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Yong-Xiao Wang *Editor*

Lung Inflammation in Health and Disease, Volume I

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Editor

Lung Inflammation in Health and Disease, Volume I

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Preface

Inflammation is a ubiquitous natural cellular process in virtually all types tissues, organs, or systems of the human body. This process can be acute and chronic. Acute inflammation is an immediate healthy response to protect and repair the body from harmful stimuli. Usually it occurs within a couple of hours. Chronic inflammation is a lengthy cellular process that is not part of natural healing and thus may lead to diseases such as arthritis, asthma, pulmonary hypertension, etc.

Inflammation can also be classified as systemic or localized. The former affects the entire human body, which is a pathogenetic component in numerous acute and chronic diseases including atherosclerosis, diabetes, sepsis, trauma, and others with a significant morbidity and mortality. The latter is localized as in a specific organ. For example, inflammation caused by asthma or pulmonary hypertension is localized in the lungs.

Lung diseases are very common and can also be very severe. It is well known that lung infections are the greatest single contributor to the overall global health burden. For instance, lung diseases are the most common causes of death among children under 5 years of age – more than 9 million annually. Indeed, pneumonia is the leading killer of children worldwide. Asthma is the most common chronic disease, affecting about 14% of children globally and continuing to rise. Likewise, COPD is recognized to be the fourth leading cause of death in the world and the numbers are rising. The lung is not only the largest internal organ in the human body, but also the only internal organ that is exposed constantly to the external environment; as such, no other organ is more vital and vulnerable than the lung. This may explain the common morbidity and mortality of lung diseases.

Systemic inflammation may induce and even exacerbate local inflammatory diseases. Likewise, local inflammation can cause systemic inflammation. Indeed, there is increasing evidence of coexistence of systemic and local inflammation in patients with asthma, COPD, and other lung diseases. Moreover, the comorbidity of two and even multiple local inflammatory diseases occurs often. For instance, rheumatoid arthritis not only occurs frequently together with pulmonary hypertension, but also promotes development of the latter. The local and systemic comorbidity as well as two or more inflammatory diseases significantly deteriorate the quality of life and may even exacerbate death in patients.

The current treatment options for lung diseases are neither always effective nor specific at all. Development of new therapeutics is earnestly needed.

Equally desperately, the molecular mechanisms and physiological significance of lung diseases are still not fully understood. Apparently, this despondent fact is a major encumberment to creating new efficacious drugs in the treatment of lung diseases. This scenario is even worse in two and more lung diseases accompanied with other inflammatory diseases due to their complexity and diversity.

Despite the current state being unsatisfactory, great advancements have been made in many aspects of lung diseases from the molecular geneses to regulatory mechanisms, signaling pathways, cellular processes, basic and clinical technologies, new drug discoveries, clinical manifestations, laboratory and clinical diagnoses, treatment options, and predictive prognosis. To the best of our knowledge, however, no one cohesive book is available to present these state-of-the-art advances in the field. Thus, as one of the major aims, we compile this timely and much-needed book to provide a high-quality platform in which well-known scientists and emerging pioneers in basic, translational, and clinical settings can present their latest, exciting findings in the studies of lung inflammation in health and disease. The contents from multiple outstanding authors with unique expertise and skills in molecular and cell biology, biochemistry, physiology, pharmacology, biophysics, biotechnology, translational biomedicine, and medicine will provide new knowledge, concepts, and discoveries in the field. The second major aim is to help direct future research in lung diseases and other inflammatory diseases. The scope of this book includes nearly all new and important findings from very recent basic, translational, and clinical research in the studies of the molecular genesis, networks, microdomains, regulation, functions, elimination, and drug discoveries of inflammation in lung health and disease, which are involved in animal and human lung epithelial cells, smooth muscle cells, *endothelial* cells, adventitial cells, fibroblasts, neutrophils, *macrophages*, *lymphocytes*, and stem/progenitor cells. Lastly, but importantly, the book will offer the latest and most promising results from clinical trials in terms of exploring interventions of local and systemic inflammation in the treatment of lung diseases.

This book features contributions from numerous basic, translational, and physician scientists in the field of pulmonary vasculature redox signaling in health and disease, and as a result offers a widespread and comprehensive overview for academic and industrial scientists, postdoctoral fellows, and graduate students who are interested in redox signaling in health and disease and/or normal and pathological functions of the pulmonary vasculature. The book may also be valuable for clinicians, medical students, and allied health professionals.

We are sincerely grateful for the overwhelming support from leading scientists and experts who responded to our request to contribute chapter articles. Due to their contributions, we are now pleased to be able to share Volumes I and II. Volume I includes 20 chapters that report the latest and most important findings on the molecular genesis, networks, microdomains, regulations, functions, and drug discoveries of inflammation from basic, translational, and clinical research.

I want to express my wholehearted gratitude to all of the authors for their dedication and diligence in contributing book chapters, particularly during the challenging and unprecedented times of the global COVID-19 pandemic. Many of the authors in this book have not only performed exceptional roles as writer, but also reviewer. Their selfless contributions are sincerely appreciated. I also want to thank Ms. Alison Ball and Mr. Arjun Narayanan at Springer Nature for their assistance, patience, and enthusiasm in seeing this book to fruition.

Albany, NY, USA Yong-Xiao Wang

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1 Potential Role of Mast Cells in Regulating Corticosteroid Insensitivity in Severe Asthma

Abdulrahman Alzahrani, Aamir Hussain, Fahad Alhadian, Jameel Hakeem, Sana Douaoui, Omar Tliba, Peter Bradding, and Yassine Amrani

Abstract

The mechanisms driving corticosteroid insensitivity in asthma are still unclear although evidence points toward a potential role of lung mast cells. Indeed, a number of in vitro studies using various cell types showed that different mediators produced by activated mast cells, including cytokines, have the capacity to interfere with the therapeutic action of corticosteroids. In patients with severe allergic refractory asthma, the anti-IgE monoclonal antibody (mAb), Omalizumab, has been shown to be associated with a marked reduction in inhaled and systemic use of corticosteroids, further suggesting a key role of mast cells in the poor response of patients to these drugs. The present chapter will discuss the possible underlying mechanisms by which mast cells could contribute to reducing corticosteroid sensitivity seen in patients with severe asthma.

