

Inflammation Induces Glucocorticoid Resistance in Patients with Bronchial Asthma

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Abstract: Glucocorticoids (GCs) represent the cornerstone of treatment of patients with bronchial asthma; however, inflammation in bronchial asthma is sometimes incompletely controlled. GCs switch on the expression of anti-inflammatory genes by binding to DNA and recruiting transcriptional coactivator molecules. In contrast, they can switch off activated inflammatory genes by recruiting transcriptional repressor molecules such as histone deacetylase (HDAC) 2.

Proinflammatory transcriptional element activator protein-1 (AP-1) and transcription factor nuclear factor kappa B (NF- κ B), and upstream kinase p38 and c-Jun-N-terminal kinase (JNK) amplify inflammation and resistance to the actions of GCs. The activity of histoneacetyltransferase (HAT) and HDAC influences the expression of inflammatory genes. Cytokines, inflammatory mediators, allergens, viral or bacterial infections, oxidative stress, smoking, and vitamin D deficiency may all lead to a worsened clinical outcome by influencing these pathways.

Conventional therapy acts by inhibiting NF- κ B, enhancing glucocorticoid receptor (GR) functions, and restoring HDAC activity, resulting in helpful add-on therapy. Targeting kinases such as inhibitor of κ B kinase (IKK)2, mitogen activated protein (MAP) kinase (MAPK)s and phospho-inositol (PI)3 kinase (PI-3K) should be effective as therapy. Decoy oligonucleotides for AP-1 and NF- κ B are also candidates for the treatment of glucocorticoid-resistant (GC-R) asthma.

Since various factors affect GC response, the pathogenesis of GC-R asthma is considered to be heterogeneous. Most GC nonresponsiveness in these patients is relative and not absolute, suggesting that resistance is dependent on the intensity of localized inflammation. A better understanding of the inflammatory mechanisms of asthma may signal the management of GC-R asthma.

Keywords: Glucocorticoid (GC), glucocorticoid-resistant (GC-R) asthma, glucocorticoid receptor (GR), MAP kinase (MAPK), c-Jun-N-terminal kinase (JNK), activator protein-1 (AP-1), nuclear factor kappa B (NF- κ B), histone deacetylase (HDAC)

INTRODUCTION

GCs are the most effective anti-inflammatory therapy for bronchial asthma and are widely used via inhaled, oral, and intravenous routes. Steroids have various roles in bronchial asthma. Inhibition of the recruitment of inflammatory cells to the airways and lungs, inactivation of inflammatory cells, reduction of vascular permeability, inhibition of airway secretion, inhibition of airway hypersensitivity, enhancement of β 2-adrenergic receptor gene transcription, inhibition of arachidonate metabolism, and inhibition of leukotrienes (LTs) and prostaglandins have been demonstrated. Control of the synthesis of cytokines has attracted attention. GCs downregulate the m-RNA of various inflammatory cytokines and chemokines and upregulate the m-RNA of molecules that negatively control inflammatory cytokines.

A small proportion of asthmatic patients have persistent symptoms and poor control despite receiving GC therapy. Corticosteroid-insensitive asthma is found in about 5% of asthmatic patients, but resistance is very rare and affects less than 0.1% of patients [1]. Many processes involved in in-

flammation escape modulation by GCs, and resistance to the anti-inflammatory effects of GC is mediated by several mechanisms [1-3].

Before diagnosing a patient with GC-R asthma, several underlying clinical conditions should be taken into account, since other diseases may masquerade as GC-R asthma and co-administration of certain drugs, such as rifampicin, phenytoin, and phenobarbital, may reduce steroid availability by affecting steroid metabolism through CYP3A4.

Although the evaluation criteria of GC-R asthma may differ from trial to trial, most clinical manifestations of GC-R asthma largely emphasize the definition of persistence of airway obstruction and failure of FEV₁ to improve by 15% of baseline after 10 to 14 days of high-dose oral corticosteroids, typically 40 mg prednisolone daily, when evaluated mainly by reversibility of airflow obstruction [4]. However, when considering the current widespread use of inhaled GC, a definition referring to the inhalation route should also be taken into account [5].

There are various underlying clinical manifestations in GC-R asthma, and considering respiratory function is not always presumed to represent the GC-R status, so here, GC-R asthma is discussed not only according to traditional strict criteria but also according to the wide concept that includes clinical failure to demonstrate a satisfactory response to ster-

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oid therapy. This paper is an overview of factors associated with GC-R asthma from the view of inflammation and is structured as the following sections: introduction, anti-inflammatory action of GCs, protein kinase pathways in GC resistance, roles of transcription factors in GC resistance, profile of cytokine expression: pathogenesis of both Th1 and Th2 inflammation, environmental and other factors, the process of airway remodeling, biomarkers for GC-R asthma, available agents to improve steroid response and new therapeutic agents, and conclusion.

ANTI-INFLAMMATORY ACTION OF GCs

GCs act by binding to GR. The GR is located in the cytoplasm and in complexes containing heat shock proteins. Upon binding to its ligand, the GR undergoes dissociation from heat shock proteins, homodimerizes and translocates to the nucleus. It binds to GC response elements (GREs) in the promoter region of steroid-sensitive genes and also directly or indirectly to coactivator molecules such as cyclic adenosine monophosphate response element binding protein (CREB)-binding protein (CBP), p300/CBP activating factor (pCAF) and glucocorticoid receptor interacting protein-1 (GRIP-1), which have intrinsic HAT activity. Acetylation of lysines on histone H4 is followed by activation of genes encoding anti-inflammatory proteins, such as secretory leuko-protease inhibitor (SLPI), MAPK phosphatase 1 (MKP-1), inhibitor of nuclear factor- κ B (I κ B)- α , and glucocorticoid-induced leucine zipper protein (GILZ). These cause inhibition of p38 MAP kinase, AP-1, and NF- κ B.

GC-mediated suppression of inflammation mostly occurs without binding to DNA, by inhibiting the ability of transcription factors such as NF- κ B and AP-1 activated by cytokines to induce proinflammatory gene transcription. A dimer of p50 and p65 NF- κ B proteins translocates to the nucleus and binds to specific κ B recognition sites and to coactivators, such as CBP or pCAF, which have intrinsic HAT activity and are able to recruit other HAT enzymes. Following the acetylation of lysines of core histone H4, the chromatin structure transforms to an activated open form. This allows binding of TATA box-binding protein (TBP) and polymerase RNAII and initiation of transcription of genes encoding multiple inflammatory proteins. Repression of genes is conversely associated with a reversal of this process by histone deacetylation, mediated by HDACs and other corepressors [1].

GCs' action to suppress inflammatory cytokine production is a combination of direct inhibition of HAT activity and recruitment of HDAC activity to the activated transcriptional complex [6]. In asthmatic subjects there is an increase in HAT activity and a reduction in HDAC activity [7-9].

Anti-inflammatory gene activation is unlikely to explain the anti-inflammatory actions of GCs, since high concentrations of GCs are required to increase the transcription of anti-inflammatory genes. Switching off inflammatory genes through interaction with transcription factors may be the major effect of corticosteroids.

GR is a ligand-regulated transcription factor, widely distributed in the airways and expressed on inflammatory and structural cells, such as endothelial and epithelial cells,

smooth muscle cells, fibroblasts, eosinophils, T lymphocytes, dendritic cells, and macrophages [10].

GR belongs to the family of intracellular ligand-inducible transcription factors termed the steroid/vitamin D/retinoic acid superfamily. The essential structural and functional features are an amino-terminal transactivation domain, a central zinc-finger DNA binding domain, and a carboxyl-terminal ligand binding domain. GR phosphorylation is associated with modulation of ligand binding, nuclear translocation, DNA binding, receptor dimerization, and interaction with general transcription factors.

GR β has been implicated in GC-R asthma [11-14]. GR β is an alternatively spliced form that binds to DNA but cannot be activated by GC, and has been reported to antagonize the transactivating activity of GR α .

Whether GR β has a physiologic role in modulating steroid responsiveness in GC-R asthma is controversial [15,16], since its level of expression is very low compared to that of GR α (typically 10- to 100 fold less, at least at the level of mRNA expression).

Based on recent accumulated evidence, new actions of GR β have been revealed. GR β can widely regulate gene expression and the action of the receptor is modulated by the GR antagonist RU-486 in the U-2 OS cell line [17], GR β is able to act as a transcriptional repressor of cytokine genes and mediates its function through the recruitment of HDAC complex. GR α and GR β act in a similar manner on interleukin (IL)-5 and IL-13 promoters, serving to repress transcription [18]. GR β has intrinsic, GR α -independent transcriptional activity [19]. Taking these observations together, if GR β contributes to GC-R asthma, GR β might have a more important role, other than acting as a dominant-negative inhibitor of GR α .

PROTEIN KINASE PATHWAYS IN GC RESISTANCE

Kinase pathways are essential in the expression and activation of inflammatory mediators and in immune cell function. MAPK, JNKs, IKK, and PI-3Ks regulate inflammation either through activation of proinflammatory transcription factors such as AP-1 and NF- κ B, or through regulation of mRNA half-life. Janus kinases (JAK)s, signal transducer and activator of transcription (STAT)s pathways also play a role in inflammation [20].

