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Mechanisms and biomarkers of airway epithelial cell damage in asthma: a review

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Abstract:

Bronchial asthma is a heterogeneous disease with complex pathological mechanisms representing different phenotypes, including severe asthma. The airway epithelium is a major site of complex pathological changes in severe asthma due, in part, to activation of inflammatory and immune mechanisms in response to noxious agents. Current imaging procedures are unable to accurately measure epithelial and airway remodeling. Damage of airway epithelial cells occurs is linked to specific phenotypes and endotypes which provides an opportunity for the identification of biomarkers reflecting epithelial, and airway, remodeling. Identification of patients with more severe epithelial disruption using biomarkers may also provide personalized therapeutic opportunities and/or markers of successful therapeutic intervention. Here, we review the evidence for ongoing epithelial cell dysregulation in the pathogenesis of asthma, the sentinel role of the airway epithelium and how understanding these molecular mechanisms provides the basis for the identification of candidate biomarkers for asthma prediction, prevention, diagnosis, treatment and monitoring.

Keywords: asthma; airway epithelial cells (AECs); pathogenesis; immune regulation; biomarkers

Introduction:

Bronchial asthma, or asthma for short, is a chronic disease of the airway characterized by symptoms such as respiratory distress, chest tightness, wheezing, coughing, sputum formation, and exercise intolerance. The asthmatic airway is chronically inflamed due to the activation and/or recruitment of a variety of tissue resident and infiltrating cells including eosinophils, mast cells, T lymphocytes, macrophages, airway epithelial cells (AECs), fibroblasts and airway smooth muscle (ASM) cells. The AEC, which sits at the interface between the host and the external environment, is not only an efficient physical barrier but also represents the first line of defence against microorganisms, airborne irritants and allergens [1]. The airway epithelium maintains the health of the respiratory tract mucosa through its physical barrier function, cilia removal function and natural immune defense function. It plays a central role in the pathogenesis of asthma. On the one hand, changes in its structure and function directly affect the inflammatory response and promote disease formation. On the other hand, the airway epithelium is also significantly damaged by inflammation. At present, the epithelial barrier has been placed in the forefront of the pathophysiology of airway inflammation. Epigenetic studies have confirmed that airway epithelial damage involves structural and functional changes and plays an important role in the pathogenesis of asthma[2]. Therefore, a variety of methods for diagnosing and targeting epithelial barrier defects have emerged. In addition, the development of single-cell transcriptome technology is gradually revealing the new face of airway epithelium.

The current diagnosis of asthma mainly depends on the patient's clinical symptoms, lung function, bronchial challenge and variability in peak expiratory flow (PEF). However, some patients are not suitable for lung function tests because of pulmonary bullae, cardiac insufficiency, bronchodilator allergy [3], low lung function, suboptimal effort, active bleeding, etc. Over the years, clinicians have defined several different phenotypes based on the patient's symptoms, age of onset, severity of the disease, and the presence of other conditions, such as allergies, and also biochemical features including sputum or blood eosinophilia. Despite recognizing these phenotypes of asthma, the asthma management

method recommended by the International Asthma Global Initiative (GINA) guidelines is still based on the severity of the disease, using a tiered treatment plan, which is to add drugs on the basis of asthma control. The development of the concept of precision medicine with the goal of individualized treatment has emphasized the need for improved biomarkers of asthma phenotypes, sub-phenotypes and endotypes.

Therefore, researchers have investigated the expression of numerous markers such as eosinophilic cationic protein, exhaled nitric oxide, 8-isoprostane, leukotrienes and periostin in sputum, exhaled breath condensate (EBC) and peripheral blood of asthma patients in an attempt to identify a suitable specific biomarker [4]. Most of these markers were pre-selected and aimed at monitoring asthma status and guiding medication by reflecting the level of airway inflammation and did not necessarily act as an early warning signal of AEC damage in the early stages of asthma. In view of the fact that the airway epithelium is the core part of the local immune response, it connects innate and acquired immune functions, resists all types of harmful invaders from entering the respiratory system, and is at the center of the process leading to the formation, development and acute exacerbation of asthma. The media derived from airway epithelium may provide resources for the study of asthma biomarkers.

1. Structure and function of the airway epithelium:

At least ten epithelial cell lineages exist across the upper and lower airways and lung parenchyma [5]. Single cell RNA-sequencing analysis of bronchial biopsies from healthy subjects revealed that AECs consist of basal cells, club cells, ciliated cells, goblet cells, type 1 and type 2 alveolar cells, and rare but highly specialized cells (e.g. neuroendocrine cells, Tuft cells, microfold (M) cells), and the recently described ionocytes. The larger, proximal airways feature a pseudostratified columnar epithelium, in which all cells contact the basement membrane, while in smaller airways, the epithelium becomes columnar and cuboidal [6]. Viera-Braga and colleagues [5] mapped the cellular landscape of the lower airways and found an additional 4 cell states, including mucous ciliated cells, activated basal

cells, cycling cells and serous cells from the submucosal glands, in asthma patients. Moreover, the airway wall tissue has increased numbers of goblet cells, intraepithelial mast cells, and pathogenic effector Th2 cells. However, analysis of the intercellular communication between healthy and asthmatic airway walls reveals a remarkable loss of structural cell communication and a concomitant increase in Th2 cell interactions.

1.1. Physical barrier function: The airway epithelium is the first physical barrier against inhaled harmful stimuli from the external environment. AECs link the intracellular skeletal structure with the cytoskeleton structure of its neighboring cells to form structural adhesion of airway mucosa and maintain a complete barrier[7]. This connection is mainly achieved through three types of intercellular epithelial connections, including adhesion junctions (AJ), hemidesmosomes and tight junctions (TJ). TJ is composed of transmembrane proteins of claudin family, occludin, tricellulin and connection adhesion molecules, which are connected to actin filaments through cingulin and ZO proteins 1,-2,-3[8]. It is the most apically located intercellular junctions and regulates the permeability of the cells[7]. The AJ located directly below the TJ, composed of a cadherin-catenin complex, connects the actin filaments of the adherent cells, and performs a variety of functions, such as regulating the cytoskeleton and signal transduction to maintain the integrity of the epithelium[9]. Similarly, hemidesmosomes can attach the epithelial layer to the basement membrane. These intercellular and intracellular connections regulate airway epithelial permeability, cell proliferation, differentiation, cell repair and barrier function. In asthma patients, related functions have been found to be impaired due to epithelial destruction.

1.2. Biochemical barrier system: The mucus layer formed by submucosal glands, epithelial cell secretions and tissue exudates contains immune factors such as anti-proteases, antioxidant factors, antibacterial peptides including defensins and cathelicidins and mucins that together exert antimicrobial activity against bacteria, fungi and certain viruses [10]. The

inhaled particulate matter adheres to the mucus layer whilst the ciliated cells move rhythmically within the serous layer to enable mucociliary clearance. The proteins that make up mucus have a strong capacity to absorb water and can form a gel-like structure. Most mucin genes are constitutively expressed at low levels, however, under diverse pathological conditions their expression is rapidly and dramatically increased leading to significant hypersecretion of mucus and/or compositional changes and thus altering the physical properties of the mucus [11, 12]. Among them, the most prominent changes are the up-regulation of MUC5AC and the down-regulation of MUC5B. In particular, patients with eosinophilic type 2 asthma phenotype showed a shift in proportion to higher MUC5AC concentrations[13, 14].

