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REVIEW ARTICLE

Biologics and airway remodeling in severe asthma

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Abstract

Asthma is a chronic inflammatory airway disease resulting in airflow obstruction, which in part can become irreversible to conventional therapies, defining the concept of airway remodeling. The introduction of biologics in severe asthma has led in some patients to the complete normalization of previously considered irreversible airflow obstruction. This highlights the need to distinguish a "fixed" airflow obstruction due to structural changes unresponsive to current therapies, from a "reversible" one as demonstrated by lung function normalization during biological therapies not previously obtained even with high-dose systemic glucocorticoids. The mechanisms by which exposure to environmental factors initiates the inflammatory responses that trigger airway remodeling are still incompletely understood. Alarmins represent epithelial-derived cytokines that initiate immunologic events leading to inflammatory airway remodeling. Biological therapies can improve airflow obstruction by addressing these airway inflammatory changes. In addition, biologics might prevent and possibly even revert "fixed" remodeling due to structural changes. Hence, it appears clinically important to separate the therapeutic effects (early and late) of biologics as a new paradigm to evaluate the effects of these drugs and future treatments on airway remodeling in severe asthma.

KEYWORDS
airway remodeling, biologics, biomarkers, immunotherapies, severe asthma

Abbreviations: AHR, airway hyperresponsiveness; ANGPT, angiopoietin; ASM, airway smooth muscle; BAL, brocho-alveolar lavage; βc, common receptor β subunit; COPD, chronic obstructive pulmonary disease; CRSwNP, chronic rhinosinusitis with nasal polyps; DC, dendritic cell; EBUS, endobronchial ultrasound; ECM, extracellular matrix; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EGF, epidermal growth factor; EMT, epithelial-mesenchymal transition; EPX, eosinophil peroxidase; FcεRI, high affinity IgE; FeNO, fractional exhaled nitric oxide; FDA, food and drug administration; FGF, fibroblast growth factor; GINA, global initiative for asthma; GM-CSF, granulocyte-macrophage colony-stimulating factor; HLM, human lung macrophage; HLMC, human lung mast cell; HRCT, high-resolution computed tomography; IL, interleukins; IL-5Rα, IL-5 receptor α; ILC, innate lymphoid cell; i.v., intravenously; lfTSLP, long form TSLP; mAb, monoclonal antibody; MBP, major basic protein; MMP, matrix metalloproteinase; NET, neutrophils extracellular trap; NHR, nuclear magnetic resonance; NK cell, natural killer cell; NKT cell, natural killer T cell; PDGF, platelet-derived growth factor; PGD_2, prostaglandin D_2; RCTs, randomized clinical trials; sfTSLP, short form TSLP; TGF-β, transforming growth factor β; TSLP, thymic stromal lymphopoietin; T2-high, type 2-high; T2-low, type 2-low; VDP, ventilation defect; VEGF, vascular endothelial growth factors.

1 | INTRODUCTION

Asthma is a heterogeneous chronic inflammatory disease of the respiratory system that affects approximately 10% of adults.[1] A typical feature of asthma is a variable airflow limitation associated with symptoms such as dyspnea, cough, wheezing, and chest tightness.[2] The heterogenicity of the immunologic disorder is reflected in different phenotypes that differ in etiology, pathogenic mechanisms, symptoms, and severity.[3] Based on airway inflammation, asthma has been subdivided into type 2-high (T2-high) and type 2-low (T2-low), although the latter form is rare in clinical practice.[3–5] A similar distinction is made between eosinophilic and non-eosinophilic asthma (GINA 2021).[6] Severe asthma is defined as asthma that is not well controlled despite the adherence to maximal optimized pharmacological therapy and treatment of contributory factors.[7] Up to 6% of asthma patients have severe asthma, with a reduced quality of life, and increased risk of exacerbations, hospitalizations, and death.[8,9]

In T2-high asthma, immunologic stimuli (e.g., allergens, and viral and bacterial superantigens) activate primary effector cells of allergic disorders (i.e., mast cells and basophils) through the engagement of specific IgE to release a plethora of interleukins (ILs) (e.g., IL-3, IL-4, IL-5, and IL-13).[10,11] Eosinophils and their mediators contribute to the pathogenesis of IgE-mediated asthma and play pivotal roles in eosinophilic asthma. T2-low asthma is heterogeneous, incompletely defined and understood and presumably includes different phenotypes characterized by the involvement of mast cells, macrophages, neutrophils, and/or a mixture of these immune cells.[3,5] Bronchial epithelial cell-derived alarmins (e.g., TSLP, IL-33, and IL-25) are upstream cytokines that initiate immunologic events culminating in airway remodeling.[12–14] The latter is a complex process requiring a timely expression of fibrogenic[15] and angiogenic factors causing profound structural alterations of the bronchial walls and blood vessels.[16,17] These alterations contribute to the reduction of airway caliber and stiffening, resulting clinically in airflow limitations and respiratory symptoms.[18]

2 | AIRWAY REMODELING

Airway remodeling can affect both large and small airways[19] and is characterized by structural changes including goblet cell metaplasia, subepithelial matrix protein deposition and fibrosis, overexpression of angiogenic factors, and hyperplasia/hypertrophy of airway smooth muscle cells.[15–17,20–23] Increased deposition of extracellular matrix (ECM) proteins in the reticular basement membrane (RBM), lamina propria, and submucosa is a characteristic of asthmatic airways and contributes to the airway wall thickening and airflow obstruction.[24,25] Collagen fibers, fibronectin, and tenascin are the most abundant elements of the ECM in the asthmatic lung.[26–29] Aberrant accumulation of ECM proteins leads to alterations in tissue structure and function, contributing to airway remodeling in asthma.[30–32] ASM hypertrophy/hyperplasia (e.g., increased ASM mass) are features of asthmatic airway remodeling.[18,3334,35] ASM cells in asthmatic individuals also produce increased amounts of collagen and fibronectin.[36,37] The increase in the ASM mass contributes to bronchial obstruction,[38] loss of lung function,[21] and greater susceptibility to external triggers.[20,39,40] Airway remodeling has been implicated in irreversible airflow limitation with consequent poor symptom control and lack of response to treatment.[21,41,42]

