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Paucigranulocytic asthma: The uncoupling of airway obstruction from inflammation

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Abstract

Among patients with asthma, heterogeneity exists regarding the pattern of airway inflammation and response to treatment, prompting the necessity of recognizing specific phenotypes. Based on the analysis of inflammatory cell count in induced sputum, patients with asthma can be classified in four unique phenotypes; eosinophilic, neutrophilic, mixed granulocytic, and paucigranulocytic asthma (PGA). PGA is an asthma phenotype with no evidence of elevated numbers of eosinophils or neutrophils in sputum or blood, and in which anti-inflammatory therapies are ineffective in controlling symptoms. While under-investigated, PGA is the most common asthma phenotype in patients with stable asthma. However, PGA is sometimes underestimated due to the exclusive reliance on induced sputum cell count which is variable among cohorts of studies prompting the necessity of developing improved biomarkers. Importantly, investigators have reported that inhaled corticosteroids had limited effect on airway inflammatory markers in patients with PGA defining, therefore, PGA as a potentially “steroidinsensitive” phenotype that requires exploration of alternative therapies. PGA manifests as an uncoupling of airway obstruction from airway inflammation that can be driven by structural changes within the airways such as airway smooth muscle (ASM) tissue hypertrophy. Animal models provide evidence that processes evoking airway hyperresponsiveness and ASM thickening occur independent from inflammation and may be a consequence of a loss of negative homeostatic processes. Collectively, further understanding of PGA with focus on the characterization, prevalence, clinical significance and pathobiology derived from animal studies will likely provide precision therapies that will improve PGA clinical outcomes.

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The authors have nothing to disclose

Keywords

Irreversible airway obstruction; airway remodeling; structural cells; airway smooth muscle; steroid insensitivity; asthma phenotypes; asthma endotypes; precision medicine; biomarkers

Background.

Among patients with asthma, heterogeneity exists regarding the pattern of airway inflammation and response to treatment, prompting the necessity of recognizing specific phenotypes (1). Based on the analysis of inflammatory cell count in induced sputum, patients with asthma can be classified into four subtypes (2, 3) (**Table 1**). "Eosinophilic asthma (EoA)" describes patients with elevated sputum eosinophils (>3%) and whose symptoms are controlled by treatments aimed at suppressing eosinophils (4, 5). "Neutrophilic asthma (NeuA)" characterizes patients with elevated sputum neutrophils (≥61%[5] or ≥64%[6]) (4, 6). When sputum eosinophils and neutrophils are both increased, patients can be classified as having a "mixed granulocytic asthma (MGA)". Finally, patients can be determined to have "paucigranulocytic asthma (PGA)" if there is no evidence of elevated sputum eosinophils or neutrophils, and if treatments aimed at suppressing eosinophils and neutrophils are ineffective in controlling symptoms (2, 6). Occasionally, NeuA together with PGA are referred to as "non-eosinophilic asthma (NEoA)" (7). This review addresses current evidence on PGA, with focus on pathobiology derived from animal studies, characterization, prevalence, clinical significance and treatment strategies.

Pathobiology of PGA: Insights from animal studies.

We and others have reported an uncoupling of airway obstruction (AO) from airway inflammation (AI) in several animal studies and demonstrated the role of neuronal factors, non-immunological mediators, signaling molecules and susceptible genes **(Table 2)**. While most animal studies reported a different degree of overlap and interactions between AI and AO, we will focus mainly on animal studies reporting a significant uncoupling of AO from AI.

We and others previously demonstrated that changes in airway smooth muscle (ASM) contractile properties play an important role in the development of AHR associated with chronic airway diseases such as asthma. Indeed, the shortening of ASM in response to G-proteincoupled receptor (GPCR) stimulation regulates airway caliber and bronchomotor tone. Such shortening is initiated by increases in cytosolic Ca^{2+} concentrations and the subsequent activation of the contractile apparatus, including the phosphorylation of myosin light chain $(MLC)_{20}$. Interestingly, a variety of stimuli such as inflammatory cytokines, pollutants, mechanical strain and some therapeutic agents can prime the ASM to become 'non-specifically' hyperresponsive to contractile agonists (comprehensively reviewed in (8)). Collectively, this evidence suggests that ASM contractile function can mediate AHR in chronic airway inflammatory diseases.

