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REVIEW

Epithelial alarmins: a new target to treat chronic respiratory diseases

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ABSTRACT

Introduction: In response to injury, epithelial cells release alarmins including thymic stromal lymphopoietin (TSLP), high mobility group-box-1 (HMGB1), interleukin (IL)-33 and −25 that can initiate innate immune responses. These alarmins are recognized as activators of T2-immune responses characteristic for asthma, but recent evidence highlighted their role in non-T2 inflammation, airway remodeling, and pulmonary fibrosis making them an attractive therapeutic target for chronic respiratory diseases (CRD).
Areas covered: In this review, firstly we discuss the role of TSLP, IL-33, IL-25, and HMGB1 in the pathogenesis of asthma, COPD, idiopathic pulmonary fibrosis, and cystic fibrosis according to the published data. In the second part, we summarize the current evidence concerning the efficacy of the antialarmin therapies in CRD. Recent clinical trials showed that anti-TSLP and IL-33/R antibodies can improve severe asthma outcomes. Blocking the IL-33-mediated pathway decreased the exacerbation rate in COPD patients with more important benefit for former-smokers.
Expert opinion: Despite progress in the understanding of the alarmins' role in the pathogenesis of CRD, all their mechanisms of action are not yet identified. Blocking IL-33 and TSLP pathways offers an interesting option to treat severe asthma and COPD, but future investigations are needed to establish their place in the treatment strategies.

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KEYWORDS
Alarmins; biologics; chronic respiratory diseases; exacerbations; lung function

1. Introduction

The respiratory epithelium constitutes the first line of defense against inhaled environmental threats such as allergens, microbes, cigarette smoke (CS), and other pollutants [1]. In response to injury, airway epithelial cells (AECs) orchestrate the immune response and mediate clearance of invading pathogens to facilitate resolution of inflammation and restore tissue homeostasis. However, this process may become altered in many respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), and cystic fibrosis (CF), resulting in a persistent inflammatory response, pathological repair with loss of functional tissue architecture, and increased susceptibility for exacerbations (Figure 1) [1,2,3].

Following exposure to noxious agents, dysfunctional epithelium releases three cytokines nicknamed 'alarmins,' including thymic stromal lymphopoietin (TSLP), interleukin 33 (IL-33), interleukin 25 (IL-25), and high mobility group 1 (HMGB1) that act as danger signals and promote/exacerbate the inflammatory response [4]. These cytokines are well recognized to primarily drive type 2 (T2) inflammation, but there is increasing evidence for their role in non-T2 mediated inflammation [5]. The alarmins are key players in asthma pathogenesis, by stimulating Th2-cells, innate lymphoid group 2 cells (ILC2s), mast cells, basophils, and eosinophils to release large quantities of T2 cytokines (e.g. IL-4, IL-5 and IL-13) and growth factors promoting airway inflammation and remodeling [6]. Emerging data highlight the role of alarmins in the pathogenesis of COPD, IPF, and CF [2,4,5,7].

TSLP, a member of the IL-2 cytokine family constitutively expressed by AECs, is rapidly released following cell injury and exerts its effects by binding to a high-affinity heterodimeric receptor complex composed of TSLPR and IL-7α. There are two isoforms: the long isoform actively involved in inflammation and the short isoform, a potential splice variant [5,8]. TSLP promotes allergic responses and airway remodeling by acting on dendritic cells (DCs) with the differentiation of naïve CD4+ T-cells into Th2-cells, promoting the survival of ILC2s and inducing epithelial-mesenchymal transition (EMT) in AECs. TSLP stimulates goblet cell metaplasia, mucus secretion, collagen production by fibroblasts inducing reticular basement membrane fibrosis and promotes the proliferation of airway smooth muscle (ASM) [8]. Beyond Th2 differentiation, TSLP could activate eosinophils, mast cells,

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Article highlights

- The airway and alveolar epithelium act as the first line of defense for the lungs against many environmental triggers.
- In response to injuries, the epithelial cells release the alarmins which are important mediators of inflammation.
- Dysfunctional epithelium and persistent inflammation are the key points in the pathogenesis of many chronic respiratory diseases.
- Current evidence showed important contribution of the epithelial alarmins in the pathogenesis of asthma, COPD, IPF, and CF.
- Targeting the epithelial alarmins and their receptors by biotherapies showed benefits for the severe asthma patients and selected patients with COPD.
- Further investigation is needed to clarify all the mechanisms involving the epithelial alarmins in chronic respiratory disease pathogenesis and to develop adequate therapeutic approaches.

macrophages, neutrophils, NK T-cells, CD8+ T-cells and B-cells, emphasizing that this cytokine plays a role in both T2 and non-T2 inflammation (Figure 2) [5].

IL-33, a member of the IL-1 superfamily expressed by AECs and alveolar type-2 epithelial cells, is a dual function cytokine that plays a key role in both innate and adaptive immune responses. The full-length IL-33 is localized in the nucleus and acts as gene regulator, while the cleaved mature IL-33 isoform is the extracellular cytokine passively released after inflammatory and apoptotic protease processing, secondary to CS, pollutants, allergens, viral, or bacterial exposure [5,9,10]. Cleavage by proteases from various cell types could lead to differing effects according to the microenvironment. If the cleavage with caspase-1 in necrotic cells induces a very active proinflammatory cytokine, the cleavage with caspase-3 and −7 in apoptotic cells results in an inactive peptide [5,10]. Constitutive signaling without additional protease processing was also described in asthma and COPD. Inactivation of IL-33 activity occurs by binding to soluble suppression of tumorigenicity 2 (ST2) receptor that acts as decoy inhibiting the expansion of inflammation or by oxidation of cysteine residues with formation of disulfide bridges [9,10]. Following the attachment of IL-33 to ST2, the corrector IL-1 receptor accessory protein IL-1RAcP is recruited with the formation of a heterodimeric signaling complex that activates NF-kB and mitogen-activated protein kinase pathways promoting cell proliferation/survival, cytokine secretion (e.g. IL-4, -5 and -13), and amphiregulin expression [10]. ST2 is expressed by ILC2s, mast cells, DCs, myeloid cells, natural killer cells, Th1 and Th2 cells, cytotoxic T-cells, NK T-cells, basophils, and endothelial cells

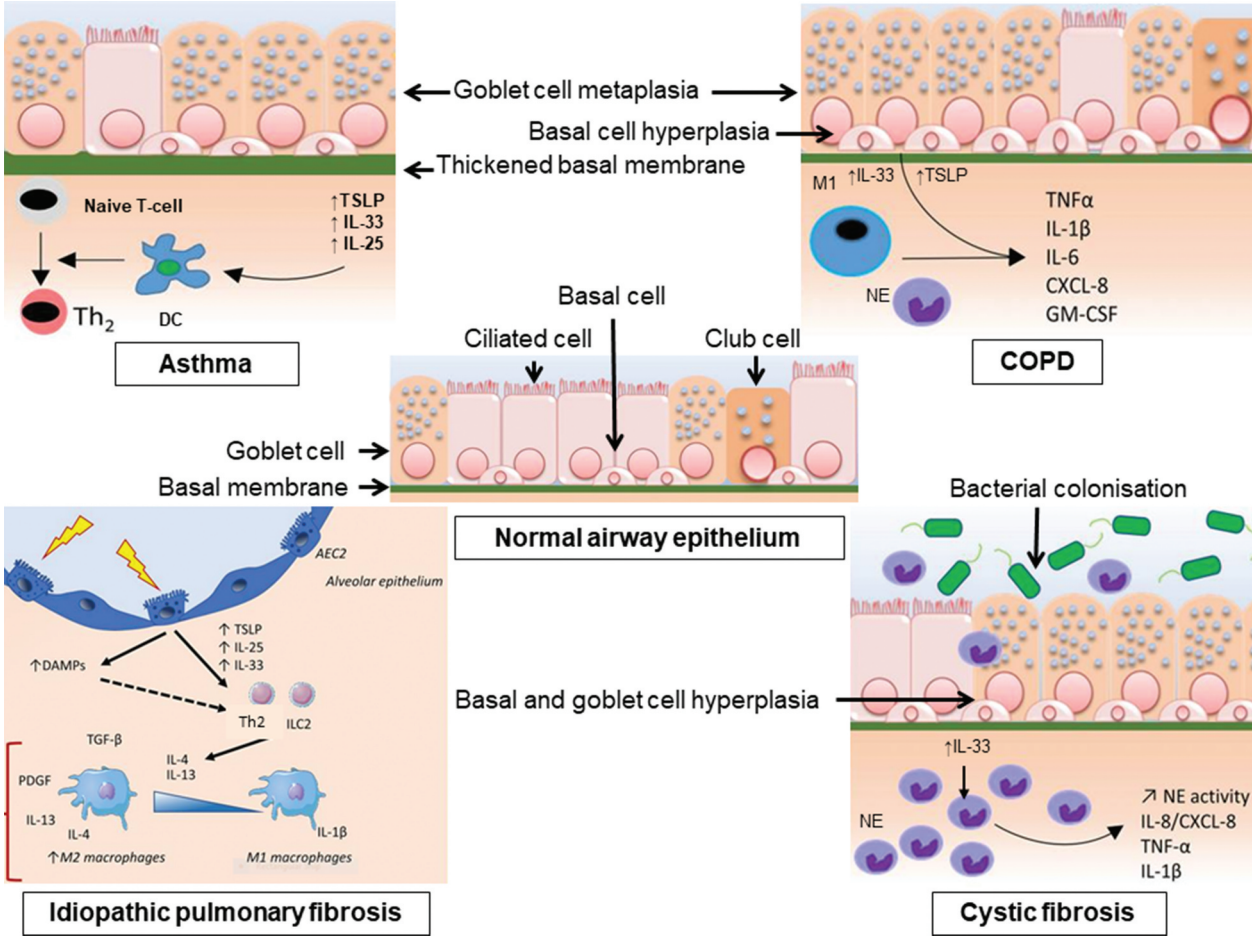

Figure 1. Epithelial airway altered structure in asthma, COPD, idiopathic pulmonary fibrosis and cystic fibrosis (adaptation of two previously published figures from [1,2].

DC: dendritic cells; Th: T-helper cells; TSLP: thymic stromal lymphopoietin; IL-33: interleukin 33; IL-25: interleukin 25; M1: macrophage type 1; NE: neutrophil; TNFα: tumor necrosis factor alpha; IL-1β: interleukin-1 beta; IL-6: interleukin 6; CXCL-8: chemokine (C-X-C motif) ligand 8; GM-CSF: granulocyte-macrophage colony-stimulating factor; AEC2: alveolar type 2 epithelial cells; DAMPs: damage-associated molecular patterns; ILC2: type 2 innate lymphoid cells; IL-4: interleukin 4; IL-13: interleukin 13; TGFβ transforming growth factor beta; PDGF: platelet-derived growth factor.

