Chronic Stress and Glucocorticoid Receptor Resistance in Asthma

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ABSTRACT

Purpose: Chronic and persistent exposure to negative stress can lead to adverse consequences on health. Particularly, psychosocial factors were found to increase the risk and outcome of respiratory diseases like asthma. Glucocorticoids (GCs) are the most efficient anti-inflammatory therapy for asthma. However, a significant proportion of patients don't respond adequately to GC administration. GC sensitivity is modulated by genetic and acquired disease-related factors. Additionally, it was proposed that endogenous corticosteroids may limit certain actions of synthetic GCs, contributing to insensitivity. Psychological and physiological stresses activate the hypothalamic-pituitary-adrenal axis, increasing cortisol levels. Here, we review the mechanism involved in altered GC sensitivity in asthmatic patients under stressful situations. Strategies for modulation GC sensitivity and improving GC therapy are discussed.

Methods: PubMed was searched for publications on psychological chronic stress and asthma, GC resistance in asthma, biological mechanisms for GC resistance, and drugs for steroid-resistant asthma, including highly potent GCs.

Findings: GC resistance in patients with severe disease remains a major clinical problem. In asthma, experimental and clinical evidence suggests that chronic stress induces inflammatory changes, contributing to a worse GC response. GC resistant patients can be treated with other broad-spectrum anti-inflammatory drugs, but these generally have major side effects. Different mechanisms of GC resistance have been described and might be useful for developing new therapeutic strategies against it. Novel drugs, such as highly potent GCs, phosphoinositide 3-kinase-delta inhibitors that reestablish histone deacetylase-2 function, decrease of GC receptor phosphorylation by p38 mitogen-activated protein kinase inhibitors, or phosphatase activators, are currently in clinical development and might be combined with GC therapy in the future. Furthermore, microRNAs (small noncoding RNA molecules) operate as posttranscriptional regulators, providing another level of control of GC receptor levels. Empirical results allow postulating that the detection and study of microRNAs might be a promising approach to better characterize and treat asthmatic patients.

Implications: Many molecular and cellular pathobiological mechanisms are responsible of GC resistance. Therefore detecting specific biomarkers to help identify patients who would benefit from new therapies is crucial. Stress consitutes a negative aspect of current lifestyles that increase asthma morbidity and mortality. Adequate stress management could be an important and positive intervention. (Clin Ther. 2020; 42:XXX–XXX) © 2020 Elsevier HS Journals, Inc. (*Clin Ther*. xxxx;xxx:xxx) © 2020 Elsevier Inc.

Key words: asthma, chronic stress, glucocorticoid resistance, pharmacologic strategies.

Accepted for publication March 4, 2020
https://doi.org/10.1016/j.clinthera.2020.03.002
0149-2918/$ - see front matter

INTRODUCTION

In the last decades, stress has become an important aspect of modern life. Exposure to adverse situations affects virtually everyone worldwide. Stress is defined as any situation capable of perturbing the physiological or psychological homeostasis. Although response to stress is a necessary survival mechanism, prolonged stress can have several repercussions affecting behavioral, endocrine, and immunologic parameters.[1] Many of these effects are mediated through stress actions on the immune system.[2–5] In an interesting review, Dhabhar[6] described the different effects that stress has on immune response, taking into account key factors such as stress duration and its timing regarding the immune response activation and course. Whether the stress effect on immune response has a beneficial or harmful effect on health depends on the end-effect of the immune response. Thus, short-term stress can be favorable by enhancing the immune response in wound healing and vaccination but promotes pathological, pro- inflammatory, or autoimmune responses. In contrast, chronic stress can suppress protective immune responses and/or exacerbate pathological ones. Chronic psychological stress has been associated with a higher risk of depression, cardiovascular disease, diabetes, autoimmune diseases, upper respiratory tract infections, and poorer wound healing.[7]

Asthma is a highly prevalent, chronic respiratory condition characterized by reversible airflow obstruction, airway hyperresponsiveness, and airway inflammation producing breathlessness, coughing, and wheezing. Currently, it is known that in asthma there is a complex interplay between inflammatory cells such as eosinophils, mast cells, basophils, dendritic cells and lymphocytes, and structural cells (namely epithelial cells, fibroblasts, and smooth muscle cells). The chronic airway inflammation has been associated with the accumulation and activation of inflammatory cells such as type 2 T-helper (Th2) cells that secrete interleukin (IL)-4, IL-5, IL-13, eosinophils, and mast cells within the bronchial mucosa.[8]

