



Why do some asthma patients respond poorly to glucocorticoid therapy?

Ishbel Henderson^{a,1}, Elisabetta Caiazzo^{a,b,1}, Charles McSharry^a, Tomasz J. Guzik^{c,d}, Pasquale Maffia^{a,b,c,*}

^a Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom

^b Department of Pharmacy, University of Naples Federico II, Naples, Italy

^c Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom

^d Department of Internal Medicine, Jagiellonian University, Collegium Medicum, Kraków, Poland

ARTICLE INFO

Keywords:

Asthma
Glucocorticoids
Glucocorticoid receptor
Steroid-resistant asthma

ABSTRACT

Glucocorticosteroids are the first-line therapy for controlling airway inflammation in asthma. They bind intracellular glucocorticoid receptors to trigger increased expression of anti-inflammatory genes and suppression of pro-inflammatory gene activation in asthmatic airways.

In the majority of asthma patients, inhaled glucocorticoids are clinically efficacious, improving lung function and preventing exacerbations. However, 5–10 % of the asthmatic population respond poorly to high dose inhaled and then systemic glucocorticoids. These patients form a category of severe asthma associated with poor quality of life, increased morbidity and mortality, and constitutes a major societal and health care burden. Inadequate therapeutic responses to glucocorticoid treatment is also reported in other inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease; however, asthma represents the most studied steroid-refractory disease. Several cellular and molecular events underlying glucocorticoid resistance in asthma have been identified involving abnormalities of glucocorticoid receptor signaling pathways. These events have been strongly related to immunological dysregulation, genetic, and environmental factors such as cigarette smoking or respiratory infections. A better understanding of the multiple mechanisms associated with glucocorticoid insensitivity in asthma phenotypes could improve quality of life for people with asthma but would also provide transferable knowledge for other inflammatory diseases. In this review, we provide an update on the molecular mechanisms behind steroid-refractory asthma. Additionally, we discuss some therapeutic options for treating those asthmatic patients who respond poorly to glucocorticoid therapy.

1. Introduction

Glucocorticosteroids (GCs), also called glucocorticoids, corticosteroids or steroids, are natural regulators of a wide range of biological processes including the hypothalamic-pituitary-adrenal (HPA) axis, immunity and energy metabolism, primarily to maintain homeostasis. In humans, the hormone cortisol is the primary endogenous glucocorticoid, synthesized and secreted by the adrenal cortex. It interacts with the GC

receptor (GR) to regulate a plethora of signaling pathways [1]. GCs are associated with potent anti-inflammatory activity, which can be exploited for therapeutic drug use. Synthetic GCs (e.g. prednisolone, dexamethasone) can be synthesized in bulk and designed for higher affinity binding to GR. They are used in medicine, to mimic this natural pathway of immune suppression and attenuate inappropriate inflammation. They are the mainstream therapy for a wide range of acute and chronic inflammatory diseases, including asthma. The Global Initiative

Abbreviations: AP1, activator protein-1; CSF 3, colony-stimulating factor 3; FE_{NO}, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; GCs, glucocorticosteroids; GR, GC receptor; GRE, GC response elements; GWAS, genomic wide association studies; HDAC, histone deacetylase; HPA, hypothalamic-pituitary-adrenal; IAV, influenza A virus; ICS, inhaled corticosteroids; IL, interleukin; JNK, c-Jun N-terminal kinase; MAP, mitogen-activated protein; MAPK, mitogen-activated protein kinase; NF-κB, Nuclear factor-κB; NLRP3, NLR Family Pyrin Domain Containing 3; PBMcs, peripheral blood mononuclear cells; RSV, respiratory syncytial virus; TF, transcription factors; Th, T helper; VDBP, vitamin D-binding protein.

* Corresponding author at: Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Sir Graeme Davies Building, 120 University Place, G12 8TA, Glasgow, United Kingdom.

E-mail address: Pasquale.Maffia@glasgow.ac.uk (P. Maffia).

¹ These authors made an equal contribution.

<https://doi.org/10.1016/j.phrs.2020.105189>

Received 22 July 2020; Received in revised form 1 September 2020; Accepted 2 September 2020

Available online 8 September 2020

1043-6618/© 2020 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

for Asthma (GINA) defines asthma as “a heterogeneous disease that is characterized by chronic inflammation of the airways and a clinical history of wheezing, cough, tightness of chest and shortness of breath varying with time and intensity, as well as expiratory airflow limitation” (<https://ginasthma.org/>). Asthma affects over 300 million people worldwide, with increasing incidence. The heterogeneity of ‘asthma’ suggests it should be an umbrella term for different phenotypes. There are two recognizable endotypes: allergic asthma, driven by a T helper 2-subset (T2-type) response, or non-allergic, non-atopic (non-T2) asthma, driven by other immune cells such as neutrophils [2–4]. GCs represent first-line treatment for asthma but there are two major limitations: firstly, toxic high dose-dependent side effects and secondly the refractory response seen among asthma patients, predominantly those with severe disease. Early asthma studies, mostly in children, characterized asthma as an IgE-dependent inflammatory response to allergens associated with eosinophilia [5]. This led to treatments centralized around inhibiting Th2 cytokines, and this delayed the recognition and understanding of non-T2 asthma phenotypes and hindered development of appropriate treatment options. T2 asthma is the dominant sub-group, and generally responds effectively to GC treatments. In contrast, non-T2 severe asthma is associated with GC insensitivity.