Keywords

Mast cells · IgE · Airway inflammation · Receptor · Airway smooth muscle · Cytokines · Growth factors · Alarmins

1.1 Introduction

Mast cells are playing a key role in asthma pathogenesis via their ability to initiate and perpetuate the type2 (or Th2) cytokine-dependent allergic inflammation in the lung. This occurs via the secretion of various key cytokines such as interleukin 4 (IL-)4 and IL-13 which induce Th2 cell proliferation and the production of allergen-specific IgE by B-cells, and IL-5 which promotes eosinophilic inflammation [1]. Mast cells in asthma are activated through many mechanisms including the high-affinity IgE receptor FcεRI, Toll-like receptors, in response to the secretion of alarmins (TSLP, IL-33, IL-25) by airway epithelium. There is evidence of ongoing mast cell activation in severe asthma, irrespective of the clinical phenotype [2]. Different studies have shown infiltration of mast cells within the epithelium, submucosa layer, and airway smooth muscle and the ability of various mast cells mediators to induce key structural/clinical features of asthma such as mucus hypersecretion, epithelium permeability,

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airway hyper-responsiveness (AHR), bronchoconstriction and airway remodeling [3]. This book chapter will *discuss the capacity of mast cells to modulate the response of asthmatic patients to corticosteroid therapy*. Evidence from our group showed that mast cells can blunt the response to bronchodilator agonists in airway smooth muscle via the paracrine action of secreted TGFβ [4] or following cell–cell physical interaction [5, 6]. Dysfunction of ß2-agonists in mast cells can also be induced following the autocrine action of secreted SCF [7]. These studies clearly suggest that mast cells can alter the therapeutic response of lung structural cells to current therapies.

Here, we will not describe the biology of mast cells nor its role in asthma pathogenesis (summarized in Fig. 1.1) as these have been extensively discussed in our last review [3]. Rather, we will briefly summarize the evidence from clinical studies that have linked mast cells to corticosteroid therapy and focus most of the discussion around the mechanisms that explain how mast cells contribute to the reduced corticosteroid responses in severe asthma and the latest inhibitory strategies targeting mast cells.

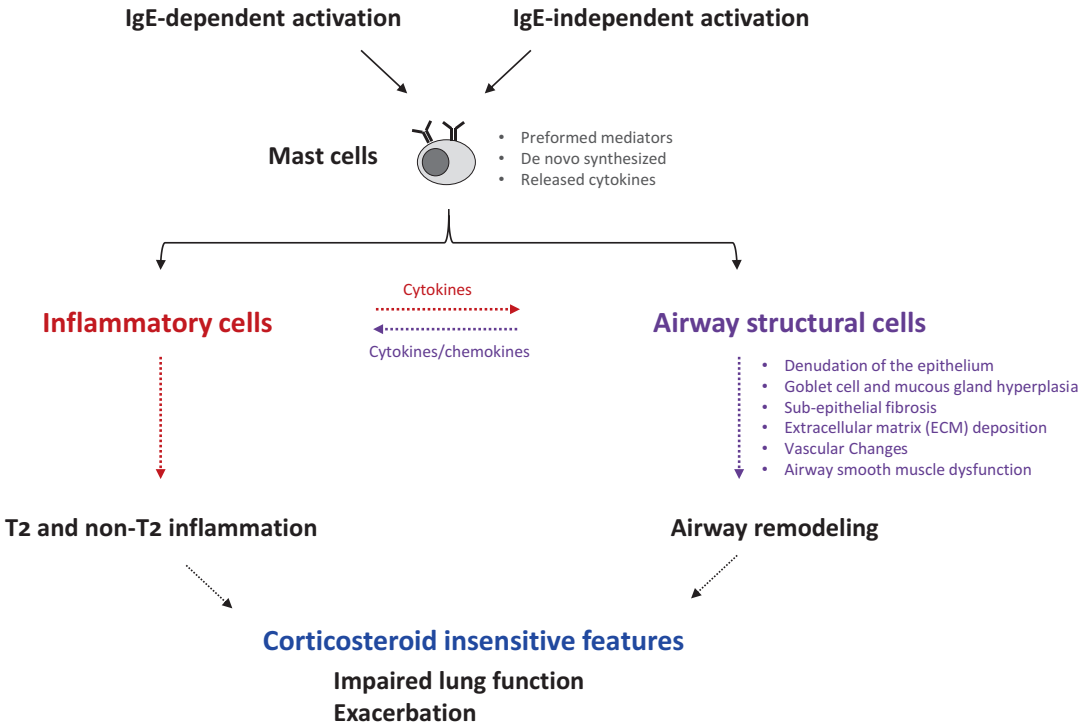

Fig. 1.1 Role of mast cell in asthma pathogenesis. Activation of mast cells via either IgE (allergen) or non-IgE mechanisms (such as TLR ligands, IgG, cytokines, complement components, neuropeptides, chemokines) can lead to the production of **preformed mediators** (chymase, tryptase, histamine), **de novo synthesized mediators** (leukotriene and prostaglandin lipid mediators), and **released cytokines** (Th2-Th1 cytokines, alarmins, growth factors). These mediators can regulate key features of asthma such as airway inflammation and airway remodeling by two main mechanisms: (i) their capacity to recruit and/or activate inflammatory cells (eosinophils, innate lymphoid cells, and lymphocytes) into the lungs and (ii) to alter the function of airway structural tissues associated with increased mast cell infiltration (epithelium, airway smooth muscle, goblet cells, vasculature). In addition, a bidirectional interaction between recruited inflammatory cells within the lung and airway structural tissues via the secretion of inflammatory and chemoattractant mediators or cell-cell interactions can also indirectly contribute to airway inflammation and airway remodeling. The overall activation of mast cells within the lung leads to impaired lung function and increase rate of exacerbation, features that are clearly insensitive to corticosteroids and improved with Omalizumab therapy