According to the World Health Organization (https://www.who.int/news-room/fact-sheets/detail/asthma), ~235 million people are living with asthma. Independently of asthma severity and despite optimal medical therapy, patients may experience acute exacerbations of symptoms and a loss of disease control. Asthma exacerbations are a major cause of disease morbidity, increases in health care costs, and, in some patients, a greater progressive loss of lung function. Inhaled corticosteroids, long-acting beta-agonists, and biologic therapy reduce asthma exacerbations and improve disease control. However, when exacerbations occur, systemic corticosteroids remain the primary intervention when bronchodilator therapy is not effective.[9]

It is known that GCs in pharmacologic doses have anti-inflammatory and immunosuppressive properties, and synthetic GCs are widely prescribed for inflammation, autoimmune disorders, and malignancies of lymphoid origin treatment. However, a substantial proportion of patients do not adequately respond to GC therapy. The biologic effects of GC are determined not only by their concentration but also by individual and tissue sensitivity to the hormone.[10]

In general, GC sensitivity refers to the response of a GC-responsive system to different concentrations of hormone, and it is dependent of GC receptor (GR) expression and affinity, and many intracellular mediators that can regulate the signal transduction cascade. Individual differences in GC sensitivity have been described in health and disease and are clinically relevant because they can influence both the outcome and the adverse effects of GC therapy.[10] GC resistance, on the other hand, refers to a decrease in the sensitivity of immune cells to GC hormones that would normally end the inflammatory response. Evidence for GC resistance has been found mainly in rheumatoid arthritis,[11] systemic lupus erythematosus, bronchial asthma, inflammatory bowel disease, and immune thrombocytopenia.[12] GC resistance in patients with severe disease remains a major clinical problem. GC sensitivity can be modulated by genetic and acquired disease-related factors.[13] In recent years, several lines of evidence have suggested that environmental factors such as cigarette smoke, exposure to bacterial toxins or allergens, viral infection, and chronic stress could influence GC response.[13,14] It has been proposed that prolonged stressors result in GC resistance, which, in turn, interferes with appropriate inflammatory regulation. In particular, chronic stress exposure could be influencing asthma exacerbations and treatment response.[14]

The present article reviews the evidence supporting the participation of stress in asthma exacerbations and the mechanisms involved in altered GC sensitivity in this group of patients. Strategies for GC sensitivity modulation and therapy improvement are also discussed.

MATERIALS AND METHODS

PubMed was searched for publications on psychological chronic stress and asthma, GC resistance in asthma, molecular and cellular mechanism for GC resistance, and drugs for steroid-resistant asthma, including highly potent GCs.

MECHANISMS OF GC ACTIONS

Exogenously administered GCs diffuse across the plasma membrane due to high lipophilicity. In the cytoplasm, GCs bind to the alpha subunit of the GR, which is part of a multiprotein complex with chaperone proteins. In the classic signal transduction pathway, GC binding induces a molecular rearrangement of the GR heterocomplex that results in subsequent homodimerization, nuclear translocation of the GC–GR complex, and interaction with specific DNA elements named GC response elements (GREs) modulating the gene transcription. The direction of the transcriptional response depends partially on the nature of the GRE. Positive GREs mediate transcriptional upregulation (transactivation), whereas negative GREs mediate transcriptional downregulation (cis-repression). Conversely, GC–GR can also regulate gene expression by physically interacting with other transcription factors such as nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1), a phenomenon called "transrepression."

Thus, GCs exert their anti-inflammatory and immunosuppressive effects through the induction of synthesis of several molecules such as lipocortin-1, secretory leukocyte inhibitory protein, an inhibitor of NF-κB (IκB-α), an inhibitor of mitogen-activated protein kinase (MAPK) pathways (MKP1), GC-inducible leucine zipper (GILZ) and anti-inflammatory cytokines (IL-10, IL-12, and IL-1 receptor antagonists). Moreover, it inhibits the synthesis of inflammatory cytokines (IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-13, IL-15, tumor necrosis factor-α [TNF-α], and granulocyte-macrophage colony-stimulating factor), chemokines (CCL1, CCL5, CCL11, CXCL8, monocyte chemotactic protein-*1*, eotaxin, RANTES [regulated upon activation normal T cell expressed and secreted], and macrophage inflammatory protein-1α); inflammatory enzymes (inducible nitric oxide [NO] synthase, inducible cyclo-oxygenase, and inducible phospholipase A2), inflammatory peptides (endothelin-1), and adhesion molecules (intercellular cell adhesion molecule 1 and vascular cell adhesion molecule 1).[15] In addition, GCs have multiple effects that do not require GC–GR complex binding to GRE or direct protein–protein interactions and occur seconds to minutes after stimulation, namely nongenomic effects.[16] Three mechanisms were described for nongenomic effects: nonspecific interactions of GCs with cellular membranes, mediated by the release of different chaperones; following binding of GC to the cytosolic GR; and specific interactions with a membrane-bound GR.

