Cross-talk between vitamin D, estrogen and corticosteroids in glucocorticoid resistant asthma

N Kalra¹, FT Ishmael²*

Abstract

Introduction

Glucocorticoids (GC) are mainstay of therapy in asthma but over 50% of asthmatics do not respond to inhaled or oral glucocorticoids. This makes glucocorticoid resistance in asthma a challenging healthcare problem associated with significant morbidity and life-threatening disease progression.

Physiologic and pharmacologic actions of glucocorticoids are mediated through binding to their receptors. Interaction of GC with glucocorticoid response elements regulates transcription of anti-inflammatory genes. Glucocorticoid actions are affected by post-translational modification of glucocorticoid receptor (GR), alterations in GR levels, or by actions that counteract its effects on downstream targets. Interplay between sex steroids and secosteroids such as vitamin D, has been shown to alter anti-inflammatory action of glucocorticoids. Given the structural similarities of steroid hormone molecules and their receptors, their co-localization in cells, and similar mechanisms on gene regulation and signalling, it is possible that cross talk between these molecules affects GC function and GC resistance. In patients with steroid resistant asthma, there appear to be defects in GC induced gene transcription of anti-inflammatory mediators such as IL10 and MKP-1. Anti-inflammatory actions of glucocorticoids may differ by gender. In women asthma may be less responsive to corticosteroid treatment, as oestrogen alters GR levels through protein degradation and suppression of GC mediated transcription of anti-inflammatory proteins, such as MKP1 and glucocorticoid-induced leucine zipper protein (GLIZ). We discuss mechanism of glucocorticoid actions and how oestrogen and vitamin D may affect its function and resistance.

Conclusion

Glucocorticoid resistant asthma is a complex phenotype and exact mechanism of its resistance is still not clearly understood. Study of interactions of major steroid hormone systems may help us understand the impact of individual hormones in regulation of inflammation in asthma.

Introduction

Glucocorticoids (GC) regulate many physiological processes and play an essential role in treatment of inflammatory diseases. In airway inflammatory disorders such as asthma, inhaled corticosteroids (ICS) are mainstay of therapy. However, up to 50% of asthmatics may not respond well to ICS, and up to 25% patients with difficult to control asthma may not respond well to oral glucocorticoids.¹²³ This makes glucocorticoid resistance in asthma a challenging healthcare problem associated with significant morbidity and life-threatening disease progression.

Although, multiple molecular mechanisms that contribute to GC resistance have been described, the exact mechanism of insensitivity has not been fully elucidated and is an area of active research. Many factors that act at level of glucocorticoid receptor and its signalling pathway can influence response to glucocorticoid therapy. Given the structural similarities of steroid hormone molecules and their receptors, their co-localization in cells and tissues, and similar mechanisms on gene regulation and signalling, it is possible that cross talk between these molecules affects their function in inflammatory diseases. This could affect how glucocorticoids function and carry implications for GC resistance. Recent studies have revealed interplay between glucocorticoid receptors and other steroid hormone receptors such as sex steroids and secosteroids such as Vitamin D (Vit D), which may alter anti-inflammatory action of glucocorticoids.

Physiologic & pharmacologic actions of glucocorticoids are mediated through its binding to glucocorticoid receptor (GR), which translocates to cell nucleus from cytoplasm. Interactions of GCs with glucocorticoid response elements (GREs) on DNA or transcription factors regulate transcription of specific genes, including anti-inflammatory genes. Actions of GCs can be regulated by several mechanisms such as post-translational modification of GR, alterations in GR levels, or by actions that counteract its effects on downstream targets.

Effects of oestrogen and Vit D on these pathways have clinical significance in asthma. Anti-inflammatory actions of glucocorticoids may differ by gender. In women, asthma may be less responsive to corticosteroid treatment suggesting oestrogen may play a role in glucocorticoid resistance.⁴⁵⁶ Vit D deficiency has been associated with poor asthma control and glucocorticoid resistance in several epidemiological studies⁷⁸⁹. In patients with steroid resistant asthma there appear to be defects in GC mediated gene transcription of anti-inflammatory...
mediators such as IL10 and mitogen-activated protein kinase phosphatase-1 (MKP-1). These changes may be reverted by Vit D. In this review, we will discuss mechanisms of how oestrogens and Vit D affect GC function and its implications in GC resistance.