Animal-based models suggested an altered neuronal control of ASM contractility as a possible mechanism promoting airway hyperresponsiveness (AHR) uncoupled from AI. For

instance, mice treated with nerve growth factor promoted AHR to a similar extent as allergensensitized mice but without any AI (9) implicating a neurogenic mechanisms in the pathophysiology of AHR. Interestingly, the role of non-immunological mediators has also been proposed in the regulation of AHR uncoupled from AI. We previously showed that ozone acutely induced AHR to carbachol independently from AI, an induction that is insensitive to corticosteroid (CS) treatment (10). This was associated with the release of eicosanoids/oxidized lipids, e.g. 20-HETE directly modulating ASM contractile function (10).

Other studies demonstrated the role of critical signaling molecules in promoting AHR dissociated from AI. The loss of the Regulator of G protein signaling 5 (RGS5) using knockout mice dramatically enhanced constitutive AHR and calcium mobilization in ASM, and had little effect on AI suggesting that i) the dysregulation of pro-contractile G protein-coupled receptor (GPCR) signaling, expressed "selectively" in ASM, promotes AHR, ii) inflammation was not a prerequisite for AHR, and iii) that underlying abnormalities in ASM contribute to asthma diathesis (11). Other studies established the involvement of caveolin-1 (Cav-1), a transmembrane protein acting as a structural scaffold for organization of cytoplasmic signal complexes, in the modulation of AHR uncoupled from AI. Indeed, allergen-challenged Cav-1 knockout mice had an increase in AHR that was independent of inflammation, an effect that was more apparent as the mice aged (12). Further, the contribution of transcription factors involved in cell growth such as early growth response-1 (Egr-1) has been also suggested in the control of AHR uncoupled from AI. Using TGF-alpha-transgenic mice crossed with Egr-1 knockout mice, Kramer and colleagues showed that the lack of Egr-1 markedly worsened TGF-alpha-induced pulmonary disease, evoking AHR to methacholine and no AI (13). A role for sex hormones in inducing AHR without AI was also reported. Using a murine model of allergen-induced AHR, the absence of estrogen receptor-alpha increased AHR without affecting AI after allergen sensitization and challenge (14). *Collectively, these studies provide evidence in animal models that processes evoking AHR and ASM thickening "without" inflammation may be due to the loss of negative homeostatic processes.*

In other studies, mice overexpressing genes present in Chromosome 17q21, a chromosome linked with asthma susceptibility, developed spontaneous increased AHR without AI (15–17). Mice overexpressing Gasdermin B (GSDMB), a gene highly linked to asthma, showed spontaneous increases in AHR and airway remodeling, with increased ASM mass in the absence of AI (16). This was mainly due to GSDMB-induced activation of TGF-β1 and 5lipooxgenase pathways (16). Similarly, mice overexpressing human Orosomucoid-like (ORMDL3), a gene also highly linked to asthma, developed spontaneous increased AHR, ASM mass, subepithelial fibrosis and mucus in the absence of AI (17). This is potentially due to an increase in mediators promoting airway remodeling such as TGF-β1, and a disintegrin and metalloproteinase-8. These molecules selectively activate an unfolded protein response pathway that includes Activating Transcription Factor-6 and its target gene Sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA2b) (17). In support of these observations, precision cut lung slices (PCLSs) derived from hORMDL3Zp3-Cre mice manifested increased airway luminal narrowing through mechanisms involving ASM SERCA2b (15). Of note, hPCLS is an *ex-vivo* alternative to *in vivo* models for studying

basic mechanisms affecting bronchoconstriction. This model provides a novel *ex vivo* system that retains *in situ* structural cell interactions, mast cells, and ciliary beat frequency providing a physiologically relevant platform for understanding how contractile signaling pathways are regulated in vivo. Further, studies using hPCLS recapitulate clinical observations of AHR in the absence of circulating immune cells. Using this model, we identified critical molecular signaling events that promote AHR (11, 18). Collectively, current evidence suggests that AHR can be uncoupled from AI by upregulating the in vivo expression of specific asthma susceptible genes.

Together, these preclinical studies provide insight into the pathobiology of the uncoupling of AO from AI that will likely provide therapeutic targets that will improve PGA outcomes. However, since some discrepancies between animal and human systems exist, caution needs to be taken regarding the extrapolation of findings stemmed from animal studies to human asthma pathobiology. A conceptual model of the pathobiology of PGA that includes additional potential mechanisms is depicted in **Figure 1**.

Characterization/Identification of PGA.