MAPKs are a family of Ser/Thr kinases that transduce extracellular signals to the nucleus. Three major groups of MAPK, extracellularly regulated kinase (ERK), JNK and p38, are known. MAPKs receive signals from a diverse range of extracellular stimuli such as stress and mitogens, thereby controlling the cellular response to environmental changes. The synthesis of many inflammatory mediators is regulated through activation of p38 MAPK. mRNAs encoding tumor necrosis factor (TNF)- α , IL-1 β , IL-6, IL-8, granulocyte-macrophage colony stimulating factor (GM-CSF) and cyclo-oxygenase (COX)-2 share common AU-rich elements (ARE) which make the mRNA unstable with rapid degradation. ARE-binding proteins (AREBP) are responsible for stabilization of those mRNAs. Activation of p38 MAP kinase results in activation of AREBP, so that synthesis of

inflammatory proteins is increased. Changes in the binding affinity of nuclear GR induced by exposure to IL-2/IL-4 may be caused by phosphorylation of GR at serine 226 secondary to the resulting activation of p38 MAP kinase [21]. The combination of IL-2 and IL-4 inhibits GR nuclear translocation in human T cells, and this effect is reversed by interferon (IFN)- γ via inhibition of p38 MAPK activation [22]. TNF reciprocally inhibits glucocorticoid actions, through suppression of GR function by activation of p38 [23-24].

JNK represents a subgroup of the MAPK family that is activated primarily by cytokines and environmental stresses such as osmotic or redox stress and UV radiation. JNK is required for the regulation of inflammatory genes including those of cytokines, growth factors, cell surface receptors, cell adhesion molecules and proteases such as matrix metalloproteinase (MMP)-1. JNKs are phosphorylated and activated by MAPK kinases, which are activated by multiple upstream MAPKK kinases including the mixed lineage kinases (MLKs). JNK and ERK inhibited GR-mediated transcriptional activation, which could be attributable to GR phosphorylation at Ser-246 by JNK but not ERK [25-27]. Cross talk of JNK signaling pathways with GR has been observed. GR may bind directly to the active AP-1 transcriptional complex, regulate JNK through MKP-1, prevent association of JNK with its upstream activators MAP kinase kinase (MKK)4 and MKK 7 by binding cytoplasmic JNK, and form inactive JNK which may compete with active JNK for binding to c-Jun in the nucleus [28,29].

GCs not only induce the MKP-1 gene, but also reduce its degradation [30,31]. MKP-1 inhibits MAPK pathways and therefore inhibits JNK and to a lesser extent ERK. One of the effects of such inhibition is reduced stability of AU-rich element-containing mRNA, which activates inflammation.

PI3Ks play a prominent role in fundamental cellular responses of various inflammatory cells, including proliferation, differentiation, and cell migration. PI3Ks might be involved in allergic airway inflammation, airway hyperresponsiveness (AHR), and airway remodeling by regulating the challenge/effector phase of allergic response [32]. PI3K may be involved in corticosteroid sensitivity through reducing HDAC activity [33].

JAKs are a class of tyrosine kinases that associate with cytokine receptors. Upon ligand binding, they activate members of the STAT family. JAK-STAT signaling controls pulmonary inflammation and AHR. Most of such studies have focused on STAT6, given its involvement in directing Th2 responses and IgE production [34].

Thus, GCs exert their anti-inflammatory effect through inhibiting kinase pathways. On the other hand, GC resistance is associated with increased activation of protein kinases, which might attenuate GR function or reduce HDAC activity. This may explain why GC resistance is dependent on the intensity of inflammation.

ROLES OF TRANSCRIPTION FACTORS IN GC RESISTANCE

Transcription factors such as AP-1, NF- κ B, STAT, nuclear factor of activated T cell (NF-AT), GATA, and GR modulate inflammatory genes.

The proinflammatory transcriptional element AP-1 is comprised of variable heterodimers of Jun (c-Jun, JunB and JunD) and Fos (c-Fos, FosB, Fra1 and Fra2) family members. It is activated through the phosphorylation of c-Jun and the transcriptional regulation of c-Fos. Phosphorylated JNK phosphorylates and activates c-jun. AP-1 increases the transcription of a number of asthma-relevant cytokine genes.

Th2 cytokines can enhance AP-1 expression, which is enhanced in the asthmatic airway [35]. Mononuclear cells of GC-R patients show defective inhibition of AP-1 in response to GCs [36]. Failure to suppress JNK phosphorylation leading to failure to suppress c-Jun phosphorylation, leading to dysregulation of AP-1 in GC-R asthma has been suggested in skin biopsy specimens from a tuberculin-induced model [37] and bronchial biopsy sections [38].

The transcription factor NF- κ B [39] consists of heterodimers and homodimers of related proteins belonging to the Rel family of transcription factors, which consists of five family members: p65 (Rel A), Rel B, c-Rel, NF- κ B1 (consisting of p50 and its precursor p105), and NF- κ B2 (p52 and its precursor p100). NF- κ B regulates the genes encoding intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E selectin, GM-CSF, TNF- α , IL-6, and chemokines belonging to the C-C and C-X-C families. NF- κ B is also involved in IL-4 signaling and CD40 signaling, which are important for the IgE production process. NF- κ B is activated by various stimulation such as TNF- α , IL-1 β , IL-2, LTB4, allergens, mitogens, lipopolysaccharide (LPS), viral infections, oxidative stress, and exposure to reactive oxygen. In resting cells, NF- κ B is retained in the cytoplasm, complexed to an inhibitor protein from the I κ B family. Stimulation activates the IKK complex, which then phosphorylates I κ B. This allows NF- κ B to translocate into the nucleus where it can bind to κ B sequences in the promoters of NF- κ B-dependent genes to up-regulate transcription.

GCs may inhibit the action of NF- κ B by increasing I κ B production in certain cell lines [40,41], by inhibiting upstream kinase pathways or by reversing histone acetylation at the site of inflammatory gene transcription, either by direct binding of activated GR to NF- κ B-associated co-activators or by recruitment of HDACs to the activated transcription complex.

NF- κ B is closely related to the pathogenesis of asthma. In patients with bronchial asthma, NF- κ B activity is increased in airway epithelial cells, submucosal cells, and macrophages from sputum [42-44]. Increased levels of activated p65, phosphorylated I κ B α (p-I κ B α), and IKK β are found in peripheral blood mononuclear cell (PBMC) of subjects with severe uncontrolled asthma [45]. The excess of active NF- κ B in severe uncontrolled asthma may impair the antiinflammatory action of GCs.

STAT plays an important role in asthma. Functional interactions have been described between STAT family members and GR or its cofactors [46]. At present, it remains unclear how these interactions influence GC resistance in asthma.

Corticosteroids inhibit GATA-3 function through a rapid inhibitory effect on GATA-3 nuclear translocation by preferential binding to the shared nuclear import protein importin-

α and also by inhibiting p38 MAP kinase through induction of MAP kinase phosphatase-1 [47].

Interaction of CCAAT/enhancer binding protein α (C/EBP α) with GR by complex formation may mediate important physiological actions of GCs. There is accumulating evidence that a decrease in the levels of C/EBP α is responsible for inflammation in the airway smooth muscle in asthmatics. The loss of C/EBP α in airway smooth-muscle cells of asthmatics may be critical for the loss of GR function since, in these cells, GR forms a critical complex with C/EBP α that enables the induction of key anti-inflammatory mediators [48].

Recent investigations suggest the participation of interferon regulatory factor-1 (IRF-1) in steroid resistance. IRFs mediate infection-induced signaling pathways and as such play a critical role in antiviral defense, immune response, cell growth regulation and apoptosis. IFN- γ and IRF-1 affect the Th1/Th2 cytokine balance, and influence the differentiation of Th2 cells, which influence the development of asthma. Expression of IRF-1 was increased after viral infections [49]. This may explain the reduced steroid responsiveness seen in patients with asthma experiencing viral infections [50,51]. IRF-1 interferes with steroid signaling in airway smooth muscle cells. IRF-1 is critical not only for regulation of the transcriptional induction of CD38 which plays a role in bronchial hyperresponsiveness and airway inflammation, but is also responsible for cytokine-induced steroid resistance in part via suppression of GR activities [52].

Multiple signals mediate activation or inhibition of transcription factors. Signals may be amplified or altered by various conditions before reaching transcription factors. Transcription factors may physically interact with each other and modify GC action [53]. These complicated pathways are underlying factors in inflammation and GC-R asthma.

PROFILE OF CYTOKINE EXPRESSION: PATHOGENESIS OF BOTH TH1 AND TH2 INFLAMMATION

The profile of cytokine expression might underlie poor responsiveness to GCs. In patients with GC-R asthma, a combination of increased IL-2 and IL-4 reduced GR binding affinity in PBMC, and the effects were reversible and blocked by IFN- γ [54,55]. Study of bronchoalveolar lavage (BAL) fluid showed significantly greater numbers of cells expressing IL-2 and IL-4 mRNA in GC-R asthmatics as compared with sensitive asthmatics [56]. Bronchial biopsy specimens from patients with GC-R asthma revealed overexpression of IL-2, IL-4, and IL-13 and a reduction in affinity of GRs in inflammatory cells [57,58].