The airway epithelium is a key component of the innate immune system and the initiator of airway remodeling in asthma.[14] A plethora of environmental insults (e.g., allergens, cytokines, microbial proteins, smoke extracts, and chemical and physical insults) damage and/or activate epithelial cells to release several cytokines, including TSLP,[13,43] IL-33,[14,44] IL-25/IL-17E,[12] TGF-β, and granulocyte-macrophage colony-stimulating factor (GM-CSF), which can recruit/activate dendritic cells (DCs), type 2 innate lymphoid cells (ILC2s), mast cells, eosinophils, and other immune cells.[45]

Thymic stromal lymphopoietin (TSLP), constitutively expressed by human bronchial epithelial cells,[46–49] can be rapidly released as a result of cell injury in response to a variety of inflammatory stimuli.[46,50–54] TSLP is also released by DCs,[55] mast cells,[56] human lung macrophages (HLMs),[57] and fibroblasts.[58,59] TSLP exerts its effects by binding to a high-affinity heterodimeric receptor complex composed of TSLPR and IL-7Rα.[13] There are two isoforms of TLSP: the long (lf) and the short (sf) isoforms; the former is also known as TSLP and its expression increases during inflammation.[57,60] The sfTSLP, constitutively expressed in epithelial cells, lung fibroblasts, and HLMs,[57,61] is not upregulated by inflammation. For instance, house dust mites induce lfTSLP but not sfTSLP in human bronchial epithelial cells.[62] TSLP immunostaining is increased in the airway epithelium in asthmatic patients,[49] and its concentrations are increased in the broncho-alveolar lavage (BAL) fluid of asthmatics.[48] TSLP is overexpressed in the airways of severe asthma patients,[48] and bronchial allergen challenge of asthmatics increases the expression of $TSLP^+$ cells in the epithelium and submucosa.[47]

TSLP promotes allergic responses and airway remodeling[63] by acting on DCs,[55] thereby promoting the differentiation of naive $CD4^+$ T cells into Th2 cells[64,65] and by inducing the survival of ILC2s[13,66,67] and the induction of epithelial-mesenchymal transition (EMT) in airway epithelial cells.[68] Human lung fibroblasts are also a significant source of TSLP.[59,69] Through an autocrine mechanism, TSLP can activate human lung fibroblasts[70] to release type I collagen[71] and promote the proliferation of ASM cells.[72] TSLP has also been shown to cause goblet cell metaplasia and mucus production.[73–75] TSLP activates human eosinophils,[13] mast cells,[56,76] and HLMs.[57] The multiple activating properties of TSLP on a plethora of immune and structural cells indicate that this cytokine plays a role in T2-high and T2-low asthma.

IL-33, an IL-1 superfamily alarmin released by airway epithelial cells and endothelial cells,[77] activates the ST2 receptor on several cells of the innate and adaptive immunity.[77] IL-33 activates ILC2s[78–80] and induces type 2 cytokines (i.e., IL-5 and IL-13) in human mast cells,[81] collagen, and fibronectin release from airway fibroblasts.[82,83] IL-33 and superantigenic activation of human lung mast cells (HLMCs) induce the release of angiogenic and lymphangiogenic factors.[84] IL-33, alone and

in combination with IL-3, activates human basophils to release a wide spectrum of cytokines (i.e., IL-4, IL-13, CXCL8, and VEGF-A).[85,86] IL-33 expression is associated with enhanced RBM thickness in bronchial biopsies of tissues severe asthmatic children.[83] In addition, it has been reported that airway remodeling is absent in $ST2^{-/-}$ mice in a model of house dust mite (HDM)-induced asthma.[83] Collectively, IL-33 and IL-33/ST2 signaling pathways might be involved in both airway inflammation and asthma remodeling through the activation of several immune and structural cells.

IL-25, also known as IL-17E, is a unique cytokine of the IL-17 family produced by a subset of airway epithelial cells (i.e., brush cells).[12,87,88] Airborne allergens, ATP, and viral infections upregulate IL-25 and its receptor IL-17RB in airway epithelial cells and submucosa.[89,90] IL-25 activates ILC2s,[91] modulates EMT of alveolar epithelial cells and local tissue remodeling,[92] and upregulates cytokine expression in lung fibroblasts.[93] IL-25 drives lung fibrosis in several mouse models.[92,94,95]

A plethora of cytokines (transforming growth factor β [TGF-β], platelet-derived growth factor [PDGF], fibroblast growth factor [FGF], epidermal growth factor [EGF], vascular endothelial growth factors [VEGFs]), and chemokines (e.g., CXCL2, CXCL3, IL-8/CXCL8) contributes directly and indirectly to airway remodeling in asthma.[96–98] In particular, TGF-β, produced by macrophages and eosinophils, is a main mediator responsible for airway remodeling by inducing epithelial-mesenchymal transition (EMT).[99] IL-4 activates ASM cells, causing an increase in actin and collagen synthesis as well as TGF-β release by the bronchial epithelium.[20,100] IL-5 promotes subepithelial and peri-bronchial fibrosis through the recruitment and activation of eosinophils, a major source of TGF-β.[101,102] IL-13 induces the release of TGF-β from bronchial epithelial cells, which increases goblet cell metaplasia.[20,103–106]