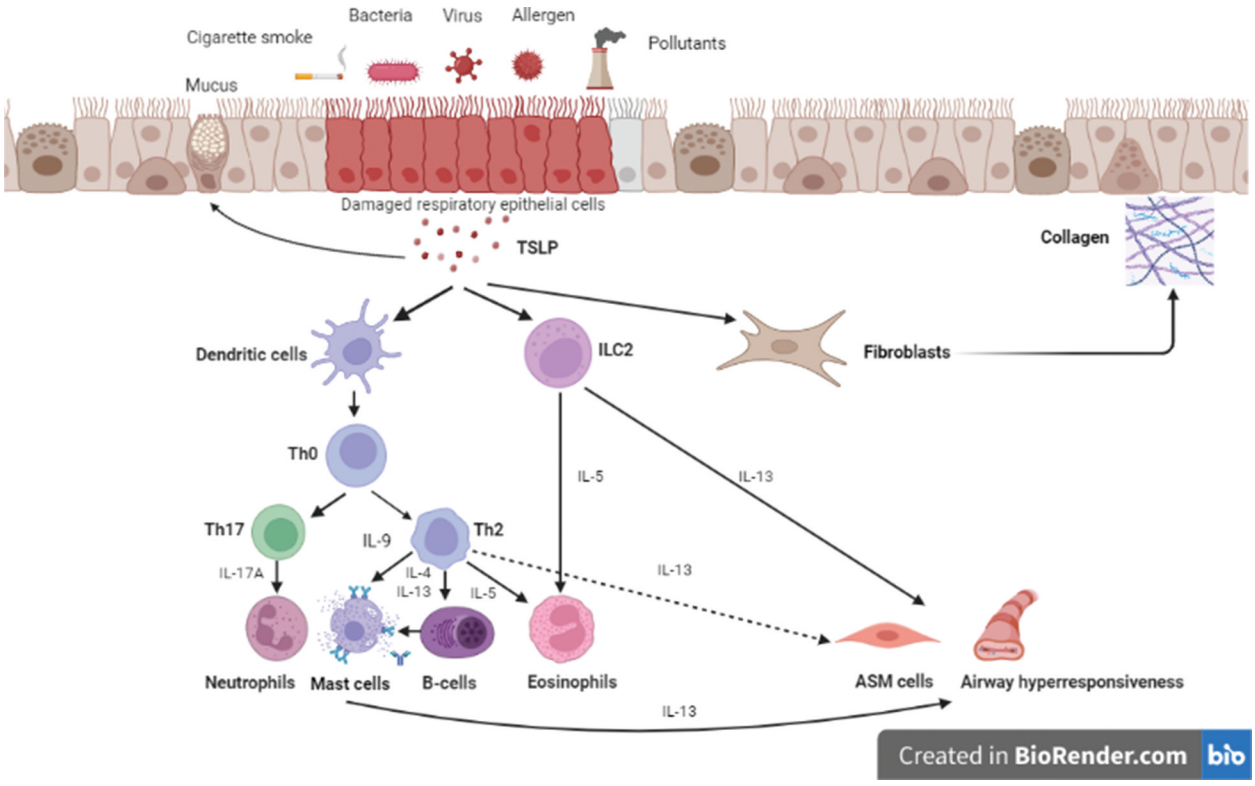

Figure 2. Role of thymic stromal lymphopoietin (TSLP) in airway inflammation, remodeling and fibrosis.

ILC2: type 2 innate lymphoid cells; Th: T-helper cells; ASM: airway smooth muscle cell; IL-4: interleukin 4; IL-5: interleukin 5; IL-13: interleukin 13; IL-9: interleukin 9; IL-17A: interleukin 17A.

[5]. IL-33 activates ILC2s, induces T2 cytokines (e.g. IL-5 and IL-13) but also non-T2 mediators (e.g. TNF-α, IL-8) production in mast cells, collagen, and fibronectin release from fibroblasts [5,8]. According to stimuli, IL-33 promotes both T1 and T2 inflammation and contributes to airway remodeling and lung fibrosis (Figure 3) [2,5,10].

IL-25, also named IL-17E, is the unique cytokine of the IL-17 family produced by AECs [2,8]. Airborne allergens, ATP, and virus infections increase IL-25 and its receptor IL-17RB expression in AECs [11–13]. IL-25 activates ILC2s, modulates EMT of alveolar epithelial cells and local tissue remodeling, stimulates fibroblast differentiation, and upregulates cytokines and growth factor secretion (Figure 4) [2,8].

HMGB1 is a highly conserved nuclear protein present in all cell types exerting functions both inside and outside of cells [14]. Inside the nucleus, it helps nucleosome development, operates as a DNA chaperone supporting DNA duplication and repair, but is also involved in transcription and programmed cell death [15]. Mechanisms inducing HMGB1 release after cellular injury include vesicular transport, inflammasome activation, necrosis, and necroptosis [16]. Excessive quantities of extracellular HMGB1, released passively after cell death or via active secretion from immune cells, produce inflammation by interactions with innate immunity effectors (e.g. ILC2, macrophages, DCs, neutrophils) [14]. The bioactivity of HMGB1 is determined by the redox state of the three cysteines present in the molecule. Gentle oxidation of the fully reduced form by a disulfide bond between Cys23 and Cys45 converts extracellular HMGB1 to a potent activator of pro-inflammatory cytokine production (e.g. IL-4, IL-6, and TNF-α) via Toll-like receptors 4 (TLR4) stimulation by binding MD-2 [14,17]. Disulfide HMGB1 loses its capacity to activate TLR4 when it is either reduced or further oxidized, and its additional oxidation generating sulfonyl groups converts it in a molecule without any pro-inflammatory capacity [14]. HMGB1 operates also via the receptor for advanced glycation end products (RAGE) leading to a direct NF-kB activation and subsequent cytokine formation [14,16]. RAGE provides a transport route for HMGB1 and its partners by endocytosis. Under acidic conditions, HMGB1 could act as a detergent in the lysosomal membrane leading to a leak out from the permeabilized lysosomes into the cytosol with the activation of cytoplasmic receptors inducing inflammation [18]. By these mechanisms, HMGB1 is an alarmin with dual functions: warning the extracellular environment about cell distress and informing cells about hazardous extracellular surrounding. Experimental data suggest the activation of HMGB1 pathway in AECs after exposure to allergens, viruses, and reactive oxygen species with T2 and non-T2 inflammatory responses [15].

The present review summarizes the current knowledge on the role of these epithelial alarmins in chronic respiratory disease (CRD) pathogenesis and the impact of biologics targeting these molecules on clinical outcomes.

2. The role of epithelial alarmins in CRD physiopathology

2.1. Asthma

Alarmins have been extensively studied for more than a decade in asthma, a chronic inflammatory airway disease (usually T2-mediated) characterized by airway hyperresponsiveness (AHR) and variable expiratory airflow limitation [8].

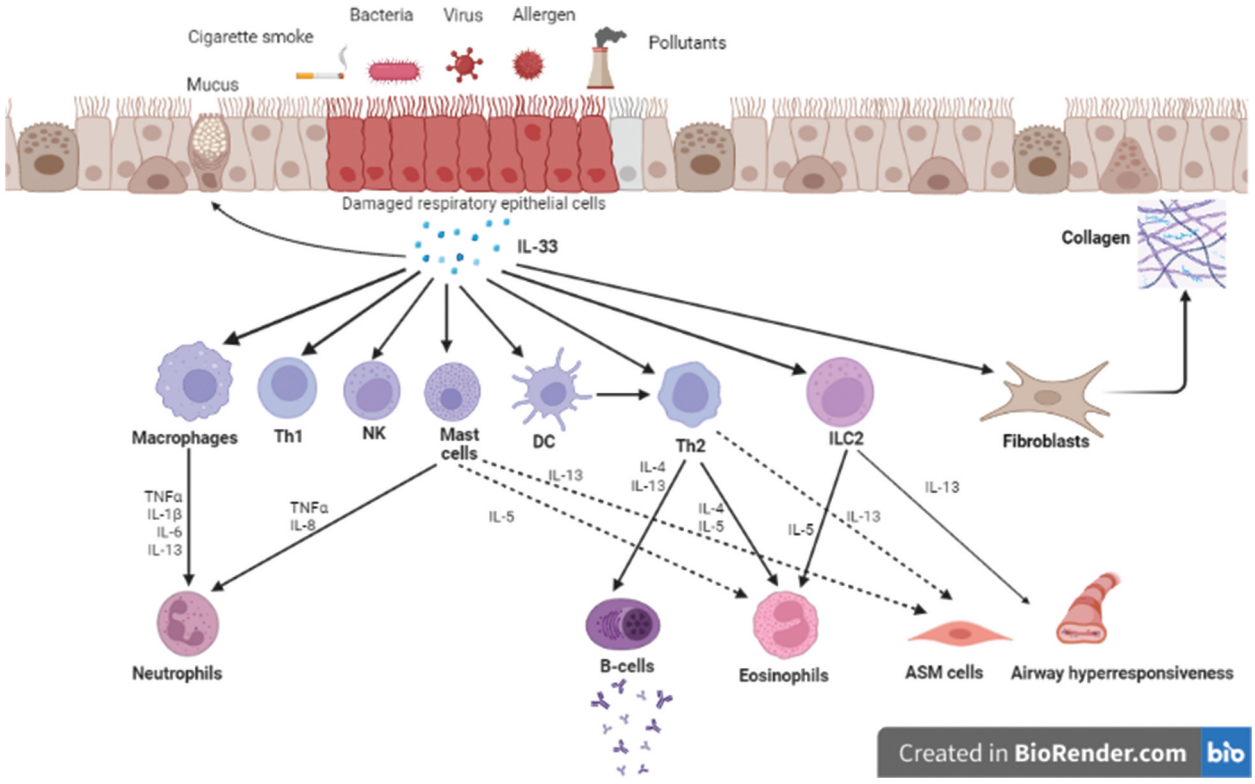

Figure 3. Role of interleukin 33 (IL-33) in airway inflammation, remodeling and fibrosis.

Th: T-helper cells; NK: natural killer; DC: dendritic cells; ILC2: type 2 innate lymphoid cells; TNFα: tumor necrosis factor alpha; IL-1β: interleukin-1 beta; IL-6: interleukin 6; IL-13: interleukin 13; IL-8: interleukin 8; IL-4: interleukin 4; IL-5: interleukin 5; ASM: airway smooth muscle cell.