Finally, it was proposed that different physiological contexts result in a variety of signaling pathways activated in concomitance with GR signaling.[17] These signaling pathways interacting with GRs can modify the sensitivity and efficacy of GC responses, with implications for physiology, diseases, and treatments.[17]

CELLULAR AND MOLECULAR MECHANISMS INVOLVED IN CORTICOSTEROID RESISTANCE IN SEVERE ASTHMA

Severe bronchial asthma is a very heterogeneous clinical syndrome in which the GC-resistant inflammatory nature is due to different cellular and molecular pathobiologic mechanisms called endotypes.[18] This concept of disease is particularly important because defining different disease-driving mechanisms may provide insights for targeted and personalized treatment for each population with corticoid-resistant severe asthma.

Currently, the endotype of asthma is divided into Th2-high and Th2-low inflammation. The Th2-high endotype is characterized by airway eosinophil counts and increased Th2 inflammatory cytokines (eg, IL-4, IL-5, IL-13). Th2-high endotype eosinophilic asthma is often responsive to steroid therapy, but 10%–20% of these patients exhibit GC insensitivity. The Th2-low endotype patients have high airway neutrophil counts and type 17 T-helper (Th17) cytokines. This endotype is a less GC-responsive disease variant and has lower lung function clinically.[19] However, this simplified

classification does not correctly reflect the underlying pathobiologic processes of GC-resistant inflammation in severe asthma. There are disease-associated mechanisms that could promote, sustain, and/or aggravate low steroid sensitivity within the population and even in the same individual over the time. In addition, a clustering analysis involving 112 clinical, physiologic, and inflammatory variables found that combined eosinophilic/neutrophilic inflammation may be a biomarker of the most severe form of asthma.[20]

The following discussion covers different cellular and molecular mechanisms that could be involved in GC resistance in severe asthma (Fig. 1).

Genetic Susceptibility to GC Resistance

There is evidence that GC resistance could have a genetic component. Hakonarson et al[21] analyzed the gene expression profiles of freshly isolated peripheral blood mononuclear cells (PBMCs) from patients with GC-sensitive and GC-resistant asthma. The authors tested the effect of GCs in resting cells and cells stimulated with IL-1β and TNF-α combined. They found a panel of 11 genes that most accurately distinguished GC responders from nonresponders, predicting the GC response phenotype with 84% accuracy.[22] Moreover, a functional variant GC-induced transcript 1 gene (GLCCI1) was associated with a substantial reduction in the inhaled GC response in patients with asthma.[23]

Changes in GR Expression

The cellular GR expression levels have been correlated with the magnitude of the biologic effects induced by GCs. Diminished GR number and binding affinity were found in peripheral blood