1.3. Innate immune defense function: Furthermore, the airway epithelium is also a central participant in innate and adaptive immunity. AECs express many pattern recognition receptors (PRRs) (Figure 1) which rapidly detect and respond to internal or external environmental agents, pathogen-associated molecular patterns (PAMPs) found in microbes and damage-associated molecular patterns (DAMPs) released upon tissue damage, cell death or cellular stress [15]. PRRs include Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectins and protease-activated receptors. Upon recognition of PAMPs or DAMPs, PRRs activate downstream signaling pathways that promote the release of pro-inflammatory cytokines/chemokines including interleukin (IL)-6, IL-8, CCL20, CCL17, thymic stromal lymphopoietin (TSLP), IL-25, IL-33 and granulocyte-macrophage colony-stimulating factor (GM-CSF). These, in turn, attract and activate a wide range of cell types important in innate and adaptive immune responses [16].

1.4. Novel, rare airway epithelial cells:

Novel epithelial cell types are being identified using single cell RNA sequencing that might play a role in chronic airway disease. Neuroendocrine cells are strategically located at the

branch of the airway where allergens and other harmful substances accumulate [17]. They serve as airway chemoreceptors that monitor airway status and release calcitonin gene–related peptide, which activates group 2 innate lymphoid cells (ILC2s) that further promote Th2 allergic responses [18, 19].

Tuft cells and M cells are highly specialized cell types within the bronchial epithelium. Tuft cells are involved in chemo-sensing of luminal signals and the initiation/regulation of immune responses [20]. In contrast, M cells sample antigenic structures and enable their transfer to lymphoid structures in the airways [21].

Ionocytes express high levels of the cystic fibrosis transmembrane conductance regulator (CFTR), which regulates TJ assembly and epithelial cell differentiation [22]. Ionocytes are the major source of CFTR suggesting its crucial role in regulating epithelial barrier function.

Above all, the airway epithelium is not only a physical barrier, it is also a key sensor and integrator of the surrounding environment that undergoes precise and strict regulation. It maintains the integrity of the immune system and the steady state of the airway microenvironment by well-balanced and coordinated activities of its cellular and biochemical components [10].

2. The role of airway epithelium in different asthma phenotypes:

In 2009 the GINA proposed the concept of asthma phenotypes. Current researches on asthma phenotypes mainly focus on two aspects namely different inflammatory phenotypes and how they may link to previously described clinical phenotypes [23]. Inflammatory phenotypes are based on the type of granulocytic cells present in induced sputum and is divided into eosinophilic, neutrophilic, mixed granulocytic and pauci-granulocytic, of which eosinophilic type is the most common. Clinical phenotyping uses multiple clinical variables including age, gender, age of onset, BMI, symptoms, atopic status, and lung function tests to cluster patients [23]. More recently, there have been combined approaches undertaken whereby the gene

expression profiles determine whether specific genes or pathways are associated with clinical phenotypes [24].

There are many types of clinical phenotypes and GINA lists some of the most common phenotypes including allergic, non-allergic, late-onset, fixed airflow limitation and obese asthma [https://ginasthma.org/]. Additional asthma endotypes have also been proposed reflecting increased knowledge regarding asthma pathogenesis. However, these endotypes are still be broadly regarded as either type 2-high ($T2_{high}$) and type 2-low ($T2_{low}$) [25]. It is evident that the current status of asthma phenotypes and endotypes is complex with overlaps and subtypes present. This may reflect the following problems: (1) The evaluation standards adopted by researchers are different, and the conclusions drawn are also very different, so that there is no unified standard. (2) Asthma phenotypes sometimes overlap, which makes it difficult to distinctly classify affected patients [26]. (3) The use of cross-sectional data that does not indicate stability over time or with treatment. (4) The impact of respiratory tract infections and allergen exposures on the airway inflammation phenotype.

In general, the current understanding of asthma has increasingly highlighted the characteristics of different phenotypes, endotypes and heterogeneity, which helps to clarify its underlying immunological mechanisms and pave the way for the development of targeted biological treatments.

2.1. Type 2 (T2)-High asthma:

Much of the currently available knowledge regarding the contribution of epithelial cells to asthma comes from investigating its endotype. Eosinophilic, $T2_{high}$ airway inflammation is present in around 50% of adults with asthma and 37% of severe asthma, and atopy is present in 50–60% of adults and children with asthma [27, 28]. $T2_{high}$ asthma phenotypes include three groups namely, early-onset allergic asthma, late-onset eosinophilic asthma, and aspirin-exacerbated respiratory disease. The airway epithelium is a dynamic orchestrator of the immune responses in $T2_{high}$ asthma. It responds rapidly to external stimuli with release

of cytokines such as IL-25, IL-33 and TSLP, which are central regulators of T2 immunity and drive a broad array of allergic responses [29]. (Figure 1). They are described as "alarmins", alerting the immune system to external insults and regulating tissue restoration and repair after injury.

While IL-33 and IL-25 mainly activate ILC2s, TSLP also primes antigen-presenting cells (APCs), typified by dendritic cells (DCs), to promote type 2 immunity by activating T cells and B cells [25]. Tuft cells are the main producers of IL-25 in the airways, suggesting a specific role of this cell type in the control of T2 immune mechanisms [30]. IL-33 and its receptor (IL1RL1 or ST2) are both related genetically to atopic asthma particularly in children [31]. Research has shown that a TSLP/ILC axis may also mediate steroid resistance in asthma and the TSLP-STAT5 pathway could be a new therapeutic target in severe, corticosteroid-resistant asthma. [32]. After allergen sensitization and consequent activation of DCs, these alarmins activate ILC2s and adaptive Th2 cells releasing IL-4, IL-5 and IL-13. Of note, ILC2s produce 10-fold more IL-5 and IL-13 compared with activated Th2 cells [33] suggesting that ILC2s are main source of these cytokines in the airway and explaining the relative paucity of ILC2 cells in the human asthmatic respiratory tract. IL-5 is a vital cytokine for the survival and maturation of eosinophils, and also supports the development of mast cells and basophils. IL-4 drives B-cell isotype switching, IgE synthesis, Th2 cell differentiation and production of downstream cytokines including IL-5 and IL-13. In addition, IL-13 and IL-4 promote goblet cell overexpression, increased mucus secretion, as well as airway hyperresponsiveness [25].