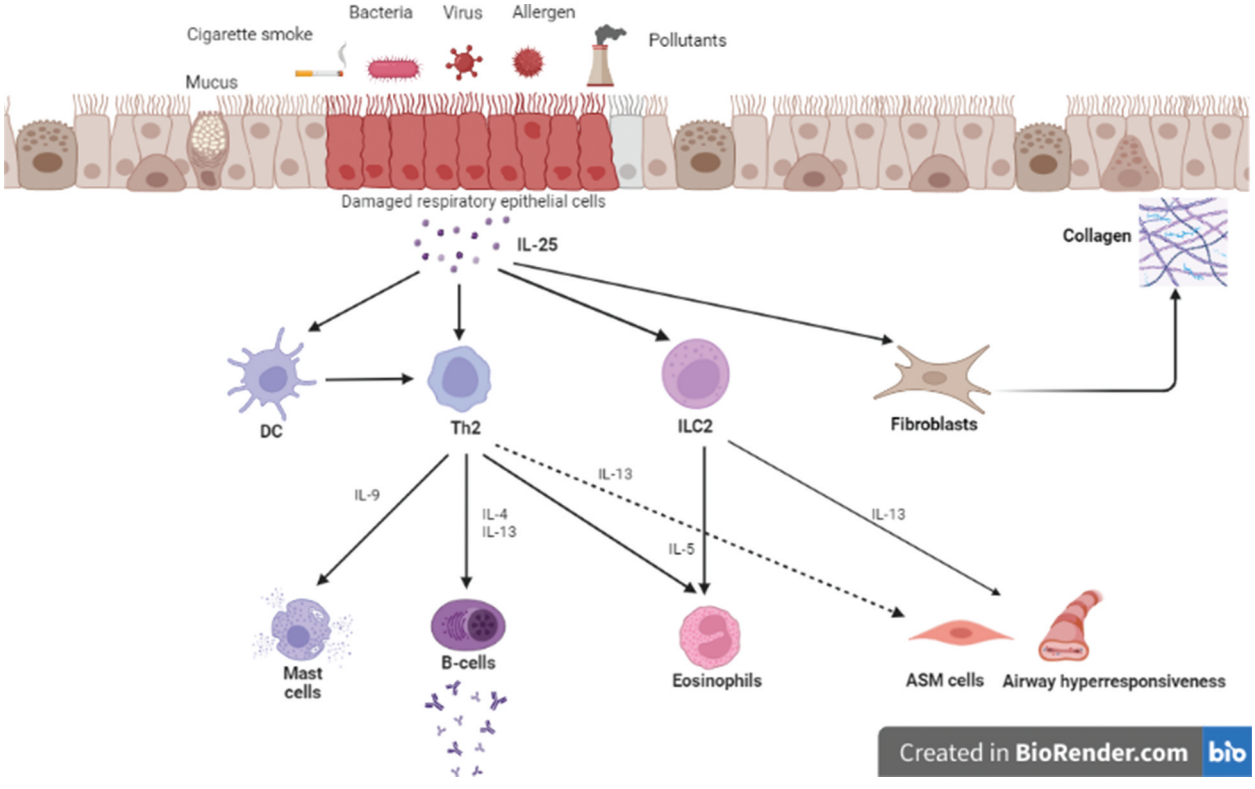

Figure 4. Role of interleukin 25 (IL-25) in airway inflammation, remodeling and fibrosis.

DC: dendritic cells; Th: T-helper cells; ILC2: type 2 innate lymphoid cells; ASM: airway smooth muscle cell; IL-4: interleukin 4; IL-5: interleukin 5; IL-9: interleukin 9; IL-13: interleukin 13.

Increased number of cells expressing IL-25, IL-33, and TSLP was demonstrated in bronchial biopsies from asthmatic patients by clinical and experimental studies [19–22]. Both airway structural cells and incoming inflammatory cells were shown to produce alarmins. The expression of IL-33 and TSLP was mainly detected in AECs, endothelial cells, fibroblasts, mast cells, and neutrophils [19,21,22]. Elevated expression of IL-25 was observed in AECs, fibroblasts, eosinophils, basophils, and mast cells of asthmatic bronchial biopsies [19]. In severe asthma patients, increased expression of IL-33 was also found in ASM cells [23]. In asthmatic patients elevated concentration of IL-25, IL-33, TSLP, and HMGB1 can be detected in blood, sputum, and bronchoalveolar lavage (BAL) fluid [24–33]. Similarly, enhanced expression of alarmin receptors, including IL-17RB, ST2, TSLPR, and RAGE, was demonstrated on cells from sputum or blood of asthmatic patients [24,31,34].

Several clinical and experimental studies showed that the elevated expression of the alarmins in blood and airways was associated with poor asthma control and outcomes [20,25,28–37,39,40]. Elevated concentration of IL-33 in induced sputum of asthmatic children and adults correlated with asthma severity and fraction of exhaled nitric oxide (FeNO) [25,35]. Higher serum IL-33 concentration (measured by standardized enzyme-linked immunosorbent assay) was found in patients with eosinophilic asthma than those with non-eosinophilic asthma, and in patients with allergic asthma compared to non-allergic asthma [36]. Conversely, low serum IL-33 level was shown to be useful for predicting that an adult's asthma will remain in control for months to years after a 50% reduction in the daily inhaled corticosteroid (ICS) dose [37]. Similarly, increased TSLP concentration in sputum correlated with intensity of eosinophilic inflammation in asthmatic children and adults [29,34]. Children with asthma have higher serum TSLP concentrations than healthy controls, but TSLP does not seem to be a biomarker of disease exacerbation or useful in asthma phenotyping [38]. Asthmatic patients with higher IL-25 concentration in sputum and bronchial biopsies have poor asthma control and lung function parameters, greater AHR, eosinophilic inflammation, and increased serum IgE levels [39,40]. Serum IL-25 levels correlated with epithelial IL-25 expression, airway eosinophilia, and good responses to ICS treatment [40]. Higher levels of HMGB1 and endogenous secretory RAGE in the sputum were correlated with disease severity in adults and children with asthma [31,32,41]. Several data showed positive correlations between higher levels of HMGB1 in the sputum with the percentage of neutrophils, while others with the number of sputum eosinophils [31,42]. In asthmatic children, positive correlations were found between sputum levels of HMGB1 and total serum IgE levels, while a good response to ICS was associated with the decrease of the levels of HMGB1 in the sputum [32,41].

Higher concentrations of IL-33 and TSLP in the BAL fluid were inversely correlated with the forced expiratory volume in the first second (FEV1) in asthmatics independently of corticosteroid therapy, and higher expression of TSLP in bronchial biopsies was associated with airway obstruction [20,29]. An inverse correlation between blood levels of IL-25 and IL-33 and FEV1 was demonstrated in some clinical studies [30,35]. Similarly, higher levels of HMGB1 in the sputum were negatively correlated with FEV1 values in both children and adults with asthma [32,41,43].

Studies on asthma exacerbation induced by allergen challenge or experimental rhinovirus infection provided further evidence of strong association between alarmin expression and lung function in asthmatic patients [24,27,44,45]. Allergen challenge produced robust increase of IL-25, IL-33 and TSLP expression in the epithelium, submucosa, eosinophils, and basophils [19,45]. The magnitude of this increase correlated with the extent of the late phase of airway obstruction [19]. Inhalation of a relevant allergen by allergic asthmatics led to a rapid release of IL-33 by AECs and enhanced expression of ST2 on eosinophils in blood and sputum [19,21,44]. Rhinovirus infection enhanced expression of IL-25 and IL-33 on AECs in patients with asthma already treated by ICS and the levels of these alarmins in the BAL is correlated with Th2 cytokines: IL-4, IL-5 and IL-13 [27]. Experimental data showed a high expression of HMGB1 by bronchiolar cuboidal cells during the respiratory syncytial virus infection promoting neutrophilic inflammation at 8–10 days and T2-inflammation via ILC2 activation in the late stage (from days 14 to 30 post-infection) [16,46]. HMGB1 signaling via the RAGE and TLR4 is an important pathway for the sensitization of animals to inhaled dust mite allergens [15]. RAGE production by lung cells is essential for the accumulation of ILC2 and production of IL-5, IL-13, respectively, IL-33 in a murine model of eosinophilic airway inflammation [47].

Additional support for the role of alarmins in asthma comes from the genetic studies which linked mutations within the alarmin genes with risk of asthma [48–50]. The presence of a rare mutation which resulted in loss of function of IL-33 was associated with decreased risk of asthma [49]. Similarly, subjects who carry mutations of TSLP gene leading to decreased expression of TSLP have lower risk to develop asthma [50].

Asthmatic patients are characterized by elevated expression of epithelial alarmins and their receptors. Genetic and environmental factors increasing alarmin expression are associated with altered asthma control, airway inflammation, and worse lung function.

2.2. COPD

COPD is another chronic respiratory disease characterized by progressive irreversible airway obstruction reflecting inflammation (predominantly T1) and remodeling associated with alveolar destruction usually caused by significant exposure to noxious particles or gases [5].

Despite that TSLP and its receptor are highly expressed in the AECs of patients with COPD, its role in COPD is not yet well understood and almost all current data are issues from experimental studies. Overproduction of TSLP was observed in AECs after viral infection, highlighting a role for TSLP in COPD exacerbations [51]. Th17 may mediate TSLP production in AECs in COPD, and anticholinergics could exert an anti-inflammatory effect *via* TSLP-mediated pathway [52]. CS induces TSLP release and TSLPR expression by ASM cells, and these interactions are associated with increased airway

contractility [53]. In COPD patients, TSLP release could be induced by IL-1β and TNF-α, while TSLPR-mediated ASM activation promoted IL-6, –8, and eotaxin-1 production indicating that TSLP pathway plays an important role in both Th2 and Th1 airway inflammation [5].

Experimental studies suggest that IL-33 signaling in COPD is complex. Epithelial damage signals *via* CS, viral infection, or oxidative stress enhance the expression and release of IL-33 in AECs [10,54–56]. IL-33 stimulates apical IL-8 release from goblet cells with an influx of neutrophils to the airways, promotes mucus production, but also the differentiation of macrophages toward an alternatively activated phenotype involved in airway remodeling [9,10,54,57]. CS exposure and oxidative stress highlighted an IL-33-driven Th1 inflammatory response whose mechanisms remain unclear. The effect does not seem to be ILC2-dependent as CS decreased ST2 expression on these cells but enhanced its expression on macrophages and natural killer cells [56]. The exposure to ultrafine particles induces the release of IL-33 in a caspase-4-dependent manner from peripheral blood mononuclear cells of unstable COPD patients compared with healthy subjects, suggesting a possible role in noninfectious acute exacerbations and in pathogenesis of COPD independently from CS [58]. IL-33 seems to play a key role in virus-induced exacerbations. Mice infected with influenza virus after CS exposure had increased release of epithelial IL-33 and enhanced viral-induced responses compared to virus infection alone [56]. A recent experimental study showed that IL-33 induces the production of autoantibodies against AECs in mice and humans, suggesting its involvement in another possible mechanism for COPD pathogenesis [59]. Clinical studies found increased levels of IL-33 in the serum and sputum from stable non-atopic COPD patients that correlates with BEC, while IL-33 gene polymorphism (rs1891385) was associated with impaired lung function and early onset COPD [60–62].

Increased serum levels of IL-25 were found in COPD patients and its production seems to be promoted by TSLP [4,63].

Clinical studies found increased levels of HMGB1 in plasma, sputum, BAL, and bronchial biopsies from patients with stable COPD compared to healthy-subjects smokers and nonsmokers [33,64]. Experimental data reported increased levels of HMGB1 and TLR4 in lung tissues and airways of mice exposed to CS due to extracellular translocation from the cytoplasm, while the HGMB1 inhibition decreased the CS-induced inflammatory response [65]. A clinical study showed that patients with COPD active smokers have higher concentrations of HGMB1-positive cells in the bronchial mucosa and BAL than healthy smokers. The expression of HMGB1 on AECs was associated with an overexpression of RAGE in the AECs and the smooth muscle. A positive correlation was found between BAL levels of HMGB1 and TNF-RII and IL-1β [64]. There were positive correlations between HMGB1 levels in sputum and plasma with neutrophil counts/percentage of neutrophils in patients with COPD and negative correlation with FEV1 [33]. A randomized clinical trial showed increased serum levels of HMGB1 in women with COPD during exacerbations [66].