IgE itself could play a role in airway remodeling by stimulating the production of interleukins.[107] Several investigators have reported that human monomeric IgE, in the absence of cross-linking, can induce the release of cytokines (e.g., IL-4) and chemokines (e.g., CXCL8) from mast cells.[108,109] Moreover, Roth et al have shown that in vitro incubation of serum containing IgE obtained from allergic asthmatics caused ASM proliferation and marked production of type I collagen.[110]

Similarly, a large number of cells of the innate and adaptive immune system contribute directly and indirectly to airway remodeling in asthma. Human eosinophil granules are armed with cytotoxic major basic protein (MBP), eosinophil peroxidase (EPX), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), and galectin-10 (also known as Charcot-Leyden protein).[111] ECP and MBP induce the release of preformed (histamine and tryptase) and de novo synthesized mediators (prostaglandin D_2: PGD_2) from human mast cells.[112–114] Activated human eosinophils secrete LTC_4 and a wide array of type 2 cytokines (e.g., IL-5, IL-4, and IL-13) and TGF-β.[96,115] Altogether, current data indicate that eosinophils play a globally pathogenic role in airway remodeling.

Macrophages (alveolar and interstitial) are the most abundant immune cell type in human lung[116–118] and are involved in immune responses as well as tissue remodeling.[119] HLMs contribute to airway remodeling through the release of TGF-β, matrix metalloproteinases (MMPs), angiogenic (e.g., VEGF-A and ANGPT2), and lymphangiogenic factors (i.e., VEGF-C).[57,120] HLMCs are important lung-resident immune cells involved in asthmatic airway remodeling.[121] IgE- and non-IgE-mediated activation of HLMCs induces the release of several profibrotic cytokines (e.g., IL-13 and TNF-α), as well as inflammatory mediators (e.g., PGD_2 and tryptase).[113,114] Tryptase induces fibroblast, endothelial and epithelial cell proliferation, further fueling airway remodeling in asthmatic individuals.[122] Neutrophils have also been shown to produce MMP-9,[123,124] angiogenic factors,[125] and neutrophil extracellular traps (NETs)[126–128] and can be associated with severe asthma.[126]

Angiogenesis is fundamental to providing the blood vessels to maintain tissue homeostasis,[125] whereas inflammatory angiogenesis is a critical factor in the development of a disease process.[84,129,130] Blood vessel density and vascular area are increased in patients with asthma.[16,17]

A recent study in a mouse model of asthma demonstrated dynamic changes in the respiratory microbiota at different stages of the disease. In particular, *Staphylococcus* and *Cupriavidus* were more abundant during airway remodeling.[131] Additional studies are urgently needed to investigate whether the dysbiosis of airway microbiota could also play a role in the progression from allergic inflammation to airway remodeling in humans.

2.1 | Imaging of airway remodeling

The direct assessment of human airway remodeling can be performed in vivo on bronchial biopsies in asthmatic patients.[115,132,133] Bronchial biopsies identify useful information on bronchial and pulmonary alterations, such as thickening of the bronchial tissue, infiltration and density of inflammatory cells, aberrant accumulation of elements of ECM, and ASM hyperplasia and hypertrophy.[15] However, this procedure has several limitations that should be pointed out. First, it is not part of the routine clinical evaluation of asthmatic patients. Second, it does not easily facilitate serial biopsies in the same patient. Third, the results can be significantly influenced by the operator and the techniques used to assess tissue remodeling.

During the last decade, high-resolution computed tomography (HRCT) and nuclear magnetic resonance (NHR) are gaining a place as non-invasive techniques to examine different aspects of airway remodeling in asthma. Hoshino found increased airway wall thickening in asthmatics assessed by HRCT.[134] Bronchial wall thickness assessed by endobronchial ultrasound (EBUS) was increased in asthma patients than healthy controls.[135] Hartley et al found that the proximal airway wall area was increased in asthmatics compared to controls.[136] The loss of the peripheral pulmonary vasculature, also termed pruning, was associated with asthma severity.[137] Recently, Eddy and co-workers demonstrated that the total number of CT-visible airways was correlated to asthma severity.[138]

Preliminary results derive from imaging studies that have evaluated the effects of biological therapies on airway remodeling

using HRCT. Hoshino et al reported that 16-week treatment with omalizumab reduced the airway wall thickness and the number of sputum eosinophils.[139] In another study, 48-week treatment with omalizumab reduced the airway wall area corrected for body surface, but no changes in percentage wall area, without changes in the luminal area.[140] In two studies conducted by Haldar et al[141,142] on airway remodeling by HRCT, it was found that the biological treatment determined a greater variation in pre-/post-treatment luminal area compared to the placebo group. A recent study evaluated the impact of one-year mepolizumab therapy on airway remodeling through EBUS and HRCT. Improved airway remodeling (e.g., reduction in bronchial wall thickness) was better noticeable in invasive EBUS than in non-invasive HRCT.[143] In the phase 2 CASCADE study, tezepelumab increased the CT scan-determined lumen area across airway generations.[132]

Hyperpolarized helium-3 MRI of the lung has demonstrated regional heterogeneity of lobar ventilation in asthma,[144] which is correlated with asthma severity.[145] MRI ventilation defects (VDP are correlated to sputum eosinophilia in severe asthma[146] and are a predictor of exacerbation).[147] Collectively, studies with CT and MRI in asthma have shown some structural and functional changes in airways and pulmonary vasculature associated with more severe disease but are unable to differentiate between reversible and potentially irreversible changes.