1.2 Increased Airway Infiltration of Mast Cells Is a Key Feature in Asthma Pathogenesis

The key contribution of mast cells in asthma pathogenesis can be explained by their high abundance within dysfunctional airway subcompartments of patients as reported by different studies. The authors that have stained mast cells in endobronchial biopsies using antibodies against tryptase have found that infiltration of mast cells in the airways often correlates with various clinical aspects of the disease (Table 1.1). Pesci and colleagues were among the first to demonstrate a greater infiltration of activated mast cells within human bronchial mucosa compared to healthy subjects [8]. Carroll and colleagues later extended these findings in patients with fatal asthma by reporting increased degranulated mast cells in various locations including submucosal mucous glands [9, 10] as well as in the airway smooth muscle [9, 10]. This unexpected infiltration of mast cells within the airway smooth muscle tissue was reported to strongly correlate with disease severity (as mostly seen in fatal cases) [9, 10], abnormal lung function (when assessing PC_{20} for methacholine) [11], or levels of TGF-β1 expression in the airway smooth muscle itself [12]. Other studies confirmed the greater number of mast cells in different structural compartments including epithelium [13], as well as in airway smooth muscle in both allergic and nonallergic asthmatics, with their number and activation state (assessed by extracellular deposition of mast cell products) to be significantly higher in smooth muscle bundle in patients with allergic asthma [14]. Brightling and colleagues found that the number of mast cells (positively stained for both CXCR3 and tryptase) was greater in the airway smooth muscle compared to that in bronchial submucosa in patients with asthma [15]. The concept of infiltration of tryptase-positive mast cells within the airway smooth muscle tissue has since been validated in different cohorts of asthma patients [12, 16–18].

The mechanisms leading to mast cells infiltration within the airways have not been completely

Table 1.1 Studies describing features of mast cell infiltration within the airways in asthma patients

References	Mast cell infiltration in the lung	Features of mast cells in asthma
[8] n = 13 stable asthma n = 8 healthy controls	Airway epithelium and Lamina propria	Degranulation greater in asthmatics
[9, 10] n = 8 fatal asthma n = 8 non-fatal asthma n = 8 healthy controls	ASM bundle and mucous glands	Degranulation related to disease severity
[11] n = 17 asthma n = 13 eosinophilic bronchitis (EB) n = 11 healthy controls	ASM bundle	Greater number in asthma vs EB and controls Correlation with impaired lung function (PC_{20}) in asthma
[12] n = 9 controls n = 10 intermittent asthma n = 9 persistent asthma	ASM bundle	Greater number in asthma Number related to TGFβ expression in ASM
[14] n = 29 allergic and non-allergic asthma	Epithelium, lamina propria and ASM bundle	Greater number in ASM of allergic asthma vs non-allergic asthma
[15] n = 16 asthma n = 14 controls	ASM bundle and submucosa	Greater number in asthma Greater number of CXCR3+ mast cells in ASM versus submucosa
[16] n = 5 controls n = 9 persistent asthma	ASM bundle	Greater number in asthma Correlation with vasoactive intestinal peptide (VIP) staining in ASM
[17] n = 10 controls n = 16 asthma	ASM bundle and submucosa	Greater number in asthma Greater degranulation in muscle vs submocusa No correlation with asthma severity

(continued)

Table 1.1 (continued)

References	Mast cell infiltration in the lung	Features of mast cells in asthma
[18] n = 18 controls n = 12 asthma	ASM bundle	Correlation with α-smooth muscle actin staining in ASM
[19] n = 34 controls n = 53 mild asthma n = 21 moderate asthma n = 57 severe asthma	Airway epithelium and submucosa	Greater number of double positive (chymase and tryptase) in the epithelium in severe asthma

elucidated. In vitro studies have shown that various factors produced by the epithelium or airway smooth muscle are capable of exerting chemoattractive effects toward mast cells including fractalkine/vasoactive intestinal peptide axis [16], CXCL10 via CXCR3 receptor [12], and TGFß/SCF [15]. The observation that all these mediators were found to be produced in vivo by ASM bundles strongly support the essential role of structural lung cells in the recruitment of mast cells within the lungs in patients with asthma.

As patients with severe asthma (5–10%) poorly respond to the current asthma management guideline therapies including corticosteroids [20], it is important to understand the mechanisms driving this poor response to corticosteroids. A number of different clinical trials conducted in severe asthmatics have shown that the monoclonal antibody omalizumab, that targets specifically circulating IgE, leads to improved asthma symptoms, pulmonary function (%FEV1 predicted), morning peak expiratory flow, rates of exacerbations, and a reduction in markers of inflammation and airway remodeling [21]. These observations reinforce the concept that IgE-dependent release of mast cell mediators contributes to the pathogenesis of severe asthma. Therefore, a legitimate question that remains to be answered is whether mast cells can play a role in asthma severity by interfering with corticosteroid therapy via secreted (stored and newly synthesized) mediators. In the following sections, we will summarize the growing literature that links mast cells to the impaired corticosteroid sensitivity seen in asthma.