Despite that all the mechanisms involving epithelial alarmins in COPD pathogenesis are not yet identified, according to the current knowledge, blocking them or their receptors represents a promising therapeutic avenue for COPD patients.

2.3. Other CRD

T2 immune responses mediated by TSLP, IL-33, and IL-25 have been shown to play an important role in pulmonary fibrosis in experimental models; however, their clinical implications remain poorly understood [2,67–69]. Staining for TSLP in IPF identified this protein in alveolar epithelial cells and fibroblasts in fibrotic foci [2]. Bleomycin instillation induces the expression of TSLP in AECs and alveolar epithelial cells, and TSLP participated in the macrophage-induced EMT of alveolar epithelial cells promoting pulmonary fibrosis in mice [2,70]. Similarly, IL-33 is upregulated in experimental lung fibrosis and induces collagen deposition in both ST2 dependent and independent manner [69]. However, peripheral recruitment of ST2 positive cells by IL-33 seems to be very important, as selective bone-marrow ST2 deficiency was sufficient to protect mice from bleomycin-induced lung fibrosis [71]. TSLP and IL-33 were found in increased concentrations in the BAL of patients with IPF compared to controls [72]. Similarly, IL-33 levels in exhaled breath condensate (EBC) in patients with IPF are significantly higher compared to controls and correlated to lung diffusion capacity of carbon monoxide [7]. IPF patients have elevated TSLP concentrations in the serum and EBC, and in stable patients, the treatment by pirfenidone for 12 months significantly decreased its levels in the lung compartment [72]. There is also increased pulmonary expression of IL-25 in human IPF, and IL-25-deficient animals are protected from bleomycin-induced lung fibrosis due to ILC2-related IL-13 production [2]. Clinical and experimental data showed higher expression of HMGB1 in bronchiolar and alveolar epithelial cells in BAL and biopsies from patients with IPF as well in the mice model of bleomycin-induced pulmonary fibrosis [73]. A recent clinical study reported high serum levels of HMGB1 in patients with stable IPF vs controls with a significant increase of its level during exacerbations. In addition, higher levels of HMGB1 were associated with earlier onset of acute exacerbations and with shorter survival in IPF patients [74]. Experimental data showed that HMGB1 enhances epithelial permeability via p63/TGF-β signaling in lung and induces fibroblast to myofibroblast differentiation of lung fibroblasts via NFκBmediated TGFβ1 release [75,76]. Taken together, these data support the contribution of epithelial alarmins in pulmonary fibrosis and several experimental data suggest that targeting these molecules could be an interesting therapeutic option in this pathology.

Several data suggest a role of IL-33 in the pathogenesis of cystic fibrosis (CF), an autosomal recessive disease associated with dysfunctional epithelium, due to altered conductance of chloride across membrane [77–79]. An increased expression of IL-33 in the nucleus of AEC was reported in lung explant biopsies from patients with CF compared to controls. Experimental data on bacteria-stimulated AEC media showed that IL-33 enhanced 1.4-fold the neutrophil recruitment indirectly by stabilizing

the CXCR2 receptor [79]. Pseudomonas aeruginosa exposure increased the expression of IL-33 in CF AEC line through Toll-like receptors 2 and 5 signaling pathways [77]. A clinical study showed that patients with CF have higher levels of IL-33 in the LBA compared to controls that positively correlated with levels of IL-8 and IL-13 but negatively correlated with forced vital capacity (FVC) supporting its role in CF airway inflammation and remodeling [80]. Targeting IL-33 pathway could be an interesting way to treat CF patients with frequent exacerbations despite current therapies. Clinical data showed increased serum and sputum levels of HMGB1 in patients with CF compared to nonsmoking healthy subjects. In addition, the elevation of serum levels was associated with 5% increased risk of pulmonary disease progression, whereas the augmentation of sputum levels was related to a 10% increased risk of lung function decline [81]. Several experimental data suggest that the inhibition of HMGB1 could enhance the bacterial clearance and protect against Pseudomonas aeruginosa pneumonia in CF [82].

Table 1 summarizes the current data on the expression of epithelial alarmins in humans with CRD in vivo.

The next section is dedicated to discuss the efficacy and safety of biological therapies targeting epithelial alarmins in CRD according to current published data. At the moment, there is no biotherapy targeting HMGB1 available and most of the other biologics were studied in asthma and COPD.

3. The efficacy of biologics targeting epithelial alarmins in CRD

3.1. Asthma

Many therapeutic options are currently available for patients with refractory severe asthma driven by T2 inflammation. Biologics including omalizumab, mepolizumab, reslizumab, benralizumab, and dupilumab targeting IgE, IL-5 and IL-4/13 pathways showed benefits by reducing the annualized asthma exacerbation rate (AER) and oral corticosteroids (OCS) use, improving the FEV1 and asthma control [83]. However, there is a proportion of patients with T2 asthma, who do not respond to these biologics. In addition, there are limited treatment options, and biologics are lacking for T2-low asthma. Considering the central, upstream role of alarmins like TSLP, IL-33 and IL-25, they became strong potential therapeutic targets for addressing the treatment needs for patients with both T2-high and T2-low asthma.

Table 1. Expression of epithelial alarmins in patients with chronic respiratory diseases in vivo.

Alarmin	Compartment	Asthma	COPD	IPF	CF
TSLP	Biopsy	↑	↑	↑	-
	Sputum	↑	-	-	-
	BAL	↑	-	↑	-
	Serum	↑	-	↑	-
	EBC	-	-	↑	-
IL-33	Biopsy	↑	↑	↑	↑
	Sputum	↑	↑	-	-
	BAL	↑	-	↑	↑
	Serum	↑	↑	-	-
	EBC	-	-	↑	-
IL-25	Biopsy	↑	-	↑	-
	Sputum	↑	-	-	-
	BAL	↑	-	↑	-
	Serum	↑	↑	-	-
	EBC	-	-	-	-
HMGB1	Biopsy	↑	↑	↑	-
	Sputum	↑	↑	-	↑
	BAL	↑	↑	↑	↑
	Serum	↑	↑	↑	↑
	EBC	-	-	-	-

BAL : bronchoalveolar lavage; EBC : exhaled breath condensate.

3.1.1. Anti-TSLP therapy

Tezepelumab, a fully human monoclonal antibody (IgG2λ) that specifically binds to TSLP, blocking it from interacting with its heterodimeric receptor, was investigated in several clinical trials.

The phase 2b PATHWAY trial evaluated the efficacy and safety of tezepelumab in patients with moderate-to-severe asthma that remained uncontrolled despite treatment with long-acting beta-agonists (LABA) and medium-to-high doses of ICS. This study included 550 patients randomized in four groups, treated by subcutaneous injections of tezepelumab at different doses of 70 mg every 4 weeks, 210 mg every 4 weeks, or 280 mg every 2 weeks or placebo every 2 weeks for 52 weeks. The primary efficacy end point was the AER (events per patient-year). Treatment with tezepelumab led to lower AER in all groups, but higher efficacy was found for the dose of 210 mg administered every 4 weeks with a reduction by 71% (90% CI: 54–82; $p < 0.001$) compared with placebo. Similar results were observed in patients with both T2-low and high disease using as threshold 250 cells/μL for blood eosinophil count (BEC) or 24 ppb for FeNO. The prebronchodilator FEV1 significantly increased in all three groups receiving tezepelumab compared with placebo (by ≥0.12 L, all $p <$ 0.015), effect observed as early as week 4 and maintained throughout the duration of the study. The levels of BEC, FeNO, and serum IgE were decreased in all treatment groups. There was no significant difference between all treatment groups and placebo regarding incidence of adverse events (AEs) ([65.0–67.4%] vs 65.9). The most common reported AEs were asthma symptoms 19.7–27.7%, nasopharyngitis 10.9–13.9%, headache 3.6–8.0%, and bronchitis 3.6–6.6%. Three serious AEs were deemed by the investigator to be related to the trial agent: pneumonia and stroke occurred in the same patient in the low-dose tezepelumab group and a Guillain–Barré syndrome in the medium-dose tezepelumab group. The patient with stroke died 8 weeks after the treatment period ended. The rates of discontinuation due to adverse events were 1.2% among patients receiving tezepelumab and 0.7% in the placebo group. No product-related anaphylactic reactions were reported, neither neutralizing antibodies detected. The maximum rate of injection-site reactions was 3.6% for the patients receiving the treatment similar to those reported in the placebo group [84].

The subsequent phase 3 NAVIGATOR trial investigated the effect of tezepelumab on AER, lung function, Asthma Control Questionnaire-6 (ACQ6), and Asthma Quality of Life Questionnaire (AQLQ). Study enrolled 1061 patients of ≥12-years old with severe uncontrolled asthma, treated by tezepelumab at a dose of 210 mg or placebo every 4 weeks for 52

weeks. The treatment significantly improved the primary end point with an AER of 0.93 (95% CI: 0.80–1.07) with tezepelumab vs 2.10 (95% CI: 1.84–2.39) with placebo (rate ratio [RR], 0.44; 95% CI: 0.37–0.53; $p < 0.001$). In patients with a BEC of <300 cells/μL, the AER was 1.02 in the tezepelumab group and 1.73 in the placebo group (RR, 0.59; 95% CI: 0.46–0.75; $p < 0.001$). The benefit was greater in patients who had T2-high asthma with BEC ≥ 300 cells/μL and FeNO ≥25 ppb. In addition, there were significant improvements in prebronchodilator FEV1 (difference, 0.13 L; 95% CI: 0.08–0.18), ACQ-6 score (difference, −0.33; 95% CI: −0.46 to −0.20) and AQLQ score (difference, 0.34; 95% CI: 0.20–0.47) (all $p < 0.001$). The studied biomarkers BEC, FeNO, and serum IgE were reduced in patients treated with tezepelumab. There was no difference concerning the AEs reported in both groups [85].

The results from the phase 3 SOURCE trial that evaluated the effect of tezepelumab on OCS reduction without loss of asthma control at 48 weeks of treatment in adults with corticosteroid-dependent asthma were disappointed with comparable results for tezepelumab and placebo in the overall population (odds ratio [OR] 1.28; 95% CI: 0.69–2.35, $p = 0.43$). However, 54% of the patients treated by tezepelumab and 46% in the placebo group discontinued OCS at week 48, while 74% of patients in the tezepelumab group and 70% in the placebo group were able to decrease the dose by ≥50%. Greater effect was recorded for the patients with BEC ≥ 150 cells/μL than with placebo (2.58 [1.16–5.75]). Treatment with tezepelumab led to reduction of AER at week 48 by 31% compared to placebo [86]. There are two other ongoing studies investigating the OCS sparing effect of tezepelumab in patients with OCS-dependent asthma. WAYFINDER (NCT05274815) is a single arm, open-label study, enrolling adults with OCS-dependent asthma (prednisone/prednisolone 5–40 mg/day or equivalent for at least 3 months before inclusion) with the aim to reduce OCS dose during the 48 weeks of treatment without loss of asthma control, based on a predefined rapid titration schedule with the assessment of adrenal insufficiency. SUNRISE (NCT05398263) is phase 3 placebo-controlled study evaluating the safety and efficacy of tezepelumab in reducing OCS at week 28 with maintaining asthma control in adult patients with corticosteroid-dependent asthma.