Nuclear receptor super family of steroid hormone receptors
Both steroid hormone receptors and Vitamin D receptors belong to nuclear receptor super family and have homologous receptors (Figure 1). Steroid hormone receptors are classified into five groups based on receptors to which they bind. These five groups are glucocorticoids, mineralocorticoids, androgens, oestrogens, and progesterone.

Mechanism of glucocorticoid action
Glucocorticoids are lipid soluble molecules, synthesized from cholesterol.

Endogenous glucocorticoids are secreted by adrenal cortex and their production is regulated by hypothalamic pituitary adrenal axis. The secretion of GC in body follow circadian rhythm, levels are high in morning, decrease during day and increase with stress. Immuno-suppressive and anti-inflammatory actions of GC are due to their genomic and non-genomic effects. Genomic actions are mediated after binding to glucocorticoid receptors.

Like most nuclear receptors, GR is a homodimer and shares common structure that includes N-terminal activation function-1 domain (AF-1), highly conserved DNA-binding domain (DBD) and C-terminal ligand binding domain (LBD). In addition to ligand recognition, LBD also contains ligand dependent activation function (AF-2), which is regulated by hormone binding. GR gene is located on chromosome 5 and has nine exons. Alternate splicing of GR primary transcript (pre mRNA) produces four different isoforms: GRα, GRβ, GRδ and GRy of which GRα and GRβ have been studied more extensively. GRα is the only biologically active isoform. It is widely expressed in tissues and mediates genomic action of GC. GRβ has non-functional ligand binding domain which is incapable of binding GC. Increased expression of GRβ may interfere with binding of GR to DNA and influence sensitivity of glucocorticoids due to its dominant negative inhibitor function.

The glucocorticoid receptor undergoes many post-translational modifications including phosphorylation that further modulates receptor activity. Human GRα has 6 known phosphorylation sites (Serines 113, 141, 203, 211, 226, 404) located on N terminal binding domain of the receptor.

Receptor displays basal level of phosphorylation, which increases after binding of glucocorticoids. The activity is differentially affected by phosphorylation at different sites. For instance, phosphorylation at Ser-211 correlates with transcriptionally active form of GRα, while phosphorylation at Ser-226 impairs its activity. Major kinases responsible for receptor phosphorylation include MAPK, cyclin dependent kinases, glycogen synthase kinases and c-Jun N-terminal kinase (JNK). The p38 MAPK-mediated phosphorylation of GR has been associated with GC resistance in asthma. On the other hand protein phosphatases (PP) such as 1, PP2a and PP5 dephosphorylate the receptor. Phosphorylation of GRα occurs after binding to the ligand and may affect its turnover and translational activity. It also determines cofactor interaction and may alter receptor signalling such as duration and strength.

Ubiquination, another post-translational modification of GR has been described to regulate GR turnover. Ubiquitin is 76 amino acid protein which when attached to appropriate residue on the target protein marks it for turnover by proteasome. Ubiquination of GRα at specific lysine residue (Lys 419) has been shown to tag the receptor for proteasomal degradation. Studies show degradation of GRα is enhanced by glucocorticoid dependent phosphorylation of the receptor. Because phosphorylation is a signal for ubiquination and proteasomal degradation it plays a major role in turnover of GR.

Genomic and Non Genomic actions of glucocorticoids
In its inactive state, GR is present as a cytoplasmic complex and associated with different chaperone proteins such as heat shock protein (hsp) 90, hsp70, hsp56, hsp40, p23 and src. After binding to GC, cytoplasmic GR undergoes conformational change that includes dissociation of chaperone proteins and dimerization of receptor. This allows translocation of hormone receptor complex into the nucleus. In nucleus, GR binds to palindromic...
nucleotide sequences in promoter region of target genes (GC response elements, GRE) and this interaction activates (transactivation) or inhibits (transrepression) gene transcription depending on type of GRE sequence (Figure 2). Activation of glucocorticoid-responsive genes involves recruitment of co-activator proteins such as Cyclic AMP response element-binding protein (CREB) binding proteins, which have histone acetyltransferase activity that acetylates core histones. This initiates chromatin remodelling and initiation of transcription by RNA polymerase II resulting in gene activation.