Finally, fractional exhaled nitric oxide (FeNO) has been proposed to assess airway structure variations in asthma patients, especially in the distal airway. FeNO was associated with bronchial wall thickening in the third to the sixth generation of bronchial trees.[148]

2.2 | Single-cell transcriptomics of airway remodeling

The human lung is a complex tissue which comprises more than 40 cell populations, including immune and structural cells.[149,150] Previous investigations of airway remodeling in asthma were limited by the examination of a restricted profile of immune and structural cells. Next-generation sequencing technologies (RNA-Seq) are now available to identify transcriptomic map of the human lung.[150,151] Transcriptomic analysis of flow cytometry-sorted cell population from the human lung and single-cell RNA-Seq (scRNA-Seq) allows reliable identification of even closely related cell population.[152] scRNA-Seq also allows the identification of known or novel cell populations for which there are no reliable surface markers during health and disease.[153] scRNA-Seq has been recently employed to reveal ectopic and aberrant lung-resident cell populations in patients with pulmonary fibrosis.[150,151] Recently, single-cell transcriptomic of mouse lung revealed a population of inflammatory memory neutrophils with specific molecular features in a model of allergic asthma.[154] Vieira Braga and collaborators used single-cell transcriptomics to chart the cellular landscape of airways and lung parenchyma in healthy and asthmatic lungs. These authors discovered a novel subset of $CD4^+$ T cells in asthmatic airway wall and a novel mucous ciliated cell in the airway epithelium that contributes to goblet cell hyperplasia.[133] We anticipate that the use of scRNA-Seq will provide fundamental information to chart the landscape of immunological and structural cells involved in airway remodeling in different pheno-endotypes of asthma and the effects of biologics in this heterogeneous disorder.

2.3 | Targeting airway remodeling

Experimental and human studies have demonstrated that glucocorticoids and certain anti-inflammatory drugs can reduce inflammation and morbidity in mild to moderate asthma. However, evidences of beneficial effects of inhaled (ICS) or oral (OCS) glucocorticoids on airway remodeling have not been demonstrated, with several reports showing contradictory results with ICS.[17,155,156] The introduction of several biological immunotherapies (e.g., anti-IgE, anti-IL-5/IL-5Rα, anti-IL-4Rα, and anti-TSLP)[2,157] has contributed to the development of a personalized medicine approach for the treatment of patients with severe asthma.[158–161] There is some evidence that these biologics can improve not only clinical symptoms but also certain features of airway remodeling and functional decline of FEV_1. It is important to emphasize that FEV_1 and other spirometric indices are only surrogate markers of airway remodeling in asthma and any improvement in FEV_1 after treatment with biologics could be attributable not only to the attenuation of any remodeling processes but also due to the changes in bronchial hyperreactivity, autonomic nervous system modulation and other factors. Finally, it will be important to evaluate the effects of biological immunotherapies in randomized clinical trials (RCTs) and real-life settings.[162,163]

2.4 | Omalizumab

Omalizumab is a humanized IgG1-κ monoclonal antibody (mAb) that binds to the Fc fragment of IgE.[164] This mAb inhibits the binding of IgE to the high-affinity IgE (FcεRI) receptor on human mast cells, basophils,[165,166] and DCs.[167] Several studies have documented the efficacy of omalizumab in improving allergic asthma and reducing symptoms and exacerbations[168–170] also in children and pregnant women.[171–173] Although omalizumab did not cause FEV_1 improvement in RCTs,[174,175] there is some evidence that this mAb can improve FEV_1 in real-life settings[176,177] and can reduce the thickness of the basement membrane[139,178] and fibronectin deposits in asthmatic airways[179] in addition to preventing exacerbation-induced inflammatory alterations in the airways. IgE-containing serum from asthmatic patients stimulated in vitro mesenchymal cell proliferation and accumulation of collagen and fibronectin. Both proliferation and matrix deposition were prevented by preincubation of the cells with omalizumab.[110]

2.5 | Mepolizumab

Mepolizumab is an IgG1-κ anti-IL-5 mAb approved as add-on treatment for severe eosinophilic asthma (SEA).[180,181] IL-5 is the most important growth, differentiation, and activation factor of human eosinophils.[182] This cytokine acts on eosinophils by binding to the specific IL-5 receptor (IL-5R), which consists of an IL-5 receptor α (IL-5Rα) subunit and the common receptor β subunit (βc).[183] IL-5, together with IL-3 and GM-CSF,[184] is crucial for the maturation of human eosinophils in the bone marrow.[57,183] IL-5 is mainly produced by type-2 ILC2s, Th2 cells, mast cells, invariant NKT cells, and eosinophils themselves.[182] Human eosinophils can also be activated by IL-33[77] and TSLP.[13]

The efficacy and safety of mepolizumab have been demonstrated in several RCTs.[185–188] The DREAM study, conducted with mepolizumab at 3 different doses administered intravenously (*i.v.*), showed the clinical efficacy but there were no statistically significant changes in $FEV_{1,}$ although there was an improvement, compared to baseline, in FEV_1 in the mepolizumab group versus placebo.[185] In the MENSA study, the FEV_1 increase was rapid, starting from the first administration, and persisted over time.[188] Two additional studies showed a rapid and long-lasting improvement in pre-bronchodilator FEV_1 in the mepolizumab group compared to placebo.[189,190] There is some evidence that mepolizumab can also improve FEV_1 in real-life settings.[191–193]

Interestingly, some patients first enrolled in the COSMOS study and subsequently in the long-term COSMEX study, with a suspension of more than 12 weeks of mepolizumab between the two, reported a transient worsening of their symptoms and $FEV_{1,}$ which rapidly improved upon reintroduction of mepolizumab.[187] A clinical trial (NCT03797404) is evaluating the effects on airway remodeling during mepolizumab treatment.