Whereas the EoA and NeuA phenotypes likely reflect ongoing adaptive immune responses to allergens and innate immune responses to environmental triggers respectively, PGA remains poorly understood and its characterization is less well defined (19) (*Table 3*).

Induced sputum.

Analysis of induced sputum is commonly used to characterize asthma phenotypes. As mentioned earlier, PGA lacks elevation in eosinophils and neutrophils above certain thresholds. This needs to be appreciated within the context that variable cut-off points have been proposed to define neutrophilia. Threshold values commonly used for eosinophilia are ≥2% (20, 21), >2% (22) or ≥3% (4, 6, 23–25), of which ≥3% is the most sensitive biomarker of eosinophilic asthma (26). Importantly, eosinophil counts in sputum correlate with eosinophil numbers in bronchial tissue, suggesting that sputum eosinophils may serve as a surrogate for eosinophilic AI (27). Blood eosinophils can also provide a practical alternative to predict sputum eosinophilia, whereas blood neutrophils relate poorly to sputum numbers (23), (28). In contrast to eosinophil counts, to date there is little consensus regarding the cut-off value of sputuminduced neutrophils that defines NeuA as those proposed are extremely variable: >40% (29, 30), ≥50% (31), ≥61% (5), ≥64% (6), >65% (32), and ≥76% (23). Apparently, the higher the threshold value used to define NeuA, the greater the proportion of patients with PGA.

ASM phenotypic changes.

Considering the relative lack of increase in either eosinophils or neutrophils, PGA is considered to be non-inflammatory or at best a syndrome of low grade AI (28) that is associated with ASM dysfunction and AHR (19). In PGA patients, Zhang and colleagues proposed that the lack of increase in airway eosinophils and neutrophils was associated with airway remodeling, a possible consequence of “burnt-out” inflammation: past extensive inflammation having exhausted the pool of inflammatory cells, which ultimately manifests

as a paucity of immunocytes (33). Such patients may have persistent airflow limitation and less variability in their disease (34). Undoubtedly, ASM mass increases, especially in those patients with severe disease (35–38). Thickening of the ASM layer is a pivotal component of airway remodeling that underpins exaggerated airway narrowing in asthma (39). However, the nature of this increased ASM mass remains unclear, but could involve myocyte hyperplasia, hypertrophy, and/or migration, driven by synergistic and additive effects of a number of micro-environmental cues (1). Interestingly, changes in ASM mass may exist independent of inflammation (35). Conceivably, in the absence of eosinophilia and neutrophilia, specific markers directly associated with increased ASM, such as thickening of the subepithelial basement membrane and elevated levels of TGFβ (33), could define PGA (40),(41).

Oxidative stress markers.

Studying disturbances in oxidative stress pathways may be an approach to phenotype PGA. Glutathione (GSH), the main pulmonary antioxidant, maintains the reduced state of protein thiols, mainly through covalent and reversible binding (42). The latter occurs under physiological conditions and is known as S-glutathionylated proteins (PSSG). GSH can be removed from proteins by glutaredoxins (Grx1) that restores the function of proteins targeted by PSSG (43). Evidence suggests that decreased lung function correlates with lower Grx1 and higher PSSG in induced sputum (44). Interestingly, such enzymes are altered in asthma (42). Indeed, sputum PSSG levels were significantly reduced in patients with EoA and NeuA but not in PGA patients (44). In addition, Grx1 protein levels were specifically enhanced in sputum supernatants of patients with EoA and PGA, but not in those with NeuA (44). Arguably, unaltered PSSG and increased Grx1 levels in sputum could specifically define PGA.

Galectin-3 and IL-1RA.

Analysis of sputum revealed reduced levels of galectin-3 (gal-3) that serve to recruit, activate, and remove neutrophils, in NeuA compared to EoA and PGA. In addition, while gal-3 binding protein (gal-3BP) and IL-1β levels were increased, the ratios of gal3/gal-3BP ratio and IL-1 receptor antagonist (RA)/IL-1β were significantly reduced in NeuA compared to EoA and PGA (45). Even though these markers do not discriminate between EoA and PGA, it does provide a provocative framework for exploring pathophysiological mechanisms characterizing unique asthma phenotypes.

MMP and neutrophilic elastase.