Herein we provide a state-of-the-art overview of the proposed mechanisms leading to glucocorticoid refractory asthma and potential therapeutic strategies.

2. Mechanisms of action of glucocorticoids

Although GCs have been widely used for many decades, the complete understanding of their multiple molecular mechanisms of immune modulation is still elusive. It is known that they act through genomic and nongenomic mechanisms. The strong suppression of airway inflammation is mainly due to the genomic mechanism. GCs act by binding to intracellular receptors of the target cell (glucocorticoid receptors; GRs). Genomic mechanisms derive from glucocorticoids binding to glucocorticoid receptors in the cytoplasm and the translocation of the GC/GR complex into the nucleus. In the nucleus, the GC/GR complex modifies transcription of specific genes through direct DNA binding or transcription factor inactivation (Fig. 1). This is either done by binding to small motifs known as GC response elements (GRE) in the promoter regions of susceptible genes. GRs consist of different subunits, with a variable N-terminal domain, C-terminal domain, and a DNA binding domain, with zinc fingers to assist genomic interactions. GRs are located

in an inactive form in the cytoplasm, as a multi-protein complex, attached to a chaperone protein. Originally, upon activation, it was believed that the GR and chaperone protein dissociated allowing the GR to translocate into the nucleus. However, research now indicates that the chaperone complex is required for nuclear transportation [6]. The GR can function as a monomer, homo, or hetero-dimer, and recently described as a tetramer [7]. There are two variants of the receptor, GR- α , and GR- β , with subtle splicing differences in the C-terminal domain. The GR- β isoform is most abundantly expressed; however, it is unable to bind to GCs and therefore cannot transduce GC-induced functions. It is believed to regulate GC activity, through antagonizing the GR- α isoform and regulation through GR- α/β heterodimers [8,9]. GR- α is subject to different post-translational modifications, which include phosphorylation, acetylation and other modifications that affect GR signaling pathways [10].

Once inside the nucleus, the GC/GR complex functions by regulating up to 20 % of genes expressed by immune cells. Specifically, GCs act by trans-repressing inflammatory genes and stimulating the transcription of anti-inflammatory genes leading to reduced activation, recruitment and survival of inflammatory and epithelial cells [1,11,12]. Glucocorticoids may also regulate the immunomodulatory function of smooth muscle cells and affect airway remodeling in asthma [13]. Other genomic mechanisms such as regulation of mRNA stability have been also described [14]. Nongenomic actions are mediated by specific interaction with membrane-bound or cytoplasmic GRs, or nonspecific interactions with the cell membrane [15].

Synthetic GCs are designed with optimal characteristics for potent, high affinity binding to the GR only, making them more specific than natural GC, which bind to both the GR and the closely related mineralocorticoid receptor [16,17]. GCs also possess pro-inflammatory effects, under stress conditions [18].

3. Glucocorticoid resistance

The term glucocorticoid resistance is formally used to describe the resistance to adrenal suppression by dexamethasone, as in Cushing’s syndrome. In asthma, patients generally respond well to GCs, but in some this can vary and when responses to inhaled and then oral GCs are inadequate this is called steroid unresponsive, refractory or resistant asthma. According to international guidelines, for adults and children patients with persistent asthma low-dose inhaled corticosteroids (ICS) with or without long-acting bronchodilator (β_2 -agonists) represent the

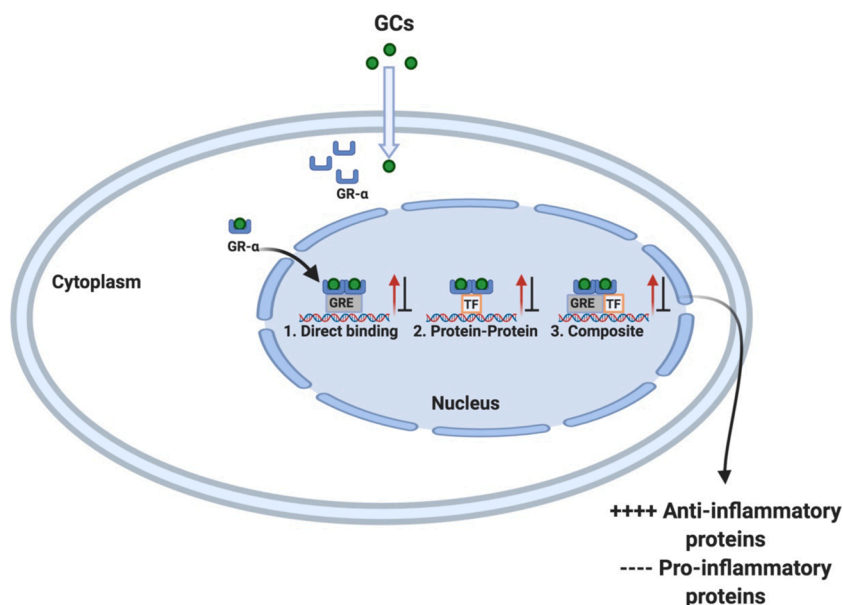


Fig. 1. Glucocorticoid transcriptional regulation. The lipid-soluble glucocorticoid (GC) passively diffuses through the cell membrane and binds to the glucocorticoid receptor (GR- α) in the cytoplasm. Once ligated the GC/GR complex translocate into the nucleus through the nuclear pore or exerts non-genomic effects in the cytoplasm. In the nucleus, the complex can either: 1. Bind directly to DNA and regulate transcription using positive GC response elements (GREs) or negative GREs; 2. Interact with other transcription factors (TF) to mediate their activity or; 3. Compositely bind to both DNA and TFs to regulate gene transcription. Notably, all three of these mechanisms can positively and negatively regulate gene expression. Figure created in BioRender (<https://biorender.com/>).

first-line therapy. Besides, they are recommended in patients with intermittent or mild asthma requiring a short-acting β_2 -agonist more than twice a week or twice a month (<https://ginasthma.org/>).