GCs attenuate airway eosinophilia by inducing eosinophil apoptosis and inhibiting the response to IL-5 and GM-CSF survival signals [59,60]. On the other hand, non-eosinophilic asthma is associated with a poor response to GC [61,62]. Recently, in a mouse model, involvement of Th17 cells in GC-R asthma was reported [63]. Th17 cells represent a distinct population of CD4(+) Th cells that mediate neutrophilic inflammation and are characterized by the production of IL-17, IL-22, and IL-6. Th17 cytokine responses are not sensitive to dexamethasone (DEX) treatment. Th17 cell-mediated airway inflammation and AHR are steroid resis-

tant, indicating a potential role of Th17 cells in GC-R asthma.

The anti-inflammatory cytokine IL-10 inhibits pro-inflammatory cytokine production, antigen presentation, T cell activation and mast cell and eosinophil function. CD4+ T cells from GC-R asthmatics show a marked deficiency in their capacity to synthesize IL-10 following *in vitro* stimulation in the presence of DEX, as compared with those from GC-sensitive patients with similar disease severity [64].

A Th2-mediated disease process is undoubtedly important in many patients with asthma, but even this concept has some problems. For example, Th1 cells and cytokines have also been shown to play a critical role in AHR. Increased Th2 cells in the airways of mice mediate eosinophilic inflammation and AHR that can be suppressed by treatment with GCs. Th1 cells, on the other hand, induce steroid-resistant AHR through an INF- γ /TLR4-MyD88-dependent mechanism after priming of the innate host defense system by LPS [65].

Cytokines induce immune activation, which leads to reduced GR binding affinity. GC-R asthma is associated with alterations in Th2/Th1 type cytokine gene expression profiles; that is, failure to repress the production of inflammatory cytokines and to induce the production of anti-inflammatory cytokines.

ENVIRONMENTAL AND OTHER FACTORS

Factors that induce asthma attacks or exacerbate asthma, such as allergens, viral and bacterial infections, and oxidative stress, attenuate GR function or activate transcription factors through kinase pathways, and some of them also influence HDAC2 activity.

A reduction in GR binding affinity was observed in ragweed-allergic asthmatics during ragweed pollen season. *In vitro*, GR binding in PBMC from atopic asthmatics, incubated with antigens derived from ragweed or cat, but not *Candida albicans*, was significantly reduced [66] suggesting that the reduction was allergen specific.

Rhinovirus infection is the one of the main causes of asthma exacerbations, provoking steroid refractory and abnormally intense neutrophilic inflammation. Interestingly, patients with asthma do not always have more frequent infections, but rather more intense reactions. Recently, interplay of the epidermal growth factor receptor (EGFR) with MMP and ERK signaling [67] and primary or acquired IFN α and λ deficiency [68,69] has been reported as an explanation. Rhinovirus infection induces activation of NF- κ B, which leads to cytokine production and expression of adhesion molecules [70-72]. A study of BAL fluid from subjects with GC-R asthma demonstrated classical macrophage activation and induction of LPS signaling pathways, suggesting a contribution of endotoxin exposure to GC resistance [73]. Two distinct pathologic, physiologic, and clinical subtypes of severe asthma, based on the presence or absence of eosinophils, have been reported. Neutrophils are increased in both groups [74]. However, neutrophilic inflammation in acute exacerbations of asthma tends to be resistant to treatment with GCs, by causing impaired nuclear recruitment of HDAC2 [9].

External influences such as exposure to cigarette smoke, an oxidative stress, inhibit the anti-inflammatory actions of GC by reducing HDAC expression and activity [75]. Clinically, patients with bronchial asthma who smoke have an impaired response to GC therapy compared with those who do not smoke [76]. Smoking increases NF- κ B activity, resulting in increased expression of inflammatory genes such as IL-8, MMP and MCP. Smoking can also inhibit GR function by suppression of GR-associated HDAC2 activity and expression [77].

There has been growing interest in the role of vitamin D. A higher vitamin D intake by pregnant mothers reduces asthma risk by as much as 40% in children 3 to 5 years old [78,79]. Local conversion of inactive to active vitamin D alters immune function in the lung [80]. Addition of vitamin D3 and DEX to regulatory T (Treg) cells enhances IL-10 secretion, and administration of vitamin D3 to patients with GC-R asthma enhanced the subsequent responsiveness of induction of IL-10 to DEX [81]. Vitamin D also modulates Treg function and IL-10 production, which may increase the therapeutic response to GC in GC-R asthma [82]. Thus, improving the vitamin D status holds promise for the primary prevention of asthma, decreasing exacerbations of the disease, and treating steroid resistance.

These observations together suggest that GC-R asthma may be in part an acquired heterogeneous disease, and explain the clinical manifestation that GC resistance is relative and not absolute.

THE PROCESS OF AIRWAY REMODELING

In asthma, lymphocytes and eosinophils constitute most of the inflammatory cells infiltrating the bronchial mucosa [83]. Patients with GC-R asthma have persistent airway inflammation despite treatment with steroids, and therefore could be predisposed to increased airway remodeling and irreversible lung disease. Airway remodeling in asthma refers to structural changes in the airways, including subepithelial fibrosis, smooth muscle hypertrophy, and blood vessel hyperplasia.

Reticular basement membrane (RBM) thickening is considered a hallmark for airway remodeling in airway diseases [84]. Abnormal epithelial and lamina reticularis (LR) thickening is observed in subjects with severe asthma. An abnormal proliferative process mediated by the Bcl-2 family of genes may play a role in the balance of proliferative and death responses. This response in the airway may result in epithelial hyperplasia and thickening that appears uncontrolled despite treatment with a high dose of GC [85].

Imbalance between a remodeling-associated mediator, MMP-9, which is capable of degrading extracellular matrix (ECM) components, and tissue inhibitor of metalloproteinases (TIMP)-1, which inhibits MMP-9, is observed in asthma. An excess of MMPs may be responsible for structural degradation of tissues, whereas an excess of TIMPs may promote excessive tissue repair processes and fibrosis. In patients with severe asthma, there is a positive correlation between improvement in FEV₁ with GC treatment and serum MMP-9/TIMP-1 ratio [86,87]. DEX upregulates TIMP-1 mRNA in BAL fluid cells from patients with GC-sensitive

asthma, but not in cells from GC-R asthma patients. Inability of GC to enhance TIMP-1 production causes a shift in the MMP-9/TIMP-1 ratio in GC-R asthma, potentially promoting proteolytic activity in the airways and contributing to chronic airway remodeling [88]. Thus, the MMP/TIMP ratio may be a promising candidate for a prognostic indicator in GC-R asthma.

BIOMARKERS FOR GC-R ASTHMA

Studies have tried to identify the clinical phenotype of asthma [89-91]. To accurately distinguish between GC-sensitive asthma and GC-R asthma patients, a search for biomarkers based on mRNA expression profiles in PBMC has been attempted. It is compelling that the gene that confers the best prediction by the RT-PCR method is the gene encoding the NF- κ B DNA binding subunit. NF- κ B is an exciting candidate as one of the key culprit genes responsible for GC-R asthma. STAT-4 and IL-4R were also good predictors (5). Expression of FK506-binding protein 51 (FKBP51), which is an immunophilin chaperone protein, a subunit of the multiprotein GR "aporeceptor" complex that resides in the cytoplasm before hormone binding, might affect the clinical responsiveness to corticosteroids in asthma, and may be a biomarker of corticosteroid responsiveness [92].

AVAILABLE AGENTS TO IMPROVE STEROID RESPONSE AND NEW THERAPEUTIC AGENTS

Various antiasthmatic agents have been reported to affect NF- κ B, enhance GR functions and enhance GR-associated histone deacetylase activity. These have been shown to be effective as add-on therapies.