Whereas increased matrix metalloproteinase-9 (MMP-9) activity was found in EoA, higher neutrophil elastase activity and inactive MMP-9 levels were observed in NeuA. Within the specific asthma cohorts, an inverse relationship was observed between active MMP-9 (characteristic of EoA) and active neutrophil elastase (characteristic of NeuA) (3, 46). In PGA patients, however, levels of active MMP-9 and neutrophils elastase were unchanged compared to heathy controls (46).

Omics markers.

Analysis sputum transcriptomics may hold promise in identifying different asthma phenotypes. For instance, Baines and colleagues subjected whole-genome gene expression profiles from induced sputum of adults with stable asthma to unsupervised hierarchical cluster analysis (47). Interestingly, three distinct Transcriptional Asthma Phenotypes (TAPs) were identified that had similarities to previously defined sputum inflammatory phenotypes of EoA (TAP1), NeuA (TAP2), and PGA (TAP3) (47). In addition to transcriptomics, proteomics have recently been explored as a tool in phenotyping asthma. A large study by the Severe Asthma Research Program investigators focused on 18 cytokines detectable in bronchoalveolar lavage (BAL) and discriminated mild-to-moderate and severe asthmatic groups based on cytokine expression and its association with methacholine responsiveness (48). Whether PGA can similarly be identified using a specific protein profile in BAL remains, however, to be determined. Other studies employed protein microarray platforms using induced sputum and revealed differentially increased inflammatory protein markers among asthma phenotypes (49). However, the focus in this study was on phenotypes displaying either increased eosinophils and/or neutrophils and therefore, further analysis on markers relevant to PGA is warranted.

Fractional Exhaled nitric oxide (FeNO) and AHR.

FeNO levels can phenotype patients with asthma. Porsbjerg and colleagues showed that NeuA was associated with low levels of FeNO, whereas patients with PGA had levels of FeNO lower than those with EoA, but comparable to those with mixed granulocytic asthma (MGA) (50). Interestingly, when AHR response to mannitol was determined, similar results were observed: low degree of AHR in NeuA, moderate and comparable response in PGA and MGA, and highest degree of AHR was observed in EoA. These findings support that further sub-classification of asthma subtypes can be useful, as marked differences in FeNO and AHR were observed among these phenotypes (50).

Prevalence and Clinical Significance of PGA (Table 4).

Up to 50% of patients with asthma have a non-eosinophilic (NEoA) phenotype (7, 23, 29, 51). Different threshold values applied to determine sputum cell counts, as discussed earlier, may explain the variation in prevalence of different asthma subtypes. A large retrospective study conducted in 508 patients with asthma (cut-off values for eosinophils and neutrophils were ≥3% and ≥76%, respectively) revealed while 16% of patients were of the neutrophilic phenotype, the majority of asthma patients manifest an eosinophilic (42%) and paucigranulocytic (40%) phenotype (23). Similarly, in a study population of 93 patients with asthma (cut-off values for eosinophils and neutrophils were ≥1% and ≥61%, respectively), 31% of patients were identified as having PGA, 41% as having EoA, and 20% as having NeuA (3). An even higher proportion of NEoA phenotype (50%) was found in a multicenter study that enrolled 995 patients with asthma (21). More recently, a study conducted in 240 subjects revealed that 47.9% had PGA, while 40% manifested an eosinophilic subtype (2). Collectively, these studies suggest that PGA and EoA are the most common asthma phenotypes.

Wang and colleagues studied the relative frequency of asthma phenotypes (cut-off values for eosinophils and neutrophils were >3% and >61%, respectively) in stable and acute asthma in adults and children (52). In adults with stable asthma, the most frequent asthma phenotype was PGA (51.7%) followed by NeuA (27.6%). In children with stable asthma, the most frequent inflammatory phenotype was also PGA (49%) but was followed by EoA (28.6%). In contrast, acute exacerbations of asthma were predominantly eosinophilic in children (50%) and neutrophilic in adults (81.8%). Surprisingly, acute asthma was not associated with PGA in children or adults. Collectively, these findings suggest that PGA represents the most common asthma phenotype in stable asthma (52). Since these studies are a snapshot in the life cycle of asthma, it is possible that PGA, represents a cross sectional view related to disease activity rather than a stable phenotype. Further longitudinal studies, however, are needed to examine the stability of this phenotype.