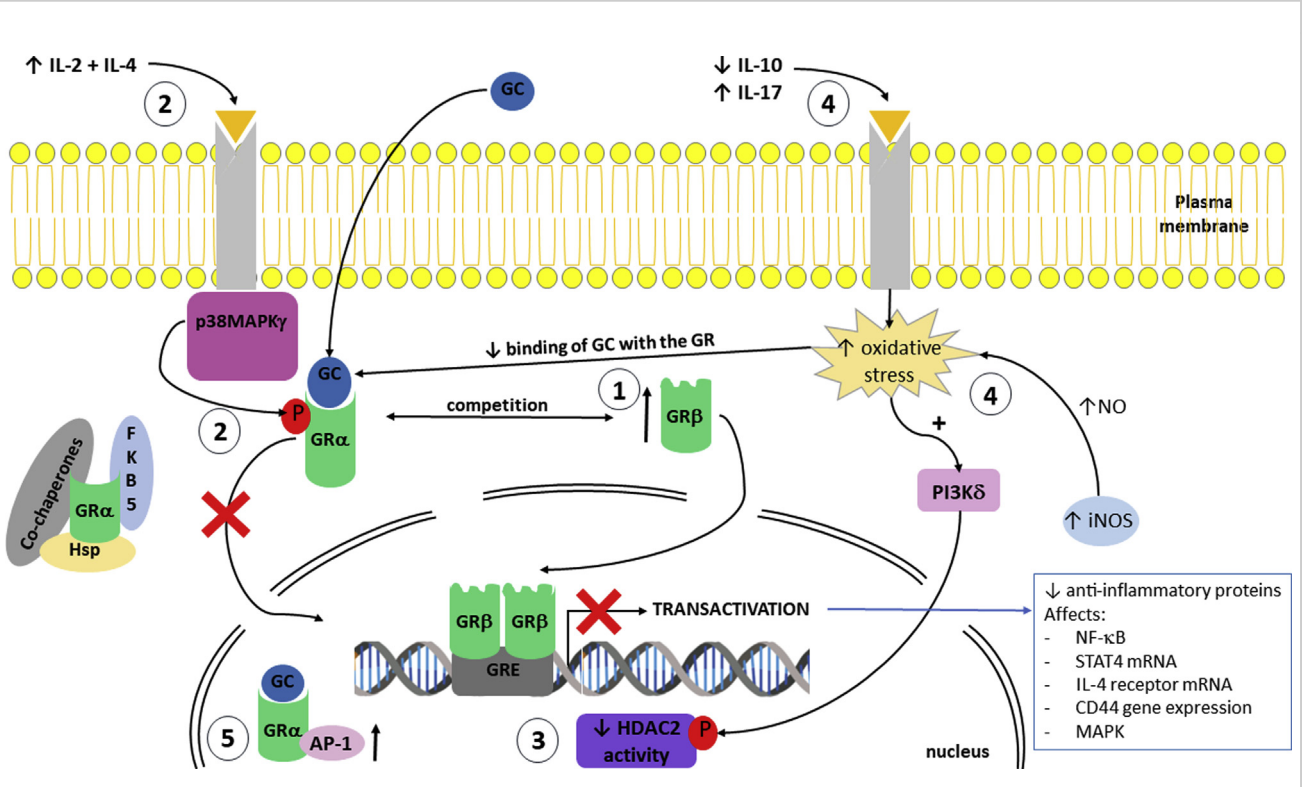

Figure 1. This mechanism may cause a decrease in anti-inflammatory proteins and affects nuclear factor kappa B (NF-κB) function, STAT4 mRNA expression, interleukin (IL)-4 receptor mRNA expression , CD44 gene expression, and mitogen-activated protein kinase (MAPK) activity among others instead of This mechanism may cause a decrease in anti-inflammatory proteins and affects nuclear factor kappa B (NF-κB), STAT4 mRNA, interleukin (IL)-4 receptor mRNA , CD44 gen, and mitogen-activated protein kinase (MAPK) among others.

monocyte cells from patients with GC-resistant asthma.[24] Most cell types showed a downregulation of the amount of GR upon GC exposure, probably as a mechanism to maintain GC homeostasis. Another level of fine adjustment GR levels is provided by microRNAs (miRs). miRs are a class of naturally occurring, small noncoding RNA molecules, ~21 to 25 nucleotides in length that operate as posttranscriptional regulators, reducing translation and increasing mRNA degradation. Some miRs, such as miR-18 and mi-124, decrease GR protein levels in the brain.[25] In particular, it was shown that GC treatment induces expression of miR-124, which, in turn, could be downregulating GR-α in T cells, limiting anti-inflammatory effects of GCs.[26] Further investigations proved the involvement of miR in the inflammatory and immunological mechanisms of asthma genesis.[27]

Increased Expression of GR-β Isoforms

Functional polymorphisms of the GR partially determine differences in sensitivity to GCs. In particular, GR-β isoform is a nonligand-binding receptor, and it reportedly is a dominant-negative inhibitor of GR-α that competitively binds GREs, influencing transactivation.[28] Several studies found augmented GR-β gene expression in both infiltrating blood cells and in airway epithelial cells of patients with GC-insensitive asthma.[29–31] Furthermore, RNA silencing of GR-β mRNA in bronchoalveolar lavage macrophages of patients insensitive to GC increases transcriptional activity of GR-α.[31] Vazquez-Tello et al[32] showed that IL-17/IL-23 cytokines promoted GR-β upregulation, which is associated with induced GC insensitivity in PBMCs. Conversely, GC resistance induced by IL-2/IL-4 was associated with decreased GR-α expression.[24,32,33] These results support the possibility that Th-17 lymphocytes and associated cytokines have a role in the mechanism of steroid hyporesponsiveness in patients with severe asthma.

Defective Nuclear Translocation and Binding Affinity

GR can be phosphorylated at multiple serine/threonine residues, and different phosphorylation patterns can alter its GC binding to receptor, stability, translocation to the nucleus, binding to DNA, and interaction with other proteins such as transcription factors and molecular chaperones.[34] Several kinases have been identified as being capable of phosphorylating the GR (glycogen synthase kinase-3β, MAPKs, and cyclin-dependent kinase). Cellular conditions modulate the activation of these signaling pathways, resulting in different GR phosphorylation patterns that modulate its transcriptional activity within cells.[35]

Bhavsar et al[36] found a greater activation degree of p38 MAPK in alveolar macrophages from patients with asthma and GC insensitivity compared with those with normal response. In addition, a p38 MAPK inhibitor induced an increase of GC sensitivity in PBMCs from patients with severe asthma.[37] It was reported that IL-2 and IL-4 synergistically reduce nuclear translocation and binding affinity in T cells, orchestrated via the p38 MAPK pathway; this effect can be reversed by a p38 inhibitor.[33] Similarly, MAPK c-Jun N-terminal kinase (JNK), which is activated by TNF-α and other proinflammatory cytokines, directly phosphorylates GR at Ser226 and inhibits GRE binding too.[38] Moreover, it was reported that inhibition of the extracellular receptor kinase (ERK) pathway restored dexamethasone sensitivity in some GC-resistant T cells.[39]

Ubiquitination and sumoylation are other posttranslational GR modifications. Both induce GR degradation and consequently affect its transcriptional activity.[40] Furthermore, in vitro GR may be nitrosylated by NO donors. This action results in reduced binding affinity for GC.[41] In severe asthma, there is increased expression of inducible NO synthase, which produces large amounts of NO that, in turn, could reduce GC responsiveness.