1.3 Clinical Evidence Suggesting a Role of Mast Cells in Corticosteroid Insensitivity

Many clinical studies have confirmed that Omalizumab was effective in improving asthma control [22–24]. Omalizumab led to improved asthma symptoms, reduced rates of exacerbations, and improved features of airway remodeling as evidenced by the reduction in airway wall thickness seen in CT scans [22, 25, 26]. In addition, omalizumab treatment improved pulmonary function such as forced expiratory volume in 1 s (FEV1) and morning peak expiratory flow [22, 25, 27]. The question was whether omalizumab was associated with changes in patients' response to either inhaled (ICS) or oral (OCS) corticosteroids. Previous reports have revealed a strong association between IgE levels and a high usage of ICS [28]. Most studies focusing in severe asthma have demonstrated that the marked reduction in peripheral blood IgE levels induced by omalizumab therapy was also associated with a reduction in the use of both ICS and OCS [22, 27, 28]. An elegant review by MacDonald colleagues has recently summarized the overall clinical impact of omalizumab observed from 42 different studies [29]. The authors concluded that omalizumab therapy for >2 months or longer led patients to either reduce or stop their ICS/OCS usage suggesting a role of mast cells in mediating corticosteroid insensitivity in severe allergic asthma. The underlying mechanisms by which mast cells could drive corticosteroid insensitivity have not been elucidated. A number of mediators produced by activated mast cells have been reported to interfere with corticosteroid responses in various cell types associated with asthma.

1.4 Mediators Produced by Mast Cells and Associated Mechanisms Shown to Blunt Corticosteroid Sensitivity

Numerous clinical and preclinical reports have supported the critical implication of mast cells in the pathogenesis of severe asthma [3]. Not only the number of mast cells increased in the airways of severe asthmatics but their number correlated with markers of disease severity [30]. As stated before, severe allergic asthma patients treated with omalizumab, the anti-IgE monoclonal antibody, show clear improvement of various clinical outcomes [21]. A number of studies have demonstrated the clinical values of targeting various mast cell mediators in severe asthmatics including pro-inflammatory cytokines such as TLSP, IL-4, IL-5, and IL-13 known to regulate eosinophilic inflammation [31], or IL-17 reported to drive neutrophilic inflammation [32]. Interestingly, several in vitro studies have reported some of these mast cell cytokines have the capacity to induce corticosteroid insensitivity in various cells associated with asthma (summarized in Table 1.2).

Table 1.2 Mediators produced by activated mast cells that are capable of altering corticosteroid response in various cell types involved in asthma pathogenesis

Mast cell mediators	Target cells	Mechanisms of steroid insensitivity
TNFα/ IFNγ	Airway smooth muscle cells	Dominant negative effect of GRβ Competition for the transcriptional co-activator GRIP-1 PP5-dependent GRα dephosphorylation
IL-2/ IL-4	PBMCs T lymphocytes (CD4+ and CD8+ T cells) PBMCs PBMCs Eosinophils	Reduced nuclear GRα ligand binding activity Reduced GRα nuclear translocation and dependent gene expression (MKP-1) Downregulation of GRα levels p38MAPK-γ dependent GRα phosphorylation PP5-dependent GRα dephosphorylation
IL-2	Th2 lymphocytes Murine cell line (HT-2)	Downregulation of GRα levels (mRNA) Reduced GRα binding to FKBP5 promoter STAT5-dependent pathways
IL-13	Human bronchial epithelial cells PBMCs (monocyte fraction)	Not investigated Decreased GRα binding activity
IL-17A IL-17/ IL-23	Airway epithelial cells PBMCs	PI3K-dependent reduction in HDAC2 activity GRβ upregulation
TGFβ	Airway epithelial cells	ALK5-dependent inhibition of GRα dependent gene expression
IFNγ	Airway epithelial cells	Activation of JAK/ STAT1 pathways
TSLP	Natural helper cells Innate lymphoid cells (ILC2)	STAT5 pathways and expression of Bcl-xL MEK- and STAT5-dependent pathways

1.4.1 Interleukin 2 and 4 (IL-2/IL-4)

A number of original studies carried out in isolated peripheral blood mononuclear cells (PBMCs) or alveolar macrophages were the first to support the existence of corticosteroid insensitive features in immune cells in patients within steroid-resistant asthma (defined by their FEV1% changes following a course of corticosteroids) and with severe asthma (defined based on the GINA guidelines) [33–39]. A more recent report showed that neutrophils derived from steroid-resistant asthmatics exhibited a blunted ex vivo response to dexamethasone [40]. IL-2 and IL-4 were among the first cytokines known to be produced by mast cells (at least from mouse work for IL-2) to have been tested for their ability to modulate corticosteroid responses in asthma. Although IL-2 is typically produced by activated T-lymphocytes, evidence have suggested a critical role of IL-2-derived from mast cells in the suppression of allergic dermatitis [41], in part via the ability of IL-2 to regulate the expansion of regulatory cells [42]. Most studies focusing on

PBMCs and T-cells have reported IL-2 and IL-4 exposure for 48 h can blunt the anti-inflammatory actions of dexamethasone [43–45]. The precise mechanisms underlying cytokine-induced steroid insensitivity in these cells involved mostly changes in GRα function occurring at multiple levels: (i) a reduction in nuclear GRα translocation [44], (ii) decreased in GRα expression [45], or (iii) reduction in nuclear GRα binding affinity in T-cells [43]. Similar effects of IL-2 and IL-4 were as well seen in eosinophils treated for shorter time (16 h) which led to reduced GRα expected responses to dexamethasone such as receptor phosphorylation and the ability to stimulate the expression of anti-inflammatory proteins such as GILZ and MKP-1 [46]. Only one report showed that IL-2 on its own could reduce the pro-apoptotic effect of dexamethasone in human Th2 cells, an effect possibly due to the decreased levels of GRα and interaction with FKBP5 [47].