Whether PGA is associated with asthma severity remains unclear. Evidence suggests that airway granulocytic inflammation is a common finding in severe asthma (53), (54). Since PGA manifests no sputum eosinophilia or neutrophilia, one may posit that PGA can manifest as a mild disease. Supporting this hypothesis, investigators showed that PGA patients had better lung function based on post-bronchodilation FEV1 (% predicted) and FEV1/FVC ratio as compared to patients with other asthma phenotypes (2). Further, PGA patients expressed lower levels of inflammatory markers in exhaled air and sputum, and severe refractory asthma occurred less frequently in PGA than in EoA and MGA (2). Similarly, others demonstrated that lung function was less altered in patients with PGA, while EoA phenotype patients exhibited higher FeNO levels, higher AHR and lower asthma control (23). Despite the fact that PGA was related to better lung function, a substantial proportion of patients with PGA (21.7%) was characterized as having severe refractory asthma and 14.8% of patients with PGA had an asthma control test score less than 19, suggesting that this subpopulation of PGA is not well controlled despite the absence of inflammatory cells in their sputum (2). Surprisingly, this latter cohort (i.e. PGA patients with severe refractory asthma), presented higher levels of FeNO, and sputum IL-13 and IL-8 compared to those with mild/moderate asthma (2). The presence of surrogate inflammatory markers in the absence of any granulocytic infiltration is intriguing and supports the need for a more comprehensive approach in the evaluation of AI in PGA patients with severe refractory asthma in order to develop adequate therapeutic strategies.

Importantly, airway remodeling is significantly more pronounced in patients with severe asthma than in patients with mild asthma (55). Evidence suggests that airway remodeling is relatively insensitive to ICS, as compared to allergen-induced airway inflammation (56, 57). Since airway remodeling represents a prominent histopathological finding of PGA, we speculate that PGA is more common in patients with severe asthma than patients with mild asthma and may contribute to inadequate ICS responsiveness seen in patients with severe asthma. Additional studies, however, are still needed to further explore the distinct difference of PGA in severe and mild asthma.

PGA: Current and Potential Future Therapeutic Strategies.

The identification of different asthma phenotypes based on sputum cell counts has supported the development of a variety of therapeutic strategies to control asthma symptoms.

Inhaled corticosteroids (ICS).

ICS are particularly effective against Th2-driven inflammation featuring mast cell and eosinophilic airway infiltration. Indeed, numerous studies in asthma revealed that ICS effectively reduces the percentage of sputum eosinophils (58, 59), represses the release of Th2 cytokines from lymphocytes (60) and eotaxin from epithelial cells (61). Unfortunately, ICS' effects on innate immunity driven neutrophilic inflammation is rather poor (19).

Studies in a large asthma cohort (n=995) reported that in repeated measures analyses of patients with asthma not taking ICS, 22% of subjects had sputum eosinophilia on every occasion (persistent eosinophilia); 31% had eosinophilia on at least one occasion (intermittent eosinophilia); and 47% had no eosinophilia on every occasion (persistently non-eosinophilic) (21). Strikingly, anti-inflammatory therapy caused significant improvements in airflow obstruction in EoA, but not in persistently NEoA (21). Importantly, Ntontsi and colleagues provided strong evidence that such NEoA (which includes NeuA and PGA) represents a phenotype that does not benefit from CS treatment and that the CS dosing and adherence is not an explanation for poor CS responsiveness in this subgroup (2, 21). Accordingly, others compared the effects of ICS on sputum cell counts in PGA (28) and showed no significant differences between patients treated and untreated with ICS (28). Collectively, these studies suggest that ICS had limited effects on airway inflammatory markers in patients with PGA, thus defining PGA as a potentially "CS-insensitive" phenotype where the exploration of alternative therapeutic interventions is needed.

Therapies targeting airway remodeling.

Since granulocytic infiltration is not observed in PGA patients, symptoms in these patients may be primarily driven by ASM phenotypic changes or neuronal dysfunction. Thus, therapies directed towards ASM such as mast-cell directed therapies or in the most severe cases, bronchial thermoplasty, may benefit these patients. Although the mechanism of action is uncertain, bronchial thermoplasty aims to decrease ASM mass through the delivery of localized thermal energy (62, 63). Bronchial thermoplasty is indicated when AHR is severe ($PC20 < 0.25$) and when frequent exacerbations persist despite absent or controlled airway inflammation (62); this treatment may benefit the PGA subpopulation with severe refractory asthma. Since ICS-insensitive airway remodeling, the main driving component of PGA pathogenesis, is more prominent in patients with severe asthma than in patients with mild asthma, it is plausible that therapies such as bronchial thermoplasty aimed at targeting airway remodeling will be more beneficial in PGA patients with severe asthma than patients with mild asthma. Additional remodeling treatment options that could also be beneficial to this subpopulation were comprehensively reviewed elsewhere (64–66).