For the majority of asthma patients, ICS work well, improving lung function and reducing exacerbations. Patients with difficult asthma require higher doses of oral GCs to manage their asthma, and these include 5–10 % of patients who don't respond satisfactorily to these drugs, resulting in difficult to manage asthmatic symptoms thus denoted as having steroid-resistant asthma [19]. Th2-low asthma phenotypes are less responsive to steroid therapies and have a higher prevalence of severe asthma cases. However, glucocorticoid insensitivity in patients with persistent eosinophil inflammation has also been described [20]. They are a disproportionately large burden on health care; the 10 % with refractory asthma cost 80 % of health care costs [21], along with a significantly increased morbidity and mortality [22]. This emphasizes the importance of a better understanding of this poor responsiveness and the need for new therapeutic options.

Steroid resistance is defined as <15 % improvements in forced expiratory volume in 1 s (FEV_1), after 2 weeks of appropriate dose steroid treatment. These patients, however, do respond well to β_2 -adrenergic agonist-mediated vasodilation [23,24]. There are currently no clinically accepted biomarkers or phenotypes for resistance; meaning diagnosis is based on the clinical history and lung function after sufficient steroid treatments. This results in patients receiving increasing doses of steroids for extended periods, until it is recognized that this is ineffective for treating the severity of asthma. Indeed, a key cause of morbidity in these patients comes from the toxic side effects of long-term high-dose steroids; these include increased susceptibility to infections, osteoporosis, hyperglycemia and cardiovascular disease [25,26].

3.1. Understanding mechanisms of the inadequate responses to glucocorticosteroids

To gain a better understanding of steroid resistance an important distinction must be made between patients who poorly manage their asthma and the group of genuinely refractory asthmatic patients. Several studies highlighted that about one third of difficult asthma cases that did not respond to CS was due to poor compliance and not steroid insensitivity [27,28]. The clinical pattern of exacerbations and poor asthma control was similar to the GC-refractory patients. Fortunately, poor-compliance can be assessed by measuring fractional exhaled nitric oxide (FE_{NO}) levels which is high in asthma and is reduced confirming compliance with steroid treatment unless the patient is bluntly resistant to the treatment [29].

Successful adherence to medications is a complex issue, with many patients either self-assessing their condition and coming off long-term treatments when they feel healthy or become overly concerned about the side effects of the treatments. Another major issue is poor inhalation technique, considered non-intentional non-adherence [30], which can be resolved with better training. Once the true refractory patient cohort is identified studies can be more accurate, focusing on the causes of insensitivity and alternative therapeutic interventions.

Refractory patients have typically severe asthma, with low percent-predicted FEV_1 indicating higher levels of fixed airway obstruction and reduced lung function [31,32]. A consideration here is whether the lack of efficacy of GCs results in severe symptoms or whether the inflammatory mechanisms maintaining severity also drives resistance.

3.2. Cellular and molecular basis of glucocorticoid-resistance

Poor steroid responsiveness can be inherited or acquired. Inherited genetic mutations specifically associated with refractory asthma remain poorly described [33]. Many genomic wide association studies (GWAS) and pharmacogenomic studies have been carried out to investigate the relationship between genetic variations and response to steroids. High-density oligonucleotide microarray studies of peripheral blood

mononuclear cells (PBMCs) from patients with glucocorticoid-sensitive asthma and those with glucocorticoid-resistant asthma revealed that 11 genes accurately predicted corticosteroid resistant asthma [34]. Further pharmacogenomic studies including an appropriately large patient cohort would be useful to power a study to fully differentiate glucocorticoid-resistant and glucocorticoid-sensitive asthmatic patients. Single nucleotide polymorphisms in the *GLCCI1* gene, encoding the glucocorticoid-induced transcript 1 protein, were associated with response to GC therapy in asthma [35]. These findings were replicated in multiple candidate gene analysis studies [36,37]. The functional single nucleotide polymorphism, rs37973, was associated with reduced ICS sensitivity. Children with two copies of the mutant allele are less responsive to GC therapy [35], and increased expression of *GLCCI1* demonstrated better responses to ICS [38]. Several additional genes have significant associations with GC insensitivity. However, these findings were not well replicated between studies, perhaps due to variation in protocols, heterogeneity of asthma phenotype, or the complexity of the GC pathways. It is feasible that GC insensitivity is not caused by a singular mutation, and more likely involves a range of genetic variations that remain to be determined.

Multiple molecular mechanisms have been identified associated with GC dysfunction including: reduced GR- α expression [39], defective binding between the GC and the GR or between the GR complex and DNA [40], and increased antagonism, either from increased pro-inflammatory transcription factors or increased GR- β expression [41]. Additionally, GR phosphorylation by e.g. p38 mitogen-activated protein kinase (MAPK) and by reduced activity of histone deacetylase 2 (HDAC2) can reduce the expression various anti-inflammatory genes induced by GCs [42,43].