1.4.2 TNFα

Activated mast cells represent a crucial source of TNFα in asthma [48, 49]. A number of preclinical studies using blocking strategies have indeed confirmed the contribution of TNFα in driving some corticosteroid resistance features seen in severe asthmatics including infiltration of various inflammatory cells [50], or neutrophilic inflammation [51]. The mechanisms by which TNFα promotes corticosteroid resistance have not been completely elucidated but in vitro studies performed on structural cells isolated from the lungs have led to some interesting observations. Studies conducted in human ASM cells, for example, have demonstrated that the production of fluticasone-resistant chemokines/cytokines (i.e., CXCL10, CCL5, and CXCL8) can be induced by TNFα when associated with IFNγ [52]. The underlying mechanisms likely result from the modulation of GRα transactivation function caused by three different inhibitory pathways: (i) the antagonistic action of GRβ, dominant negative isoform of GRα, (ii) the competition for GRα essential transcriptional co-activator GRIP-1 and, (iii) protein phosphatase PP5-dependent dephosphorylation of GRα (reviewed in [52]). The "GRβ" hypothesis has been investigated in asthma, although its role remains still controversial [53–55]. The ability of IFNγ to render lung structural cells refractory to fluticasone when combined to TNFα may likely related to the synergistic activation of IFNγ-associated steroid insensitive pathways. We showed that activation of the transcription factor IRF-1 became resistant to fluticasone when induced by TNFα in the presence of IFNγ [56]. Similarly, we also reported that in lung epithelial cells, the ability of fluticasone to inhibit steroid-insensitive genes induced by IFNγ could be restored when JAK pathways were blocked using siRNA strategy aimed at the downstream signaling molecule STAT-1 [57]. Targeting the JAK/STAT axis may therefore represent a novel therapeutic option for reversing corticosteroid insensitivity in asthma.

1.4.3 TGFβ

Growth factors produced by mast cells have been also associated with steroid insensitivity in asthma. Elegant studies from Stewart's group in Melbourne provided the first evidence that TGFβ is able to reduce dexamethasone-induced GRE-dependent gene expression not only in A549 lung adenocarcinoma-derived epithelial cell line [58] but also in differentiated primary air–liquid interface human bronchial epithelial cells, via a mechanism involving the TGFβ type I receptor kinase (ALK5) [59]. A more recent study identified cofilin1, an intracellular actin-modulating protein, as the main downstream pathway driving TGFβ-induced corticosteroid insensitivity in lung epithelial cells [60]. The same group demonstrated that infection of human airway epithelial cells with different respiratory viruses including respiratory syncytial virus, rhinovirus, and influenza A virus led to corticosteroid insensitivity in part via autocrine action of TGFβ and associated ALK5 pathways [61]. We recently reported a role of mast cell-derived TGFβ in the inhibition of β2-receptor function in airway smooth muscle cells [4]. Whether TGFβ regulates corticosteroid responses in other lung structural cells via similar ALK5 mechanisms remains to be further investigated.

1.4.4 Interleukin 17 (IL-17)

IL-17 is also another mast cell-derived cytokine involved in asthma that has been associated with steroid insensitivity in severe asthma. McKinley and colleagues were the first to suggest a role of Th-17 cells in driving steroid resistance in asthma in a mouse of allergic asthma [62]. The authors found that both airway inflammation and airway hyper-responsiveness were resistant to dexamethasone in allergen-challenged mice following adoptive transfer of Th17 cells. Another study using of neutralizing antibody clearly indicated that some of corticosteroid insensitive features following ozone exposure in mice, such as neutrophilic inflammation and BALF cytokine levels, were mediated by IL-17 [63]. In vitro work in 16HBE14o human bronchial epithelial cells (16HBE) confirmed the capacity of IL-17 to markedly reduce the inhibitory action of budesonide on TNFα-induced IL-8 production. Mechanisms driving IL-17-induced steroid resistance involved a reduction of HDAC2 expression via phosphoinositide-3-kinase (PI3K) pathways [64]. In PBMCs, IL-17/IL-23 combination reduced dexamethasone-induced suppression of cell proliferation via the inhibition of GRα transactivation and transrepression properties [65].

1.4.5 Interleukin 13 (IL-13)

IL-13 has been considered as one of the essential cytokines involved in asthma pathophysiology which can originate from Th2 lymphocytes, innate lymphoid cells, and mast cells. Although elevated IL-13 levels have been correlated with typical asthma features including airway hyperresponsiveness, mucus hypersecretion, and airway remodeling, there is also evidence for a role in steroid resistance [66]. Administering IL-13 directly in mouse airways using an adenoviral vector resulted in airway inflammatory changes that are unresponsive to dexamethasone including neutrophils and macrophages lung accumulation [67]. In primary human bronchial epithelial cells, IL-13 stimulated the production of the profibrotic factor TGFβ2 that was unaffected by dexamethasone [68]. In PBMCs treated with IL-13, GRα binding activity was found to be impaired in the monocyte population and associated with a reduced inhibitory effect of hydrocortisone on LPS-induced IL-6 production [69]. Interestingly, none of the other cytokines tested (IL-1, IL-3, IL-5, IL-7, IL-8, IL-12, or granulocyte-macrophage-CSF) had any effect of steroid sensitivity in these cells. These studies reinforce the concept that IL-13 is an important driver of steroid-insensitive pro-remodeling and pro-inflammatory responses in the airways.