Future Perspectives.

The identification of PGA as a common phenotype in stable asthma both in adults and children has critical impact for future asthma research. Since surrogate inflammatory markers are present in the absence of any granulocytic infiltration, it is evident that in patients with PGA, other features than granulocytic infiltration should be investigated such as inflammatory mediators generated by airway structural cells such as ASM cells. Even though currently no drug convincingly reverses airway remodeling, non-pharmacological intervention by means of bronchial thermoplasty may benefit patients who suffer from severe refractory disease and are insensitive to current mainstay treatment options. Interestingly, previous studies identified a subgroup of patients with asthma insensitive to ICS treatment. Whether such CS insensitivity is caused by or a consequence of PGA, requires further investigation. While there are no compelling data to show that ICS are less effective in treating patients with PGA, we believe that ICS are less effective based on the pathogenesis of PGA where i) airway inflammation, the main target of ICS, is modest or absent in patients with PGA and ii) airway remodeling, which drives most of the pathogenesis of PGA, is relatively insensitive to ICS.

Since ICS increases airway neutrophils, we posit that ICS treatment of patients with PGA may increase airway neutrophils and switches asthma phenotype from PGA to NeuA, a possibility that also warrants further investigation. Since PGA represents a common phenotype in adults and children, studies aimed at identifying the molecular heterogeneity of PGA and its pathogenesis will likely provide precision therapies that will improve clinical outcomes of this disease.

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Abbreviations:

5-LOX	5-Lipoxygenase
20-HETE	20-Hydroxyeicosatetraenoic acid
Ach	Acetylcholine
ADAM8	A Disintegrin and metalloproteinase-8
AHR	Airway hyperresponsiveness
AI	Airway inflammation
AO	Airway obstruction
ASM	Airway Smooth Muscle
ATF6	Activating Transcription Factor-6

BAL	bronchoalveolar lavage
Cav-1	Caveolin-1
ECM	extracellular matrix
EGFR	Epidermal growth factor receptor
Egr-1	Early Growth Response-1
EoA	Eosinophilic Asthma
ER-alpha	Estrogen Receptor alpha
FeNO	Fractional exhaled nitric oxide
Gal-3	Galectin-3
Gal-3BP	gal-3 binding protein
GPCR	G Protein Coupled Receptors
Grx1	glutaredoxins
GSDMB	Gasdermin B
GSH	Glutathione
ICS	inhaled corticosteroid
IL	Interleukin
IL-1RA	Interleukin-1 Receptor Antagonist
M_2R	Muscarinic Receptor 2
M_3R	Muscarinic Receptor 3
MCP-1	Monocyte Chemoattractant Protein 1
MGA	mixed granulocytic asthma
MMP9	matrix metalloproteinase-9
MUC5AC	Mucin 5AC Oligomeric Mucus/Gel-Forming
NE	neutrophil elastase
NeuA	Neutrophilic Asthma
NGF	nerve growth factor
Orai1	Calcium release-activated calcium channel protein 1
ORMLD3	Orosomucoid-like-3
PCLS	precision cut lung slices

PGA	Paucigranulocytic Asthma
PSSG	S-glutathionylated proteins
RGS5	Regulators of G Protein Signaling-5
SERCA2b	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2b
TAPs	Transcriptional Asthma Phenotypes
TGFβ	Transforming growth factor beta