High doses of inhaled short-acting β 2-adrenoceptor agonists (SABA), salbutamol and fenoterol, have been reported to induce steroid resistance by activating CREB, which interacts with GR, resulting in reduced ability of GR to bind DNA [93]. This may explain the adverse influence of high doses of β 2-agonists on morbidity and mortality in asthma [94]. β 2-adrenoceptor agonists are potent activators of GR [95]. Inhaled long-acting β 2-adrenoceptor agonists (LABA) may modify GR nuclear localization through modulation of GR phosphorylation and furthermore through priming of GR functions within the nucleus by modifying GR or GR-associated protein phosphorylation [96]. The combination of inhaled steroid and LABA is widely used treatment today. The interaction of formoterol and budesonide results in synchronization of the activity of the glucocorticoid receptor and C/EBP α , which in turn achieves an optimum antiproliferative action on smooth muscle cells [97]. Fluticasone and salmeterol synergistically decreased p-I κ B expression in asthmatic T cells, limiting NF- κ B activation [98]. The same combination can enhance GR nuclear translocation *in vivo*, as well as *in vitro* [99]. Salmeterol and formoterol enhance glucocorticoid-induced simple GRE-dependent transcription via the cAMP-dependent protein kinase (PKA) pathway in human bronchial epithelial cells [100]. Clinically, the addition of LABA to an inhaled steroid markedly improved lung function and asthma control compared to an increased dose of inhaled steroid, which could be explained by the en-

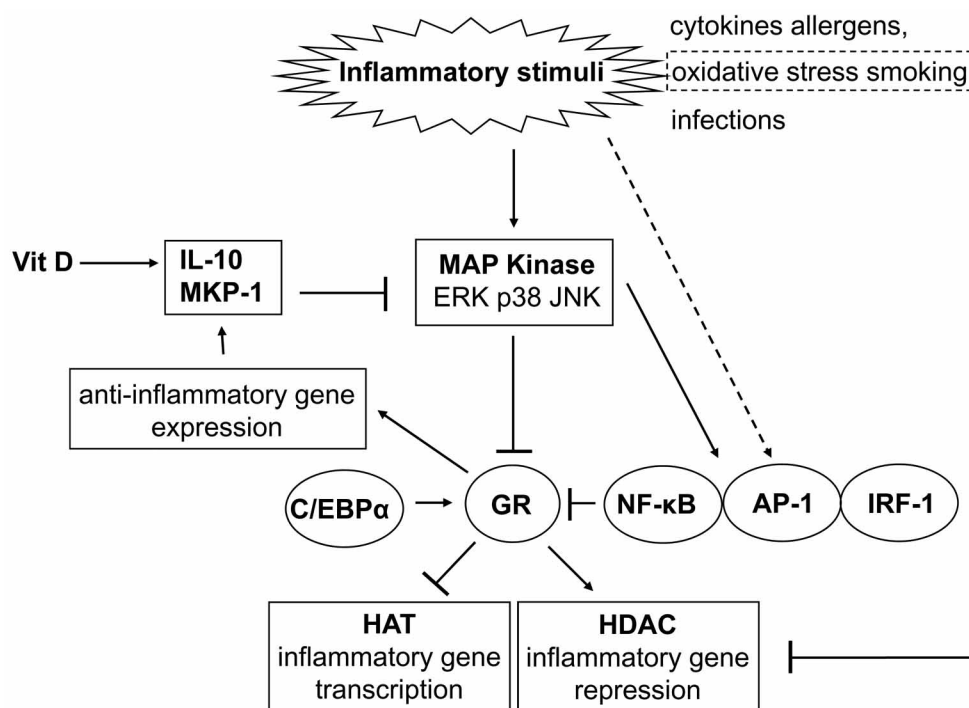


Fig. (1). Extracellular and intracellular factors and pathways of GC-R asthma. GC acts through switching on the expression of anti-inflammatory genes such as MKP-1 or switching off inflammatory genes through recruitment of co-repressor proteins and repression of the activity of pro-inflammatory transcription factors such as NF- κ B and AP-1. Inflammatory stimulation provokes activation of protein kinase pathways and transcription factors, resulting in attenuation of GR function and reduction of HDAC activity or recruitment.

hancement of GR function by LABA [101,102]. Thus, LABAs act as steroid-sparing agents.

Theophylline suppresses the production of pro-inflammatory cytokines *via* inhibition of NF- κ B activation through preservation of the I κ B α protein in monocytes/macrophages and T cells [103-105]. Its effect may be mediated through attenuating transport of NF- κ B into nuclei, as well as DEX [106]. A low concentration of theophylline enhances GC actions, by increasing the recruitment of HDACs by GR, with increased activity [107], resulting in improved anti-inflammatory actions.

The effect of a cysteinyl leukotriene receptor 1 (cysLTR1) agonist on activation of NF- κ B has been reported. Independently of cysLTR1 antagonism, micromolar concentrations of the leukotriene 1 receptor antagonist, pranlukast, suppress the production of proinflammatory cytokines *via* inhibition of NF- κ B activation in monocytes/macrophages and T cells [108]. Pranlukast attenuates allergen-induced TNF- α production by peripheral blood monocytes from atopic asthmatics concomitant with downregulation of NF- κ B, which is a distinct pathway from cysLTR1 antagonism [109]. LTD4 upregulates MUC2 gene transcription *via* a signaling pathway involving cysLTR1, G-protein, protein kinase C (PKC), mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK), ERK and NF- κ B [110]. MUC2 is one of the mucin genes. Mucins protect the epithelial surface by binding and trapping inhaled bacteria and viruses. In asthma, excessive mucus production is seen. A CysLTR1 agonist inhibits NF- κ B activation and MUC2 mucin gene expression in epithelial cells [111]. These effects may partly explain the additive effect of pranlukast with GC

[112,113]. Montelukast inhibits TNF- α -stimulated IL-8 expression through changes in NF- κ B p65-associated HAT activity [114], demonstrating an anti-inflammatory effect. By attenuating inflammation, these drugs may potentiate steroid therapy.

Targeting kinases that are overexpressed or overactive in GC-R asthma should have therapeutic effects. Selective inhibitors of these kinase pathways can modulate the expression of numerous inflammatory mediators and adhesion molecules, and control T-cell, macrophage and epithelial cell function [115,116]. Inhibitors of p38 MAP kinase may prove to be useful novel therapies in the treatment of severe asthma, because they are capable of reducing both the synthesis of pro-inflammatory cytokines and their signaling [117,118]. It is most likely that PI3K inhibitors will be more efficacious in GC-R asthma where GCs are of limited effectiveness and no alternative therapy is available [33]. Therapeutic inhibition of PI3K δ may restore GC function in oxidative stress-induced GC insensitivity [119].

Some of the transcription factors are candidates for new targets for therapy [120] Decoy oligonucleotides for AP-1 [121] and NF- κ B [122] are also candidates for the treatment of GC-R asthma. Suppression of beneficial host responses is sometimes a dilemma when targeting NF- κ B for asthma therapy, since IFNs, which are crucial for the host defense against viral infections, are induced by viruses in an NF- κ B-dependent manner. NF- κ B plays a central role in tissue and organ homeostasis, and its long-term general suppression or overexpression would cause severe side effects. It is difficult to draw conclusions [123].

The concept that NF- κ B is pro-inflammatory has been accepted, and has focused mainly on the p50:p65 dimer. Anti-inflammatory actions of different subunits of NF- κ B, NF κ B1 (p50 and its precursor p105), are emerging. Enhancing the protective effects of (p50)₂ may allow the development of novel therapeutics for the future treatment of inflammatory diseases [124].

CONCLUSION

The details of the genetic mechanism in GC-R asthma are not yet certain. The GR gene may be a candidate for a genetic basis to GC resistance. Generalized GC resistance sometimes derives from mutations in GR [125,126], whereas GC resistance in asthma appears to be localized primarily in the lungs and certain circulating cells [127]. In most cases but not all, excessive inflammation driven by various factors might produce this tissue resistance to GC. Various factors induce GC resistance, some derive from a Th2 independent [128] or non-eosinophilic process, possibly meaning that the pathogenesis of GC-R asthma is not simply Th2-mediated, but a heterogeneous disease.

The intracellular mechanism of failure to respond well to steroid therapy in asthmatics may mostly result from reduced GR function by enhanced activation of AP-1 and NF- κ B and upstream kinase pathways, or reduced HDAC activity (Fig. 1). The intensity of inflammation may explain the most frequent clinical status that the resistance is relative, and patients often respond to high doses of GCs. Current drugs are sometimes useful to potentiate the GC response through their anti-inflammatory effects, not by their original effect. Novel therapeutic agents are also now targeting inflammatory pathways.

A better understanding of the inflammatory mechanisms of asthma may hasten the advent of new diagnostic tests and effective therapy for GC-R asthma.