The long-term safety, tolerability, and efficacy of tezepelumab are evaluated in DESTINATION study, a phase 3 trial including patients who had completed either the NAVIGATOR or SOURCE studies. The period of evaluation was 104 weeks (inclusive of the treatment period of either predecessor study). There was no difference between the incidence rates of AE between the groups receiving tezepelumab and placebo. Tezepelumab reduced the AER over 104 weeks vs placebo by 58% (95% CI: 49–65) and 39% (95% CI: 4–62) in NAVIGATOR and SOURCE patients [87].

Two phase 2 studies, UPSTREAM and CASCADE, investigated the impact of tezepelumab on airway inflammation and AHR. In the UPSTREAM study, the administration of 700 mg tezepelumab intravenously every 4 weeks in patients with uncontrolled asthma allowed a nonsignificant improvement of AHR to mannitol at week 12 compared to placebo ($p = 0.06$), whereas airway biopsy and LBA eosinophils were significantly suppressed in the treatment group [88]. Conversely, in the CASCADE study, tezepelumab 210 mg administered subcutaneously every 4 weeks for 28 weeks led to a significant 1.15 doubling dose improvement in mannitol AHR ($p = 0.03$) and greater reduction in airway submucosal eosinophils vs placebo ($p < 0.001$) [89].

Tezepelumab is currently the only approved biologic targeting TSLP, that is effective in patients with uncontrolled moderate-to-severe asthma regardless of the phenotype, with good tolerability and safety profile.

An inhaled anti-TSLP antibody, ecleralimab (CSJ117), has been investigated in a phase 1 study and showed after 12 weeks of treatment significant reduction of allergen-induced bronchoconstriction, sputum eosinophilia, and FeNO levels in mild allergic asthmatics compared to placebo with good tolerability (NCT03138811) [90]. Another phase 2 study investigates the safety and tolerability of CSJ117 as add-on therapy in adults with moderate-to-severe asthma at 24 weeks of treatment (NCT04410523).

3.1.2. Anti-IL-33/R therapy

Currently, biologics targeting IL-33 or IL-33 receptor/ST2 (IL-33 R) are still under study. In a phase 2 trial, an anti-IL-33 antibody, itepekimab, administered subcutaneously 300 mg every 2 weeks was investigated as monotherapy and in combination with dupilumab in patients with moderate-to-severe asthma uncontrolled despite medium-to-high dose ICS and LABA. The LABA was discontinued at week 4, and the ICS tapered over 2 to 3 weeks starting at week 6. For the primary end point of loss of asthma control after 12 weeks, the OR compared to placebo were 0.42 (95% CI: 0.20–0.88; $p = 0.02$) in the itepekimab group, 0.52 (95% CI: 0.26–1.06; $p = 0.07$) in the combination group and 0.33 (95% CI: 0.15–0.70) in the dupilumab group. Itepekimab as monotherapy led to increase by 0.14 L (95% CI: 0.01–0.27) of pre-bronchodilator FEV1 at week 12 when compared with placebo, similarly to dupilumab group − 0.16 L (95% CI: 0.03–0.29), whereas the combination was not better than placebo. Itepekimab monotherapy or in combination significantly improved the ACQ and AQLQ scores ($p < 0.05$) compared with placebo concomitantly with decrease of BEC. Itepekimab alone was also able to reduce FeNO, serum IgE, periostin, and plasma eotaxin-4 levels, although these effects were inferior when compared with dupilumab alone or combination therapy. The incidence of AEs was similar among all four groups: 70% in each of the itepekimab, combination, and placebo groups; 66% in the dupilumab group. The most common AEs in the group treated by itepekimab were nasopharyngitis (18%), headache (8%), nausea (5%), and back pain (5%). No serious adverse event has been reported in the itepekimab group, and the incidence of injection-site reaction was lower in the itepekimab group compared to placebo (1% vs 5%) [91]. Another anti-IL-33 antibody, tozorakimab (MEDI3506) is currently investigated in a phase 2 study in patients with moderate-to-severe asthma.

The development melrilimab (GSK3772847), an anti-ST2 antibody, was discontinued following a phase 2 trial where 66% of patients with moderate-to-severe uncontrolled asthma receiving the treatment (10 mg/Kg, intravenously, every 4

weeks) experienced event indicating loss of asthma control at week 16 vs 81% in the placebo group ($p = 0.045$). There were no differences between both groups regarding FEV1, morning or evening peak expiratory flow (PEF), FeNO values, ACQ-5, and St. George's Respiratory Questionnaire (SGRQ) scores [5]. The efficacy and safety of astegolimab, a human IgG2 antibody targeting ST2, were evaluated in a phase 2b study ZENYATTA in 502 patients with severe asthma receiving 70-mg, 210-mg, 490-mg of treatment or placebo administered subcutaneously every 4 weeks for 52 weeks. The most interesting results were found for the group treated by 490-mg astegolimab with a reduction of AER by 43% compared with placebo ($p = 0.005$), a longer time to the first exacerbation and a higher proportion of patients with an improvement in the AQLQ score (OR 1.79, 95% CI: 1.01–3.18; $p = 0.046$). A greater effect of astegolimab was observed in patients with low BEC (<300 cells/μL) with a reduction of AER over placebo by 54% ($p = 0.002$). BEC was reduced in all active arms groups, and this was maintained throughout the treatment period. There was no significant improvement in prebronchodilator FEV1 or decrease in FeNO levels in any of the treatment groups. AEs were similar in astegolimab and placebo groups ([70.9–72.2%] vs 77.2%). The most common AEs reported by patients receiving the astegolimab were asthma symptoms (33.6–44.4%), nasopharyngitis (9.4–16.7%), headache (5.6–11.5%), and upper respiratory tract infection (4.9–7.1%). A higher rate of injection-site reactions was registered in the treatment groups than in placebo ([4.9–7.9%] vs 0.8%). The incidence of serious AEs was 7.4% in the treatment group compared to 6.3% in the placebo group. Two deaths considered unrelated to the study drug were reported: one due to a severe asthma exacerbation, and another unexplained [92].

Overall, these trials with biologics targeting IL-33/R showed several promising results in severe asthma population. More studies with different design and combination with other biologics could be beneficial for this group of patients.

3.1.3. Anti-IL-25 therapy

Despite that blocking IL-25 in a murine model of allergic asthma prevented allergen-induced AHR, reduced IL-5 and IL-13 production, eosinophil infiltration, goblet cell hyperplasia, and serum IgE secretion [93], the results from a phase 2a study with brodalumab, a human monoclonal antibody which blocks its receptors IL-17-RA and IL-17-RB, were disappointing. No improvements were observed in the primary end point of ACQ after 12 weeks of treatment in patients with uncontrolled moderate-to-severe asthma. Only in the subgroup with high-reversibility (postbronchodilator FEV1 improvement ≥20%), the ACQ change was nominally significant (estimated treatment difference 0.53, $p = 0.02$) in the group treated by 210 mg subcutaneously brodalumab every 2 weeks. Incidences of all AEs were comparable for the treatment groups and the placebo group ([59.2–63.5%] vs 56.6%), as well for the serious AEs ([1.3–4.1%] vs 1.3%). No fatal reaction occurred. The most frequent AEs reported in patients treated by brodalumab were asthma symptoms (18.4–24.3%), upper respiratory infection (5.3–10.8%), and nasopharyngitis (3.9–6.9%). The incidence of injection-site erythema was 6.8–9.2% in the treatment groups, higher than those reported for the placebo group 2.6% [94].

Further studies are needed to assess the efficiency of anti-IL-25 blockade in patients with asthma on other outcomes such as AER, OCS dose reduction and its effect on FeNO, BEC, and IgE, but currently there are no clinical trials in this domain.

3.2. COPD

Exacerbations of COPD represent a major cause of hospital admissions, disease-related morbidity, and mortality, so the decrease of their number is a priority in the management of this disease. A recent Cochrane systematic review including six studies with 5,542 participants showed that mepolizumab and benralizumab are likely to reduce the rate of moderate/severe exacerbations in the highly selected group of patients with COPD and high levels of BEC [95]. However, targeting the alarmins (TSLP, IL-25, and IL-33) or their receptors could be a more effective strategy than cibling the Th2 cytokines, since the blockage would occur earlier in the cascade of the immune reaction.

3.2.1. Anti-TSLP therapy

Tezepelumab is under investigation in a phase 2a randomized trial in patients with moderate-to-very severe COPD treated by triple inhaled maintenance therapy, with ≥2 acute exacerbations/year (NCT04039113). An inhaled anti-TSLP molecule (CSJ117) is also currently evaluated in COPD patients (NCT04882124).

3.2.2. Anti-IL-33/R therapy

A phase 2 study evaluated the impact of itepekimab, an anti-IL-33 antibody (300 mg administered as two subcutaneous injections every 2 weeks) in patients with moderate-to-severe COPD, as an add-on to the standard of care on the exacerbation rate at 52 weeks of treatment and failed to show a significant improvement compared to placebo [96]. However, in the analysis of the subgroup of former-smokers, this treatment significantly reduced the exacerbation rate (RR 0.58 [95% CI: 0.39–0.85]; $p = 0.006$) and improved the FEV_1 (least squares mean difference 0.09 L [0.02–0.15], $p = 0.008$) compared with placebo, so itepekimab is currently under investigation in two phase 3 trials to confirm these results in this specific population (NCT04701983 and NCT04751487). AEs occurred in 78% of patients treated by itepekimab and 80% of patients included in the placebo group, with serious events reported in 17% vs 20% of patients in the same groups. The most common AEs in the treatment group were nasopharyngitis (16%), bronchitis (10%), headache (8%), and upper respiratory tract infection (8%). Three deaths occurred in the itepekimab group and two in the placebo group during the study. The rate of the AEs leading to permanent discontinuation was comparable in the treatment and placebo groups (5% vs 4%). Tozorakimab (MEDI3506), another anti-IL-33 antibody, is in study in a phase 2a trial that evaluates its impact, compared with placebo, on lung function after 12 weeks of treatment in patients with moderate-to-severe COPD (NCT04631016). There are also two ongoing studies assessing the effect of two different doses of tozorakimab vs placebo on

the exacerbation rate at 52-week of treatment (NCT05166889 and NCT05158387).