The expression of these epithelial-derived T2 cytokines is significantly increased in the airways of asthmatics and related to the severity of the disease. This suggests that they may be useful biomarkers of asthma and also therapeutic targets. The expression of IL-33 in the sputum and blood of asthmatics was higher than that of healthy controls and positively correlated with asthma severity [34, 35]. An asthma patient who has IL-25 mRNA levels that are above the 95th percentile in normal control AECs is defined as "IL-25_{high}". These asthmatics are more sensitive to skin allergy testing and exhibit higher eosinophilia, higher

IL-13 and greater airway hyperresponsiveness to methacholine challenge supporting the concept that they have a more severe T2 asthma phenotype [36]. In a phase 2 study, the neutralizing antibody against TSLP (tezepelumab, AMGl57) demonstrated a significant reduction in the annual asthma exacerbation rate compared with placebo in patients with severe uncontrolled asthma [37]. And, starting from the fourth week, Tezepelumab can also reduce T2 inflammation markers including blood eosinophils, IgE and FeNO levels. This antibody is now in a phase 3 multicenter, randomized, double-blind, placebo controlled, parallel group trial (NAVIGATOR, NCT03347279). A humanized anti–IL-33 IgG1 antibody Etokimab has completed phase I and phase IIa trials whilst antibodies against IL-25 are under development but have not yet entered clinical trials [29].

2.2. Non-$T2_{high}$ ($T2_{low}$) asthma:

The mechanisms that contribute to pathogenesis of $T2_{low}$ asthma are not yet clear, but it represents an existing unmet need, and there are few treatment methods and biological agents for it. It is a non-eosinophilic asthma without type 2 inflammation markers and patients with this phenotype usually present with late-onset asthma [38] and have a poor response to inhaled and oral corticosteroids [39]. Existing knowledge suggests that AECs are involved in the pathogenesis of $T2_{low}$ asthma, mainly through the interaction with Th17 cells and the secretion of mediators such as IL-6, IL-8 and leukotriene B4 (LTB4). It is possible that epithelial damage inflicted by nonallergic stimuli such as bacterial and viral infections, smoking and exposure to environmental/occupational air pollutants may lead to the establishment of a delicately balanced Th1/Th17 milieu in the airways[40].

According to the type and number of cells in the sputum specimen, $T2_{low}$ asthma can be further divided into neutrophils and paucigranulocytic subtypes. T helper 1 (Th1) and T helper 17 (Th17) cells, neutrophils and proteins including IL-1ß, IL-6, IL-8, IL-17A/F, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) are implicated in its pathobiology [41]. In addition, the abnormal structure of ASM and neuronal activation may also be

involved. The underlying mechanism leading to neutrophil airway inflammation is mainly related to the activation of Th17 and Th1 cells, which secrete IL-17, IL-21, IL-22 and IFN-γ, playing a major role in neutrophil asthma. IL-17A and IL-17F are up-regulated in the lungs of asthma patients, which is related to the severity of asthma and steroid resistance [42]. And increased levels of IL-17 in sputum and serum of patients with severe asthma have been proved to be independent risk factors for severe asthma[43, 44].

AECs release IL-6 and IL-8 under the induction of IL-17 to promote the migration of neutrophils, induce mucous cell metaplasia and increase the quality of ASM, causing airway narrowing[45]. IFN-γ-induced downregulation of secretory leukocyte protease inhibitor (SLPI) in AECs causes an increase in airway hyperresponsiveness (AHR) in severe asthma [46]. As a pro-inflammatory cytokine, IL-6 has elevated levels in the serum[47], bronchoalveolar lavage fluid (BALF) [48], and sputum IL-6 concentrations in severe asthma are inversely correlated with FEV1[49].

Paucigranulocytic asthma(PGA) is considered non-inflammatory and is mainly related to ASM dysfunction and AHR[41]. It is a unique phenotype of corticosteroid-resistant asthma, showing the uncoupling of airway inflammation and airway obstruction. IL-33 has been reported to be an important target for regulating mast cell-ASM crosstalk in asthma[50]. Both bronchial epithelium and ASM express IL-33, but the latter is mainly related to AHR. For the role of AECs in PGA, further research is needed.

Glucocorticoids(GCs) are traditional, long-lasting, first-line drugs to control asthma inflammation. As T2 asthma is the main subtype, inhaled GCs are clinically effective in most asthma patients, which can improve lung function, prevent acute exacerbations, improve airway hyperresponsiveness, reduce inflammatory symptoms, and reduce asthma mortality rate[51]. Its specific mechanism is almost involved in all aspects of asthma: such as reducing the blood levels of prostaglandins and leukotrienes, inhibiting the metabolism of arachidonic acid and the synthesis of cytokines, and inactivating the chemotaxis and activity of eosinophils. Among them, the strong inhibitory effect of GCs on airway inflammation is

mainly caused by genomic and non-genomic mechanisms, especially the former[51]. They bind intracellular GC receptors to trigger increased expression of anti-inflammatory genes and suppression of pro-inflammatory gene activation in asthmatic airways. In contrast, it has been suggested that 5–10% of asthma patients are steroid resistant[52]. The mechanism has been strongly related to immunological dysregulation, genetic, and environmental factors such as cigarette smoking or respiratory infections[51].

In adults and children, PGA is a common phenotype of stable asthma. Studies have shown that inhaled corticosteroids(ICS) has a limited impact on airway inflammatory markers in PGA patients, therefore PGA is defined as a potential "steroidinsensitive" phenotype[53]. It is not yet clear whether this CS insensitivity is caused by PGA or a consequence of PGA. Some scholars believe that based on the pathogenesis of PGA, the therapeutic effect of ICS is poor[53]. On the one hand, airway inflammation, the main target of ICS, is modest or absent in patients with PGA. On the other hand, airway remodeling, which drives most of the pathogenesis of PGA, is relatively insensitive to ICS. In addition, since ICS can increase airway neutrophils, whether ICS treatment of PGA patients may increase airway neutrophils and change the phenotype of asthma from PGA to Neutrophilic asthma remains to be further studied.

Currently, there is still a lack of effective control drugs for patients with $T2_{low}$ asthma. Treatment needs to consider the patient's disease characteristics, including the type of airway inflammation, the degree of corticosteroid sensitivity, and the possible involvement of non-inflammatory pathways, such as AHR and airway remodeling. In recent years, there have also been clinical trials of a variety of new biological agents for $T2_{low}$ asthma. Although some preliminary results have been obtained, it will take a long time before they are officially used in the clinic. For example, selective C-X-C motif chemokine receptor 2 (CXCR2) antagonists (SCH527123, AZD5069) may be used for neutrophil asthma[54, 55]. In a double-blind, randomized, placebo-controlled trial, SCH527123 significantly reduced sputum and blood neutrophilia in patients with severe neutrophilic asthma and was associated with less mild exacerbations. In addition, there are anti-TNF-α (Golimumab), anti-IL-17 (Brodalumab,

CJM112), anti-IL1 (anakinra), anti-IL-6, IFN, IL-23 (Risankizumab), KIT inhibitor (imatinib), 5-lipoxygenase-activating protein (FLAP) inhibitor (GSK2190915), LC28-0126 and other novel small molecule medications are under development[41]. Currently, anti-IL-6 and LC28-0126 have no published clinical trial data for asthma. There are no active phase III clinical trials for the aforementioned molecules, and the increased risk of serious infections and malignant tumors caused by treatment with TNF-α receptor blockers is worrying[56].

Figure 1. Schematic representation of the functional crosstalk between AECs and innate and adaptive immune cells. Airway inflammation is initiated by epithelial cells. AECs express many PRRs to rapidly detect and respond to internal or external environmental agents including pollutants, viruses and allergens causing the release of remodeling factors and bronchoconstrictor agents as well as pro-inflammatory chemokines and cytokines. These latter mediators enable AECs to bridge the gap between the innate and adaptive immune systems.