REFERENCES

- [1] Barnes, P.J. Corticosteroids: the drugs to beat. *Eur. J. Pharmacol.*, **2006**, *533*, 2-14.
- [2] Ito, K.; Chung, K.F.; Adcock, I.M. Update on glucocorticoid action and resistance. *J. Allergy Clin. Immunol.*, **2006**, *117*, 522-543.
- [3] Adcock, I.M.; Barnes, P.J. Molecular mechanisms of corticosteroid resistance. *Chest*, **2008**, *134*, 394-401.
- [4] Woolcock, A.J. Corticosteroid-resistant asthma: definitions. *Am. J. Respir. Crit. Care Med.*, **1996**, *154*, 45-48.
- [5] Hakonarson, H.; Bjornsdottir, U.S.; Halapi, E.; Bradfield, J.; Zink, F.; Mouy, M.; Helgadóttir, H.; Gudmundsdóttir, A.S.; Andreason, H.; Adalsteinsdóttir, A.E.; Kristjánsson, K.; Birkiðsson, I.; Arnason, T.; Andresdóttir, M.; Gislason, D.; Gislason, T.; Gulcher, J.R.; Stefánsson, K. Profiling of genes expressed in peripheral blood mononuclear cells predicts glucocorticoid sensitivity in asthma patients. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*, 14789-14794.
- [6] Ito, K.; Barnes, P.J.; Adcock, I.M. Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1 β -induced histone H4 acetylation on lysines 8 and 12. *Mol. Cell Biol.*, **2000**, *20*, 6891-6903.
- [7] Cosío, B.G.; Mann, B.; Ito, K.; Jazrawi, E.; Barnes, P.J.; Chung, K.F.; Adcock, I.M. Histone acetylase and deacetylase activity in alveolar macrophages and blood monocytes in asthma. *Am. J. Respir. Crit. Care Med.*, **2004**, *170*, 141-147.
- [8] Bhavsar, P.; Ahmad, T.; Adcock, I.M. The role of histone deacetylases in asthma and allergic diseases. *J. Allergy Clin. Immunol.*, **2008**, *121*, 580-584.
- [9] Ito, K.; Herbert, C.; Siegle, J.S.; Vuppusetty, C.; Hansbro, N.; Thomas, P.S.; Foster, P.S.; Barnes, P.J.; Kumar, R.K. Steroid-resistant neutrophilic inflammation in a mouse model of an acute exacerbation of asthma. *Am. J. Respir. Cell Mol. Biol.*, **2008**, *39*, 543-550.
- [10] Barnes, P.J. Distribution of receptor targets in the lung. *Proc. Am. Thorac. Soc.*, **2004**, *1*, 345-351.
- [11] Leung, D.Y.; Hamid, Q.; Vottero, A.; Szeffler, S.J.; Surs, W.; Minshall, E.; Chrousos, G.P.; Klemm, D.J. Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta. *J. Exp. Med.*, **1997**, *186*, 1567-1574.
- [12] Hamid, Q.A.; Wenzel, S.E.; Hauk, P.J.; Tscicopoulos, A.; Wallaert, B.; Lafitte, J.J.; Chrousos, G.P.; Szeffler, S.J.; Leung, D.Y. Increased glucocorticoid receptor beta in airway cells of glucocorticoid-insensitive asthma. *Am. J. Respir. Crit. Care Med.*, **1999**, *159*, 1600-1604.
- [13] Goleva, E.; Li, L.B.; Eves, P.T.; Strand, M.J.; Martin, R.J.; Leung, D.Y. Increased glucocorticoid receptor beta alters steroid response in glucocorticoid-insensitive asthma. *Am. J. Respir. Crit. Care Med.*, **2006**, *173*, 607-616.
- [14] Tliba, O.; Cidlowski, J.A.; Amrani, Y. CD38 expression is insensitive to steroid action in cells treated with tumor necrosis factor-alpha and interferon-gamma by a mechanism involving the up-regulation of the glucocorticoid receptor beta isoform. *Mol. Pharmacol.*, **2006**, *69*, 588-596.
- [15] Gagliardo, R.; Chanez, P.; Vignola, A.M.; Bousquet, J.; Vachier, I.; Godard, P.; Bonsignore, G.; Demoly, P.; Mathieu, M. Glucocorticoid receptor alpha and beta in glucocorticoid dependent asthma. *Am. J. Respir. Crit. Care Med.*, **2000**, *162*, 7-13.
- [16] Torrego, A.; Pujols, L.; Roca-Ferrer, J.; Mullol, J.; Xaubet, A.; Picado, C. Glucocorticoid receptor isoforms alpha and beta in *in vitro* cytokine-induced glucocorticoid insensitivity. *Am. J. Respir. Crit. Care Med.*, **2004**, *170*, 420-425.
- [17] Lewis-Tuffin, L.J.; Jewell, C.M.; Bienstock, R.J.; Collins, J.B.; Cidlowski, J.A. Human glucocorticoid receptor beta binds RU-486 and is transcriptionally active. *Mol. Cell Biol.*, **2007**, *27*, 2266-2282.
- [18] Kelly, A.; Bowen, H.; Jee, Y.K.; Mahfiche, N.; Soh, C.; Lee, T.; Hawrylowicz, C.; Lavender, P. The glucocorticoid receptor beta isoform can mediate transcriptional repression by recruiting histone deacetylases. *J. Allergy Clin. Immunol.*, **2008**, *121*, 203-208.
- [19] Kino, T.; Manoli, I.; Kelkar, S.; Wang, Y.; Su, Y.A.; Chrousos, G.P. Glucocorticoid receptor (GR) beta has intrinsic, GRalpha-independent transcriptional activity. *Biochem. Biophys. Res. Commun.*, **2009**, *381*, 671-675.
- [20] Tatjana, T. Clarissa Gust and Arne v. Bonin kinases as drug targets in inflammation: *In vitro* and *in vivo* target validation and expression profiling. *Anti-Inflamm. Anti-Allergy Agents Med. Chem.*, **2007**, *6*, 19-27.
- [21] Irusen, E.; Matthews, J.G.; Takahashi, A.; Barnes, P.J.; Chung, K.F.; Adcock, I.M. p38 Mitogen-activated protein kinase-induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid-insensitive asthma. *J. Allergy Clin. Immunol.*, **2002**, *109*, 649-657.
- [22] Goleva, E.; Li, L.B.; Leung, D.Y. IFN-gamma reverses IL-2- and IL-4-mediated T-cell steroid resistance. *Am. J. Respir. Cell Mol. Biol.*, **2009**, *40*, 223-230.
- [23] Franchimont, D.; Martens, H.; Hagelstein, M.T.; Louis, E.; Dewe, W.; Chrousos, G.P.; Belaiche, J.; Geenen, V. Tumor necrosis factor alpha decreases, and interleukin-10 increases, the sensitivity of human monocytes to dexamethasone: potential regulation of the glucocorticoid receptor. *J. Clin. Endocrinol. Metab.*, **1999**, *84*, 2834-2839.
- [24] Szatmáry, Z.; Garabedian, M.J.; Vilcek, J. Inhibition of glucocorticoid receptor-mediated transcriptional activation by p38 mitogen-activated protein (MAP) kinase. *J. Biol. Chem.*, **2004**, *279*, 43708-43715.
- [25] Krstic, M.D.; Rogatsky, I.; Yamamoto, K.R.; Garabedian, M.J. Mitogen-activated and cyclin-dependent protein kinases selectively and differentially modulate transcriptional enhancement by the glucocorticoid receptor. *Mol. Cell Biol.*, **1997**, *17*, 3947-3954.
- [26] Rogatsky, I.; Logan, S.K.; Garabedian, M.J. Antagonism of glucocorticoid receptor transcriptional activation by the c-Jun N-terminal kinase. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 2050-2055.
- [27] Itoh, M.; Adachi, M.; Yasui, H.; Takekawa, M.; Tanaka, H.; Imai, K. Nuclear export of glucocorticoid receptor is enhanced by c-Jun N-terminal kinase-mediated phosphorylation. *Mol. Endocrinol.*, **2002**, *16*, 2382-2392.