1.4.6 Alarmins (TLSP)

An elegant report combining a mixture of in vitro and in vivo studies was the first to suggest the implication of TSLP in driving corticosteroid refractory responses in one family member of type 2 innate lymphoid cells (ILC2) called natural helper (NH) cells [70]. The TSLP-induced steroid resistance was mediated via the activation of STAT5 signaling pathways, through mechanisms that remain to be further explored. A more recent study performed in blood and lung ILC2s revealed that the ability of dexamethasone to reduce the production of type 2 cytokines was greatly impaired by TSLP or IL-7 [71]. This study suggests the involvement of common signaling pathways downstream to the IL-7 receptor α in the regulation of steroid insensitivity. Interestingly, as reported in NH cells, corticosteroid resistance in ILC2s was mediated via both MEK- and STAT5-dependent pathways. Activated mast cells are a source of TSLP in asthmatic airways, and might therefore promote steroid resistance through this mechanism [72, 73].

1.5 Potential Mast Cell Inhibitors for the Treatment of Allergic Diseases

Recent reports have uncovered a number of different strategies that are capable to inhibiting mast cells and their contribution to lung

diseases. One elegant report provided the first evidence that Imatinib, a KIT inhibitor, decreased airway hyper-responsiveness (methacholine PC_{20}), mast-cell counts, and a marker of mast cell activation (serum levels of tryptase) in severe asthmatics [74]. Inhibiting another tyrosine kinase (Spleen tyrosine kinase Syk) by SYKi has been reported to inhibit IgE-mediated contraction and production of mast cell mediators in precision cut lung slice (PCLS) model [75]. Similarly, RN983, an inhibitor of bruton's tyrosine kinase (Btk) required for mast cell activation has proven to be effective in reducing the early asthmatic response in mouse model of allergic asthma when given by inhalation [76]. More recently, a study using FDA-approved BTK inhibitors (BTKi's) demonstrated promising therapeutic actions both in vitro (allergen-induced contraction) and in vivo (IgE-mediated anaphylaxis), supporting the key role played by Btk in FcεRI-mediated mast cell degranulation [77]. The use of the pharmacological inhibitor by AGK2 allowed to demonstrate the central contribution of NAD+ (nicotinamide adenine dinucleotide)-dependent deacetylase SIRT2 pathways in mediating mast cell degranulation and allergic airway inflammation in a murine model [78]. The clinical benefit of noncompetitive inhibitory antibody against human β-tryptase in both mouse and primate models of allergic response has been described as a promising treatment of severe asthma [79]. The mitochondrial STAT3 appears to be another target as inhibitors called Mitocur-1 and Mitocur-3 significantly suppressed degranulation of cultured rodent and human mast cells and reduce key allergic features in a OVA murine model such as blood histamine and eosinophilia [80]. Activating specific pathways could also serve as a potential strategy to suppress mast cell function. Levels of Raf kinase inhibitor protein (RKIP), which has been described as a negative regulator of IgE-mediated allergic response [81], are decreased in peripheral blood of asthma patients. This suggests a possible defect of RKIP as a new mechanism underlying allergic responses in asthma.

1.6 Conclusions

Clinical trials as well as real-life studies have demonstrated that anti-IgE therapy (omalizumab) is associated with a corticosteroid-sparing effect in moderate to severe asthma. This reduction in corticosteroid usage/dependence was associated with marked improvements in different clinical outcomes including the rate of exacerbations and asthma symptoms. Unfortunately, not all severe asthmatics respond to omalizumab. It is likely that mediators released by activated mast cells via both IgE-dependent and IgE-independent pathways may play a key role in driving patients' reduced sensitivity to corticosteroid therapy. Indeed, a number of in vitro studies conducted in immune cells and lung structural cells have shown that different mediators (Th1 and Th2 cytokines, growth factors, alarmins) produced by mast cells can blunt the response to corticosteroids via multiple mechanisms. This include effects on the function of GRα ranging from impaired receptor phosphorylation, receptor DNA-binding activity, and receptor competition for transcriptional co-activator. These studies further support the capacity of mast cells to contribute to the overall mechanisms blunting corticosteroid therapy in severe asthma. Identifying how mast cells regulate corticosteroid insensitive features could led to novel therapeutic interventions for the treatment of refractory severe asthma. Potential therapeutic interventions targeting mast cells besides current anti-IgE omalizumab include soluble inhibitors of pathways to prevent mast cell degranulation (see Sect. 1.4.4 above), monoclonal antibodies against key mast cell mediators and pharmacological inhibition of signaling pathways interfering with corticosteroid receptor function (summarized in Fig. 1.2).