Glucocorticoids induce genes of anti-inflammatory proteins such as IL-10, annexin 1, MKP-1, inhibitor of nuclear factor kappa B (NfκB) and IkB. MKP-1 is a central target of GC, whose function may be modulated by other steroid hormones. It is a phosphatase that inactivates mitogen-activated protein kinase (MAPK), thus inhibits the production of proinflammatory cytokines regulated by MAPK.

Glucocorticoids also function by transrepression, where GC/GR complex directly or indirectly inhibits the function of transcription factors. These effects can be mediated by protein–protein interactions by binding of GR to proinflammatory transcription factors such as activator protein 1 (AP-1) and NFκB and Raf-1 proteins, inhibiting their transcriptional activity.

In addition, GC/GR complex can prevent gene transcription by binding to negative GRE, and examples of genes affected by this mechanism include prolactin and osteocalcin.

Non genomic and post-transcriptional actions of glucocorticoids

Glucocorticoids also have nongenomic actions that are characterized by their rapid effect. Several basic mechanisms of nongenomic action of GC have been proposed. GC may activate secondary messengers (Ca++, IP3, cAMP, protein kinase C) by binding to GC receptor in cell membrane. Proteins dissociated after steroid receptor complex formation such as heat shock protein and members of MAP kinase family play role in GC non-transcriptional signals.

Glucocorticoids may also act post-transcriptionally to regulate expression at level of mRNA. GCs induce production of RNA-binding proteins, such as tristetraprolin (TTP), which have been shown to modulate effects of hormone. We previously demonstrated that presence of TTP was crucial for effects of GC in airway epithelial cells. In addition, GR can act as an RNA-binding protein in the cytoplasm, where it can bind to the mRNA of cytokines and chemokines to repress expression.

We demonstrated that GR binds to CCL2 and other cytokine transcripts in the cytoplasm and serves to enhance their mRNA turnover. More recently, GCs have been implicated in the expression and function of microRNAs (miRNAs). MiRNAs are small, non-coding RNAs which bind to transcripts and repress their translation or promote their degradation. GCs can alter the expression of miRNAs involved in pro-inflammatory responses and T-cell differentiation to impart their anti-inflammatory effects.

Mechanisms of GC resistance

It has now become apparent that both genetic and acquired factors affect glucocorticoid sensitivity and several molecular mechanisms have been proposed to describe resistance to GCs (Table 1). Glucocorticoid resistant asthma is often found within families, suggesting genetic factors may influence glucocorticoid responsiveness. Micorarray studies of peripheral blood mononuclear cells (PBMC) identified 11 genes that discriminate between glucocorticoid resistant and glucocorticoid sensitive asthma patient groups. BMPRII gene was identified as most discriminant gene in a gene profiling study of healthy subjects that compared expression of circulating genes between 10% of participants with the highest glucocorticoid responsiveness and 10% with least steroid responsiveness.

In familial glucocorticoid resistance, several abnormalities in GR have been described including binding affinity for cortisol, reduced receptor expression...
and reduced binding to DNA, all of which are caused by mutations of glucocorticoid receptor. Splice variants of GR formed by alternative splicing may modulate glucocorticoid sensitivity. Increased expression of GRβ isoform has been reported in patients with steroid resistant asthma. It is induced by pro-inflammatory cytokines and competes with GRα to bind with GRE, thus acting as dominant negative inhibitor36,37.