- [28] Bennett, B.L. c-Jun N-terminal kinase-dependent mechanisms in respiratory disease. *Eur. Respir. J.*, **2006**, *28*, 651-661.
- [29] Davies, L.; Karthikeyan, N.; Lynch, J.T.; Sial, E.A.; Gkourtsa, A.; Demonacos, C.; Krstic-Demonacos, M. Cross talk of signaling pathways in the regulation of the glucocorticoid receptor function. *Mol. Endocrinol.*, **2008**, *22*, 1331-1344.
- [30] Clark, A.R. MAP kinase phosphatase 1: a novel mediator of biological effects of glucocorticoids. *J. Endocrinol.*, **2003**, *178*, 5-12.
- [31] Abraham, S.M.; Lawrence, T.; Kleiman, A.; Warden, P.; Medghalchi, M.; Tuckermann, J.; Saklatvala, J.; Clark, A.R. Anti-inflammatory effects of dexamethasone are partly dependent on induction of dual specificity phosphatase 1. *J. Exp. Med.*, **2006**, *203*, 1883-1889.
- [32] Takeda, M.; Ito, W.; Tanabe, M.; Ueki, S.; Kato, H.; Kihara, J.; Tanigai, T.; Chiba, T.; Yamaguchi, K.; Kayaba, H.; Imai, Y.; Okuyama, K.; Ohno, I.; Sasaki, T.; Chihara, J. Allergic airway hyperresponsiveness, inflammation, and remodeling do not develop in phosphoinositide 3-kinase gamma-deficient mice. *J. Allergy Clin. Immunol.*, **2009**, *123*, 805-812.
- [33] Ito, K.; Caramori, G.; Adcock, I.M. Therapeutic potential of phosphatidylinositol 3-kinase inhibitors in inflammatory respiratory disease. *J. Pharmacol. Exp. Ther.*, **2007**, *321*, 1-8.
- [34] Pernis, A.B.; Rothman, P.B. JAK-STAT signaling in asthma. *J. Clin. Invest.*, **2002**, *109*, 1279-1283.
- [35] Demoly, P.; Basset-Seguin, N.; Chanez, P.; Campbell, A.M.; Gauthier-Rouvière, C.; Godard, P.; Michel, F.B.; Bousquet, J. c-fos proto-oncogene expression in bronchial biopsies of asthmatics. *Am. J. Respir. Cell Mol. Biol.*, **1992**, *7*, 128-133.
- [36] Adcock, I.M.; Lane, S.J.; Brown, C.R.; Lee, T.H.; Barnes, P.J. Abnormal glucocorticoid receptor-activator protein 1 interaction in steroid-resistant asthma. *J. Exp. Med.*, **1995**, *182*, 1951-1958.
- [37] Sousa, A.R.; Lane, S.J.; Soh, C.; Lee, T.H. *In vivo* resistance to corticosteroids in bronchial asthma is associated with enhanced phosphorylation of JUN N-terminal kinase and failure of prednisolone to inhibit JUN N-terminal kinase phosphorylation. *J. Allergy Clin. Immunol.*, **1999**, *104*, 565-574.
- [38] Loke, T.K.; Mallett, K.H.; Ratoff, J.; O'Connor, B.J.; Ying, S.; Meng, Q.; Soh, C.; Lee, T.H.; Corrigan, C.J. Systemic glucocorticoid reduces bronchial mucosal activation of activator protein 1 components in glucocorticoid-sensitive but not glucocorticoid-resistant asthmatic patients. *J. Allergy Clin. Immunol.*, **2006**, *118*, 368-375.
- [39] Ghosh, S.; Hayden, M.S. New regulators of NF-kappaB in inflammation. *Nat. Rev. Immunol.*, **2008**, *8*, 837-848.
- [40] Scheinman, R.I.; Cogswell, P.C.; Lofquist, A.K.; Baldwin, A.S. Jr. Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. *Science*, **1995**, *270*, 283-286.
- [41] Auphan, N.; DiDonato, J.A.; Rosette, C.; Helmsberg, A.; Karin, M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science*, **1995**, *270*, 286-290.
- [42] Hart, L.A.; Krishnan, V.L.; Adcock, I.M.; Barnes, P.J.; Chung, K.F. Activation and localization of transcription factor, nuclear factor-kappaB, in asthma. *Am. J. Respir. Crit. Care Med.*, **1998**, *158*, 1585-1592.
- [43] Vignola, A.M.; Chiappara, G.; Siena, L.; Bruno, A.; Gagliardo, R.; Merendino, A.M.; Polla, B.S.; Arrigo, A.P.; Bonsignore, G.; Bousquet, J.; Chanez, P. Proliferation and activation of bronchial epithelial cells in corticosteroid-dependent asthma. *J. Allergy Clin. Immunol.*, **2001**, *108*, 738-746.
- [44] Caramori, G.; Oates, T.; Nicholson, A.G.; Casolari, P.; Ito, K.; Barnes, P.J.; Papi, A.; Adcock, I.M.; Chung, K.F. Activation of NF-kappaB transcription factor in asthma death. *Histopathology*, **2009**, *54*, 507-509.
- [45] Gagliardo, R.; Chanez, P.; Mathieu, M.; Bruno, A.; Costanzo, G.; Gougat, C.; Vachier, I.; Bousquet, J.; Bonsignore, G.; Vignola, A.M. Persistent activation of nuclear factor-kappaB signaling pathway in severe uncontrolled asthma. *Am. J. Respir. Crit. Care Med.*, **2003**, *168*, 1190-1198.
- [46] Rogatsky, I.; Ivashkiv, L.B. Glucocorticoid modulation of cytokine signaling. *Tissue Antigens*, **2006**, *68*, 1-12.
- [47] Barnes, P.J. Role of GATA-3 in allergic diseases. *Curr. Mol. Med.*, **2008**, *8*, 330-334.
- [48] Roth, M.; Johnson, P.R.; Borger, P.; Bihl, M.P.; Rüdiger, J.J.; King, G.G.; Ge, Q.; Hostettler, K.; Burgess, J.K.; Black, J.L.; Tamm, M. Dysfunctional interaction of C/EBPalpha and the glucocorticoid receptor in asthmatic bronchial smooth-muscle cells. *N. Engl. J. Med.*, **2004**, *351*, 560-574.
- [49] Kröger, A.; Köster, M.; Schroeder, K.; Hauser, H.; Mueller, P.P. Activities of IRF-1. *J. Interferon Cytokine Res.*, **2002**, *22*, 5-14.
- [50] Vianna, E.O.; Westcott, J.; Martin, R.J. The effects of upper respiratory infection on T-cell proliferation and steroid sensitivity of asthmatics. *J. Allergy Clin. Immunol.*, **1998**, *102*, 592-597.
- [51] Yamada, K.; Elliott, W.M.; Hayashi, S.; Brattsand, R.; Roberts, C.; Vitalis, T.Z.; Hogg, J.C. Latent adenoviral infection modifies the steroid response in allergic lung inflammation. *J. Allergy Clin. Immunol.*, **2000**, *10*, 844-851.
- [52] Tliba, O.; Damera, G.; Banerjee, A.; Gu, S.; Baidouri, H.; Keslacy, S.; Amrani, Y. Cytokines induce an early steroid resistance in airway smooth muscle cells: novel role of interferon regulatory factor-1. *Am. J. Respir. Cell Mol. Biol.*, **2008**, *38*, 463-472.
- [53] Adcock, I.M.; Caramori, G. Cross-talk between pro-inflammatory transcription factors and glucocorticoids. *Immunol. Cell Biol.*, **2001**, *79*, 376-384.
- [54] Kam, J.C.; Szefer, S.J.; Surs, W.; Sher, E.R.; Leung, D.Y. Combination IL-2 and IL-4 reduces glucocorticoid receptor-binding affinity and T cell response to glucocorticoids. *J. Immunol.*, **1993**, *151*, 3460-3466.
- [55] Sher, E.R.; Leung, D.Y.; Surs, W.; Kam, J.C.; Zieg, G.; Kamada, A.K.; Szefer, S.J. Steroid-resistant asthma. Cellular mechanisms contributing to inadequate response to glucocorticoid therapy. *J. Clin. Invest.*, **1994**, *93*, 33-39.
- [56] Leung, D.Y.; Martin, R.J.; Szefer, S.J.; Sher, E.R.; Ying, S.; Kay, A.B.; Hamid, Q. Dysregulation of interleukin 4, interleukin 5, and interferon gamma gene expression in steroid-resistant asthma. *J. Exp. Med.*, **1995**, *181*, 33-40.
- [57] Leung, D.Y.; Spahn, J.D.; Szefer, S.J. Immunologic basis and management of steroid-resistant asthma. *Allergy Asthma Proc.*, **1999**, *20*, 9-14.
- [58] Szefer, S.J.; Leung, D.Y. Glucocorticoid-resistant asthma: pathogenesis and clinical implications for management. *Eur. Respir. J.*, **1997**, *10*, 1640-1647.
- [59] Wallen, N.; Kita, H.; Weiler, D.; Gleich, G.J. Glucocorticoids inhibit cytokine-mediated eosinophil survival. *J. Immunol.*, **1991**, *147*, 3490-3495.
- [60] Lamas, A.M.; Leon, O.G.; Schleimer, R.P. Glucocorticoids inhibit eosinophil responses to granulocyte-macrophage colony-stimulating factor. *J. Immunol.*, **1991**, *147*, 254-259.
- [61] Pavord, I.D. Non-eosinophilic asthma and the innate immune response. *Thorax*, **2007**, *62*, 193-194.
- [62] Pavord, I.D.; Brightling, C.E.; Woltmann, G.; Wardlaw, A.J. Non-eosinophilic corticosteroid unresponsive asthma. *Lancet*, **1999**, *353*, 2213-2214.
- [63] McKinley, L.; Alcorn, J.F.; Peterson, A.; Dupont, R.B.; Kapadia, S.; Logar, A.; Henry, A.; Irvin, C.G.; Piganelli, J.D.; Ray, A.; Kolls, J.K. TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J. Immunol.*, **2008**, *181*, 4089-4097.
- [64] Hawrylowicz, C.; Richards, D.; Loke, T.K.; Corrigan, C.; Lee, T. A defect in corticosteroid-induced IL-10 production in T lymphocytes from corticosteroid-resistant asthmatic patients. *J. Allergy Clin. Immunol.*, **2002**, *109*, 369-370.
- [65] Yang, M.; Kumar, R.K.; Foster, P.S. Pathogenesis of steroid-resistant airway hyperresponsiveness: interaction between IFN-gamma and TLR4/MyD88 pathways. *J. Immunol.*, **2009**, *182*, 5107-5115.
- [66] Nimmagadda, S.R.; Szefer, S.J.; Spahn, J.D.; Surs, W.; Leung, D.Y. Allergen exposure decreases glucocorticoid receptor binding affinity and steroid responsiveness in atopic asthmatics. *Am. J. Respir. Crit. Care Med.*, **1997**, *155*, 87-93.
- [67] Liu, K.; Gualano, R.C.; Hibbs, M.L.; Anderson, G.P.; Bozinovski, S. Epidermal growth factor receptor signaling to Erk1/2 and STATs control the intensity of the epithelial inflammatory responses to rhinovirus infection. *J. Biol. Chem.*, **2008**, *283*, 9977-9985.
- [68] Contoli, M.; Message, S.D.; Laza-Stanca, V.; Edwards, M.R.; Wark, P.A.; Bartlett, N.W.; Keadze, T.; Mallia, P.; Stanciu, L.A.; Parker, H.L.; Slater, L.; Lewis-Antes, A.; Kon, O.M.; Holgate, S.T.; Davies, D.E.; Kotenko, S.V.; Papi, A.; Johnston, S.L. Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat. Med.*, **2006**, *12*, 1023-1026.