As examples of acquired GC resistance, inflammation or oxidative stress can negatively affect GR signaling [22]. Possible mechanisms for acquired resistance could be related to immune dysregulation; for example, interleukin (IL)-2, IL-4 and IL-13 are often overexpressed in the lungs of steroid insensitive patients [44–46]. This profile of cytokine up-regulation is associated with reduced GR affinity *in vitro* through activation of p38 mitogen-activated protein kinase resulting in the phosphorylation of GR and diminished nuclear translocation in inflammatory cells [47].

Th1 cytokines have also been associated with GC-resistance. Specifically, IFN- γ can increase GR phosphorylation and inhibit GR nuclear translocation in different experimental models of steroid-resistant airway hyperresponsiveness by up-regulating miR-9 expression in the lung and pulmonary macrophages [48]. Recently, TNF- α and IFN- γ cytokines have been shown to sustain glucocorticoid-resistance in human fetal airway smooth muscle cells by promoting the Nuclear factor- κ B (NF- κ B) pathway and Stat1 phosphorylation [49].

Kim and co-workers have suggested that in murine models of steroid-resistant allergic airway disease, the exaggerated NLR Family Pyrin Domain Containing 3 (NLRP3) inflammasome/IL-1 β activation critically contributed to glucocorticoid resistance [50]. The mechanism is not yet fully understood but included a role for IL-1 β in Th17 cell differentiation and IL-17 production [51]. It is interesting to note that asthmatic patients resistant to glucocorticoids show increased Th17 cells and IL-17A levels [52], and the adoptive transfer of Th17 cells in mice has resulted in the development of steroid insensitivity [53]. Accordingly, Th17 responses have been shown to increase the expression of GR- β , a mechanism of steroid resistance in bronchial epithelial cells [54]. Very recently, Ouyang et al. have found that IL-17A synergizes with dexamethasone in inducing colony-stimulating factor 3 (CSF 3) in both human airway smooth muscle cells and fibroblasts through transcriptional and post-transcriptional regulation. This effect is further increased in the presence of TNF- α , as described above, and has been associated with glucocorticoid resistance [55]. Additionally, it has been suggested that IL-17 produced in the lung by type 3 innate lymphoid cells, ILC3, may play a role in steroid resistance associated with the obesity phenotype of asthma [56].

GC insensitivity has been associated with dysregulated IL-10 production. Specifically, T lymphocytes from corticosteroid-resistant asthmatic patients have impaired IL-10 production following *in vitro* stimulation with dexamethasone compared to T lymphocytes from steroid-sensitive asthmatics [57]. This defect can be reversed by the combination of GC with salmeterol or the administration of vitamin D3 [58,59]. Recently, a decreased regulatory T-cell activity has been shown in older asthmatic patients that render them more vulnerable to type 2 inflammation and steroid resistance [60]; thereby, suggesting age as an important factor in determining glucocorticoid sensitivity.

Exogenous factors such as cigarette smoking, respiratory viral and bacterial infections, high-fat diet and/or obesity, may also contribute to mechanisms of steroid-resistant asthma. The airway inflammation observed in asthma patients who smoke cigarettes is typically neutrophilic rather than eosinophilic. This non-T2 endotype is consistent with the major phenotypes of steroid-refractory asthma. A mechanism proposed for cigarette smoke induced GC-insensitivity is a reduced ratio of GR- α to GR- β isoforms [61], resulting in increased antagonism of the GR- α . Cigarette smoke is associated with reduced HDAC activity in alveolar macrophages, which controls access to chromatin, a crucial stage in GC-mediated gene regulation [62,63]. This could be a potential target for novel therapies, to re-balance the isotype ratio and increase HDAC activity to re-sensitize patients to steroids. An additional mechanism included the ligation by cigarette smoke components of the aryl hydrocarbon receptor that suppresses smoke-induced inflammation, apoptosis and oxidative stress. It is proposed this is done through microRNA regulation inhibiting protein synthesis [64] and indirectly regulating the Th17 pathways, which have been linked with steroid resistance, as discussed above. Th17 cells mediate neutrophilic airway inflammation by stimulating the production of IL-8. McSharry et al. have shown the increase in neutrophils and IL-8 levels in sputum fluid from asthmatic smokers compared to that from nonsmokers with asthma, suggesting a contribution for this cytokine in glucocorticoid insensitivity [65]. Another clinically important study has shown that steroid sensitivity returns after smoking cessation suggesting mechanisms of resistance are reversible [66] and providing smoking cessation advice as a clear therapeutic strategy.

Exposure to combustion products from cigarette smoke or burning biomass fuel is a major cause of the development of chronic obstructive pulmonary disease (COPD). COPD is stubbornly refractory to mainstay corticosteroid treatment and the molecular mechanisms of steroid insensitivity in COPD are incompletely understood [67]. Similar to asthma in cigarette smokers, it has been proposed that cigarette smoke and oxidative stress in COPD may decrease HDAC2 activity [22] and increase various kinase pathways such as p38 MAPK [68]. Of note, the lung inflammation in COPD and smokers with asthma is predominantly neutrophilic [69], and is the basis of defining an asthma-COPD overlap syndrome (ACOS) sharing steroid-refractory Th17 endotype that predisposes to neutrophilia and neutrophilic asthma is associated to steroid resistance [70,71].