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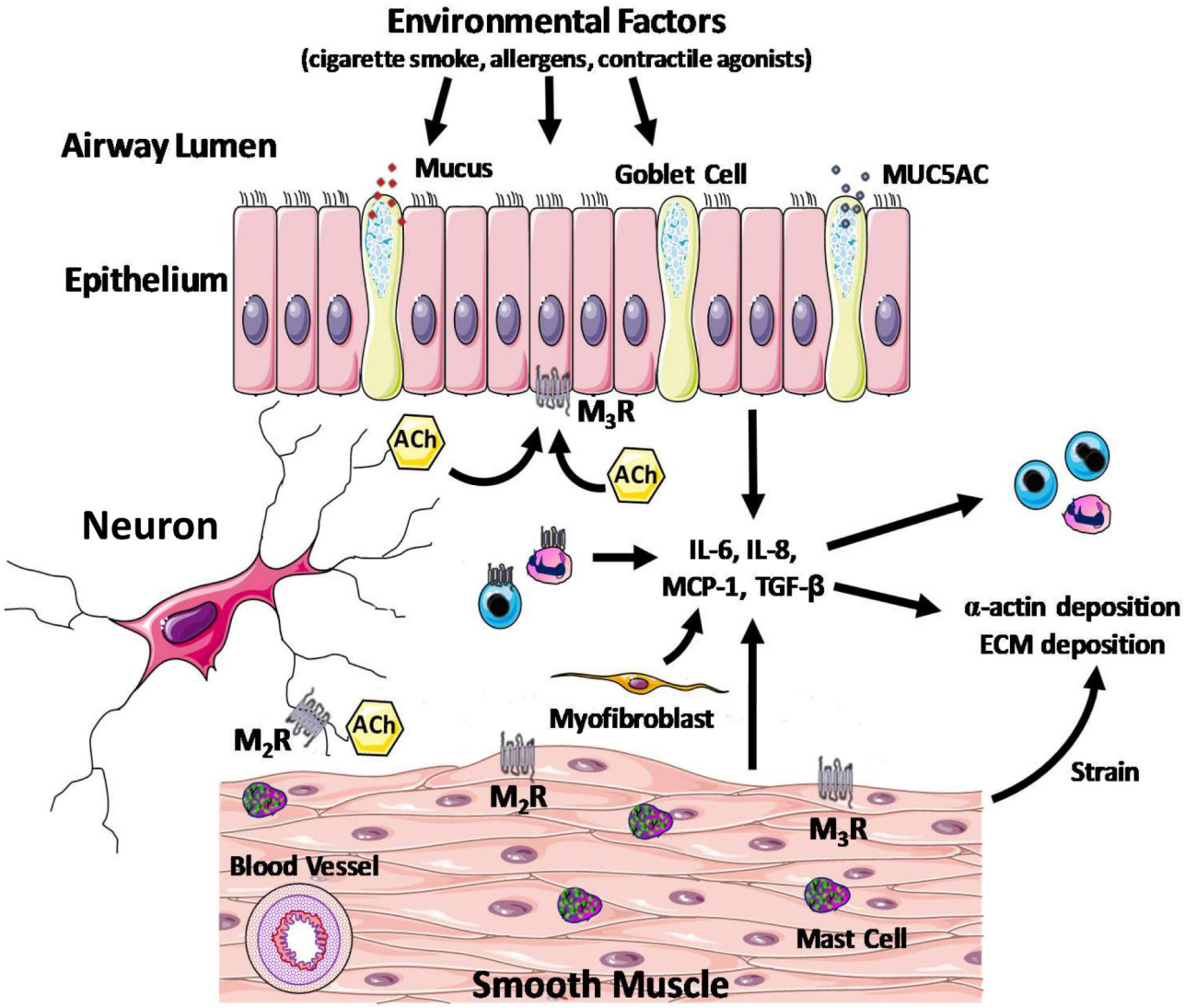

Figure 1: A conceptual model of the pathobiology of paucigranulocytic asthma (PGA).

A variety of triggers induce alterations in structural cell function (myofibroblasts, epithelial, smooth muscle and mast cells) that engenders airway hyperresponsiveness. Typically, patients with PGA manifest insensitivity to corticosteroid therapy. In a corticosteroid-insensitive manner, structural cells also modulate airway inflammatory responses such as chemokine and cytokine secretion and leukocyte trafficking. **MUC5AC**: Mucin 5AC Oligomeric Mucus/Gel-Forming; **Ach**: Acetylcholine; **M_2R**: Muscarinic Receptor 2; **M_3R**: Muscarinic Receptor 3; **IL**: Interleukin; **MCP-1**: Monocyte Chemoattractant Protein 1; **TGFb**: Transforming growth factor beta; **ECM**: extracellular matrix.

Table 1.

Asthma phenotypes.

Asthma phenotypes	Eosinophilic		Non-Eosinophilic	
	Eosinophilic	Mixed Granulocytic	Neutrophilic	Paucigranulocytic
Sputum Eosinophils*	>3%	>3%	<3%	<3%
Sputum Neutrophils*	<61% or <64%	>61% or >64%	>61% or >64%	<61% or <64%
Response to treatment**	+	+/−	-	-

*Commonly accepted thresholds;

**treatment aimed at suppressing eosinophils.

Table 2.