Altered Transcriptional Regulation

Physical association with other transcription factors is one of the mechanisms that regulates GR transcriptional response. GR is able to interact with proinflammatory transcription factors, such as NF-κβ and AP-1, inhibiting their activity and repressing inflammatory signaling pathways.[42]

It has been shown that overexpression of AP-1 contributes to GC resistance in patients with asthma.[43] A possible mechanism for the AP-1 increase is the presence of the proinflammatory cytokine TNF-α, which causes AP-1 activation through the JNK pathway.

Another important mechanism of GR transcriptional regulation involves modulation of DNA accessibility. Recruitment of histone deacetylase-2 (HDAC-2), allowing deacetylation and condensation of histones located in promoter regions of proinflammatory genes, is the major mechanism that inhibits their transcription. Reduced HDAC-2 activity was reported in patients in alveolar macrophages with refractory asthma and in the airways of patients with asthma who smoke.[44] It was reported that oxidative and nitrative stress results in the formation of peroxynitrite, which, in turn, induces increased proteasomal degradation following ubiquitination.[23,45]

GR RESISTANCE ASSOCIATED WITH EXTERNAL FACTORS THAT ALTER RECEPTOR BIOLOGY

There is evidence suggesting that some external factors, including cigarette smoke, exposure to bacterial toxins, viral infection, allergen exposure, and lifestyle, could influence GC response by altering receptor biology (Fig. 2).

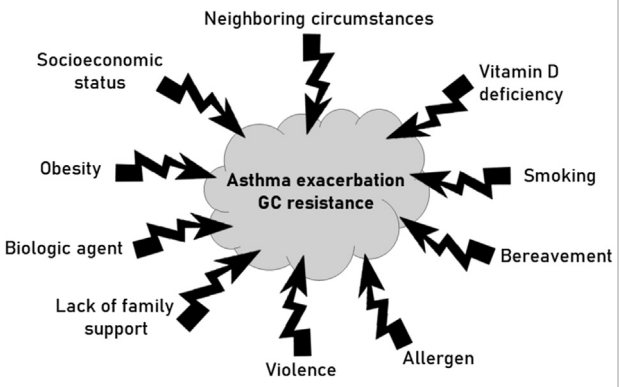

Figure 2. External factors that could alter the biology of the receptor inducing glucocorticoid receptor (GR) resistance. Among them are environmental factors such as exposure to allergen or biological agents and cigarette smoking, nutritional factors of diseases such as obesity and vitamin D deficiency and psychological stress such as family and neighboring circumstances.

Cigarette Smoking

Cigarette smoking reportedly increases asthma incidence among adolescents, enhances asthma severity, and induces airway neutrophilia.[46–48] Cigarette smoking also decreases the clinical response to inhaled and oral corticosteroids.[49] It was proposed that cigarette smoking induces an oxidative stress that in turn affects nuclear cofactors. In fact, a reduction in HDAC-2 expression was found in the airways of patients with asthma who smoke.[50] In addition, PBMCs from patients with asthma who smoke also have an elevated GR-β to GR-α ratio.[51]

Exposure to Allergen and Biological Agents

Patients with severe allergic asthma require increased amounts of GC therapy to control their asthma during the pollen season.[52] The effect of allergen exposure on GR function and GR-binding affinity in PBMCs from patients with atopic asthma was examined by Nimmagadda et al.[53] The authors found a significant reduction in the GR-binding affinity in ragweed-allergic patients with asthma during ragweed pollen season compared with PBMCs obtained before and after ragweed season. These effects seem to be mediated by an increase in IL-2 and IL-4 cytokine levels.[54]

Moreover, recurrent exacerbations are triggered by respiratory viruses and constitute a serious problem in patients with asthma.[54] Clinical and laboratory evidence indicates that GCs are strongly inefficient in treating virus-induced asthma exacerbations.[55,56] It has been reported that rhinovirus infection can reduce GR-α nuclear translocation and both transactivation and transrepression activities of GC in airway epithelial cells through a mechanism involving JNK and NF-kB pathway activation.[57] Finally, it was reported that microbial superantigens induced GC resistance in T cells in vitro via activation of ERK pathways.[39,58]

Obesity

A large body of robust epidemiologic data has revealed that obesity increases both asthma risk and asthma severity.[59,60] In addition, pharmacologic trials showed that clinical responsiveness to inhaled GCs was diminished in overweight patients.[61] High body mass index is associated with a blunted in vitro

response to dexamethasone in overweight and obese patients with asthma.[62]

Disease-Related Factors

A proportion of patients have a deficient response to GCs due to tissue-specific resistance induced by disease-related factors. As described, several factors for this acquired GC resistance are associated with significant changes in the cellular microenvironment, such as oxidative stress, cytokine production, and increased P-glycoprotein–mediated drug efflux.