A role for viral and/or bacterial respiratory infections in glucocorticoid refractivity in asthmatic patients has been also described. Specifically, *Chlamydia pneumoniae*, *Haemophilus influenzae*, rhinovirus, influenza A virus (IAV) and respiratory syncytial virus (RSV) infections have each been associated with steroid resistance [72–76]. The reduction of GR- α nuclear translocation through NF- κ B and c-Jun N-terminal kinase activation has been proposed as a molecular mechanism of glucocorticoid insensitivity in rhinovirus-infected primary human bronchial epithelial cells [77].

Non-T, neutrophilic asthma patients often have bacterial infections, which could impair steroid sensitivity [78]. Some bacterial products, such as staphylococcal endotoxins B, have been shown to increase GR- β expression [79]. Recently, Kim et al. have developed novel mouse models of steroid-resistant asthma driven by bacterial (*Chlamydia* and *Haemophilus influenzae*) and viral (influenza and RSV) respiratory tract infections. In these experimental models, the authors demonstrated a

role for miR-21 in inducing steroid insensitivity through PI3K-mediated phosphorylation and nuclear translocation of pAKT [80].

Steroid resistance has been also associated with fungus-exposed patients through induction of Th2/Th17 responses [81]. Also *Aspergillus alternata* exposure has been shown to induce IL-33 dependent steroid-resistant asthma, mediated by ILC2 and Th2 cells in neonatal mice [82]. It has been suggested that the ability of IL-33 to activate p38-MAPK in CD4 + T cells and to induce phosphorylation of GR may be a mechanism underlying glucocorticoid insensitivity [83].

As described above, GCs can exert also non-genomic actions, especially at high concentrations, and few non-genomic pathways have been identified to date. GCs can inhibit the degranulation of mast cells through stabilization of the plasma membrane or by a reduction in [Ca²⁺] elevation [84]. In addition, GCs can exert their anti-inflammatory effects by negative interference with MAPK signaling pathways [85]. Abnormalities of these non-genomic mechanisms on immune cells may contribute to GC insensitivity; however, more research is needed to fully understand how non-genomic mechanisms can influence GC sensitivity.

Key molecular mechanisms involved in steroid resistance in asthmatic patients are summarized in Table 1 and Fig. 2.

3.3. Therapeutic perspectives

From the information provided, and the little progress in the past decades towards effective treatments for non-T2, steroid-refractory asthma, it is clear that future research needs a multidisciplinary approach and knowledge collaborations to reach a deeper understanding of steroid insensitivity. Some of the main molecular mechanisms have been identified; however, a deeper understanding of these mechanisms is needed before pharmacogenomic data can be clinically utilized in predicting drug responses and effectively optimizing treatments.

Innovative solutions should consider environmental and genetic factors contributing to the resistance, better technologies for molecular imaging of inflammation [87], and, in the era of big data, the establishment of large databases to support immune research into drug discovery and therapeutics [88,89], along with omics approaches for the identification of biomarkers. Several serum biomarkers have been proposed to predict steroid resistance in asthmatic children such as vitamin D-binding protein (VDBP), miRNA-21 and OX40 ligand [90–93].

The development of more personalized approaches for steroid-resistant asthma is particularly useful because of the different mechanisms and multiple immunological and inflammatory phenotypes likely leading to steroid resistance. Traditionally steroids were considered a universal treatment, with reduced sensitivity forcing a higher dose regime, following the theory that stronger Th2 responses require higher doses of steroid suppression. It is now evident that complex asthma phenotypes highlight the need for patient stratification and individualized therapy.

Identifying the underlying endotype driving each phenotype should

Table 1
Proposed molecular mechanisms of steroid resistance.

Mechanism	References
Genetic abnormalities in GRs	[83]
Reduced GR- α expression	[39]
Defective GC binding to GR- α	[40]
Reduced GR- α translocation due to increase phosphorylation by kinases such as p38 MAPK and JNK	[43]
Reduced HDAC2 activity and expression	[42]
Increased pro-inflammatory transcription factor activation, such as NF- κ B and AP1	[41]
Increased GR- β expression	[86]

Abbreviations: GR: glucocorticoid receptor, GC: glucocorticoid, MAPK: mitogen-activated protein kinase, JNK: c-Jun N-terminal kinase, HDAC: histone deacetylase, NF- κ B: nuclear factor- κ B, AP1: activator protein-1.