Alarmins (TSLP, IL-25 and IL-33) activate ILC2s, which are potent producers of IL-5 and IL-13, participating in type 2 airway inflammation. The interaction between TSLP and antigen presenting cells such as DCs that present inhaled antigens to T and B cells resulting in Th2 differentiation and IgE production plays an important role in the sensitization of inhaled allergens. The maturation of dendritic cells promotes the generation of effector T cells and triggers the release of direct bronchial contractors and Th2 cytokines, which feed back on the epithelium and airway smooth muscle and further facilitate amplification of airway inflammation through subsequent adaptive T cell responses. AECs also release periostin, which further boosts TGF-β production, activating the underlying fibroblasts to differentiate into myofibroblasts, participating in the formation of airway remodeling. Abbreviations: AEC, airway epithelial cell; Th, helper T cell; Treg, regulatory T cell; ILC, innate lymphoid cell; DC, dendritic cell; IL, interleukin; TLR, Toll-like receptors; PLR, protease activated receptors; CLR, C-type lectine receptors; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; TGF, transforming growth factor; TSLP, thymic stromal lymphopoietin; GM-CSF, granulocyte-macrophage colony-stimulating factor; CXCL, chemokine (C-X-C motif) ligand ; CCL, chemokine ligand.

3. Pathophysiological changes in the asthmatic airway epithelium：

Asthmatic patients have different degrees of chronic persistent inflammation of the airway epithelium with airway epithelial damage occurring even in mild, early and nonfatal asthma [57]. The degree of inflammation is variable but airway edema, inflammatory cell infiltration, goblet cell hyperplasia, mucus plug formation, epithelial tissue damage and epithelial cell shedding are observed in the airways of asthma patients [58]. AEC damage and shedding are important pathological features of asthma with the abnormal epithelium being more susceptible to injury and apoptosis than that of healthy control subjects [59]. AECs from asthmatic subjects obtained by bronchial brushing appear to be less viable and more hyperreactive [60], which may be the result of inflammatory damage. Furthermore, basal AECs can preferentially differentiate into goblet cells that secrete mucus, and the number and volume of mucous glands is increased. Recent genetic evidence also emphasizes the role of goblet cells and mucus production in asthma [61, 62].

AECs have diverse and complex functions rather than the initial concept of a passive barrier and these are key to maintaining a dynamic defense against the external environment. The airways of asthmatic patients display characteristic signs of dysregulation of airway epithelial repair, leading to a chronic cycle of wound repair and subsequent lung remodeling. A new paradigm for asthma epithelial injury and aberrant repair is that it is involved in the origins of the disease and not merely as a consequence of longstanding inflammation [63]. Thus, the inherent abnormality of the ability to repair and restore an effective barrier to the external environment after epithelial injury seems to be indispensable for the occurrence and development of asthma.

There is widespread airway epithelial damage in asthma patients. The abnormal morphology and function of AECs are seen very early on in disease and this abnormal state of injury and repair is sustained throughout the patient's life. These changes result in impaired airway epithelial barrier function, which is also the cause of airway remodeling and AHR, and the

associated decline in lung function. Analysis of samples from patients who died from asthma obtained at autopsy revealed extensive airway remodeling including airway smooth muscle hypertrophy, epithelial goblet cell hyperplasia and sub-epithelial tissue collagen deposition [59]. There is a thickening of the basal lamina in both adults and children with asthma that is usually associated with subepithelial myofibroblast recruitment and fibrosis prior to the establishment of airway inflammation [64, 65].

The repair of AEC in asthmatic patients is dysregulated although it has a higher proliferative capacity than those from normal subjects [66]. Monolayers of AEC from asthmatic children fail to repair post wounding and these cells produce less fibronectin (FN) than normal children. And supplementation with exogenous FN does not completely repair these paediatric asthmatic AECs [66]. In addition, the transcriptomic map of AECs in asthma patients also provides clues to the importance of chronic epithelial damage and repair in the pathogenesis of asthma. The expression of at least 60 genes in AEC is reduced and many are related to wound healing and inflammation including the chemokine ligands (CCL)-3, CCL-5 and CCL-18; TLR2, TLR8; CD14; IL-1α and IL-1β and the receptors IL-1R and IL-8RA; galectin-1 and galectin-3, and the binding proteins for galectin-3 and galectin-9 [66].

Airway epithelial damage triggers the induction of inflammation and repair mechanisms that involve cell-to-cell communication and intracellular signal transmission [67]. Thus, airway remodeling in asthma is a consequence of dysfunctional epithelial repair post wounding, where dysregulated inflammation and an imbalance in the epithelial-mesenchymal trophic unit lead to a vicious cycle of dysfunctional wound repair and attempted resolution [15, 68]. Epidermal growth factor receptor (EGFR) immunoreactivity is significantly higher in biopsies of patients with mild and severe asthma, suggesting the potential for altered EGFR signaling and epithelial repair [69]. Extrinsic factors induce AECs to release recruitment factors such as IL-5 and epidermal growth factor (EGF), recruiting and stimulating inflammatory cells and resulting in an imbalance of epithelial mesenchymal units [70, 71]. In addition, heightened secretion of fibroblast growth factor-transforming growth factor-β (TGF-β) and EGF is an important mechanism leading to airway remodeling in refractory

asthma. Abnormal expression of cell-extracellular matrix (ECM) proteins and integrins is likely to further drive the pathological defects seen in the functional responses of the airway epithelium in asthma [67].

4. Current and future asthma biomarkers:

The National Institutes of Health (NIH, USA) defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". An ideal biomarker for asthma should have high sensitivity and specificity, can reflect intrinsic pathology, can be used to identify clinical phenotypes or treatment response phenotypes, and to assess changes in disease activity.

The above content suggests that since the bronchial epithelium plays a key role in the origin and development of asthma, proteins and genes derived from it are likely to become biomarkers for asthma. In Table 1, we list the currently proposed candidate biomarkers as well as their potential sampling compartment, advantages, disadvantages and utility for clinical use. Furthermore, we also have a more detailed discussion on substances that have been studied intensively.

Epithelium-derived biomarkers：

4.1. Periostin is an extracellular matrix protein induced by IL-4 and IL-13 in AECs and lung fibroblasts. It is a key molecule connecting T2 airway inflammation and airway remodeling, and is related to $T2_{high}$ eosinophilic asthma [72]. Mouse models suggested a role of periostin in subepithelial fibrosis, eosinophil recruitment, and mucus production from goblet cells [73]. In childhood asthma, the level of periostin was significantly higher than that of the healthy control groups [74] and was related to the degree of disease control in severe asthma. Serum periostin levels in 2-year-old children were 2-3fold higher than previously observed adult levels and could predict asthma at aged 6. In children between 4 and 11 years of age, serum periostin was the best predictor of airway eosinophilia compared with FeNO, blood eosinophil counts and serum IgE[75]. However, in children with mild asthma, the level of EBC periostin was very low and had no significant difference from healthy people[76]. Previous data in adults asthma found elevated levels of serum periostin, which were associated with fixed and more severe airflow obstruction [77] and greater decline in lung function [78]. Thus, periostin was reported to be a systemic and promising biomarker of T2, IL-13-driven, corticosteroid-responsive asthma. Furthermore, serum periostin levels were stable during disease progression in adults with asthma and did not show a seasonal variation [79]. Serum periostin was used as a biomarker in phase 2 and 3 clinical studies of the anti-IL-13 antibody lebrikizumab [80]. However, recent evidence indicated little selectivity of serum periostin for T2 asthma and it is not surprising, therefore, that phase 3 studies of lebrikizumab were not considered effective [81]. In contrast, high sputum periostin reflects $T2_{high}$ asthma [82] whereas high serum periostin is now considered indicative of omalizumab efficacy in asthma [83]. While periostin may have prognostic, predictive, and pharmacodynamic properties, the inconsistency of results, serum levels that change with age, and increased expression in other diseases limit its clinical applicability and affect its utility as an independent biomarker [84].

