 inhibits the rate-limiting step in sphingolipid synthesis, and mice genetically engineered to overexpress ORMDL3 exhibit reduced serum sphingolipids, increased AHR and asthmatic airway remodeling without airway inflammation [28•,29]. Children with 17q21 asthma risk alleles were found to have lower serum sphingolipid levels relative to those with wildtype alleles. Interestingly, T2-low asthmatic children had lower serum sphingolipid levels relative to both T2-high asthmatic and non-asthmatic children [30]. ORMDL3 overexpression may induce paucigranulocytic asthma by upregulating mediators important in airway remodeling but not inflammation [31].

Finally, guanine nucleotide-binding (G)-protein-coupled receptors (GPCRs) are the principal mediators of airway tone. ASM contraction occurs when ligands or spasmogens provoke conformational changes in GPCRs, resulting in G-alpha-q subunit signaling, which causes intracellular calcium increases and myosin light chain phosphorylation. Regulator of G-protein signaling (RGS) proteins modulate GPCRs through signal termination, and several RGS proteins have been implicated in asthma [32]. Peripheral blood mononuclear cells of asthmatic patients and lung tissue in cases of fatal asthma express lower RGS2 protein levels compared to non-asthmatic controls [33]. Interestingly, RGS2 and RGS5 knockout mice spontaneously exhibit AHR to broncho-constrictors independently of airway inflammation [34,35]. Ongoing clinical studies will clarify whether RGS dysregulation is important in paucigranulocytic asthma in humans.

Obesity and metabolism in non-T2 asthma

Obese asthma is recognized as a distinct phenotype typically seen in women with late-onset symptoms, corticosteroid resistance and non-T2 disease. When accompanied by the metabolic syndrome, asthma severity associates with systemic IL-6-mediated inflammation. A SARP3 study observed that exacerbation-prone asthma (defined as having ≥2 asthma exacerbations per year) is a persistent feature in severe asthmatic patients at 3 years of follow-up that associates with obesity and elevated plasma IL-6 levels. Further, plasma IL-6 levels predicted asthma exacerbation risk independently of T2-high biomarkers [36•]. Another connection between obesity and non-T2 asthma relates to a deficiency in the airways of the endogenous bronchodilator nitric oxide (NO) due to increased catabolism by serum arginase of the NO precursor L-arginine [37]. A recent pilot study with obese asthmatic patients demonstrated that dietary supplementation with L-citrulline increased serum L-arginine and airway NO and improved lung function and asthmatic symptoms, particularly in women with late-onset disease [38•]. This potential non-T2 asthma-specific therapy needs confirmation in larger, more rigorous trials.

Transcriptomics and proteomics in non-T2 asthma

A deeper mechanistic understanding of asthma phenotypes can be explored through cluster analysis and transcriptomics. Cluster analysis involves mathematically grouping heterogeneous cohorts through specific characteristics. Transcriptomics analyzes RNA transcripts from cells or tissues. Sputum transcriptomics in U-BIOPRED study participants identified three clusters based on differential gene expression [39]. One transcriptome-associated cluster was characterized by elevated sputum neutrophil numbers and enrichment in inflammasome and tumor necrosis factor superfamily gene transcription. Another was characterized by paucigranulocytic inflammation and was enriched in metabolic pathway gene transcription, including ubiquitination and mitochondrial function. Proteomics is similar to transcriptomics but analyzes protein production rather than RNA transcripts. Two recent studies analyzed both sputum proteomics and cytology. As with the transcriptomics study, this approach also identified a NA phenotype again associated with more severe disease. Both studies identified sputum azurocidin (a protein found in neutrophil azurophil granules) as predictive of NA [40,41]. Identifying additional biomarkers will potentially help with developing endo-type-specific therapies [22•,42,43].

Management and potential therapeutic options

There are currently few effective treatment options for non-T2 asthma, and available ones (i.e. trigger avoidance, vaccination against respiratory pathogens, smoking cessation, and weight reduction in obese asthmatics) are not mechanism-based. Corticosteroids are the cornerstone of asthma controller therapy but non-T2 asthma is typically corticosteroid-resistant. Long-acting muscarinic antagonists, beta-2 adrenergic agonists and oral macrolide therapy may improve non-T2 asthma but are not specifically indicated. Typically, non-T2 asthma is less responsive to available biologics, which target T2-high asthma. Patients with non-T2 asthma and AHR are potential candidates for bronchial thermoplasty [44]. As a

heterogeneous disease with diverse underlying endotypes, identification and characterization of these endotypes is necessary to develop effective treatments for non-T2 asthma.

Conclusion

Despite exciting advances in treatments for T2-high asthma, treatments for non-T2 asthma are limited and specific therapies have largely been disappointing. Future research endeavors should focus on defining practical clinical biomarkers, along with developing more effective therapies.

Acknowledgments

Sources of funding

This work was conducted with the support of grants K23AI125785 to JCC, and by generous contributions by the James A. Haley Veterans' Affairs Hospital in Tampa and the Culverhouse family fund to all authors.

Conflicts of interest statement

DKL reports receiving research support (paid to university) from AstraZeneca and Genentech/Roche; is a consultant of AstraZeneca; is a speaker of AstraZeneca, Genentech/Roche, Meda, Novartis, and Teva; and receives fees for legal opinions regarding drug allergy, metal allergy, radiocontrast reaction, and asthma death.

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Table 1

Potential factors or pathways and their mechanisms implicated in non-T2 asthma

Factors/pathways	Mechanisms	References
Th17 pathway	Secretion of IL-17 and IL-6 with promotion of neutrophil infiltration in the airway	[4,5,9,10•]
Th1 pathway	Secretion of IFN-γ with suppression of secretory leukocyte protease inhibitor associates with AHR development	[12]
NETs	Activation of the inflammasome and secretion of IL-1β, causing airway epithelial injury	[13,15,17•]
Airway dysbiosis	Sputum microbiome is less diverse and dissimilar in bacterial taxa, but underlying mechanism is unclear	[19•,20,21,22•]
Nitric oxide signaling	Decreased levels of NO in the airway of obese asthmatics may result in impaired bronchodilation	[37,38•]
Nerve growth factor	Sensory hyperinnervation contributing to AHR	[24,25]
ORMDL3	Reduced serum sphingolipids contributing to AHR and airway remodeling	[28•,31]
RGS	Reduced termination of GPCR signaling may result in more severe AHR	[32–35]

AHR: Airway hyperresponsiveness; GPCR: G protein-coupled receptor; NET: neutrophil extracellular traps; ORMDL3: Oromucoid-like 3; RGS: Regulator of GPCR signaling.