In a pioneering study, Flood-Page and collaborators examined the bronchial biopsies of mild atopic asthmatics, treated only with SABA, studied before and after treatment with three mepolizumab infusions.[115] They demonstrated that the thickness and density of tenascin in the RBM, the airway TGF-β1$^+$ eosinophils, and the BAL concentrations of TGF-β1 were increased in mild asthmatic patients compared to normal subjects. As expected, mepolizumab reduced bronchial eosinophil numbers but also TGF-β1$^+$ eosinophils, thickness and tenascin immunoreactivity, and the concentration of TGF-β1 in BAL fluid.[115]

2.6 | Reslizumab

Reslizumab is a humanized IgG4-κ anti-IL-5 mAb developed by drafting technology from a rat mAb with high affinity against human IL-5.[194] Reslizumab binds to a small region corresponding to amino acids 89-92 of IL-5, which are critical for binding to IL-5Rα.[195] Reslizumab administration results in clinical improvement in asthma and an increase in FEV_1 in patients with eosinophilic counts >400 cells/μl compared to placebo.[195–200] Apparently contrasting results have been reported on the effects of reslizumab on the improvement of FEV_1 compared to placebo. Although several studies reported that reslizumab significantly improved FEV_1 compared to placebo,[196,199] other investigators found that reslizumab had no improvement in FEV_1 compared to those receiving placebo.[196] A real-life study recently confirmed the efficacy of reslizumab treatment in reducing the number of exacerbations and increasing FEV_1 6 months after the beginning of treatment.[201] Further investigations are needed to evaluate the potential role of reslizumab on mechanisms implicated in the pathogenesis of airway remodeling in asthma patients.

2.7 | Benralizumab

Benralizumab is a humanized, afucosylated IgG1-κ mAb that targets the α subunit of the IL-5 receptor (IL-5Rα) and it binds via the constant Fc fragment to the FcγRIIIa receptor for IgG expressed on natural killer (NK) cells, macrophages, and neutrophils, which results in eosinophil apoptosis via antibody-dependent cell-mediated cytotoxicity.[202] Several studies have demonstrated that benralizumab administration leads to subjective and functional clinical improvement in patients with SEA. RCTs reported an improvement in FEV_1 at 12 weeks from the beginning of treatment.[203–205] A post hoc analysis conducted on SIROCCO and CALIMA data further documented that benralizumab promoted functional improvement even in patients with fixed airflow obstruction, an alteration found in approximately 16% of patients with severe asthma.[206] A recent study extended the previous results by showing that benralizumab caused a rapid (4 weeks) improvement in FEV_1, which increased after 12 weeks and persisted throughout the period (24 weeks) of observation.[205]

Cachi and collaborators evaluated the effects of benralizumab on airway remodeling by examining biopsies from patients with SEA.[207] Benralizumab reduced the number of eosinophils in the bronchial lamina propria and ASM mass compared to placebo. In the benralizumab group, there were no significant changes in the number of myofibroblasts compared to the control group. The effects of benralizumab on ASM mass were attributed to an indirect effect mediated by the depletion of local TGF-β1$^+$ eosinophils in the bronchial lamina propria.

Pelaia and collaborators emphasized the clinical efficacy of benralizumab in terms of lung function in real life after the first administration of this mAb.[208] Padilla-Galo et al reported an improvement in FEV_1 after three months of treatment with benralizumab, which lasted up to six months.[209] Similar results were obtained in a retrospective study.[210] The authors ascribed the rapid improvement of lung function to the early effects of the mAb on peripheral blood and bronchial eosinophils. Although these observations were obtained in small cohorts of patients, they emphasize that the improvement caused by benralizumab on lung function observed in real life is more evident compared to certain RCTs.[73,203,204] Finally, clinical trials (NCT04365205, NCT03953300) are evaluating the effects of benralizumab on airway remodeling.

2.8 | Dupilumab

The Th2-like cytokines IL-4 and IL-13 and the heterodimeric IL-4 receptor (IL-4R) complexes that they activate play a key pathogenic role in asthma.[211] Dupilumab is a human IgG4 mAb that targets the IL-4 receptor α chain (IL-4Rα), common to both IL-4R complexes: type 1 (IL-4Rα/γc; IL-4 specific) and type 2 (IL-4Rα/IL-13Rα1: IL-4 and IL-13 specific).[212]

In several RCTs, dupilumab reduced the annualized rate of asthma exacerbations in patients with moderate-to-severe uncontrolled asthma compared to placebo.[213–215] Dupilumab in patients with severe asthma caused rapid (2 weeks) and long-lasting improvement in FEV_1 versus placebo.[214,215] Furthermore, the trend curves of FEV_1 post-bronchodilation showed a loss of function in the placebo group and no decrease in FEV_1 over time in the dupilumab group. Moreover, in a mouse model of asthma, dual IL-4/IL-13 blockade with dupilumab prevented eosinophil infiltration into lung tissue without affecting circulating eosinophils.[216] A real-life retrospective study demonstrated that 4 weeks of treatment were necessary to achieve a significant improvement in FEV_1 compared to baseline.[217] The VESTIGE study (NCT04400318), evaluating dupilumab effects on lung function and structural airway changes using functional respiratory imaging (FRI), is ongoing.