- [69] Wark, P.A.; Johnston, S.L.; Bucchieri, F.; Powell, R.; Puddicombe, S.; Laza-Stanca, V.; Holgate, S.T.; Davies, D.E. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J. Exp. Med.*, **2005**, *201*, 937-947.
- [70] Zhu, Z.; Tang, W.; Ray, A.; Wu, Y.; Einarsson, O.; Landry, M.L.; Gwaltney, J. Jr.; Elias, J.A. Rhinovirus stimulation of interleukin-6 *in vivo* and *in vitro*. Evidence for nuclear factor kappa B-dependent transcriptional activation. *J. Clin. Invest.*, **1996**, *97*, 421-430.
- [71] Zhu, Z.; Tang, W.; Gwaltney, J.M. Jr.; Wu, Y.; Elias, J.A. Rhinovirus stimulation of interleukin-8 *in vivo* and *in vitro*: role of NF-kappaB. *Am. J. Physiol.*, **1997**, *273*, L814-824.
- [72] Papi, A.; Johnston, S.L. Respiratory epithelial cell expression of vascular cell adhesion molecule-1 and its up-regulation by rhinovirus infection via NF-kappaB and GATA transcription factors. *J. Biol. Chem.*, **1999**, *274*, 30041-30051.
- [73] Goleva, E.; Hauk, P.J.; Hall, C.F.; Liu, A.H.; Riches, D.W.; Martin, R.J.; Leung, D.Y. Corticosteroid-resistant asthma is associated with classical antimicrobial activation of airway macrophages. *J. Allergy Clin. Immunol.*, **2008**, *122*, 550-559.
- [74] Wenzel, S.E.; Schwartz, L.B.; Langmack, E.L.; Halliday, J.L.; Trudeau, J.B.; Gibbs, R.L.; Chu, H.W. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am. J. Respir. Crit. Care Med.*, **1999**, *160*, 1001-1008.
- [75] Ito, K.; Lim, S.; Caramori, G.; Chung, K.F.; Barnes, P.J.; Adcock, I.M. Cigarette smoking reduces histone deacetylase 2 expression, enhances cytokine expression, and inhibits glucocorticoid actions in alveolar macrophages. *FASEB J.*, **2001**, *15*, 1110-1112.
- [76] Chaudhun, R.; Livingston, E.; McMahon, A.D.; Lafferty, J.; Fraser, I.; Spears, M.; McSharry, C.P.; Thomson, N.C. Effects of smoking cessation on lung function and airway inflammation in smokers with asthma. *Am. J. Respir. Crit. Care Med.*, **2006**, *174*, 127-133.
- [77] Adcock, I.M.; Ford, P.; Ito, K.; Barnes, P.J. Epigenetics and airways disease. *Respir. Res.*, **2006**, *7*, 21.
- [78] Litonjua, A.A.; Weiss, S.T. Is vitamin D deficiency to blame for the asthma epidemic? *J. Allergy Clin. Immunol.*, **2007**, *120*, 1031-1035.
- [79] Litonjua, A.A. Childhood asthma may be a consequence of vitamin D deficiency. *Curr. Opin. Allergy Clin. Immunol.*, **2009**, *9*, 202-207.
- [80] Hansdotter, S.; Monick, M.M.; Hinde, S.L.; Lovan, N.; Look, D.C.; Hunninghake, G.W. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. *J. Immunol.*, **2008**, *181*, 7090-7099.
- [81] Xystrakis, E.; Kusumakar, S.; Boswell, S.; Peek, E.; Urry, Z.; Richards, D.F.; Adikibi, T.; Pridgeon, C.; Dallman, M.; Loke, T.K.; Robinson, D.S.; Barrat, F.J.; O'Garra, A.; Lavender, P.; Lee, T.H.; Corrigan, C.; Hawrylowicz, C.M. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *J. Clin. Invest.*, **2006**, *116*, 146-155.
- [82] Ginde, A.A.; Mansbach, J.M.; Camargo, C.A. Jr. Vitamin D, respiratory infections, and asthma. *Curr. Allergy Asthma Rep.*, **2009**, *9*, 81-87.
- [83] Chakir, J.; Hamid, Q.; Bossé, M.; Boulet, L.P.; Laviolette, M. Bronchial inflammation in corticosteroid-sensitive and corticosteroid-resistant asthma at baseline and on oral corticosteroid treatment. *Clin. Exp. Allergy*, **2002**, *32*, 578-82.
- [84] Bourdin, A.; Neveu, D.; Vachier, I.; Paganin, F.; Godard, P.; Chanez, P. Specificity of basement membrane thickening in severe asthma. *J. Allergy Clin. Immunol.*, **2007**, *119*, 1367-1374.
- [85] Cohen, L.; E, X.; Tarsi, J.; Ramkumar, T.; Horiuchi, T.K.; Cochran, R.; DeMartino, S.; Schechtman, K.B.; Hussain, I.; Holtzman, M.J.; Castro, M.; NHLBI Severe Asthma Research Program (SARP). Epithelial cell proliferation contributes to airway remodeling in severe asthma. *Am. J. Respir. Crit. Care Med.*, **2007**, *176*, 138-145.
- [86] Bossé, M.; Chakir, J.; Rouabhia, M.; Boulet, L.P.; Audette, M.; Laviolette, M. Serum matrix metalloproteinase-9/Tissue inhibitor of metalloproteinase-1 ratio correlates with steroid responsiveness in moderate to severe asthma. *Am. J. Respir. Crit. Care Med.*, **1999**, *159*, 596-602.
- [87] Mattos, W.; Lim, S.; Russell, R.; Jatakanon, A.; Chung, K.F.; Barnes, P.J. Matrix metalloproteinase-9 expression in asthma: effect of asthma severity, allergen challenge, and inhaled corticosteroids. *Chest*, **2002**, *122*, 1543-1552.
- [88] Goleva, E.; Hauk, P.J.; Boguniewicz, J.; Martin, R.J.; Leung, D.Y. Airway remodeling and lack of bronchodilator response in steroid-resistant asthma. *J. Allergy Clin. Immunol.*, **2007**, *120*, 1065-1072.
- [89] Lemièrre, C.; Ernst, P.; Olivenstein, R.; Yamauchi, Y.; Govindaraju, K.; Ludwig, M.S.; Martin, J.G.; Hamid, Q. Airway inflammation assessed by invasive and noninvasive means in severe asthma: eosinophilic and noneosinophilic phenotypes. *J. Allergy Clin. Immunol.*, **2006**, *118*, 1033-1039.
- [90] Brasier, A.R.; Victor, S.; Boetticher, G.; Ju, H.; Lee, C.; Bleecker, E.R.; Castro, M.; Busse, W.W.; Calhoun, W.J. Molecular phenotyping of severe asthma using pattern recognition of bronchoalveolar lavage-derived cytokines. *J. Allergy Clin. Immunol.*, **2008**, *121*, 30-37.
- [91] Haldar, P.; Pavord, I.D.; Shaw, D.E.; Berry, M.A.; Thomas, M.; Brightling, C.E.; Wardlaw, A.J.; Green, R.H. Cluster analysis and clinical asthma phenotypes. *Am. J. Respir. Crit. Care Med.*, **2008**, *178*, 218-224.
- [92] Woodruff, P.G.; Boushey, H.A.; Dolganov, G.M.; Barker, C.S.; Yang, Y.H.; Donnelly, S.; Ellwanger, A.; Sidhu, S.S.; Dao-Pick, T.P.; Pantoja, C.; Erle, D.J.; Yamamoto, K.R.; Fahy, J.V. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc. Natl. Acad. Sci. USA*, **2007**, *104*, 15858-15863.
- [93] Peters, M.J.; Adcock, I.M.; Brown, C.R.; Barnes, P.J. Beta-adrenoceptor agonists interfere with glucocorticoid receptor DNA binding in rat lung. *Eur. J. Pharmacol.*, **1995**, *289*, 275-281.
- [94] Adcock, I.M.; Stevens, D.A.; Barnes, P.J. Interactions of glucocorticoids and beta 2-agonists. *Eur. Respir. J.*, **1996**, *9*, 160-168.
- [95] Eickelberg, O.; Roth, M.; Lörx, R.; Bruce, V.; Rüdiger, J.; Johnson, M.; Block, L.H. Ligand-independent activation of the glucocorticoid receptor by beta2-adrenergic receptor agonists in primary human lung fibroblasts and vascular smooth muscle cells. *J. Biol. Chem.*, **1999**, *274*, 1005-1010.
- [96] Adcock, I.M.; Maneechotesuwan, K.; Usmani, O. Molecular interactions between glucocorticoids and long-acting beta2-agonists. *J. Allergy Clin. Immunol.*, **2002**, *110*, S261-268.
- [97] Roth, M.; Johnson, P.R.; Rüdiger, J.J.; King, G.G.; Ge, Q.; Burgess, J.K.; Anderson, G.; Tamm, M.; Black, J.L. Interaction between glucocorticoids and beta2 agonists on bronchial airway smooth muscle cells through synchronised cellular signalling. *Lancet*, **2002**, *360*, 1293-1299.
- [98] Pace, E.; Gagliardo, R.; Melis, M.; La Grutta, S.; Ferraro, M.; Siena, L.; Bonsignore, G.; Gjomarkaj, M.; Bousquet, J.; Vignola, A.M. Synergistic effects of fluticasone propionate and salmeterol on *in vitro* T-cell activation and apoptosis in asthma. *J. Allergy Clin. Immunol.*, **2004**, *114*, 1216-1223.
- [99] Usmani, O.S.; Ito, K.; Maneechotesuwan, K.; Ito, M.; Johnson, M.; Barnes, P.J.; Adcock, I.M. Glucocorticoid receptor nuclear translocation in airway cells after inhaled combination therapy. *Am. J. Respir. Crit. Care Med.*, **2005**, *172*, 704-712.
- [100] Kaur, M.; Chivers, J.E.; Giembycz, M.A.; Newton, R. Long-acting beta2-adrenoceptor agonists synergistically enhance glucocorticoid-dependent transcription in human airway epithelial and smooth muscle cells. *Mol. Pharmacol.*, **2008**, *73*, 203-214.
- [101] Greening, A.P.; Ind, P.W.; Northfield, M.; Shaw, G. Added salmeterol versus higher-dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroid. Allen & Hanburys Limited UK Study Group. *Lancet*, **1994**, *344*, 219-224.
- [102] Shrewsbury, S.; Pyke, S.; Britton, M. Meta-analysis of increased dose of inhaled steroid or addition of salmeterol in symptomatic asthma (MIASMA). *BMJ*, **2000**, *320*, 1368-1373.
- [103] Coward, W.R.; Sagara, H.; Church, M.K. Asthma, adenosine, mast cells and theophylline. *Clin. Exp. Allergy*, **1998**, *28*, 42-46.
- [104] Ichiyama, T.; Hasegawa, S.; Matsubara, T.; Hayashi, T.; Furukawa, S. Theophylline inhibits NF-kappa B activation and I kappa B alpha degradation in human pulmonary epithelial cells. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **2001**, *364*, 558-561.
- [105] Umeda, M.; Ichiyama, T.; Hasegawa, S.; Kaneko, M.; Matsubara, T.; Furukawa, S. Theophylline inhibits NF-kappaB activation in human peripheral blood mononuclear cells. *Int. Arch. Allergy Immunol.*, **2002**, *128*, 130-135.
- [106] Tomita, K.; Chikumi, H.; Tokuyasu, H.; Yajima, H.; Hitsuda, Y.; Matsumoto, Y.; Sasaki, T. Functional assay of NF-kappaB translocation into nuclei by laser scanning cytometry: inhibitory effect by dexamethasone or theophylline. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **1999**, *59*, 249-255.