4.2. Club cell secretory protein 16 (CCSP16) is produced predominantly by club cells and non-ciliated epithelial cells in the distal airways and is readily detectable in the peripheral circulation [85]. Mounting evidence suggested that this protein is critical in mediating anti-inflammatory and anti-oxidant functions within the lung and, by virtue of these activities, may protect against development of obstructive lung diseases [86].

CCSP16 is considered to be both a sign of the loss in airway epithelial barrier integrity and a common participant in the anti-inflammatory response. Low levels of CCSP16 in the serum were associated with decreased lung function in childhood, accelerated decline in lung function in adulthood and restricted airflow [87]. While studies have shown that CCSP16 and surfactant protein D (SPD) in sputum and BAL were significantly higher in patients with severe asthma compared to mild-moderate and healthy controls [88], serum CCSP16 levels were reduced in asthmatics. BAL levels of CCSP16 correlated with epithelial detachment suggested its possible role of in the remodeling process. Zhai and colleagues [86] using human data from a birth cohort, suggested that low circulating CCSP16 levels were not only a biomarker of airway pathology but may be implicated in the pathophysiology of the progressive airway damage that characterize obstructive lung diseases. Moreover, urinary CCSP16 may be a useful tool or biomarker for studying asthma and the integrity of the alveolar epithelium in children with lung injury [89].

Emerging biomarkers:

4.3. Ezrin is a membrane-associated cytoskeleton protein that plays a role in maintaining cell morphology and adhesion between cells and protects AEC barrier function. We have proposed that the downregulation of ezrin indicated AEC injury in

asthma and may be a potential marker for monitoring the severity of disease. This concept is based upon the functional effect of ezrin on AEC barrier function and the high degree of correlation between decreased ezrin levels in several asthma biosamples including EBCs and serum in humans and BAL in mice, and decreased lung function[90]. Furthermore, serum ezrin levels negatively correlated with serum periostin and IL-13 levels. Although exosome secretion from AECs was suggested as a mechanism by which ezrin localizes in EBC, BAL and serum, further work is needed to confirm this [91]. In contrast, acute bronchial challenge of patients with steroid-naïve mild allergic asthma with Dermatophagoides pteronyssinus resulted in enhanced serum levels of ezrin and IL-13 after 24 hours [92]. The authors suggested that acute asthma attacks result in heightened release of biologically active substances such as ezrin from damaged AECs, which initiates an IL-13-driven immune cascade that results in further increases in ezrin levels [92]. Further studies are required to look at temporal changes in ezrin levels in various biosamples and the impact of natural asthma exacerbations.

4.4. Chitinase-3-like protein 1 (CHI3L1), also known as YKL40, is expressed and secreted by various cells such as epithelial cells, macrophages, neutrophils, and Smooth muscle cells. It is significantly increased in asthma patients and its expression is closely related to asthma severity and airway remodeling. YKL40 may promote the airway remodeling of asthma by activating FAK and MAPK signaling pathways, inducing epithelial mesenchymal transition (EMT) and subepithelial

fibrosis [93]. Tang and colleagues [94] showed that serum YKL40 levels of Chinese patients with asthma were increased and correlated with the number of exacerbations. Serum YKL40 levels were correlated with total IgE, blood eosinophils and inversely with lung function and could predict the longitudinal decline of lung function in response to cigarette smoke exposure [95]. As to non-T2 asthma, serum YKL-40 positively correlated with non-T2 inflammatory signatures such as IL-1β and IL-6, which could predict responsiveness or insensitivity to anti-asthma medications and more exacerbations[96]. Hence, the YKL40 clusters are potentially useful for identification of severe or exacerbation-prone asthma in non-T2 patients. In addition, YKL40 may also be a blood-based biomarker in neutrophilic asthma [97] as serum levels correlate with sputum neutrophils [27]. YKL40 has also been used to distinguish asthma from chronic obstructive pulmonary disease (COPD) and healthy controls [98], as well as between patients with asthma-COPD overlap (ACO) and COPD [99].

Regarding the relationship between YKL-40 and childhood asthma, there are opposite results. Study showed that YKL-40 was elevated in children with severe and treatment-resistant asthma[100], but another study found that the serum YKL-40 levels in children with severe persistent asthma were lower than children with mild and moderate persistent asthma and adults with persistent asthma[101]. Recently, there are several studies on the application of YKL-40 in childhood asthma. In a study conducted by Hanna Knihtilä et al[102], it has been demonstrated that serum YKL-40 levels were related to small airway function in children with asthma and can be used as a potential biomarker of lung function damage. Another prospective study showed that serum periosin, YKL-40, and osteopontin levels cannot predict the occurrence of asthma in preschool children with recurrent wheezing[103]. In addition, the

YKL-40 of children with bronchopulmonary dysplasia (BPD) was higher than that of asthma, and associated with severity of the disease[104]. YKL-40 can be used to distinguish asthma and BPD, and indicate the degree of airway dysfunction of BPD.

5. Genetic and epigenetic factors of airway epithelium in asthma：

Genetic and environmental risk factors play important roles in the development of asthma. The interaction between those triggers and susceptible genetic factors can affect the epigenetic status of AECs [10]. This may result in functional and morphological remodeling of the airway epithelium causing distinct phenotypes of asthma [2]. Epigenetic factors that regulate the structure and function of airway epithelium is an attractive area for assessing asthma susceptibility with a focus on DNA methylation changes. Genome-wide association studies (GWAS)[105] and whole genome sequencing (WGS)[106] have provided evidence for the contribution of AECs in the development of asthma in addition to detecting genes related to asthma susceptibility. Elucidating the genetic and epigenetic landscape of epithelial cells in asthmatics may provide a scientific basis for further potential markers for the diagnosis and treatment of asthma.