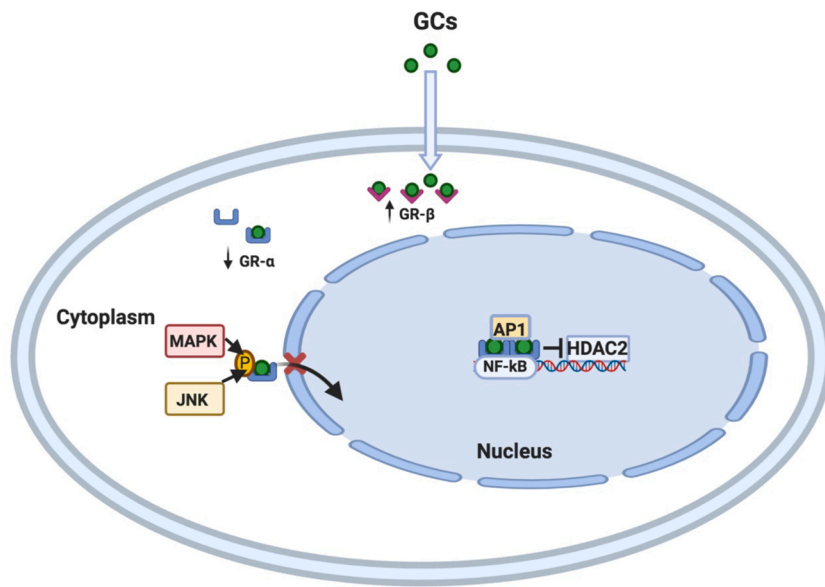


Fig. 2. Cellular mechanisms behind glucocorticoid resistance. Glucocorticoid resistance can be associated to reduced expression of GR- α that mediate the pharmacological actions of glucocorticoids, increased expression of negative isoform GR- β ; reduced GR- α translocation into nucleus due to hyperphosphorylation of GR- α p38 by kinases such as p38 MAPK and JNK; increased expression of inflammatory transcription factors like NF- κ B or AP-1 that compete for DNA binding; inhibition of HDAC2 activity that suppresses various inflammatory gene expression. Figure created in BioRender (<https://biorender.com/>).

be utilized when considering the optimal treatments. This requires multidisciplinary collaborations to identify the different aspects of insensitivity. Once a better understanding is gained optimal treatments, such as biologicals, can be used instead of non-specific immunosuppressants.

Better education should also be provided on steroid functions and inhaler technique, to limit the non-adherence patients. Most steroid-resistant research is done on asthma; however, similar insensitivity is observed, with more frequent rates, in other inflammatory respiratory diseases such as chronic obstructive pulmonary disease, and in rheumatoid arthritis, and inflammatory bowel disease [94,95]. A better understanding of multiple mechanisms associated with GC insensitivity in asthma could provide transferrable knowledge for other inflammatory diseases since similar molecular mechanisms have been proposed. To attain more accurate research a new disease model is required, to fully understand the mechanisms, as well as identifying biomarkers to use for early identification and screening for insensitivity. This will help alleviate the time lost trialing different steroid doses, as well as optimally treating the patient.

Most clinical trials to validate new therapeutics for asthma typically exclude smokers. Since steroid insensitivity is prominent in smokers [96], future clinical trial studies should include this category of asthma patients.

Therapeutic target for reversing steroid insensitivity could include blocking the underlying mechanisms e.g. with antibodies against the key cytokines such as IL-17, IL-8, and TNF- α associated with the neutrophilic airway inflammation that is strongly steroid-resistant. Regarding NLRP3 inflammasome/IL-1 β , it has been demonstrated that specific inhibition of the NLRP3 inflammasome is more advantageous than global inhibition of IL-1 β [79].

Interestingly, the combined use of different drugs can restore glucocorticoid sensitivity. Steroid-resistance in asthma has been associated with imbalanced acetylation and deacetylation of GRs variously regulating gene transcription. Increased HDAC activity using theophylline, PI3K and p38 MAPK inhibitors [96–98] may be beneficial, especially in glucocorticoid resistant asthmatic smokers. Combined therapy with long-acting beta 2 agonists has improved glucocorticoid responses by affecting GR translocation and phosphorylation. This therapeutic strategy and could be useful for asthmatic patients in whom a poor response is related to abnormal GR signaling [99,100]. Other studies suggest that macrolides such as azithromycin and clarithromycin potentiate glucocorticoid sensitivity in asthma, but the mechanism

remains unclear [101–103]. Another interesting study has shown that statins increase the anti-inflammatory effect of glucocorticoid though induction of indoleamine 2, 3-dioxygenase in alveolar macrophages [104]. A combination of these different therapeutic strategies may help to effectively reduce GC resistance.

Finally, recent new highly potent glucocorticoids have been developed for steroid-resistant severe asthma. Among them, only GCVSG158 was demonstrated to reverse steroid-resistance in a murine model of eosinophilic and neutrophilic airway inflammation [105].

4. Conclusions

In conclusion, although glucocorticoid resistance is observed in a small proportion of asthmatic patients, it represents a serious clinical and socioeconomic problem. Therefore, future research on the molecular mechanisms of multiple steroid-resistant asthma endotypes and the identification of subgroups of patients with poor responses to steroids will facilitate the selection of appropriate treatment, in a stratified fashion for those phenotypes and the development of novel therapeutic approaches.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

Our work is funded by the British Heart Foundation grants PG/19/84/34771 and RE/13/5/30177; the European Commission Marie Skłodowska-Curie Individual Fellowships661369; the Engineering and Physical Sciences Research Council (EPSRC) grant EP/L014165/1; the European Research Council (InflammaTENSION; project Identifier: 726318); the Wellcome Trust grant204820/Z/16/Z; and the University of Glasgow Scottish Funding Council and the Global Challenges Research Fund. C.McS. is funded by the BPF medical research trust. E.C. is funded by the Italian Ministry of University and Research (MIUR) PRIN 2017 (2017NKB2N4_003).