Oxidative and nitrative stress results in the formation of peroxynitrite, which in turn nitrates tyrosine residues on HDAC-2, leading to its inactivation, ubiquitination, and degradation.[23] In addition, oxidative stress activates phosphoinositide 3-kinase (PI3K) that induces phosphorylation and inactivation of HDAC-2.[45] This evidence suggests that oxidative stress might be an important mechanism of steroid resistance in patients with severe asthma.[63]

A deregulated production of cytokine was found in GC-resistant asthma. In patients with GC-resistant asthma, it was described as a defective production of IL-10 by regulatory T cells in response to GC.[64,65] On the contrary, elevated levels of IL-17 and related cytokines produced by Th17 cells were reported in patients with severe asthma.[66] IL-17 increases the expression of GR-β expression in epithelial cells, suggesting a possible role for Th17-associated cytokines in the mechanism of steroid hyporesponsiveness in patients with asthma.[32] As noted earlier, it was reported that IL-2 and IL-4 synergistically reduce nuclear translocation and binding affinity in T cells.[33]

Last but not least, vitamin D deficiency has been associated with reduced lung function, increased airway hyperresponsiveness, and reduced GC response, suggesting that vitamin D supplementation in patients with asthma may improve asthma severity and treatment response.[67] In children with asthma, reduced vitamin D levels are associated with increased markers of allergy and asthma severity.[68] Furthermore, the addition of vitamin D_3 in combination with dexamethasone in the same study restored the ability of $CD4^+$ T cells from GC-resistant patients with asthma to release IL-10 at the same level as those seen in cells from GC-sensitive patients with asthma.[69] Moreover, vitamin D_3 was able to upregulate MKP-1 to repress p38 MAPK-mediated cytokine secretion in monocytes and macrophages.

INFLUENCE OF STRESS EXPOSURE IN ASTHMA EXACERBATIONS AND GC RESISTANCE

Currently, stress and emotional factors are considered important stimuli capable of disturbing the brain–endocrine–immune interaction, strongly implicated in morbidity and mortality in asthma. In a very interesting review, Ohno[14] presents insights into the critical role of psychological stress in the development and exacerbation of allergic asthma, emphasizing the continuity from the central sensing of psychological stress to the enhanced eosinophilic airway inflammation.

Clinical studies have shown a relation between psychological stress and the exacerbation of asthma symptoms.[70,71] According to a survey performed in 3085 patients, which searched for factors contributing to asthma exacerbation, emotional stress caused 10%–15% of the asthma exacerbation. Both acute[72–74] and chronic[74,75] stress exposures have been associated with enhanced asthma exacerbations in children[73,74] and adults.[72,75,76]

Sandberg et al[74] reported that in children, harsh negative life events significantly increase the risk of experiencing more asthmatic episodes in upcoming weeks. The risk is enhanced if the child lives in conditions of chronic high stress. Furthermore, exposure to physiological stress has been found to strongly correlate with poor outcome for asthma, both in children and adults.[77]

Psychological stress caused by domestic and neighboring circumstances[78,79] such as bereavement[80] and violence[81,82] is frequently related to stress-induced asthma exacerbation. In a study of youths diagnosed with asthma aged 9–18 years, Chen et al[79] investigated the influence of family support and neighborhood problems in asthma outcomes. This study found that a lack of family support was related to increased asthma symptoms and poorer pulmonary function through allergic inflammation. In addition, evidence was consistent with the hypothesis that greater neighborhood problems were related to greater asthma symptoms by providing role models for maladaptive health behaviors, such as smoking. However, peer support was unrelated to asthma outcomes.

A link between lower socioeconomic status and asthma exacerbation has also been described. An increase was found in IL-5 and IL-13 levels and eosinophil counts in children from families that rented rather than owned their home.[83] Moreover, it was reported that the production of IL-13 in children with asthma was inversely correlated with family savings and annual family incomes.