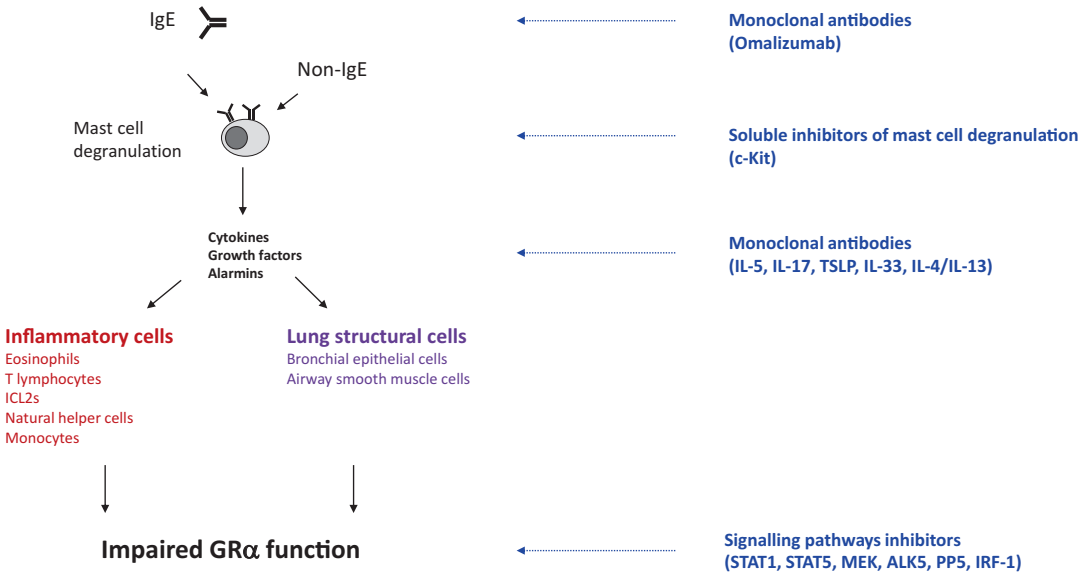

Fig. 1.2 The proposed therapeutic strategies to restore patients' response to corticosteroids in severe asthmatics. One current therapy targeting mast cells include omalizumab which has clearly demonstrated clinical benefits in patients with severe allergic asthma including the need for high doses of corticosteroids. Considering that not all patients respond to omalizumab and the fact that mast cells can be activated via IgE-independent mechanisms, other potential strategies need to be developed. These therapies are based on their ability to target mast cell activation (Kit inhibitors such as Dasatinib or Imatinib) or prevent the action of its produced mediators known to impair corticosteroid therapy in various cell types using monoclonal antibodies. Targeting also specific signaling pathways using soluble pharmacological inhibitors may also contribute in preventing the action of these mast cell mediators

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2 Galectin-3 Promotes ROS, Inflammation, and Vascular Fibrosis in Pulmonary Arterial Hypertension

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Abstract

Pulmonary Arterial Hypertension (PAH) is a progressive vascular disease arising from the narrowing of pulmonary arteries (PA) resulting in high pulmonary arterial blood pressure and ultimately right ventricular (RV) failure. A defining characteristic of PAH is the excessive remodeling of PA that includes increased proliferation, inflammation, and fibrosis. There is no cure for PAH nor interventions that effectively impede or reverse PA remodeling, and research over the past several decades has sought to identify novel molecular mechanisms of therapeutic benefit. Galectin-3 (Gal-3; Mac-2) is a carbohydrate-binding lectin that is remarkable for its chimeric structure, comprised of an N-terminal oligomerization domain and a C-terminal carbohydrate-recognition domain. Gal-3 is a regulator of changes in cell behavior that contribute to aberrant PA remodeling including cell proliferation, inflammation, and fibrosis, but its role in PAH is poorly understood. Herein, we summarize the recent literature on the role of Gal-3 in the development of PAH and provide experimental evidence supporting the ability of Gal-3 to influence reactive oxygen species (ROS) production, NOX enzyme expression, inflammation, and fibrosis, which contributes to PA remodeling. Finally, we address the clinical significance of Gal-3 as a target in the development of therapeutic agents as a treatment for PAH.

Keywords

Pulmonary · Galectin-3 · ROS · Vascular remodeling · Inflammation · Fibrosis

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2.1 Pulmonary Arterial Hypertension (PAH)

Pulmonary Arterial Hypertension (PAH) is a progressive disease of the lung vasculature that is characterized by a sustained elevation of pulmonary arterial pressure [1]. PAH is currently defined as a mean pulmonary artery pressure at rest ≥ 20 mmHg [2], which can result in increased

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pulmonary vascular resistance subsequently leading to compensatory right ventricular hypertrophy [1, 3]. Medial hypertrophy of pulmonary artery (PA) smooth muscle cells is a hallmark feature of PAH [4], which elicits vessel luminal occlusion [5]. In most forms of PAH, muscularization of small distal PA occurs [6], and is further characterized by excessive arterial cell proliferation, fibrosis, and inflammation, leading to medial remodeling, rarefaction, and a loss of compliance of the pulmonary blood vessels [5, 7–9]. Increased resistance to blood flow via loss of PA compliance contributes to the failure of the right ventricle (RV) [10, 11]. In addition, the response of the RV to the increased afterload associated with PAH increases end-diastolic volume, hypertrophy, alters contractility, induces dilation, cardiac fibrosis, and eventual decompensation [12]. Ultimately, increased RV volume (diastolic and systolic) combined with increased intraluminal cardiac pressure leads to an unsustainable increase in wall stress that culminates in right heart failure and ultimately death [13–15].

In PAH, within the vessel wall, endothelial cells become dysfunctional and fail to maintain homeostasis, and vascular smooth muscle cells undergo a phenotypic switch from a quiescent contractile phenotype to a "synthetic" phenotype that is characterized by a decrease in contractile smooth muscle genes, increased secretion of matrix and proteases, and increased proliferation [16, 17]. The subsequent increase in pro-inflammatory and pro-fibrotic molecules increases fibrosis, inflammation, and the deposition of extracellular matrix [18–22]. The signaling moieties that modify cellular properties in PAH remain ill-defined, but endothelin, PDGF, TGF-β, BMPs, hypoxia, altered metabolism, reactive oxygen species (ROS), and nitrogen species (RNS) have all been identified as contributing factors [19, 23–25].

2.2 Evidence for ROS Signaling in PAH

Previous evidence shows increased levels of ROS in both human and experimental models of PAH [26–32]. The major ROS that are produced in the pulmonary vasculature are superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), and hydroperoxyl radical ($HO_2^{\cdot}$) [33]. Numerous mechanisms have been proposed to account for increases in ROS including altered eNOS activity and increased NOX enzyme expression and activation. Steady-state levels of ROS reflect the balance between ROS generation and eradication/scavenging, and evidence supports alterations in both pathways in PAH [25]. We have previously shown that ROS contributes to the development of PAH [34] and Fig. 2.1a shows that PA contraction to angiotensin II is enhanced from rats with monocrotaline (MCT)-induced PAH. In addition, treatment of pre-contracted vessels with the antioxidant Tempol, elicits a greater relaxation of induced tone in MCT-treated vessels compared to control conditions (Fig. 2.1b), suggesting that increased oxidant tone in PA from MCT-induced PAH augments vascular contraction. Of the ROS produced, O_2^- and H_2O_2 activate multiple signaling pathways promoting cell proliferation and apoptosis, elevated vascular tone, fibrosis, and inflammation,