Furthermore, in individuals with moderate-to-severe COPD, astegolimab, an anti-ST2 antibody administered 490 mg subcutaneous every 4 weeks for 44 weeks did not significantly reduce the exacerbation rate compared to placebo but improved the SGRQ score (the mean difference between the treatment group versus placebo was −3.3[95% CI: −6.4 to −0.2]; $p = 0.039$). Significant differences in geometric mean ratios between the two groups were found for BEC 0.59 (95% CI: 0.51–0.69) and for sputum eosinophil counts 0.25 (95% CI: 0.19–0.33) (both $p < 0.001$). Incidence of treatment-emergent AEs was similar between the study groups [97]. Astegolimab is also under investigation to evaluate its efficacy compared with placebo on the exacerbation rate over a 52-week placebo-controlled treatment period (NCT05037929).

These data suggest that targeting the IL-33/R could be an interesting therapeutic option in former-smokers by improving their quality of life, the exacerbation rate, and the lung function.

Table 2. The effects of antialarmin therapies on asthma and COPD outcomes according to current data from randomized controlled trials.

Disease	Biologic	Anti-TSLP	Anti-IL33	Anti-IL33R	Anti-IL25R
Asthma	ER	↓	-	↓	-
	ACQ score	↓	↓	-	↔
	AQLQ score	↑	↑	↑	-
	FEV1 (L)	↑	↑	↔	-
	AHR	↓	-	-	-
	BEC (cells/μL)	↓	↓	↓	-
	FeNO (ppb)	↓	↓	↔	-
	Total IgE (IU/mL)	↓	↓	-	-
	OCS sparing	↔	-	-	-
COPD	ER	-	↓*	↔	-
	FEV1	-	↑*	-	-
	SGRQ	-	-	↓	-
	BEC	-	-	↓	-

ER: exacerbation rate; ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire; FEV1: forced expiratory volume in 1 second; AHR: airway hyperresponsiveness; BEC: blood eosinophil count; FeNO: fractional exhaled nitric oxide; IgE: immunoglobulin E; OCS: oral corticosteroids; SGRQ: St. George's Respiratory Questionnaire; *: former-smokers.

Table 2 summarizes the current data of the effects of antialarmin therapies on asthma and COPD outcomes.

4. Conclusion

Current evidence suggests that epithelial alarmins play an important role in CRD pathogenesis and exacerbations. However, despite progress in the understanding of their role as inducers of immune responses, all the mechanisms of action of epithelial alarmins are not yet identified and need future investigations. Several clinical trials showed benefits of biologics targeting TSLP and IL-33/R in both T2-high and T2-low severe asthma outcomes, and this is an important advantage compared to currently available biologics targeting only T2-inflammation. More limited data exists in COPD, but the former-smokers seem to have more benefits from antialarmin therapies. Despite the role of the epithelial alarmins in IPF and CF, there are no studies investigating the impact of the biologics targeting these molecules on their clinical outcomes. It could be interesting to study the impact of biologics targeting the epithelial alarmins in T2-mediated fibrosis but also in CF patients who do not respond as well to current antifibrotic therapies, respectively, to elexacaftor-tezacaftor-ivacaftor association in terms of exacerbations.

5. Expert opinion

The respiratory epithelium is considered as the first line of defense in the lungs against environmental insults such as antigens, pathogens, toxins, and pollutants. In response to injury, epithelial cells are required to coordinate the innate immune response and mediate the clearance of invading pathogens and damaged cells to promote resolution of inflammation and restoration of normal tissue homeostasis. However, evidence from the last decades showed that in asthma, COPD, CF, and pulmonary fibrosis, this process becomes dysregulated inducing a persistent inflammatory response, pathological repair, and loss of functional tissue architecture, crucial mechanisms in this CRD pathogenesis. One of the consequences of the epithelial injury is the release of danger signals called alarmins including IL-25, IL-33, TSLP, and HMGB1. These cytokines are well recognized as potent activators of T2 immune responses characteristic for asthma, but recent evidence has highlighted their role in non-T2 inflammation, airway remodeling, and pulmonary fibrosis, making them an attractive therapeutic target.

Despite that current data suggest a role of IL-25 in asthma, COPD, and lung fibrosis pathogenesis, all the mechanisms involving this cytokine are not yet understood. Viral infections, exposure to airborne allergens and oxidative stress upregulate IL-25 and its receptor expression in AECs. IL-25 activates ILC2, stimulates fibroblast differentiation but also modulates EMT of alveolar epithelial cells promoting local tissue remodeling and fibrosis. The administration of a monoclonal antibody targeting the IL-17-RA and -RB, brodalumab, in patients with moderate-to-severe asthma failed to significantly improve asthma control score after 12 weeks of treatment excepting the subgroup with a high reversibility. Future studies are needed to better explore the role of this cytokine in CRD pathogenesis and the interest to block the IL-25 mediated pathway in these specific populations.

IL-33 is a key player in both innate and adaptive immune responses, able to produce T2 and T1 inflammation according to stimuli, involved in airway remodeling and pulmonary fibrosis. IL-33 seems to be involved in allergic and non-allergic asthma, airway remodeling in asthma and COPD, as well as in T2-immunity mediated fibrosis. Current data suggest that IL-33 is involved in virus-induced exacerbations of asthma and COPD, respectively, bacteria-induced exacerbations in CF patients. In this context, blocking the IL-33 pathway represents an interesting therapeutic option for the clinicians. The results issues from phase 2 clinical trials with biologics targeting this pathway are more interesting for the patients with asthma and in the subgroup former-smoker of the patients with COPD with a significant reduction of the exacerbation rates and, for some products,

a positive impact on the lung function, symptoms' control, and/or patients' quality of life. Understanding the complex role of IL-33/ST2 pathway in CRD pathogenesis and the effects of its blockade on the disease evolution should represent a priority area of research in the future.

TSLP has a broad immune effect, activating multiple cell types involved in T2 and non-T2 inflammation, but also promotes mucus secretion by the goblet cells, smooth muscle contraction/hyperreactivity, and airway remodeling in asthma and COPD. TSLP seems to play a role in pulmonary fibrosis and virus-induced exacerbations in COPD patients. The anti-TSLP antibody, tezepelumab, showed undeniable effectiveness as an add-on therapy in severe asthma by decreasing exacerbation rates, improving asthma control, patients' quality of life and lung function but failed to prove an evident benefit on OCS sparing. Tezepelumab is therefore the only one anti-alarmin therapy approved and available as a treatment for asthma. The lack of apparent efficacy in OCS-dependent patients requires further investigation. The effect of anti-TSLP blockade in COPD is still under investigation. As for the other epithelial alarmins, all the mechanisms of action of TSLP are not yet identified, and future studies are needed to evaluate its role in CRD pathogenesis but also to understand how its blockade influences long-term disease evolution.

Increasing data showed that HMGB1 plays not only a key role in the nucleus and cytoplasm as a DNA chaperone, chromosome gatekeeper, autophagy maintainer, and protector from apoptotic cell death but can also act as extracellular alarmin. After cellular injuries, HMGB1 released passively by lytic cell death or secreted actively by viable cells that modulate cell stress responses and inflammation. Experimental data suggest its involvement in the pathogenesis of T2-lung diseases such as asthma by activating ILC2 and DCs but also in non-T2 responses induced by reactive oxygen species, bacterial and viral infections via ferroptosis or pyroptosis and secretion of proinflammatory cytokines involved in lung fibrosis, cancer, and COPD. Treatment with anti-HMGB1 antibody showed promising anti-inflammatory results in an animal model of asthma but these data should be verified by clinical trials.

Targeting epithelial alarmins in CRD seems to be an interesting avenue to treat these CRD in the future. However, many questions remain unanswered about their mechanisms of action and the effects of their blockade on CRD evolution. Concerning the anti-alarmin therapies, more data exist for severe asthma and COPD patients, so future investigations are needed for the other CRD. Head-to-head trials of monoclonal antibodies against IgE, T2 cytokines, and alarmins are required to determine their comparative effectiveness in patients with severe asthma and to help us to apply the precision medicine in this domaine.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Carlier FM, de Fays C, Pilette C. Epithelial barrier dysfunction in chronic respiratory diseases. Front Physiol. 2021;12:691227. doi: 10.3389/fphys.2021.691227
- **An interesting review concerning the epithelial dysfunction in chronic respiratory diseases.**
2. Planté-Bordeneuve T, Pilette C, Froidure A. The epithelial-immune crosstalk in pulmonary fibrosis. Front Immunol. 2021;12:631235. doi: 10.3389/fimmu.2021.631235
3. Burgoyne RA, Fisher AJ, Borthwick LA. The role of epithelial damage in the pulmonary immune response. Cells. 2021;10(10):2763. doi: 10.3390/cells10102763
4. Moermans C, Damas K, Guiot J, et al. Sputum IL-25, IL-33 and TSLP, IL-23 and IL-36 in airway obstructive diseases. Reduced levels of IL-36 in eosinophilic phenotype. Cytokine. 2021;140:155421. doi: 10.1016/j.cyto.2021.155421
5. Calderon AA, Dimond C, Choy DF, et al. Targeting interleukin-33 and thymic stromal lymphopoietin pathways for novel pulmonary therapeutics in asthma and COPD. Eur Respir Rev. 2023;32(167):220144. doi: 10.1183/16000617.0144-2022
- **A very recent review summarizing the role of IL-33 and TSLP in asthma and COPD and the interest to target these cytokines in both diseases.**
6. Gauvreau GM, Bergeron C, Boulet LP, et al. Sounding the alarmins—the role of alarmin cytokines in asthma. Allergy. 2023;78(2):402–417. doi: 10.1111/all.15609
7. Majewski S, Tworek D, Szewczyk K, et al. Epithelial alarmin levels in exhaled breath condensate in patients with idiopathic pulmonary fibrosis: a pilot study. Clin Respir J. 2019;13(10):652–656. doi: 10.1111/crj.13075
8. Varricchi G, Ferri S, Pepys J, et al. Biologics and airway remodeling in severe asthma. Allergy. 2022;77(12):3538–3552. doi: 10.1111/all.15473
9. Donovan C, Hansbro PM. IL-33 in chronic respiratory disease: from preclinical to clinical studies. ACS Pharmacol Transl Sci. 2020;3(1):56–62. doi: 10.1021/acsptsci.9b00099