Kopel et al[84] showed that children whose caregivers felt their neighborhood to be unsafe have a greater rate of uncontrolled asthma than those living in neighborhoods considered to be safe. It was also reported[85] that a perceived stress level in parents (as evaluated by a self-report questionnaire) is associated with increased levels of asthma-relevant inflammatory markers. Data of clinical studies[86,87] support the hypothesis that chronic stress leads to a reduced ability of cortisol to regulate cytokine activity and subsequent airway inflammation. Thus, acute and chronic stress exposures have been related to decreased expression of genes encoding for GR (by 5.5-fold) and the β_2-adrenergic receptor (by 9.5-fold) in leukocytes of children with asthma.[86] In addition, school-aged children who perceived a low parental presence had a reduced leukocyte response to GCs in vitro.[85]

Research performed in animal models has been used to study the pathophysiological mechanism that participates in stress-induced asthma exacerbation.[88–92] Accumulating evidence indicates that psychological stress enhanced the frequency, duration, and severity of asthma symptoms by eosinophilic increasing airway inflammatory responses, characterized by a switch toward a Th2-dominant cytokine profile.[93,94] Furthermore, a Th2 dominant cytokine profile is related to GC resistance.[24,32,33]

The canonical pathways participating in stress response include the hypothalamic–pituitary–adrenal axis and the sympathetic–adrenal–medullary axis. The stress hormones, GCs, epinephrine, and norepinephrine, induce immunologic alterations. This neuroendocrine response amplifies Th2-type immune responses in the lungs via the induction of Th1/Th2 or regulatory T cell/Th2 imbalance. This response is strongly associated with psychological stress–induced asthma exacerbation[14,95] and GC resistance.[24,32,33] Okuyama et al[96] showed that a rise of corticosterone levels during stress exposure was implicated in the exacerbated allergic airway response induced by challenge with ovalbumin. The stressed mice challenged with ovalbumin had an increase in eosinophils and lymphocytes in the airways and augmented levels of IL-13 in the lung compared with nonexposed mice. The administration of a GR antagonist and a GC synthesis inhibitor during stress exposure significantly reduced these effects in stressed mice challenged with antigen. Kawano et al[97] in a murine model of allergic asthma found that psychological stress exposure avoids the development of respiratory tolerance. The mechanism involved GC-dependent suppression of tolerogenic dendritic cells and regulatory T-cell induction, which, in turn, results in an increased susceptibility to allergic asthma. These findings suggest that psychological stress can potentially increase allergic asthma susceptibility by inhibiting immune tolerance.

All these results indicate a pathophysiological role for the neuroendocrine axis, linking psychological stress with asthma exacerbations. However, GC administration has been widely used for asthma management. To explain this apparent discrepancy of GC's role in allergic inflammation, Ohno[14] proposed that GCs may play a distinctive role in the regulation of Th2 immune responses depending on several factors, among them the timing, frequency, and location of this hormone's appearance.

THERAPEUTIC APPROACHES TO IMPROVE CORTICOSTEROID SENSITIVITY IN SEVERE ASTHMA

As noted earlier, resistance to the anti-inflammatory effects of GC is a serious concern that limits the response in asthma treatment. The use of alternative anti-inflammatory treatments is restricted by their side effects. Thus, potential therapeutic strategies involving the development of highly potent GCs or drugs able to interfere with molecular pathways involved in GC resistance are being studied (Table).

Development of Highly Potent GCs

He et al[98] have been developing a series of highly potent GCs, namely VSGC12, VSG158, and VSG159, based on the structural insight into the GR. In particular, the authors developed an extremely potent GC-denominated VSG158. This GC presented the highest potency in avoiding lung airway hyperresponsiveness and lung inflammation in a mouse model of eosinophilic and neutrophilic airway

Table. Relevant therapeutic strategies under study to reverse glucocorticoid resistance in asthma.

Therapeutic Strategy	Drugs	Pharmacological Effect
Highly potent glucocorticoids	VSG158[98]	10 times more potent than the most potent clinical glucocorticoid fluticasone
Restoration of HDAC-2 function	IC87114[99]	Inhibits PI3Kδ
	Formoterol[99]	
	Theophylline[99]	
	Antagonist miR-21[100]	
Decrease GR phosphorylation	SB203580[101]	Inhibits p38 MAPK
	Antagonist miR9[106]	Increases PP2A activity
	Formoterol[105]	

GR = glucocorticoid receptor; HDAC-2 = histone deacetylase-2; MAPK = mitogen-activated protein kinase; miR = microRNA; PI3Kδ = phosphoinositide 3-kinase-delta; PP2A = protein phosphatase 2A.

inflammation. This compound was 10 times more potent than the current most potent clinical GC, fluticasone furoate, and displayed reduced off-targeting and side effects. In addition, these GCs also display pharmacokinetic properties that are suitable for the inhalation delivery method for asthma treatment. Taking these findings into account, VSG158 could be a promising drug for treating steroid-resistant severe asthma.