References

- [1] J. Galon, D. Franchimont, N. Hiroi, G. Frey, A. Boettner, M. Ehrhart-Bornstein, J. J. O'Shea, G.P. Chrousos, S.R. Bornstein, Gene profiling reveals unknown

- enhancing and suppressive actions of glucocorticoids on immune cells, *FASEB J.* 16 (1) (2002) 61–71.
- [2] P.M. Hansbro, R.Y. Kim, M.R. Starkey, C. Donovan, K. Dua, J.R. Mayall, G. Liu, N. G. Hansbro, J.L. Simpson, L.G. Wood, J.A. Hirota, D.A. Knight, P.S. Foster, J. C. Horvat, Mechanisms and treatments for severe, steroid-resistant allergic airway disease and asthma, *Immunol. Rev.* 278 (1) (2017) 41–62.
- [3] S.E. Wenzel, Asthma phenotypes: the evolution from clinical to molecular approaches, *Nat. Med.* 18 (5) (2012) 716–725.
- [4] L.P. Tavares, H.Y. Peh, W.S.D. Tan, H. Pahima, P. Maffia, E. Tiligada, F. Levi-Schaffer, Granulocyte-targeted therapies for airway diseases, *Pharmacol. Res.* 157 (2020), 104881.
- [5] J.V. Fahy, Type 2 inflammation in asthma—present in most, absent in many, *Nat. Rev. Immunol.* 15 (1) (2015) 57–65.
- [6] M.D. Galigniana, P.C. Echeverria, A.G. Erlejan, G. Piwien-Pilipuk, Role of molecular chaperones and TPR-domain proteins in the cytoplasmic transport of steroid receptors and their passage through the nuclear pore, *Nucleus* 1 (4) (2010) 299–308.
- [7] D.M. Presman, S. Ganguly, R.L. Schiltz, T.A. Johnson, T.S. Karpova, G.L. Hager, DNA binding triggers tetramerization of the glucocorticoid receptor in live cells, *Proc. Natl. Acad. Sci. U.S.A.* 113 (29) (2016) 8236–8241.
- [8] R.H. Oakley, M. Sar, J.A. Cidlowski, The human glucocorticoid receptor beta isoform. Expression, biochemical properties, and putative function, *J. Biol. Chem.* 271 (16) (1996) 9550–9559.
- [9] E. Charmandari, G.P. Chrousos, T. Ichijo, N. Bhattacharyya, A. Vottero, E. Souvatzoglou, T. Kino, The human glucocorticoid receptor (hGR) beta isoform suppresses the transcriptional activity of hGRalpha by interfering with formation of active coactivator complexes, *Mol. Endocrinol.* 19 (1) (2005) 52–64.
- [10] K. Scheschowitsch, J.A. Leite, J. Assrey, New insights in glucocorticoid receptor signaling—more than just a ligand-binding receptor, *Front. Endocrinol. (Lausanne)* 8 (2017) 16.
- [11] M. Dennis, I.H. Itkin, Effectiveness and complications of Aerosol DEXAMETHASONE phosphate in severe asthma, *J. Allergy* 35 (1964) 70–76.
- [12] L.M. Schwiebert, L.A. Beck, C. Stellato, C.A. Bickel, B.S. Bochner, R.P. Schleimer, Glucocorticosteroid inhibition of cytokine production: relevance to anti-allergic actions, *J. Allergy Clin. Immunol.* 97 (1 Pt 2) (1996) 143–152.
- [13] S.J. Hirst, T.H. Lee, Airway smooth muscle as a target of glucocorticoid action in the treatment of asthma, *Am. J. Respir. Crit. Care Med.* 158 (5 Pt 3) (1998) S201–6.
- [14] A.A. Alangari, Genomic and non-genomic actions of glucocorticoids in asthma, *Ann. Thorac. Med.* 5 (3) (2010) 133–139.
- [15] C. Stahn, F. Buttgeriet, Genomic and nongenomic effects of glucocorticoids, *Nat. Clin. Pract. Rheumatol.* 4 (10) (2008) 525–533.
- [16] D.W. Cain, J.A. Cidlowski, Immune regulation by glucocorticoids, *Nat. Rev. Immunol.* 17 (4) (2017) 233–247.
- [17] C. van der Heijden, J. Deinum, L.A.B. Joosten, M.G. Netea, N.P. Rixsen, The mineralocorticoid receptor as a modulator of innate immunity and atherosclerosis, *Cardiovasc. Res.* 114 (7) (2018) 944–953.
- [18] D. Cruz-Topete, J.A. Cidlowski, One hormone, two actions: anti- and pro-inflammatory effects of glucocorticoids, *Neuroimmunomodulation* 22 (1–2) (2015) 20–32.
- [19] H.J. Schwartz, F.C. Lowell, J.C. Melby, Steroid resistance in bronchial asthma, *Ann. Intern. Med.* 69 (3) (1968) 493–499.
- [20] M.C. Peters, S. Kerr, E.M. Dunican, P.G. Woodruff, M.L. Fajt, B.D. Levy, E. Israel, B.R. Phillips, D.T. Mauger, S.A. Comhair, S.C. Erzurum, M.W. Johansson, N. Jarjour, A.M. Coverstone, M. Castro, A.T. Hastie, E.R. Bleecker, S.E. Wenzel, J. V. Fahy, Refractory airway type 2 inflammation in a large subgroup of asthmatic patients treated with inhaled corticosteroids, *J. Allergy Clin. Immunol.* 143 (1) (2019), 104–113.e14.
- [21] D.M. Lang, Severe asthma: epidemiology, burden of illness, and heterogeneity, *Allergy Asthma Proc.* 36 (6) (2015) 418–424.
- [22] P.J. Barnes, Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease, *J. Allergy Clin. Immunol.* 131 (3) (2013) 636–645.