It should be noted that blood eosinophilia was reported in 4%–25% of patients in the dupilumab group compared to 0.6% in the placebo group.[215,218] This paradoxical effect has also been reported in patients treated with dupilumab for moderate-to-severe atopic dermatitis.[219] However, transient early elevation of blood eosinophils in asthma, chronic rhinosinusitis with nasal polyps (CRSwNP) or atopic dermatitis patients on dupilumab declined to baseline levels over time.[220]

Additionally, indirect evidence of a combined anti-inflammatory effect as well as on tissue and structural cells can be derived from the observation that all of the abovementioned anti-Th2 cytokine or receptor antibodies have been shown to reduce the size of nasal polyposis in patients with CRSwNP.[221–224]

2.9 | Tezepelumab

Tezepelumab is a human IgG2-λ mAb, which binds with high affinity to TSLP, an epithelial cell-derived cytokine implicated in the pathogenesis of different phenotypes of asthma.[13] TSLP, a pleiotropic cytokine overexpressed in the airway epithelium of asthmatics,[49] exerts its effects by binding to a high-affinity heterodimeric receptor complex composed of TSLPR and IL-7Rα.[13] TSLP concentrations are increased in BAL fluid of asthmatics,[48] and bronchial allergen challenge increases TSLP expression in the asthmatic epithelium and submucosa. Importantly, serum concentrations of TSLP are increased during asthma exacerbations.[225] Finally, TSLP induces the release of angiogenic and lymphangiogenic factors from HLMs.[57] TSLP can promote airway remodeling via the activation of human lung fibroblasts.[70]

The FDA has recently approved tezepelumab for the treatment of severe asthma with no phenotype or biomarker limitations. Tezepelumab is the first biologic antagonizing an alarmin (i.e., TSLP), which plays a pivotal role in the pathogenesis of asthma.[13,43] The phase II PATHWAY study showed that three different doses (70 mg, 210 mg, or 280 mg s.c. every 4 weeks) of tezepelumab reduced the number of annual exacerbation rates regardless of blood eosinophil count, with a significant increase in prebronchodilator FEV_1 at 52 weeks from the start of treatment compared to the placebo group.[226] These results were extended in the phase III NAVIGATOR study in which tezepelumab (210 mg s.c. every 4 weeks) reduced asthma exacerbations at Week 52 and significantly improved FEV_1 regardless of peripheral blood eosinophils in adolescent and adult patients with severe uncontrolled asthma[227] although there was a trend toward a better improvement with higher eosinophil counts in subgroup analysis.

Studies conducted in different animal models using TSLP antibodies have demonstrated that TSLP blockade reduces airway inflammation, TGF-β1 levels, hyperreactivity, and airway remodeling.[228–231] The phase II CASCADE study evaluated the effects of tezepelumab on airway remodeling by performing bronchoscopic biopsies in moderate-to-severe asthma patients.[132] Tezepelumab caused a greater reduction from baseline to the end of treatment in airway submucosal eosinophils compared to placebo. There were no other significant changes either at the level of other immune cells (neutrophils, mast cells, and T cells) and at the structural level (e.g., RBM thickness and epithelial integrity). Interestingly, tezepelumab administration was associated with lower hyperresponsiveness to mannitol inhalation compared to placebo. The latter finding was confirmed in an independent study.[232] These preliminary results on the effects of tezepelumab on airway remodeling are of translational interest for several reasons. There is overwhelming evidence that fibroblasts are a source of TSLP[58,59] and there is evidence that a functional TSLP signaling axis plays a role in fibrotic lung disease.[59] Figure 1 schematically illustrates the mechanisms of action of different biologics and their immunological and cellular targets in the context of airway remodeling.

3 | FUTURE PERSPECTIVES

3.1 | Astegolimab

Astegolimab is an IgG2 mAb that blocks IL-33 signaling by targeting ST2, the IL-33 receptor.[77] The phase 2b ZENYATTA study[233] evaluated the safety and efficacy of astegolimab in patients with severe asthma. Astegolimab was safe, well tolerated, and effective in reducing the annualized asthma exacerbations at Week 54. Astegolimab did not show a significant benefit compared to placebo in the absolute change in FEV_1 at Week 54.