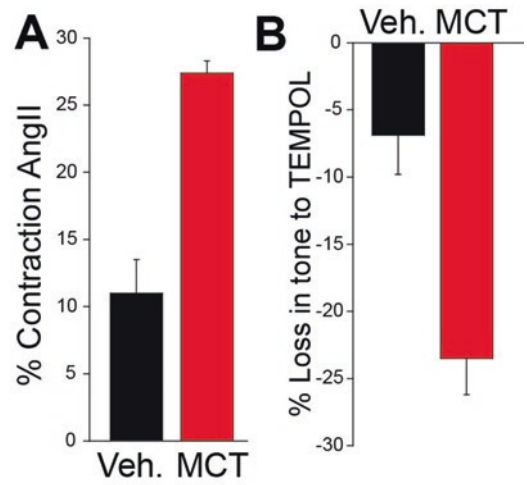

Fig. 2.1 Hypertensive PA produces greater contractile force dependent on ROS. (**a**) PA from control (vehicle) and MCT-rats were mounted on a myograph (1 g passive tension) and contracted with low dose Angiotensin (Ang) II (100 nM). (**b**) Drop in tension following addition of ROS scavenger TEMPOL (100 mM). n = 3–4 per group. (Reprinted with copyright permission from *Antioxidants and Redox Signaling*, Volume 31, Issue 14, Mary Ann Liebert, Inc., New Rochelle, NY (Publisher))

which are all hallmark signs of PAH [33]. However, the cellular basis and functional significance of ROS in PAH remain poorly described. The human genome encodes five NOX isoforms and four of these isoforms, NOX1, NOX2, NOX4, and NOX5 are expressed in pulmonary vascular cells, and NOX4 is regarded as a constitutively active enzyme that produces levels of H_2O_2 that are primarily controlled by changes in gene expression [35, 36]. Increased expression of NOX4 has been reported in human PAH [37], and several lines of evidence support an important role for NOX4 in the pathogenesis of PAH in rat and human [34, 37] but this premise is less well-defined in mice [38–40]. NOX4 has been reported to be a major NADPH oxidase homolog expressed in human pulmonary arterial smooth muscle cells (PASMCs) [41], and its expression at the mRNA and protein level is significantly increased in lungs from patients with idiopathic pulmonary arterial hypertension (IPAH) compared to healthy lungs [37], which suggests a correlation between NOX4 and the onset of PAH. NOX4 mediates the hypoxia-induced growth of human PASMCs [42], and other studies report that NOX4 expression is highest in endothelial cells and perivascular fibroblasts [34, 43, 44]. Endothelial cell NOX4 is thought to be protective, and supports endothelial nitric oxide synthase function [45, 46], whereas fibroblast NOX4 is highly upregulated by TGFβ and is pro-fibrotic [34, 47]. Collectively, these findings support the argument for NOX4 expression being integrally involved in pulmonary vascular remodeling by promoting arterial medial smooth muscle proliferation, endothelial proliferation, and adventitial fibroblast-activation in PAH. NOX4 is also upregulated in cardiac hypertrophy and myocardial remodeling [48].

Epidemiologically, PAH is more frequent in women than men, and left untreated has a survival time of 5–7 years post-diagnosis [49]. From a therapeutic standpoint, there are a number of vasodilator drugs that are indicated for the treatment of PAH, but none of the current therapeutics offers long-term success for survival due to limited effectiveness and unwanted side effects [50]. More importantly, focus is being increasingly placed on the underlying causes of the vascular remodeling that is a hallmark of the disease [51].

2.3 Galectin-3

Galectin-3 (Gal-3; *LGALS3*, Mac-2) is a member of the lectin family of proteins, which recognize and bind to specific carbohydrate motifs on glycosylated proteins as well as lipids [52]. Gal-3 protein was first identified in the 3 T3 mouse fibroblast cell line [53] and is robustly expressed in the lung [54]. The gene encoding Gal-3 was cloned in 1987 and changes in mRNA expression in fibroblasts were observed in response to growth factors [55]. Gal-3 protein is present in both the cytoplasm and nucleus of cells, with higher expression in the nucleus of proliferating cells [56]. Gal-3 cellular expression appears to be age-dependent with robust expression induced by growth factors in young cells, which deteriorates in aged cells or those with replicative senescence [57]. Approximately 30 years ago, the macrophage surface antigen, Mac-2 was determined to be identical to Gal-3 and shown to be expressed in high concentrations by specific subpopulations of pro-inflammatory macrophages and secreted into the extracellular space [58, 59]. As the name implies, Mac-2 expression was extensively used to identify or mark macrophages [60]. It is now known that Gal-3 expression is also expressed in fibroblasts (where it was originally discovered), smooth muscle cells [61], endothelial cells [62], activated T-cells [63], epithelial cells [64, 65], and select types of tumor cells [66].

Gal-3 belongs to a family of 16 related members that all share an evolutionarily conserved carbohydrate recognition domain (CRD) that can bind β-galactosides and lactose but they differ in their ability to bind more complex saccharides. Gal-3 "family" members can be broadly classified into three types: the prototypes which contain one CRD and are monomers or homodimers (includes galectins- 1, 2, 5, 7, 10, 11, 13, 14, 15, and 16), the chimeras (Gal-3 is the only member) which contain one CRD and a self-association domain, and the tandem-repeat galectins (galectin- 4, 6, 8, 9, and 12) which have two CRDs connected by a linker peptide. As the only chimeric galectin, Gal-3 is comprised of a C-terminal CRD that is present in all members of the galectin family and a unique N-terminal domain that contains glycine and proline-rich domains that enable