10. Gabryelska A, Kuna P, Antczak A, et al. IL-33 mediated inflammation in chronic respiratory diseases-understanding the role of the member of IL-1 superfamily. Front Immunol. 2019;10:692. doi: 10.3389/fimmu.2019.00692
11. Yi L, Cheng D, Zhang K, et al. Intelectin contributes to allergen-induced IL-25, IL-33, and TSLP expression and type 2 response in asthma and atopic dermatitis. Mucosal Immunol. 2017;10(6):1491–1503. doi: 10.1038/mi.2017.10
12. Zhang K, Feng Y, Liang Y, et al. Epithelial miR-206 targets CD39/extracellular ATP to upregulate airway IL-25 and TSLP in type 2-high asthma. JCI Insight. 2021;6:e148103, 148103. doi: 10.1172/jci.insight.148103
13. Beale J, Jayaraman A, Jackson DJ, et al. Rhinovirus-induced IL-25 in asthma exacerbation drives type 2 immunity and allergic pulmonary inflammation. Sci Transl Med. 2014;6(256):256ra134. doi: 10.1126/scitranslmed.3009124
14. Yang H, Wang H, Andersson U. Targeting inflammation driven by HMGB1. Front Immunol. 2020;11:484. doi: 10.3389/fimmu.2020.00484. eCollection 2020.
15. Allegra A, Murdaca G, Gammeri L, et al. Alarmins and MicroRNAs, a New axis in the genesis of respiratory diseases: possible therapeutic implications. Int J Mol Sci. 2023;24(2):1783. doi: 10.3390/ijms24021783
16. Simpson J, Loh Z, Ullah MA, et al. Respiratory syncytial virus infection promotes necroptosis and HMGB1 release by airway epithelial cells. Am J Respir Crit Care Med. 2020;201(11):1358–1371. doi: 10.1164/rccm.201906-1149OC
17. Furci F, Murdaca G, Pelaia C, et al. TSLP and HMGB1: Inflammatory targets and potential biomarkers for precision Medicine in asthma and COPD. Biomedicines. 2023;11(2):437. doi: 10.3390/biomedicines11020437
18. Deng M, Tang Y, Li W, et al. The endotoxin delivery protein HMGB1 mediates caspase-11-dependent lethality in sepsis. Immunity. 2018;49(4):740–753.e7. doi: 10.1016/j.immuni.2018.08.016
19. Wang W, Li Y, Lv Z, et al. Bronchial allergen challenge of patients with atopic asthma triggers an alarmin (IL-33, TSLP, and IL-25) response in the airways epithelium and submucosa. J Immunol. 2018;201(8):2221–2231. doi: 10.4049/jimmunol.1800709
20. Ying S, O'Connor B, Ratoff J, et al. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. J Immunol. 2005;174:8183–8190. doi: 10.4049/jimmunol.174.12.8183
21. Préfontaine D, Nadigel J, Chouiali F, et al. Increased IL-33 expression by epithelial cells in bronchial asthma. J Allergy Clin Immunol. 2010;125(3):752–754. doi: 10.1016/j.jaci.2009.12.935
22. Kaur D, Gomez E, Doe C, et al. IL-33 drives airway hyper-responsiveness through IL-13-mediated mast cell: airway smooth muscle crosstalk. Allergy. 2015;70(5):556–567. doi: 10.1111/all.12593
23. Préfontaine D, Lajoie-Kadoch S, Foley S, et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. J Immunol. 2009 Oct 15;183(8):5094–5103. doi: 10.4049/jimmunol.0802387
24. Tang W, Smith SG, Beaudin S, et al. IL-25 and IL-25 receptor expression on eosinophils from subjects with allergic asthma. Int Arch Allergy Immunol. 2014;163(1):5–10. doi: 10.1159/000355331
25. Poulsen NN, Bjerregaard A, Khoo SK, et al. Airway interleukin-33 and type 2 cytokines in adult patients with acute asthma. Respir med. 2018;140:50–56. doi: 10.1016/j.rmed.2018.05.016
26. Momen T, Ahanchian H, Reisi M, et al. Comparison of interleukin-33 serum levels in asthmatic patients with a control group and relation with the severity of the disease. Int J Prev Med. 2017;8(1):65. doi: 10.4103/ijpvm.IJPVM_179_16
27. Southworth T, Pattwell C, Khan N, et al. Increased type 2 inflammation post rhinovirus infection in patients with moderate asthma. Cytokine. 2020;125:154857. doi: 10.1016/j.cyto.2019.154857
28. Paplińska-Goryca M, Grabczak EM, Dąbrowska M, et al. Sputum interleukin-25 correlates with asthma severity: a preliminary study. Postepy Dermatol Alergol. 2018;35(5):462–469. doi: 10.5114/ada.2017.71428
29. Li Y, Wang W, Lv Z, et al. Elevated expression of IL-33 and TSLP in the airways of human asthmatics in vivo: a potential biomarker of severe refractory disease. J Immunol. 2018;200(7):2253–2262. doi: 10.4049/jimmunol.1701455
30. Guo Z, Wu J, Zhao J, et al. IL-33 promotes airway remodeling and is a marker of asthma disease severity. J Asthma. 2014;51(8):863–869. doi: 10.3109/02770903.2014.921196
31. Watanabe T, Asai K, Fujimoto H, et al. Increased levels of HMGB-1 and endogenous secretory RAGE in induced sputum from asthmatic patients. Respir med. 2011;105(4):519–525. doi: 10.1016/j.rmed.2010.10.016
32. Manti S, Leonardi S, Parisi GF, et al. High mobility group box 1: biomarker of inhaled corticosteroid treatment response in children with moderate-severe asthma. Allergy Asthma Proc. 2017;38(3):197–203. doi: 10.2500/aap.2017.38.4047
33. Hou C, Zhao H, Liu L, et al. High mobility group protein B1 (HMGB1) in asthma: comparison of patients with chronic obstructive pulmonary disease and healthy controls. Mol Med. 2011;17(7–8):807–815. doi: 10.2119/molmed.2010.00173
34. Paplińska-Goryca M, Nejman-Gryz P, Proboszcz M, et al. Expression of TSLP and IL-33 receptors on sputum macrophages of asthma patients and healthy subjects. J Asthma. 2020;57(1):1–10. doi: 10.1080/02770903.2018.1543435
35. Wang Y, Wang L, Hua S. Interleukin-33 in children with asthma: a systematic review and meta-analysis. Allergol Immunopathol (Madr). 2017;45(4):387–392. doi: 10.1016/j.aller.2016.12.007
36. Gasiuniene E, Janulaityte I, Zemeckiene Z, et al. Elevated levels of interleukin-33 are associated with allergic and eosinophilic asthma. Scand J Immunol. 2019;89(5):e12724. doi: 10.1111/sji.12724
37. Tsurikisawa N, Oshikata C, Sato T, et al. Low variability in peak expiratory flow predicts successful inhaled corticosteroid step-down in adults with asthma. J Allergy Clin Immunol Pract. 2018;6(3):972–979. doi: 10.1016/j.jaip.2017.10.036
38. Vrsalović R, Korošec P, Štefanović IM, et al. Value of thymic stromal lymphopoietin as a biomarker in children with asthma. Respir med. 2022;193:106757. doi: 10.1016/j.rmed.2022.106757
39. Seys SF, Grabowski M, Adriaensen W, et al. Sputum cytokine mapping reveals an 'IL-5, IL-17A, IL-25-high' pattern associated with poorly controlled asthma. Clin Exp Allergy. 2013;43(9):1009–1017. doi: 10.1111/cea.12125
40. Cheng D, Xue Z, Yi L, et al. Epithelial interleukin-25 is a key mediator in Th2-high, corticosteroid-responsive asthma. Am J Respir Crit Care Med. 2014;190(6):639–648. doi: 10.1164/rccm.201403-0505OC
41. Cuppari C, Manti S, Chirico V, et al. Sputum high mobility group box-1 in asthmatic children: a noninvasive sensitive biomarker reflecting disease status. Ann Allergy Asthma Immunol. 2015;115(2):103–107. doi: 10.1016/j.anai.2015.06.008
42. Shim EJ, Chun E, Lee HS, et al. The role of high-mobility group box-1 (HMGB1) in the pathogenesis of asthma. Clin Exp Allergy. 2012;42(6):958–965. doi: 10.1111/j.1365-2222.2012.03998.x
43. Zhou Y, Jiang Y, Wang W, et al. HMGB1 and RAGE levels in induced sputum correlate with asthma severity and neutrophil percentage. Hum Immunol. 2012;73(11):1171–1174. doi: 10.1016/j.humimm.2012.08.016
44. Mitchell PD, Salter BM, Oliveria JP, et al. IL-33 and its receptor ST2 after inhaled allergen challenge in allergic asthmatics. Int Arch Allergy Immunol. 2018;176(2):133–142. doi: 10.1159/000488015
45. Corrigan CJ, Wang W, Meng Q, et al. Allergen-induced expression of IL-25 and IL-25 receptor in atopic asthmatic airways and late-phase cutaneous responses. J Allergy Clin Immunol. 2011;128(1):116–124. doi: 10.1016/j.jaci.2011.03.043
46. Chen S, Yu G, Xie J, et al. High-mobility group box-1 protein from CC10+ club cells promotes type 2 response in the later stage of respiratory syncytial virus infection. Am J Physiol Lung Cell Mol Physiol. 2019;316(1):L280–L290. doi: 10.1152/ajplung.00552.2017
47. Oczypok EA, Milutinovic PS, Alcorn JF, et al. Pulmonary receptor for advanced glycation end-products promotes asthma pathogenesis through IL-33 and accumulation of group 2 innate lymphoid cells.