5.1. Asthma susceptibility genes in airway epithelium:

Previous studies have determined that more than 100 genes are related to the occurrence and development of asthma. The asthma susceptibility genes expressed in AEC may play an important role in mediating many aspects related to asthma. For example, MUC5AC, KIF3A and EFHC1 are related to the mucociliary clearance[107]; IL33, TSLP, IL1RL1 are related to the inflammatory environment; And PCDH1, SMAD3, GSDMB, ORMDL3 and PLAUR are related to cell homeostasis and epithelial integrity, including proliferation, migration, cell-cell adhesion, apoptosis and repair. Furthermore, VEGF, CHI3L1, TSLP, GSDMB, TGF-β1 and POSTN are related to the decline of lung function and/or airway remodeling in asthma patients[108]. PCDH1, a susceptibility gene for AHR, may be a potential biomarker of asthma in both children and adults[109]. In previous gene expression and RT-qPCR studies,

three genes were identified as being highly induced by IL-13 in AECs: POSTN, CLCA1, and SERPINB2. These genes are considered markers of T2 inflammation and are overexpressed in a specific subset of patients with asthma [110]. IRAKM may represent a potential biomarker for early onset of asthma [111]. ORMDL3/GSDMB is more suitable for predicting asthma risk in children [112] and CDHR3 is associated with asthma in children with severe exacerbations. CST1 can differentiate asthmatics with exercise-induced bronchoconstriction (EIB) from those without EIB [113, 114].

5.2. Epigenetic regulatory factors in airway epithelium:

The effects of different environmental challenges (such as smoking, air pollution, microbial exposures nutrients, allergens, use of drugs and infectious agents) on the development of asthma are partly mediated by epigenetic mechanisms, including: DNA modifications, histone modifications and non-coding RNAs. Their common feature is to regulate genes at transcriptional and post-transcriptional levels without affecting the nucleotide sequence of genomic DNA in response to changes in internal and external conditions.

5.2.1. **DNA methylation**:

DNA methylation refers to the covalent bonding of a methyl group to the 5th carbon position of the cytosine of the genome CpG dinucleotide under the action of DNA methyltransferase. Compared to healthy control subjects, a variety of asthma-associated genes linked to immunity were differentially methylated in nasal epithelial cells of atopic asthmatic children including IFNGR2, HLKA-DPA1, LAG3, NFIL3, PRF1, TNFSF13. Recently, CDH26 and CDHR3, associated with epithelial barrier function, were found differentially methylated [115, 116]. And ZMYND10, selectively expressed in ciliated epithelial cells was also found to be differentially methylated[117]. Other differentially methylated promoters highlighted genes involved in epigenetic regulation (ATXN7L1, H1F0, HIST1H1D, METTL1), airway obstruction (GABRG3) and obesity (C1QTNF1, GPC4) [116]. A number of DNA methylation signatures have also been identified in asthmatic AECs including cytokeratin 5 (KRT5) [118], signal transducer and activator of transcription 5A (STAT5A) [118],

cysteine-rich protein 1 (CRIP1) [118] and arginase2 (ARG2) [119].

5.2.2. **Histone modification：**

Histone modification refers to the modification of histones under the action of related enzymes, such as methylation, acetylation, phosphorylation, and ubiquitination. AECs in adult asthmatics express increased levels of histone H3 lysine 18 (H3K18) acetylation and histone H3 lysine 9 trimethylation (H3K9me3) [120]. The degree of acetylation of lysine 27 on histone 3 (H3K27ac; an active promoter and enhancer mark) was closely related to genes linked with $T2_{high}$ asthma [110]. The expression of HDAC1 and HDAC9 in bronchial epithelial cells of asthmatics increased[121], and HDAC2 activity was reduced, which was associated with corticosteroid resistance[122].

5.2.3. **Non-coding RNA：**

A number of classes of non-coding RNAs exist in mammalian cells including long non-coding RNAs (lncRNAs), piwi-interacting RNAs (piRNAs), and miRNAs [2]. Their common feature is that they can be transcribed from the genome, but not translated into protein, and perform their respective biological functions at the RNA level. Among them, miRNAs are proposed to control the expression of 30–60% [123] of human genes and are closely related to the occurrence and development of asthma.

Some differentially expressed miRNAs regulate the expression of genes related to epithelial barrier function, repair, proliferation or apoptosis[107]. For example, miR-744, miR-19a, miR221, miR-27A, miR-128, and miR-34/449 were differentially expressed in bronchial epithelial cells of asthmatic patients compared with the control group. Among them, the expression of miR-19 was increased in patients with severe atopic eosinophilic asthma, which could distinguish severe asthma from mild asthma[124]. Martinez-Nunez and colleagues [125] found that microRNAs-18a, -27a, -128 and -155 were down-regulated in asthmatic bronchial epithelial cells compared to healthy donors. These miRNAs have an inhibitory effect on IL-8 and IL-6 gene expression. Other miRNAs expressed in AEC with a potential role in asthma development include the miR-34/449 family and the miR-17 family [2].

Differentially expressed miRNAs in asthmatic AECs may be used as a potential biomarker for the "endotype" classification of asthma and as such the miR-34/449 family is considered to be related to T2 asthma [126]. Interestingly, no clear relationship was observed between these differentially expressed miRNA and serum IgE level in asthmatics [127]. Furthermore, inhaled corticosteroids only had minor effects on miRNA expression and failed to restore miRNA levels to those seen in healthy control subjects [127].

Recently, the transport of epithelial-derived extracellular vesicles (Evs) between cells have been considered to be a new communication method that plays a role in airway homeostasis, airway remodeling, and epithelial barrier function. Sabine Bartel's data indicated that secretion of miRNAs in EVs from the airway epithelium, in particular miR-34a, miR-92b, and miR-210, might be involved in the early development of a Th2 response in the airways and asthma[128]. However, it is still unclear whether the changes in asthma are consistent with those in experiments.

6.Tissue compartments for biomarker assessment：

Various compartments can be used for the assessment of biomarkers and each compartment has its own strengths and weaknesses. Using the blood to identify biomarkers is minimally invasive (the procedure can be painful and difficult in some patients) and easy to realize in the clinical setting and requires minimal patient effort. In addition, blood can be collected across the age spectrum and is a cost-effective sample type [129]. However, peripheral blood does not necessarily reflect airway biology or provide disease-relevant mechanistic insight[129].

EBC has the advantage of being non-invasive and can offer real-time monitoring, simplicity and repeatability. However, there are also limitations, such as the lack of unified standards

for the selection of biological indicators, collection methods and collection time window, the sensitivity of the detection reagent, affected by eating, drinking, smoking and the inability to conduct anatomical localization of the gas passage[129]. Nowadays, EBC is mainly used for research purposes and is not widely used in clinical practice. Studies have shown that LTB4 and 8-isoprostane reflect airway inflammation and oxidative stress [130]. Horvath and co-workers [131] published a European Respiratory Society technical standard that provides technical norms and recommendations for the collection and analysis of EBC samples. Overall, EBCs have the potential to provide useful airway-associated information to enable the detection of disease progression and therapeutic efficay. The analysis of volatile organic compounds (VOCs) using electronic noses and/or mass spectrometry may become an increasingly important noninvasive means of assessing AEC function over time in all patients with asthma [132]. In recent years, induced sputum analysis has become a more common noninvasive method to evaluate airway inflammation in respiratory diseases. The safety and tolerability of sputum induction accounts for its popularity, whilst the technique has been standardized to improve the quality and reproducibility of specimens. However, due to the time and cost of sputum induction and the failure to achieve a suitable sample in every subject, its wide application in clinical practice is limited. More importantly, although the short-term reproducibility of induced sputum cell analysis appears to be good [133], inflammatory phenotypes are unstable and can change spontaneously or with changes in treatment, and a single induced sputum test cannot reliably predict persistent airway inflammatory phenotypes [39].