Resetting HDAC-2 Function

HDAC-2 is believed to play a critical role in mediating the anti-inflammatory actions of corticosteroids. Many potential therapeutic approaches to restore HDAC-2 function have emerged from in vitro studies. HDAC-2 activity is diminished by phosphorylation induced by the PI3K pathway. The PI3Kδ isoform has recently been identified as an important upstream kinase in mediating steroid resistance in severe asthma. Inhibitors of PI3Kδ, such as such as IC87114,[99] or nonspecific drugs such as formoterol or theophylline have been shown to be capable of restoring GC response. In addition, an important role of miR-21 in the pathogenesis of asthma and in steroid resistance via PI3K activation was reported.[100] It was proposed that the development of miRNA-based drugs could constitute a promising therapy to improve treatment of GC-resistant asthma.

p38 MAPK Inhibitors

Evidence supports a critical role of the MAPK pathway in steroid resistance in severe asthma. Thus, a higher level of p38 MAPK phosphorylation was reported in the PBMCs of GC-resistant patients with asthma compared with patients with GC-sensitive asthma.[101] The treatment with the p38 MAPK inhibitor SB203580 diminished p38 phosphorylation and mitigated steroid resistance by enhancing dexamethasone-mediated suppression of IL-8 mRNA expression induced by lipopolysaccharide in PBMCs from GC-resistant patients.

Other Possible Strategies

Although the debate continues, the role of vitamin D in asthma pathogenesis and steroid responsiveness has recently gained much interest.[102] As described earlier, vitamin D deficiency has been associated with reduced lung function and lack of steroid effectiveness in vitro.[67,68] In addition, vitamin D has been shown to be effective at restoring GC responsiveness in in vitro models.[69] Clinical trials are currently underway to determine whether vitamin adjunct therapy can restore clinical GC sensitivity and asthma severity.[103,104]

It was reported that phosphorylation of the GR at Ser226 reduces GR nuclear translocation, resulting in GC insensitivity in patients with severe asthma.[105] Thus, other possible strategies could include modulating GR activity by using protein phosphatase activators. A recent study identified a protein kinase dual-specificity phosphatase involved in the regulation of corticosteroid sensitivity. In addition, the authors showed that formoterol restores impaired GC

sensitivity in an in vitro model, suggesting that this phosphatase might be a novel therapeutic target in severe asthma. Moreover, it was shown that inhibition of miR-9 increased both phosphatase 2A activity and GR nuclear translocation in macrophages and restored the GC sensitivity in multiple models of GC-resistant airway hyperresponsiveness.[106]

Finally, other possible therapies include: inhibitors of P-glycoprotein, antioxidants to revert oxidative stress effects, inhibition of pro-inflammatory cytokines (eg, interferon-γ, TNF-α, transforming growth factor-β, IL-17A, IL-27, IL-33), and kinase (eg, JNF, ERK, glycogen synthase kinase-3β) inhibitors.[107]

CONCLUSIONS

GCs are very effective agents available for the treatment of inflammatory diseases such as asthma. In clinical practice, a number of patients exhibit a poor or absent response even to high doses of GCs, and GC insensitivity in patients with severe disease remains a major clinical problem. Stress profoundly affects the course of airway inflammation. In asthma, evidence suggests that chronic exposure to negative stress induces inflammatory changes, increasing asthma exacerbations and contributing to a worse GC response.

Patients with GC resistance can be treated with other broad-spectrum anti-inflammatory drugs, but in general these drugs have major side effects. Different molecular mechanisms of GC resistance have been described that might be useful in the development of new therapeutic strategies for reversing GC resistance or to sensitize resistant disease to the anti-inflammatory effects of GCs. In this sense, it is important to detect specific biomarkers to help identify patients who are likely to benefit from new therapies. Novel drugs, including highly potent GCs, PI3Kδ inhibitors to resetting of HDAC-2 function, decrease of GR phosphorylation by p38 MAPK inhibitors, or phosphatase activators, are already in clinical development and therefore might be combined with corticosteroid therapy in the future. In addition, miRs (small noncoding RNA molecules) operate as posttranscriptional regulators providing another level of fine adjustment of GR levels. The experimental findings allow postulating that detection and study of miRNAs seem to be a promising approach to better characterize and treat patients with asthma.

Finally, taking into account that stress is an important external factor that increases asthma exacerbations and contributes to asthma morbidity and mortality, the adequate management of stress could be an important and positive intervention.

CONFLICTS OF INTEREST

The authors have indicated that they have no conflicts of interest regarding the content of this article.

ACKNOWLEDGMENTS

This work was supported by grants to Dr. Genaro from the National Research Council of Argentina (CONICET, PIP 00163) and from the National Agency for Science and Technology (ANPCYT, PICT 2016-2727).

Dr. Palumbo was responsible for visualization and writing/review and editing; Mr. Prochnik and Dr. Wald were responsible for writing/review and editing; and Dr. Genaro was responsible for conceptualization and writing/review and editing.

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