Potential mechanisms underlying the uncoupling of airway obstruction from airway inflammation in animal studies.

Factors		Mechanisms mediating AHR increase	References
Neuronal Factors	*Nerve Growth factor*	*Alters ASM neuronal control*	9
Non-lmmunological Mediators	*Eicosanoids/Oxidized lipids: 20-HETE*	*Enhances ASM contractility*	10
Critical Signaling Molecules	*Regulator of GPCR: RGS5*	*Its absence enhances calcium mobilization*	11
	Transmembrane proteins: Cav-1	*Its absence induces TGFβ1 and Orail expressions*	12
	Growth transcriptional factors: Egr-1	*Its absence worsens TGFa-induced pulmonary pathology via EGFR-dependent pathways*	13
	Sex hormone receptors: ERa	*Its absence upregulates M_2R*	14
Susceptible asthma genes present in chromosome 17q21	*GSDMB*	*Activates TGFβ1 and 5-LOX pathways*	16
	ORMDL3	*Induces TGFβ1 and ADAM8 dependent activation of ATF-6 and SERCA2b*	15,17

ASM: Airway Smooth Muscle;

20-HETE: 20-Hydroxyeicosatetraenoic acid;

GPCR: G Protein Coupled Receptors;

RGS5: Regulators of G Protein Signaling-5;

Cav-1: Caveolin-1;

TGFβ1: Transforming Growth Factor Beta 1;

Orai1: Calcium release-activated calcium channel protein 1;

Egr-1: Early Growth Response- 1;

EGFR: Epidermal growth factor receptor;

ERα: Estrogen Receptor alpha;

GSDMB: Gasdermin B;

5-LOX: 5-Lipoxygenase;

M_2R: Muscarinic receptor 2;

ORMLD3: Orosomucoid-like-3;

ADAM8: A Disintegrin And Metalloproteinase-8;

ATF-6: Activating Transcription Factor-6;

SERCA2b: Sarcoplasmic/endoplasmic reticulum calcium ATPase 2b.

Table 3.

Potential markers discriminating asthma phenotypes.

Asthma Phenotypes Potential Markers		EoA	NeuA	PGA
Oxidative Stress enzymes*	PSSG	*Reduced*	*Reduced*	*Unaltered***
	Grx1	*Enhanced*	*Unaltered***	*Enhanced*
Gal-3 & Gal-3BP	Gal-3	*Higher than NeuA*	*Lower than EoA and PGA*	*Higher than NeuA*
	Gal-3BP	*Lower than NeuA*	*Higher than EoA and PGA*	*Lower than NeuA*
IL1RA & IL1 p	I LIRA	*Higher than NeuA*	*Lower than EoA and PGA*	*Higher than NeuA*
	IL1β	*Lower than NeuA*	*Higher than EoA and PGA*	*Lower than NeuA*
MMP9 & NE	MM9	*High*	*Low*	*Unaltered***
	NE	*Low*	*High*	*Unaltered***
FeNO & AHR	FeNO	*High*	*Low*	*Moderate*
	AHR	*High degree*	*Low degree*	*Moderate degree*

EoA: Eosinophilic Asthma;

NeuA: Neutrophilic Asthma;

PGA: Paucigranulocytic Asthma;

PSSG: S- glutathionylated proteins;

Grx1: glutaredoxins;

Gal-3: Galectin-3;

Gal-3BP: gal-3 binding protein;

IL-1RA: Interleukin-1 Receptor Antagonist;

MMP9: matrix metalloproteinase-9;

NE: neutrophil elastase;

FeNO: Fractional exhaled nitric oxide;

AHR: airway hyperresponsiveness to Mannitol;

*Measured in sputum;

**unaltered compared to healthy subject.

Table 4.

Clinical characteristics associated with PGA patients in comparison to EoA patients:

Clinical characteristics	PGA versus EoA
FEV_1%	Similar (3) or higher (2, 23)
Atopy	Similar (3) or lower but still significantly present (2, 23)
Symptoms, emotions and activity	Similar (23)
Airway hyperresponsiveness (AHR)	Lower (23, 51)
Body Mass Index (BMI)	Lower (29)

EoA: Eosinophilic Asthma;

PGA: Paucigranulocytic Asthma;

AHR: airway hyperresponsiveness;

BMI: Body Mass Index;

FEV_1: Force Expiratory Volume during the first second.