Post translational modification of GR may influence GC sensitivity via several mechanisms. In vitro, it is theorized that GR nuclear translocation and binding affinity is reduced by GR phosphorylation. Although several kinases can cause GR phosphorylation, p38 MAPK is considered important in its ability to alter GR nuclear translocation and binding38. p38 MAPK are expressed in greater degree in alveolar macrophages of GC resistant asthma patients39. IL13, IL2 and IL4 are also overexpressed in patients with GC resistant asthma and thought to reduce GR binding and translocation via p38MAP kinases38,40,41. Defective GR binding and nuclear translocation mediated via phosphorylation of GR by p38MAP kinase is another potential mechanism of GC resistance in asthmatic patients. IL13 is up regulated in patients with GC resistant asthma40. Upon cell entry, glucocorticoid is converted into active or inactive form by enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD)-1 and 11β-HSD-2, respectively, thus regulating bioavailability of GC substrate for GR. IL13 can up-regulate 11β-HSD-1 and acts as negative feedback loop to curtail inflammation through steroid anti-inflammatory effects42. It is possible this pathway plays a role in steroid-resistant asthma.

Acetylation of GR occurs prior to nuclear translocation43. The acetylated GR is deacetylated by histone deacetylase 2 (HDAC 2), which is necessary for GR to be able to inhibit NF-κB activation of inflammatory genes hence promoting its anti-inflammatory actions43. Reduced activity and expression of HDAC2 is found in some diseases in patients who respond poorly to glucocorticoid treatment44. HDAC2 expression is markedly reduced in PBMCs and alveolar macrophages of patients with refractory asthma and in the airways of smoking asthmatic patients23,45. In smokers there is decreased HDAC2 expression and activity, which correlates with the disease. It has been shown that oxidative stress also inactivates HDAC2, which may be an important mechanism of GC resistance in asthma patients who smoke46,47,48. Air pollutants such as diesel exhaust particles (DEP) stimulate release of TH2-specific cytokines (i.e., IL4, IL5 and IL13) which play a role in airway inflammation in asthma49. Studies show that exposure to DEP results in increased expression of allergen specific IgE and cytokines in nasal lavage fluid. Diaz Sanchez et al. found that topical treatment with fluticasone inhibited local cytokine expression induced by ragweed but was unable block response to DEP challenge50.

GR can also be acetylated at Lys 494 and 495, which reduces its ability to bind to NF-κB, thereby losing its ability to repress targets of transcription factor and thus increasing GC insensitivity51. GR can be ubiquitinated and tagged for proteasomal degradation, which may promote its down regulation and lead to rapid turnover of the receptor. GC-resistance may also be driven by multiple immune mechanisms. IL10 is a potent anti-inflammatory and immune-regulatory cytokine which is secreted by regulatory T (Treg) cells in response to

Table 1: Molecular mechanisms of Glucocorticoid resistance in asthma.

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<thead>
<tr>
<th>Mechanism Genomic</th>
<th>Change</th>
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<td>Mutation in GR</td>
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<td>Familial glucocorticoid resistance (non-functional GR)</td>
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<td>Increased histone acetylation</td>
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<td>Reduced HDAC-2</td>
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<td>IL-13</td>
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<td>IL-2</td>
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<td>Cigarette smoke</td>
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corticosteroids. In patients with steroid resistant asthma, there is a reduced IL10 secretion by Tregs, which is restored by vitamin D3 in vitro. TH17 cells, which secrete IL17 appear to be increased in patients with severe asthma which is associated with neutrophilic inflammation. TH17 cells appear to be steroid resistant in mice studies.

**Vitamin D**

Vitamin D3 group consists of sterol derivatives which include 7-dehydrocholesterol, cholecalciferol and calcitriol 1,25(OH)2 vitamin D3. Vitamin D3 is either synthesized in skin or derived from nutritional sources and metabolized in liver and kidneys. In cytoplasm, Vitamin D3 binds to its receptor and forms a heterodimer with retinoid-X receptor (RXR), which subsequently translocates to the nucleus. In nucleus, VDR–RXR complex binds to specific DNA sequence on gene promoter region called vitamin D response element (VDRE). Binding of VDR to VDRE recruits co-activators and enzymes with histone acetylation activity, causing structural changes in chromatin, thereby facilitating gene transcription.

In the body, intracellular vitamin D receptors are present in diverse tissues and respond to its active form 1,25 dihydroxy vitamin D3, indicating its pleiotropic actions. Vitamin D is essential for immunomodulation, homeostasis of calcium and bone mineralization. Studies show serum Vitamin D levels and asthma and allergic diseases may be related, and Vitamin D plays a role in airway inflammatory response and remodelling. In vitro studies suggest Vitamin D may affect mechanism of development of steroid resistant asthma.