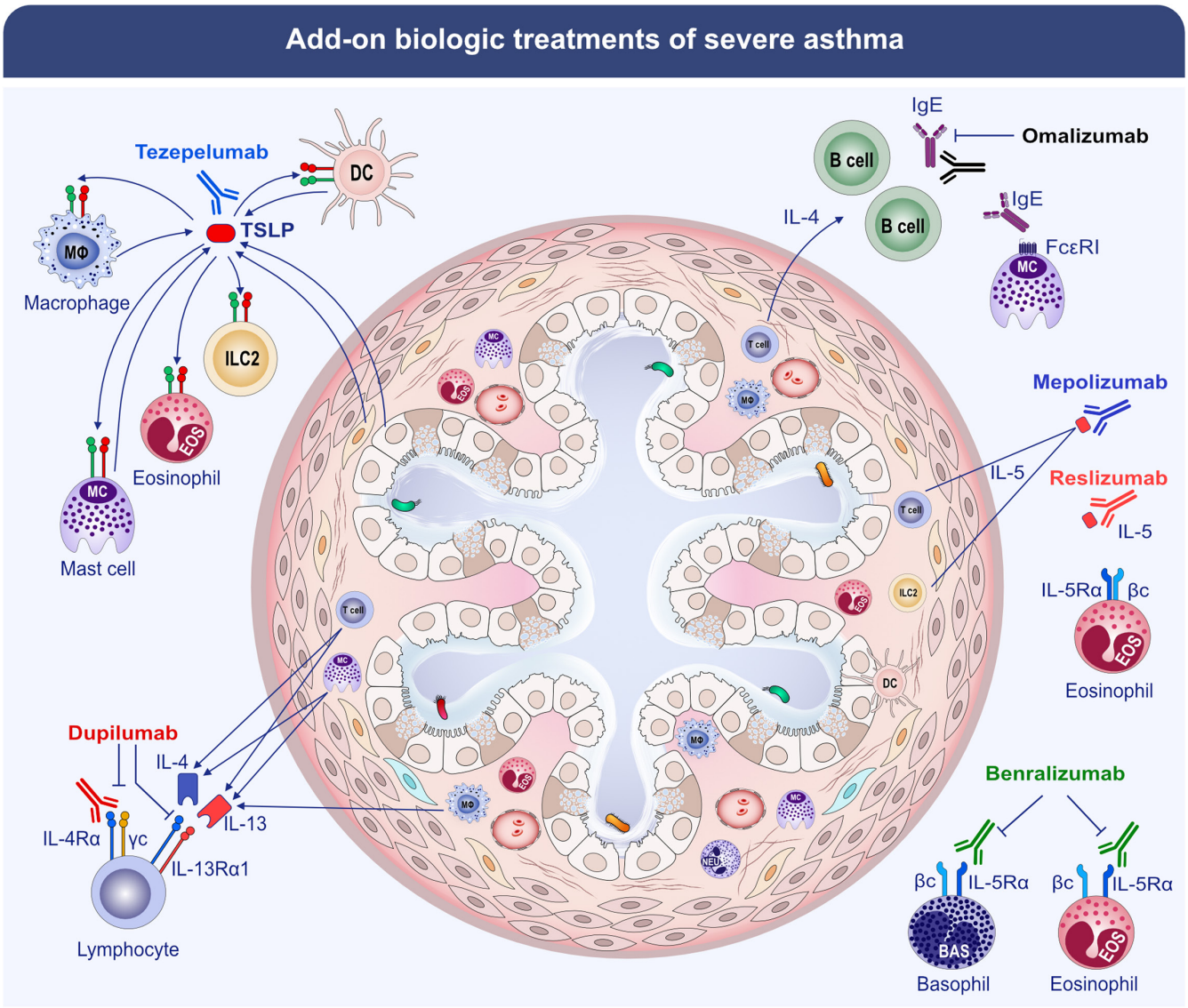

FIGURE 1 Monoclonal antibodies (mAbs) effective in the treatment of patients with severe asthma are listed, and their known immunological mechanisms are summarized. The targets of approved add-on biologic treatments of severe asthma include IgE (omalizumab), IL-5 (mepolizumab and reslizumab), IL-5 receptor (benralizumab), IL-4/IL-13 receptor complex (dupilumab), and TSLP (tezepelumab).

3.2 | Itepekimab

Itepekimab is a human IgG4 anti-IL-33 mAb. A phase 2 trial compared the safety and efficacy of itepekimab (300 mg s.c. every 2 weeks), dupilumab (300 mg. s.c. every 2 weeks), itepekimab plus dupilumab, or placebo in patients with moderate-to-severe asthma.[234] The primary endpoint, loss of asthma control, was similar in the itepekimab (22%), combination (27%), and dupilumab (19%) groups and lower than in the placebo (41%) group. Prebronchodilator FEV_1 increased with itepekimab and dupilumab monotherapies but not with combination therapy. Ipetekimab improved asthma control and quality of life compared to placebo and reduced peripheral blood eosinophils. The latter results are consistent with a role for IL-33 in the pathogenesis of asthma exacerbations and airflow limitations in asthma. Further investigations are needed to investigate whether blockade of the IL-33/ST2 axis can modify airway remodeling in patients with asthma.

4 | CONCLUSIONS AND PERSPECTIVES

Immunotherapy with mAbs targeting IgE, several cytokines, or their receptors has revolutionized the treatment landscape for patients with severe asthma. mAbs that block IgE (omalizumab), the IL-5/IL-5Rα axis (mepolizumab, reslizumab, benralizumab), IL-4Rα (dupilumab), or TSLP (tezepelumab) can produce durable responses in the majority of patients with severe asthma.

Airway remodeling is a cardinal feature of bronchial asthma and is responsible for structural alterations of the airways and lung parenchyma, determining airway hyperresponsiveness and the

development of fixed airflow obstruction.[15] Damaged epithelial barrier,[235,236] subepithelial matrix proteins and collagen deposition,[24,25,29] infiltration and activation of inflammatory cells,[115,124,133] goblet cell metaplasia,[23] overexpression of inflammatory angiogenesis,[16,17] and hyperplasia and hypertrophy of ASM cells[18,33] are major features of airway remodeling in asthma.[41,237]

At present, we have incomplete knowledge on the short- and long-term effects of biological therapies on airway remodeling in asthma. However, biological therapies targeting IgE, IL-5/IL-5Rα, IL-4Rα, TSLP, and IL-33/ST2 can improve not only clinical symptoms but also certain indirect features (e.g., FEV_1) of airway remodeling in asthma.[215,226,238–241] These findings allow us to speculate that biologics could promote the resolution of allergic and non-allergic inflammation. Late phases of airway remodeling are associated with infiltration/activation of both profibrotic immune cells (e.g., mast cells, eosinophils, and macrophages) and structural cells (e.g., fibroblasts, myofibroblasts, ASM cells, and endothelial cells). Biologics may have late effects on immune and structural cells in addition to early effects on airway inflammation. The effects of each biologic on specific features of airway remodeling are summarized in Table 1.