Bronchoalveolar lavage is an invasive technique with poor patient compliance, especially for critically ill patients, and it is difficult to accept repeated invasive examinations[129]. Therefore, the application of this method in clinical practice is limited.

The nasal mucosa is a readily accessible site for the study of inflammatory processes. The fluid from nasal washings can reflect the intensity of the inflammatory process and provide a parallel between symptoms of the upper and lower respiratory tracts [134]. However, nasal epithelial cells and washes are not exactly the same as those from the airway of asthmatics,

and there are differences in epithelial cell states and immune cell composition [135]. Further comparisons may reveal which aspects of nasal epithelial cell function provide insight into processes deeper within the airway.

Among the candidate biomarkers mentioned above there are various sampling compartments that may be used in future clinical applications. Reduced ezrin expression is detected in both EBC and serum of asthma patients [90, 92] and elevated levels of claudin 4 [136] and YKL40 are detected in asthmatic blood [97-99]. Epithelial brushings from patients with asthma have significantly lower claudin18 levels than healthy controls [137]. CCSP16 [87, 88], periostin [74, 75, 77-79], osteopontin [138-140], IL-33 [35, 141, 142], IL-25 [143, 144] and fibrinogen [145, 146] can all be measured in the sputum and blood of asthma patients. Except for CCSP16, the expression of all of these biomarkers is increased in asthma. In contrast, the levels of CCSP16 in asthmatic sputum and BAL are elevated compared to controls but levels are reduced in asthmatic blood. In addition, serum periostin is far less predictive of T2 asthma than sputum periostin [82]. TSLP [141, 147, 148] and osteopontin [149] expression levels are elevated in nasal secretions and in serum whilst that of MMP-9 [150, 151] is elevated in EBC and sputum. Reduced Sec14l3 [152] expression has only been studied in the BAL and lung tissue of asthmatic mice.

7. SUMMARY:

Bronchial asthma is more like a complex group of clinical diseases than a single disease. The core importance of airway epithelium in asthma is now widely accepted. The airway epithelium constitutes an important barrier at the interface between the external environment and the lung. A disordered barrier allows allergens to enter the body and trigger a sensitization reaction, which is the starting point of allergic asthma. A variety of factors that induce AEC damage and dysfunction are also initiating factors in asthma. This overlap highlights the key role of these cells in asthmatic airway inflammation, airway hyperresponsiveness, airway remodeling, and airway mucus hypersecretion. Compared with

healthy individuals, the epithelium of asthma patients shows several structural and functional abnormalities, which provides important mechanistic insight into how asthma is initiated and perpetuated and could provide a framework by which to select new therapeutic strategies that prevent exacerbations and alter the natural course of the disease. In addition, the study of asthma susceptibility genes and epigenetic regulatory mechanisms reveals the key role of AECs in asthma.

Asthma has great heterogeneity in etiology, triggers, clinical characteristics, and response to treatment indicating that identifying asthma patients with different phenotypes and endotypes is of great significance for accurate diagnosis and treatment. At present, the clinical treatment mainly adopts a step-by-step symptom-based approach, which is derived from a simplified view of asthma and the heterogeneity of asthma is not recognized at the clinical and molecular levels. At the same time, the emergence of expensive targeted monoclonal therapies has further prompted the expansion of research on asthma biomarkers. In view of the key role of airway epithelium in the occurrence, development and worsening of asthma, it has become a research hotspot. The development of new serum/sputum biomarker panels with higher sensitivity and specificity may lead to rapid more-accurate diagnosis of asthma subtypes. This will identify patients who may benefit from novel epithelial-focused therapies and find new therapeutic strategies targeted to correct dysregulated epithelial barrier.

The evaluation value of a single biomarker is also transformed into a combination of various markers. It is likely that combinations of analytes derived from different “omics” approaches may provide a better biomarker panel to indicate epithelial damage in asthma prior to any changes that may be detectable by enhanced imaging capabilities. Combining biomarkers with clinical parameters and new information from the fields of genomics, transcriptomics and proteomics, will further promote our understanding of AECs in asthma.

Conflict of interest:

The authors have no conflicts of interest to declare.

Abbreviations:

AECs airway epithelial cells

ASM airway smooth muscle

GINA International Asthma Global Initiative

EBC exhaled breath condensate

PRRs pattern recognition receptors

TSLP thymic stromal lymphopoietin

T2high type 2-high

T2low type 2-low

AHR airway hyperresponsiveness

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Table 1. Candidate biomarkers and a summary of major biomarker characteristics. Potential biomarkers are described, and references are listed in the table. (Red represents studies in human specimens)

Biomarker	Biosample							Advantage	Limitation	Utility	Ref
	Sputum	Blood	BALF	Biopsies/brushings	EBCs	Nasal secretions	Nasal cells				
Ezrin		+	+	+	+			• Present in multiple biosamples • Useful in early diagnosis	• Variable expression level	• Predict greater decline in lung function • Indicate early epithelial injury • Indicate asthma control	[90, 92]
Claudin 4		+		+				• A major claudin expressed in ECs • A selective sodium barrier protein • Increased in serum and significantly higher in exacerbated asthmatics	• Mechanistic role unclear • Lack of research data in people with asthma	Directly correlated with IgE • • Inversely correlated with FEV1(%predicted), FEV1/FVC • Predict airway inflammation and asthma clinic severity	[136]

Claudin 18				+				• Only lung-specific TJ protein • Associate with increased sensitization to aeroantigens and AHR • Regulated by Th2 inflammation	• Mechanistic role unclear • Lack of research data in people with asthma	• Indicate epithelial permeability • Reduced in IgE-high and eosinophilic T2high asthma	[137]
CCSP16	+	+	+					• Correlate with disease duration • Easy to detect in the circulation • The most abundant proteins in BALF • Implicated in the pathophysiology of the progressive airway damage of obstructive lung diseases	• Secreted by nonciliated terminal bronchiolar epithelial cells • Not applicable in all lung diseases • Some conflicting results	Predict accelerated lung function decline in adult life • • Marker of airway pathology • Deficition at age 6 years were associated with lower levels of FEV1 by age 1	[86, 153-156]
Sec14l3			+	+				• Specifically expressed in airway epithelium	• Difficult to obtain in clinical setting • Lack of research data in people with asthma	• Reflect ciliated epithelial cell integrity • Predict airway inflammation	[152, 157]
Osteopontin	+	+	+	+		+		• As a central mediator in the pathogenesis of LOA • A key cytokine in the modulation of inflammation and fibrotic events in asthmatic airways	• Multicellular sources • Mechanistic role unclear • In consistency of results	• Measure disease onset and treatment • Important indicator of childhood asthma • Discriminate patients with LOA and those with adult asthma from HCs • Bronchial OPN levels were correlated	[138-140, 149, 158]