Interaction of Vitamin D with other steroid hormone receptors including GR is not clearly understood, but studies show Vitamin D may enhance GC responsiveness by increasing its anti-inflammatory activity. Data suggests that vitamin D interacts with glucocorticoid signalling pathways and may potentially improve glucocorticoid responsiveness in severe asthmatics by up-regulation of IL10 production from CD4+ cells. Vitamin D enhances GC mediated transcription of genes of anti-inflammatory proteins IL10 and MKP-1 (mitogen-activated protein kinase phosphatase-1). IL10 is a potent anti-inflammatory cytokine produced by Tregs. It has been shown to regulate sensitivity of cells to GC, MKP1 inhibits production of MAPK regulated pro-inflammatory mediators, and this function is essential for anti-inflammatory actions of GC. The induction of MKP-1 by GC has been well documented and several GREs have been reported in the MKP-1 promoter.

To study the effect of GCs in steroid sensitive and steroid resistant asthmatic subjects, Hawrylowicz et al. investigated dexamethasone induced production of IL10 by CD4 T cells and found levels of IL10 in GC resistant subjects were significantly lower as compared to GC sensitive subjects. Evidence of crosstalk between the vitamin D pathway and glucocorticoids pathway also comes from the Searing et al. study, which demonstrated vitamin D has steroid-sparing effects on the cellular response to glucocorticoids.

In this in-vitro study, PBMCs isolated from asthmatic subjects were treated with dexamethasone alone and dexamethasone with Vitamin D3. Enhanced induction of MKP-1 and IL10 was seen in dexamethasone with Vitamin D3 treatment group as compared to dexamethasone alone treatment group.

The study by Xystrakis et al. showed that higher levels of IL10 are produced in presence of Vitamin D3. When CD4 T cell from GC resistant asthma patients were cultured with dexamethasone alone IL10 secretion was not enhanced, but addition of vitamin D3 increased IL10 up to the levels of the cells from patients with GC sensitive asthma, which were cultured with dexamethasone alone. Vit D enhanced activity of dexamethasone by more than 10 times. Xystrakis and colleagues concluded that supplementation of vitamin D may increase therapeutic response to corticosteroids in steroid-resistant asthma.

In an in-vitro study by Zhang et al., steroid sparing effects of vitamin D were examined which revealed vitamin...
D may improve anti-inflammatory response of GCs. They demonstrated dexamethasone induced MKP-2 expression significantly increases as serum Vit D levels rise. Significantly lower concentration of GCs was required to suppress proinflammatory cytokine production by PBMCs in the presence of Vit D. This suggests Vit D may enhance GC responsiveness and lower dose of GC required to reduce inflammation, thereby reducing GC-mediated side effects.

In another in vitro study, Zhang and colleagues demonstrated that Vit D has anti-inflammatory and corticosteroid enhancing effects in steroid resistant (SR) and steroid sensitive (SS) asthma patients. The response to corticosteroids in SR asthmatics was significantly lower than SS asthmatics. Vitamin D enhanced MKP-1 mRNA expression by cells by increasing GR binding to MKP-1 promoter, but it could not overcome decreased GR phosphorylation at Ser211 in the cells from SR asthmatics.

Epidemiological studies also suggest association between VitD and use of inhaled corticosteroids. Synergistic effect of vitamin D on corticosteroids observed in vitro may translate to pulmonary outcomes in clinical studies. In a cohort of children with mild to moderate persistent asthma who received inhaled corticosteroid, lung function improvement, as measured by pre-bronchodilator forced expiratory volume in 1 second, was the greatest in vitamin D sufficient group (>30 ng/ml) as compared to vitamin D-deficient group (≤20 ng/ml), vitamin D insufficient group faring in between (20–30 ng/ml).

A paediatric study from Costa Rica showed, higher Vit D levels were associated with lower use of inhaled corticosteroids as well as leukotriene antagonists. Higher levels were also associated with low total IgE levels and eosinophil count.