Biologics are presently used for the treatment of patients with severe asthma who are likely to have a prolonged history of persistent and/or repetitive immunological insults. Repetitive or prolonged injury can lead to a pathological state of fibrosis associated with reduced lung function. Experimental studies indicate that there is a limited time window in which stopping inflammation avoids fibrosis.[242,243] Beyond this time window, tissue remodeling inevitably occurs even if inflammation is resolved. In this circuit, macrophages, the most abundant hematopoietic cells in the human lung parenchyma, can transition between different states, including proinflammatory, anti-inflammatory, and profibrotic states.[244] Although there are no clinical and experimental data, we would like to speculate that perhaps early treatment of mild/moderate asthma with biologics might represent an innovative strategy to limit the irreversible airway remodeling of severe asthma.[245] On the contrary, this preventive approach might reduce the direct and indirect heavy socio-economic consequences of severe asthma and their exacerbations thus counterbalancing the cost of biologic therapies.

There are several limitations in studying in vivo the effects of biological therapies on airway tissue remodeling in asthmatic patients. Bronchial asthma is a highly heterogeneous disorder and different forms of airway remodeling could likely underlie several asthma pheno-/endotypes.[3] The quantitative identification of immune cells in the airway epithelium and submucosa should also take in consideration the functional heterogeneity of eosinophils,[246] macrophages,[57,118,133] mast cells,[84,247–250] neutrophils,[125,127,251] and basophils.[85,86,125,252] Considering the technical and ethical difficulties to evaluate in vivo bronchial remodeling through invasive methods, particularly in patients with severe asthma, efforts are underway to identify peripheral blood biomarkers of airway remodeling in asthma.[245]

In conclusion, a deeper understanding of the immunological mechanisms of the formation of different forms of airway tissue remodeling in various asthma phenotypes is needed. The use of single-cell transcriptomics will be of paramount importance to chart

TABLE 1 Effects of biologics on specific features of airway remodeling

Biologics	Form	Target	Biological effects	Effects on airway remodeling
Omalizumab	Humanized IgG1-κ mAb	IgE	• ↓ circulating total IgE • Downregulation of FcεRI receptors on basophils, mast cells, and DCs	• ↑ FEV_1 • ↓ RBM thickness • ↓ airway wall thickness in CT • ↓ fibronectin deposition • Prevents IgE-mediated ECM deposition in vitro
Reslizumab	Humanized IgG4-κ mAb	IL-5	Blockage of IL-5/IL-5R binding	• ↑ FEV_1
Mepolizumab	Humanized IgG1-κ mAb	IL-5	Blockage of IL-5/IL-5R binding	• ↑ FEV_1 • ↓ airway eosinophils and TGF-β1$^+$ eosinophils • ↓ tenascin expression
Benralizumab	Humanized IgG1-κ mAb	IL-5 receptor (IL-5Rα)	↓ eosinophils and basophils via antibody-dependent cell-mediated cytotoxicity (ADCC)	• ↑ FEV_1 • ↓ airway eosinophils • ↓ ASM mass
Dupilumab	Human IgG4 mAb	IL-4 receptor α chain (IL-4Rα)	• Blockage of IL-4/IL-4Rα binding • Blockage of IL-13/IL-4Rα binding	• ↑ FEV_1 • prevents eosinophil infiltration into lung tissue in a mouse model of asthma
Tezepelumab	Human IgG2-λ mAb	TSLP	Blockage of TSLP/TSLPR binding	• ↑ FEV_1 • ↓ airway eosinophils • ↓ AHR to mannitol • ↓ airway inflammation • ↓ TGF-β1 • ↑ CT scan-determined lumen area

the cellular landscape and specific signaling networks of upper and lower airways in healthy and asthmatic subjects.[133,154] The results emerging from these studies could help in the generation of new reliable diagnostic biomarkers and targeted therapeutic approaches to improve asthma treatment.

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CONFLICTS OF INTEREST

G.V., S.F., J.P., R.P., and G.S. have not potential conflicts of interest to declare. J.C.V. is a full time employee of the University of Rostock as a full time professor and chair of the Departments of Pneumology and Intensive Care Medicine has given independent advice, lectured for and received honoraria from AstraZeneca, Avontec, Bayer, Bencard, Bionorica, Boehringer-Ingelheim, Chiesi, Essex/Schering-Plough, GSK, Janssen-Cilag, Leti, MEDA, Merck, MSD, Mundipharma, Novartis, Nycomed/Altana, Pfizer, Revotar, Sandoz-Hexal, Stallergens, TEVA, UCB/Schwarz-Pharma, Zydus/Cadila, has participated in advisory boards for Avontec, Boehringer-Ingelheim, Chiesi, Essex/Schering-Plough, GSK, Janssen-Cilag, MEDA, MSD, Mundipharma, Novartis, Regeneron, Revotar, Roche, Sanofi-Aventis, Sandoz-Hexal, TEVA, UCB/Schwarz-Pharma and has received research grants from the Deutsche Forschungsgesellschaft, Land Mecklenburg-Vorpommern, GSK, MSD. E.H. received grants and personal fees from AstraZeneca, Sanofi, Regeneron, Novartis, GSK, Circassia, Nestlè Purina, Stallergenes-Greer outside the submitted work. G.W.C. received honoraria for lectures, presentations, speakers from AstraZeneca, GSK, Novartis, Sanofi, Stallergenes, Greer, Hal Allergy, Menarini, Chiesi, Mylan, Valeas, Faes.

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