- [107] Ito, K.; Lim, S.; Caramori, G.; Cosio, B.; Chung, K.F.; Adcock, I.M.; Barnes, P.J. A molecular mechanism of action of theophylline: Induction of histone deacetylase activity to decrease inflammatory gene expression. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 8921-8926.
- [108] Ichiyama, T.; Hasegawa, S.; Umeda, M.; Terai, K.; Matsubara, T.; Furukawa, S. Pranlukast inhibits NF-kappa B activation in human monocytes/macrophages and T cells. *Clin. Exp. Allergy*, **2003**, *33*, 802-807.
- [109] Tomari, S.; Matsuse, H.; Machida, I.; Kondo, Y.; Kawano, T.; Obase, Y.; Fukushima, C.; Shimoda, T.; Kohno, S. Pranlukast, a cysteinyl leukotriene receptor 1 antagonist, attenuates allergen-specific tumour necrosis factor alpha production and nuclear factor kappa B nuclear translocation in peripheral blood monocytes from atopic asthmatics. *Clin. Exp. Allergy*, **2003**, *33*, 795-801.
- [110] Suzuki, S.; Takeuchi, K.; Ishinaga, H.; Basbaum, C.; Majima, Y. Leukotriene D4 upregulates MUC2 gene transcription in human epithelial cells. *Pharmacology*, **2008**, *81*, 221-228.
- [111] Ishinaga, H.; Takeuchi, K.; Kishioka, C.; Suzuki, S.; Basbaum, C.; Majima, Y. Pranlukast inhibits NF-kappaB activation and MUC2 gene expression in cultured human epithelial cells. *Pharmacology*, **2005**, *73*, 89-96.
- [112] Laviolette, M.; Malmstrom, K.; Lu, S.; Chervinsky, P.; Pujet, J.C.; Peszek, I.; Zhang, J.; Reiss, T.F. Montelukast added to inhaled beclomethasone in treatment of asthma. Montelukast/Beclomethasone Additivity Group. *Am. J. Respir. Crit. Care Med.*, **1999**, *160*, 1862-1868.
- [113] Tomari, S.; Shimoda, T.; Kawano, T.; Mitsuta, K.; Obase, Y.; Fukushima, C.; Matsuse, H.; Kohno, S. Effects of pranlukast, a cysteinyl leukotriene receptor 1 antagonist, combined with inhaled beclomethasone in patients with moderate or severe asthma. *Ann. Allergy Asthma Immunol.*, **2001**, *87*, 156-161.
- [114] Tahan, F.; Jazrawi, E.; Moodley, T.; Rovati, G.E.; Adcock, I.M. Montelukast inhibits tumour necrosis factor-alpha-mediated interleukin-8 expression through inhibition of nuclear factor-kappaB p65-associated histone acetyltransferase activity. *Clin. Exp. Allerg.*, **2008**, *38*, 805-811.
- [115] Manning, A.M.; Davis, R.J. Targeting JNK for therapeutic benefit: from junk to gold? *Nat. Rev. Drug Discov.*, **2003**, *2*, 554-565.
- [116] Karin, M. Inflammation-activated protein kinases as targets for drug development. *Proc. Am. Thorac. Soc.*, **2005**, *2*, 386-390; discussion 394-5.
- [117] Newton, R.; Holden, N. Inhibitors of p38 mitogen-activated protein kinase: potential as anti-inflammatory agents in asthma? *Bio. Drugs*, **2003**, *17*, 113-129.
- [118] Hitti, E.; Kotlyarov, A. The ERK and p38MAPK pathways as targets for anti-inflammatory therapy. *Anti-Inflamm. Anti-Allergy Agents Med. Chem.*, **2007**, *6*, 85-97.
- [119] Marwick, J.A.; Caramori, G.; Stevenson, C.S.; Casolari, P.; Jazrawi, E.; Barnes, P.J.; Ito, K.; Adcock, I.M.; Kirkham, P.A.; Papi, A. Inhibition of PI3Kdelta restores glucocorticoid function in smoking-induced airway inflammation in mice. *Am. J. Respir. Crit. Care Med.*, **2009**, *179*, 542-548.
- [120] Kracht, M. Targeting strategies to modulate the NF-kB and JNK signal transduction network. *Anti-Inflamm. Anti-Allergy Agents Med. Chem.*, **2007**, *6*, 71-84.
- [121] Desmet, C.; Gosset, P.; Henry, E.; Garz , V.; Faisca, P.; Vos, N.; Jaspard, F.; M lotte, D.; Lambrecht, B.; Desmecht, D.; Pajak, B.; Moser, M.; Lekeux, P.; Bureau, F. Treatment of experimental asthma by decoy-mediated local inhibition of activator protein-1. *Am. J. Respir. Crit. Care Med.*, **2005**, *172*, 671-678.
- [122] Desmet, C.; Gosset, P.; Pajak, B.; Cataldo, D.; Bentires-Alj, M.; Lekeux, P.; Bureau, F. Selective blockade of NF-kappa B activity in airway immune cells inhibits the effector phase of experimental asthma. *J. Immunol.*, **2004**, *173*, 5766-5775.
- [123] Roth, M.; Black, J.L. Transcription factors in asthma: are transcription factors a new target for asthma therapy? *Curr. Drug Targets*, **2006**, *7*, 589-595.
- [124] Pereira, S.G.; Oakley, F. Nuclear factor-kappaB1: regulation and function. *Int. J. Biochem. Cell Biol.*, **2008**, *40*, 1425-1430.
- [125] Hurley, D.M.; Accili, D.; Stratakis, C.A.; Karl, M.; Vamvakopoulos, N.; Rorer, E.; Constantine, K.; Taylor, S.I.; Chrousos, G.P. Point mutation causing a single amino acid substitution in the hormone binding domain of the glucocorticoid receptor in familial glucocorticoid resistance. *J. Clin. Invest.*, **1991**, *87*, 680-686.
- [126] Charmandari, E.; Kino, T.; Ichijo, T.; Chrousos, G.P. Generalized glucocorticoid resistance: clinical aspects, molecular mechanisms, and implications of a rare genetic disorder. *J. Clin. Endocrinol. Metab.*, **2008**, *93*, 1563-1572.
- [127] Kino, T.; Chrousos, G.P. Tissue-specific glucocorticoid resistance-hypersensitivity syndromes: multifactorial states of clinical importance. *J. Allergy Clin. Immunol.*, **2002**, *109*, 609-613.
- [128] Anderson, G.P. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. *Lancet*, **2008**, *372*, 1107-1119.