Another important role of Vit D in asthma is airway remodelling due to presence of VDR in bronchial smooth muscle. Gupta et al showed in their study on children with asthma that lower Vit D levels were associated with worse lung function, poor asthma control and more steroid use. Authors concluded that airway remodeling was associated with increased smooth muscle mass. It appears there is relationship between serum concentrations of 25(OH)D3 and airway remodelling. Small prospective study of children with asthma showed that Vit D add on to budesonide treatment reported less number of respiratory infections induced asthma exacerbation than the steroid treatment alone.

**Oestrogen**

Asthma has greater prevalence and severity in women than in men, and there is mounting evidence, this may in part be due to oestrogen. Early menarche is a risk factor for asthma development.

Increased airway responsiveness to inhaled methacholine challenge coinciding with secondary sexual characters further reinforces role of oestrogen in asthma. Studies show oestrogen promotes TH2 polarization and class switching to IgE. However oestrogen’s role in glucocorticoid resistance in asthma is not well studied.

Although, glucocorticoid receptors and oestrogen receptors are both members of steroid hormone receptor family, molecular mechanism of cross talk between them is not clear. Interactions between these two hormones may play role in many clinical settings. To our knowledge, interaction between glucocorticoid and sex steroid receptors has not been demonstrated in in-vitro asthma studies, and many breast cancer studies demonstrate ineffectiveness of glucocorticoid therapy in inhibiting cell growth, suggesting possible role of oestrogen in steroid resistance. Some allergic, autoimmune and malignant diseases are also less responsive to glucocorticoid therapy in females, suggesting role of oestrogen in glucocorticoid resistance. Oestrogen exerts its effects by binding to two types of oestrogen receptors ERα and ERβ. They are present in cell membrane, cytoplasm and nucleus. After oestrogen binds to its receptor in the nucleus, the receptors dimerize. Its mechanism to regulate gene transcription is similar to that of glucocorticoids. It binds to DNA at oestrogen-response elements (EREs) located in promoters of target genes or it indirectly interacts through other DNA-binding transcription factors in nucleus.

Crosstalk between ER and GR may modify glucocorticoid receptor expression and function leading to GC resistance. Proteasomal degradation of GR enhances GR turnover and may cause GC resistance. Breast cancer studies show, in breast cancer cell line, MCF-7, oestrogen agonists downregulate GR protein levels via proteasomal degradation of GR70. This effect was mediated by enhanced ubiquitination of GR by oestrogen treatment, resulting in enhanced GR turnover. These data were supported in another breast cancer study, where oestrogen down regulated GR expression and attenuated functional responses to glucocorticoids thereby causing relative glucocorticoid resistance.

Down-regulation of GR expression led to substantial suppression of GR-mediated transcriptional activity in MCF-7 cells. Studies by Hall et al. and studies by DeFazio et al. have indicated that oestrogens might negatively influence GR levels. In small series of breast cell lines, ER-negative cell lines expressed significantly more GR mRNA than ER-positive lines. DeFazio et al. reported inverse relationship between basal levels of expression of GR mRNA and ER mRNA among several breast cancer cell lines. Oestrogen may affect function of GCs by altering posttranslational modifications of GR or by affecting expression of GC-gene targets. GR is subject to homone-dependent and independent phosphorylation on several serine and threonine residues by several kinases. Phosphorylation of GR may alter its binding, stability, translocation to the nucleus, binding to DNA and interaction with other proteins, such as transcription factors and molecular chaperones. Zhang et al demonstrated...
that oestrogens induce serine protein phosphatases (PP5), which inhibits GR phosphorylation and prevents glucocorticoid mediated induction of MKP-1 and glucocorticoid-regulated kinase genes.

Oestrogen also possibly inhibits glucocorticoid-induced leucine zipper protein (GLIZ), an anti-inflammatory protein induced by GC. This protein inhibits NF-kB and AP-1 and is associated with anti-inflammatory functions of glucocorticoids.

Oestrogen was found to antagonize induction of GLIZ expression in human uterine epithelial cells which may be a mechanism of GC resistance.