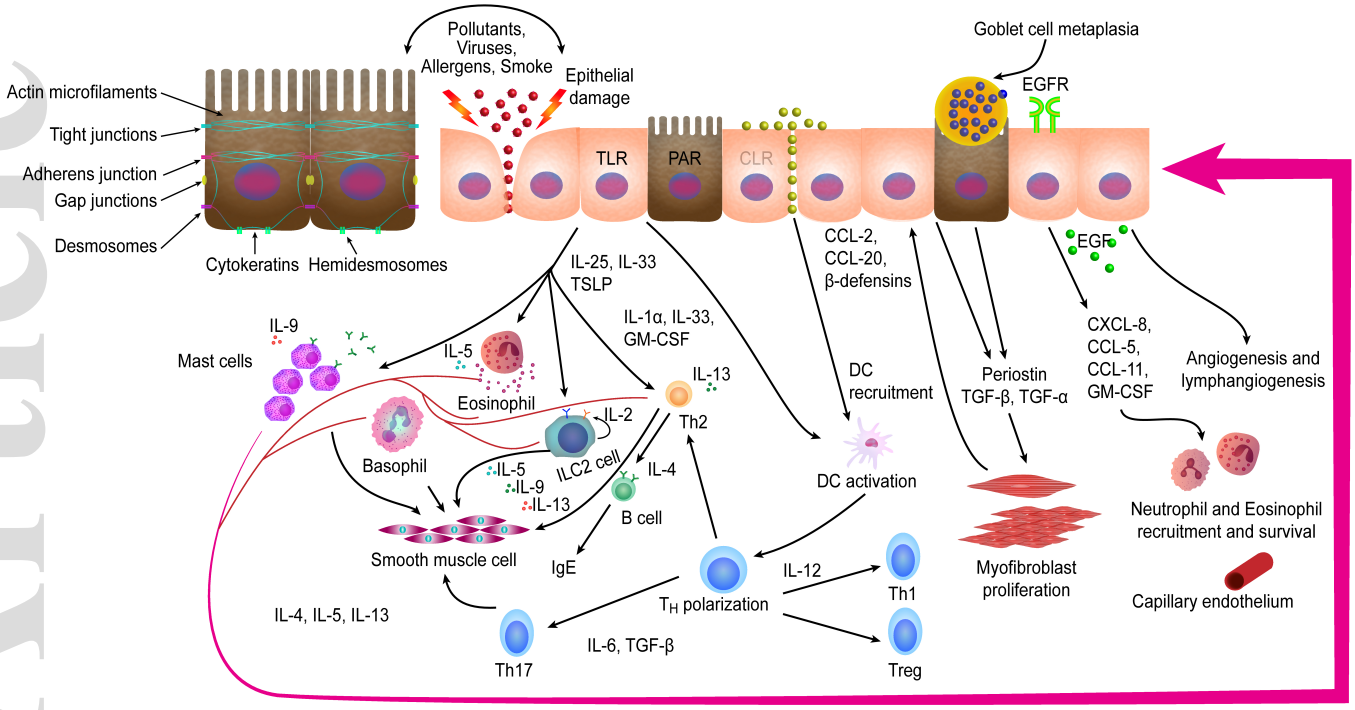
								• Sputum OPN levels were associated with the degree of neutrophilic inflammation		with impaired lung function in patients with severe refractory asthma early diagnosis of LOA in adults	
YKL-40	+	+	+	+		+	+	• Related to disease severity and decline in lung function • Present in multiple biosamples • Critical in mediating anti-inflammatory and anti-oxidant functions	• Require replication in multicenter cohorts and longitudinal studies • Conflicting results in children asthma	• Distinguishe ACO and COPD • Distinguishe Asthma from COPD and healthy controls • A potential biomarker of lung function damage in children asthma • Correlate with total IgE, blood eosinophils and inversely with lung function • Identification of severe or exacerbation-prone asthma in non-T2 patients • Predict the longitudinal decline of lung function in response to cigarette smoke exposure	[99, 101, 159, 160]
Fibrinogen	+	+						• Associated with spirometry values	• Lack of research data in people with	• Predict frequent asthma exacerbations	[145,

								• Related to asthma severity and airway inflammation • FCP-TLR4 signaling induces both antifungal responses and responsiveness to Th2 cytokines such as IL-13 • Participate in the development of bronchial remodeling	asthma	• Predict thromboembolic events and atherosclerosis in asthma	146, 161, 162]
IL-33	+	+	+	+	+	+	+	• Genetic associations with asthma risk • Related to the asthma severity • IL-33 and its ligand IL1RL1 induce airway inflammation, mucus production and airway remodeling • The expression of IL-33 in ECs of asthma patients is related to age	• Lack of sensitive and specific assay in serum	• Predict asthma risk • Disease onset and asthma prevention • Response to environment and treatment • Anti–IL-33 IgG1 antibody Etokimab • Contribute to present less type 2, but severe phenotypes of EA	[35, 141, 142, 163, 164]
IL-25	+	+	+	+	+		+	• Related to asthma severity and airway inflammation • Associated with lung function • Present in multiple biosamples	• Inconsistent between upper and lower airways • Multicellular sources	• Define asthma phenotypes • Response to ICS therapy • Predict asthma atopy: greater AHR, more airway and blood eosinophils, higher serum	[36, 143, 144]

									IgE, more subepithelial thickening, and higher expression of Th2 signature genes	
MMP-9	+	+	+	+	+	+	+	• Related to airway remodeling, inflammation and lung function • Present in multiple biosamples	• Susceptible to many factors • Multicellular sources • Difficult to detect	• Response to glucocorticoid treatment • Asthmatics exposed to cigarette smoke may be more susceptible to MMP-9-mediated airway remodeling	[150, 151, 165, 166]
Periostin	+	+	+	+	+	+	+	• Used in phase 2/3 studies of lebrikizumab • Associated with persistent airflow limitation • Serum periostin levels were stable during disease progression	• Serum levels that change with age • Inconsistency of results • Increased expression in various disease • EBC periostin was very low and had no significant difference from healthy people	• Phenotyping of severe asthma-$T2_{high}$ • Response to ICS therapy • Indicate omalizumab efficacy in asthma • In childhood asthma: related to the degree of disease control in severe asthma; the best predictor of airway eosinophilia at age 4 and 11;	[75, 76, 81-83, 167]
TSLP	+	+	+	+	+	+	+	• Genetic risk for asthma • Correlated with airflow obstruction • Play a pivotal role in the initiation and persistence of airway inflammation and	• Inconsistent between upper and lower airways • Multicellular sources	• Predict asthma risk • Treat severe uncontrolled asthma: Tezepelumab, blocking the activity of TSLP • TSLP mRNA expression is inversely	[141, 147, 148, 168-1

								remodeling		correlated with FEV1%	71]

Abbreviations: CCSP, clara cell secretory protein; Sec14l3, sec14-like protein 3; YKL-40, chitinase-3-like protein 1; MMP-9, matrix metallopeptidase 9; ACO, asthma-COPD overlap; ICS, inhaled corticosteroid; OPN, osteopontin; FCP, fibrinogen cleavage product; EC, epithelial cell; TJ, tight junction; BALF, bronchoalveolar lavage fluid; AHR, airway hyperresponsiveness; TLR, Toll-like receptor; LOA, late-onset asthma; EA, eosinophilic asthma; EBC, exhaled breath condensate; HC, healthy control.



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