J Allergy Clin Immunol. 2015;136(3):747–756.e4. doi: 10.1016/j.jaci.2015.03.011
48. Aneas I, Decker DC, Howard CL, et al. Asthma-associated genetic variants induce IL33 differential expression through an enhancer-blocking regulatory region. Nat Commun. 2021;12(1):6115. doi: 10.1038/s41467-021-26347-z
49. Smith D, Helgason H, Sulem P, et al. A rare IL33 loss-of-function mutation reduces blood eosinophil counts and protects from asthma. PLoS Genet. 2017;13(3):e1006659. doi: 10.1371/journal.pgen.1006659
50. Moorehead A, Hanna R, Heroux D, et al. A thymic stromal lymphopoietin polymorphism may provide protection from asthma by altering gene expression. Clin Exp Allergy. 2020;50(4):471–478. doi: 10.1111/cea.13568
51. Calvén J, Yudina Y, Hallgren O, et al. Viral stimuli trigger exaggerated thymic stromal lymphopoietin expression by chronic obstructive pulmonary disease epithelium: role of endosomal TLR3 and cytosolic RIG-I-like helicases. J Innate Immun. 2012;4(1):86–99. doi: 10.1159/000329131
52. Anzalone G, Albano GD, Montalbano AM, et al. IL-17A-associated IKK-α signaling induced TSLP production in epithelial cells of COPD patients. Exp Mol Med. 2018;50(10):1–12. doi: 10.1038/s12276-018-0158-2
53. Smelter DF, Sathish V, Thompson MA, et al. Thymic stromal lymphopoietin in cigarette smoke-exposed human airway smooth muscle. J Immunol. 2010;185(5):3035–3040. doi: 10.4049/jimmunol.1000252
54. Byers DE, Alexander-Brett J, Patel AC, et al. Long-term IL-33–producing epithelial progenitor cells in chronic obstructive lung disease. J Clin Invest. 2013;123(9):3967–3982. doi: 10.1172/JCI65570
55. Aizawa H, Koarai A, Shishikura Y, et al. Oxidative stress enhances the expression of IL-33 in human airway epithelial cells. Respir Res. 2018;19(1):52. doi: 10.1186/s12931-018-0752-9
56. Kearley J, Silver JS, Sanden C, et al. Cigarette smoke silences innate lymphoid cell function and facilitates an exacerbated type I interleukin-33-dependent response to infection. Immunity. 2015;42(3):566–579. doi: 10.1016/j.immuni.2015.02.011
57. Tanabe T, Shimokawaji T, Kanoh S, et al. IL-33 stimulates CXCL8/IL-8 secretion in goblet cells but not normally differentiated airway cells. Clin Exp Allergy. 2014;44(4):540–552. doi: 10.1111/cea.12283
58. De Falco G, Colarusso C, Terlizzi M, et al. Chronic obstructive pulmonary disease-derived circulating cells release IL-18 and IL-33 under ultrafine particulate matter exposure in a caspase-1/8-independent manner. Front Immunol. 2017;8:1415. doi: 10.3389/fimmu.2017.01415
59. Li Q, Hu Y, Chen Y, et al. IL-33 induces production of autoantibody against autologous respiratory epithelial cells: a potential mechanism for the pathogenesis of COPD. Immunology. 2019;157(2):137–150. doi: 10.1111/imm.13054
60. Kim SW, Rhee CK, Kim KU, et al. Factors associated with plasma IL-33 levels in patients with chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2017;12:395–402. doi: 10.2147/COPD.S120445
61. Sun BB, Ma LJ, Qi Y, et al. Correlation of IL-33 gene polymorphism with chronic obstructive pulmonary disease. Eur Rev Med Pharmacol Sci. 2019;23(14):6277–6282. doi: 10.26355/eurrev_201907_18449
62. Tworek D, Majewski S, Szewczyk K, et al. The association between airway eosinophilic inflammation and IL-33 in stable non-atopic COPD. Respir Res. 2018;19(1):108. doi: 10.1186/s12931-018-0807-y
63. Deng C, Peng N, Tang Y, et al. Roles of IL-25 in type 2 inflammation and autoimmune pathogenesis. Front Immunol. 2021;12:691559. doi: 10.3389/fimmu.2021.691559
64. Ferhani N, Letuve S, Kozhich A, et al. Expression of high-mobility group box 1 and of receptor for advanced glycation end products in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2010;181(9):917–927. doi: 10.1164/rccm.200903-0340OC
65. Cheng Y, Wang D, Wang B, et al. HMGB1 translocation and release mediate cigarette smoke–induced pulmonary inflammation in mice through a TLR4/MyD88-dependent signaling pathway. MBoC. 2017;28(1):201–209. doi: 10.1091/mbc.E16-02-0126
66. Pouwels SD, Nawijn MC, Bathoorn E, et al. Increased serum levels of LL37, HMGB1 and S100A9 during exacerbation in COPD patients. Eur Respir J. 2015;45(5):1482–1485. doi: 10.1183/09031936.00158414
67. Lee JU, Chang HS, Lee HJ, et al. Upregulation of interleukin-33 and thymic stromal lymphopoietin levels in the lungs of idiopathic pulmonary fibrosis. BMC Pulm Med. 2017;17(1):39. doi: 10.1186/s12890-017-0380-z
68. Luzina IG, Lockatell V, Courneya JP, et al. Full-length IL-33 augments pulmonary fibrosis in an ST2- and Th2-independent, non-transcriptomic fashion. Cell Immunol. 2023;383:104657. doi: 10.1016/j.cellimm.2022.104657
69. Luzina IG, Kopach P, Lockatell V, et al. Interleukin-33 potentiates bleomycin-induced lung injury. Am J Respir Cell Mol Biol. 2013;49(6):999–1008. doi: 10.1165/rcmb.2013-0093OC
70. Li D, Guabiraba R, Besnard AG, et al. IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice. J Allergy Clin Immunol. 2014;134(6):1422–1432.e11. doi: 10.1016/j.jaci.2014.05.011
71. Zhao Y, De Los Santos FG, Wu Z, et al. An ST2-dependent role of bone marrow-derived group 2 innate lymphoid cells in pulmonary fibrosis. J Pathol. 2018;245:399–409. doi: 10.1002/path.5092
72. Majewski S, Szewczyk K, Białas AJ, et al. Epithelial alarmins in serum and exhaled breath in patients with idiopathic pulmonary fibrosis: a prospective one-year follow-up cohort study. JCM. 2019;8(10):1590. doi: 10.3390/jcm8101590
73. Hamada N, Maeyama T, Kawaguchi T, et al. The role of high mobility group box1 in pulmonary fibrosis. Am J Respir Cell Mol Biol. 2008;39(4):440–447. doi: 10.1165/rcmb.2007-0330OC
74. Yamaguchi K, Iwamoto H, Sakamoto S, et al. Serum high-mobility group box 1 is associated with the onset and severity of acute exacerbation of idiopathic pulmonary fibrosis. Respirology. 2020;25(3):275–280. doi: 10.1111/resp.13634
75. Kodera Y, Kohno T, Konno T, et al. HMGB1 enhances epithelial permeability via p63/TGF-β signaling in lung and terminal bronchial epithelial cells. Tissue Barr. 2020;8(4):1805997. doi: 10.1080/21688370.2020.1805997
76. Wang Q, Wang J, Wang J, et al. HMGB1 induces lung fibroblast to myofibroblast differentiation through NF-κB-mediated TGF-β1 release. Molecular Medicine Reports. 2017;15(5):3062–3068. doi: 10.3892/mmr.2017.6364
77. Farias R, Rousseau S. The TAK1→IKKβ→TPL2→MKK1/MKK2 signaling cascade regulates IL-33 expression in cystic fibrosis airway epithelial cells following infection by Pseudomonas aeruginosa. Front Cell Dev Biol. 2015;3:87. doi: 10.3389/fcell.2015.00087
78. Manti S, Parisi GF, Papale M, et al. Type 2 inflammation in cystic fibrosis: new insights. Pediatr Allergy Immunol. 2022;33(Suppl 27):15–17. doi: 10.1111/pai.13619
79. Roussel L, Farias R, Rousseau S. IL-33 is expressed in epithelia from patients with cystic fibrosis and potentiates neutrophil recruitment. J Allergy Clin Immunol. 2013;131(3):913–916. doi: 10.1016/j.jaci.2012.10.019
80. Tiringer K, Treis A, Kanolzer S, et al. Differential expression of IL-33 and HMGB1 in the lungs of stable cystic fibrosis patients. Eur Respir J. 2014;44(3):802–805. doi: 10.1183/09031936.00046614
81. Chirico V, Lacquaniti A, Leonardi S, et al. Acute pulmonary exacerbation and lung function decline in patients with cystic fibrosis: high-mobility group box 1 (HMGB1) between inflammation and infection. Clinical Microbiology And Infection. 2015;21(4):368.e1–9. doi: 10.1016/j.cmi.2014.11.004
82. Entezari M, Weiss DJ, Sitapara R, et al. Inhibition of high-mobility group box 1 protein (HMGB1) enhances bacterial clearance and protects against Pseudomonas Aeruginosa pneumonia in cystic fibrosis. Mol Med. 2012;18(3):477–485. doi: 10.2119/molmed.2012.00024
83. Chan R, RuiWen Kuo C, Lipworth B. Pragmatic clinical Perspective on biologics for severe refractory type 2 asthma. J Allergy Clin Immunol Pract. 2020;8(10):3363–3370. doi: 10.1016/j.jaip.2020.06.048

84. Corren J, Parnes JR, Wang L, et al. Tezepelumab in adults with uncontrolled asthma. N Engl J Med. 2017;377(10):936–946. doi: 10.1056/NEJMoa1704064
85. Menzies-Gow A, Corren J, Bourdin A, et al. Tezepelumab in adults and adolescents with severe, uncontrolled asthma. N Engl J Med. 2021;384(19):1800–1809. doi: 10.1056/NEJMoa2034975
•• **A very important RCT evaluating the efficacy ot anti-TSLP antibody in severe asthma.**
86. Wechsler ME, Menzies-Gow A, Brightling CE, et al. Evaluation of the oral corticosteroid-sparing effect of tezepelumab in adults with oral corticosteroid-dependent asthma (SOURCE): a randomised, placebo-controlled, phase 3 study. Lancet Respir Med. 2022;10(7):650–660. doi: 10.1016/S2213-2600(21)00537-3
87. Menzies-Gow A, Wechsler M. DESTINATION: tezepelumab long-term safety and efficacy versus placebo in patients with severe, uncontrolled asthma. Thorax. 2022;77:A32.
88. Sverrild A, Hansen S, Hvidtfeldt M, et al. The effect of tezepelumab on airway hyperresponsiveness to mannitol in asthma (UPSTREAM). Eur Respir J. 2022;59(1):2101296. doi: 10.1183/13993003.01296-2021
89. Diver S, Khalfaoui L, Emson C, et al. Effect of tezepelumab on airway inflammatory cells, remodeling, and hyperresponsiveness in patients with moderate-to-severe uncontrolled asthma (CASCADE): a double-blind, randomised, placebo-controlled, phase 2 trial. The Lancet Respiratory Medicine. 2021;9(11):1299–1312. doi: 10.1016/S2213-2600(21)00226-5
90. Novartis Pharmaceuticals. A randomized, subject- and investigator-blinded, placebo-controlled, parallel design, broncho-provocation study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of multiple doses of inhaled CSJ117 in adult subjects with mild atopic asthma. Report No. NCT03138811. Available from: https://clinicaltrials.gov/ct2/show/NCT03138811
91. Wechsler ME, Ruddy MK, Pavord ID, et al. Efficacy and safety of itepekimab in patients with moderate-to-severe asthma. N Engl J Med. 2021;385(18):1656–1668. doi: 10.1056/NEJMoa2024257
•• **An interesting RCT investigating the efficacy of an anti-IL-33 antibody in moderat-to-severe asthma.**
92. Kelsen SG, Agache IO, Soong W, et al. Astegolimab (anti-ST2) efficacy and safety in adults with severe asthma: a randomized clinical trial. J Allergy Clin Immunol. 2021;148(3):790–798. doi: 10.1016/j.jaci.2021.03.044
93. Ballantyne SJ, Barlow JL, Jolin HE, et al. Blocking IL-25 prevents airway hyperresponsiveness in allergic asthma. J Allergy Clin Immunol. 2007;120(6):1324–1331. doi: 10.1016/j.jaci.2007.07.051
94. Busse WW, Holgate S, Kerwin E, et al. Randomized, Double-blind, placebo-controlled study of brodalumab, a human anti–IL-17 receptor monoclonal antibody, in moderate to severe asthma. Am J Respir Crit Care Med. 2013;188(11):1294–1302. doi: 10.1164/rccm.201212-2318OC
95. Donovan T, Milan SJ, Wang R, et al. Anti-IL-5 therapies for chronic obstructive pulmonary disease. Cochrane Database Of Systematic Reviews. 2020;12(12):CD013432. doi: 10.1002/14651858.CD013432.pub2
96. Rabe KF, Celli BR, Wechsler ME, et al. Safety and efficacy of itepekimab in patients with moderate-to-severe COPD: a genetic association study and randomised, double-blind, phase 2a trial. Lancet Respir Med. 2021;9:1288–1298. doi: 10.1016/S2213-2600(21)00167-3
97. Yousuf AJ, Mohammed S, Carr L, et al. Astegolimab, an anti-ST2, in chronic obstructive pulmonary disease (COPD-ST2OP): a phase 2a, placebo-controlled trial. Lancet Respir Med. 2022;10(5):469–477. doi: 10.1016/S2213-2600(21)00556-7