Based on data, it is possible pro-inflammatory effects of oestrogen by GR modification and inhibition of anti-inflammatory gene transcription mediates glucocorticoid resistance in adult women with severe medication resistant asthma.

**Discussion**

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

Anti-inflammatory effects of GCs are complex and require multiple steps such as GR expression, GR activation by GC binding and regulation of gene transcription. Resistance to anti-inflammatory effects of GCs is major barrier to treatment of asthma and related to GR dysfunction leading to altered signalling pathway. Interplay of GR with other steroid hormone receptors contribute to spectrum of clinical and pathological outcomes in steroid resistant asthma. We believe Vit D augments anti-inflammatory actions of glucocorticoids. Both, in vitro and clinical studies suggest Vit D plays protective role in asthma and enhances immunosuppressive actions of GC by up regulating IL10 and MKP1 (Figure 3). Vitamin D deficiency reduces IL10 production by Treg cells, thus reducing cellular sensitivity to steroid in asthma. We believe Vit D supplement may revert GC resistance and provide better asthma control by enhancing GC mediated production of anti-inflammatory cytokines such as IL10 and MKP1.

Gender may modulate GC sensitivity. In females, GC insensitivity may be due to cross talk between oestrogen and GCs pathway at cellular level. Breast cancer studies show oestrogen down regulates GC levels through protein degradation, while both breast cancer and uterine studies show oestrogen suppresses GR mediated transcriptional activity and inhibits anti-inflammatory proteins such as MKP1 and GLIZ (Figure 3).

These studies a) Highlight potential cross talk between oestrogen and GC, b) Suggest that ability of oestrogen to suppress GC may play important role in many clinical settings such as asthma and c) Open up new directions in exploring gender differences in response to corticosteroid therapy.

**Conclusion**

Glucocorticoid resistant asthma is a complex phenotype and exact mechanism of its resistance is still not clearly understood. Study of interactions of major steroid hormone systems may help us understand the impact of individual hormones in regulation of inflammation in asthma.

Altered GR signalling due to interplay of other steroid hormones is a potential mechanism of GC responsiveness in asthma.

**Abbreviations list**

CREB Cyclic AMP response element-binding protein
Ca Calcium
cAMP Cyclic adenosine monophosphate
CCL2 Chemokine (C-C motif) ligand 2
DBD DNA-binding domain
ERE Estrogen-response elements
ERK Extracellular-regulated kinase
GC Glucocorticoids
GR Glucocorticoid Receptor
GRE Glucocorticoid response elements
GLIZ Glucocorticoid-induced leucine zipper
HDAC 2 Histone deacetylase 2
HSP Heat shock protein
icdB Inhibitor of NFkB alpha
IJKN c-Jun N-terminal kinase
LBD Ligand binding domain
IgE Immunoglobulin E
Ikb Inhibitor of NFkB
IL Interleukin
IP3 Inositol trisphosphate
ICS Inhaled corticosteroids
MKP1 Mitogen-activated protein kinase phosphatase-1
MAPK Mitogen-activated protein kinase
MCF 7 Michigan Cancer Foundation 7
miRNAs microRNAs
NFkB Nuclear factor kappa B
PBMC Peripheral blood mononuclear cells
PP5 Protein phosphatases 5
SR Steroid-resistant
SS Steroid-sensitive
STAT Signal transduction-activated transcription
TReg Regulatory T cell
TTP Tristetraprolin
Vit D Vitamin D3 or 1α,25(OH)2D3
VDR Vitamin D receptor
11β-HSD 11beta hydroxysteroid dehydrogenase

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**Conflict of interests**

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**References**

2. Martin RJ, Szefler SJ, King TS et al. The Predicting Response to Inhaled Corticosteroid Efficacy (PRICE) trial. J...
Allergy Clinical Immunology. 2007 Jan;119(1):73-80.


31. Smith LK, Shah RR, Gidlowski JA. Glucocorticoids modulate microRNA expression and processing during...

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74. Mittelstadt PR, Ashwell JD. Inhibition ofAP-1 by the glucocorticoid-inducible protein GILZ. J.Biol.Chem. 2001, 276, 29603-10.