- [23] J. Carmichael, I.C. Paterson, P. Diaz, G.K. Crompton, A.B. Kay, I.W. Grant, Corticosteroid resistance in chronic asthma, *Br. Med. J. (Clin. Res. Ed.)* 282 (6274) (1981) 1419–1422.
- [24] P.J. Barnes, A.P. Greening, G.K. Crompton, Glucocorticoid resistance in asthma, *Am. J. Respir. Crit. Care Med.* 152 (6 Pt 2) (1995) S125–40.
- [25] G. Ozen, S. Pedro, F. Wolfe, K. Michaud, Medications associated with fracture risk in patients with rheumatoid arthritis, *Ann. Rheum. Dis.* 78 (8) (2019) 1041–1047.
- [26] P. Welsh, G. Grassia, S. Botha, N. Sattar, P. Maffia, Targeting inflammation to reduce cardiovascular disease risk: a realistic clinical prospect? *Br. J. Pharmacol.* 174 (22) (2017) 3898–3913.
- [27] R.S. Irwin, F.J. Curley, C.L. French, Difficult-to-control asthma. Contributing factors and outcome of a systematic management protocol, *Chest* 103 (6) (1993) 1662–1669.
- [28] L.G. Heaney, E. Conway, C. Kelly, B.T. Johnston, C. English, M. Stevenson, J. Gamble, Predictors of therapy resistant asthma: outcome of a systematic evaluation protocol, *Thorax* 58 (7) (2003) 561–566.
- [29] D.M. McNicholl, M. Stevenson, L.P. McGarvey, L.G. Heaney, The utility of fractional exhaled nitric oxide suppression in the identification of nonadherence in difficult asthma, *Am. J. Respir. Crit. Care Med.* 186 (11) (2012) 1102–1108.
- [30] L.G. Heaney, J. Busby, P. Bradding, R. Chaudhuri, A.H. Mansur, R. Niven, I. D. Pavord, J.T. Lindsay, R.W. Costello, Remotely monitored therapy and nitric oxide suppression identifies nonadherence in severe asthma, *Am. J. Respir. Crit. Care Med.* 199 (4) (2019) 454–464.
- [31] A. ten Brinke, A.H. Zwinderman, P.J. Sterk, K.F. Rabe, E.H. Bel, Factors associated with persistent airflow limitation in severe asthma, *Am. J. Respir. Crit. Care Med.* 164 (5) (2001) 744–748.
- [32] S.A. Little, K.J. MacLeod, G.W. Chalmers, J.G. Love, C. McSharry, N.C. Thomson, Association of forced expiratory volume with disease duration and sputum neutrophils in chronic asthma, *Am. J. Med.* 112 (6) (2002) 446–452.
- [33] N.C. Nicolaides, E. Charmandari, Novel insights into the molecular mechanisms underlying generalized glucocorticoid resistance and hypersensitivity syndromes, *Hormones (Athens)* 16 (2) (2017) 124–138.
- [34] H. Hakonarson, U.S. Bjornsdottir, E. Halapi, J. Bradfield, F. Zink, M. Mouy, H. Helgadottir, A.S. Gudmundsdottir, H. Andrason, A.E. Adalsteinsdottir, K. Kristjansson, I. Birkisson, T. Arnason, M. Andresdottir, D. Gislason, T. Gislason, J.R. Gulcher, K. Stefansson, Profiling of genes expressed in peripheral blood mononuclear cells predicts glucocorticoid sensitivity in asthma patients, *Proc. Natl. Acad. Sci. U.S.A.* 102 (41) (2005) 14789–14794.
- [35] K.G. Tantisira, J. Lasky-Su, M. Harada, A. Murphy, A.A. Litonjua, B.E. Himes, C. Lange, R. Lazarus, J. Sylvia, B. Klanderman, Q.L. Duan, W. Qiu, T. Hirota, F. D. Martinez, D. Mauger, C. Sorkness, S. Szefer, S.C. Lazarus, R.F. Lemanske Jr, S. P. Peters, J.J. Lima, Y. Nakamura, M. Tamari, S.T. Weiss, Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma, *N. Engl. J. Med.* 365 (13) (2011) 1173–1183.
- [36] O. Keskin, Ü. Uluca, E. Birben, Y. Coşkun, M.Y. Ozkars, M. Keskin, E. Kucukosmanoglu, O. Kalayci, Genetic associations of the response to inhaled corticosteroids in children during an asthma exacerbation, *Pediatr. Allergy Immunol.* 27 (5) (2016) 507–513.
- [37] M. Rijavec, M. Žavbi, A. Lopert, M. Fležar, P. Korošec, GLCCI1 polymorphism rs37973 and response to treatment of asthma with inhaled corticosteroids, *J. Invest. Allergol. Clin. Immunol.* 28 (3) (2018) 165–171.
- [38] C. Hu, Q. Xun, X. Li, R. He, R. Lu, S. Zhang, X. Hu, J. Feng, GLCCI1 variation is associated with asthma susceptibility and inhaled corticosteroid response in a Chinese Han population, *Arch. Med. Res.* 47 (2) (2016) 118–125.
- [39] S. Ramamoorthy, J.A. Cidlowski, Ligand-induced repression of the glucocorticoid receptor gene is mediated by an NCoR1 repression complex formed by long-range chromatin interactions with intragenic glucocorticoid response elements, *Mol. Cell. Biol.* 33 (9) (2013) 1711–1722.
- [40] N.L. Weigel, N.L. Moore, Steroid receptor phosphorylation: a key modulator of multiple receptor functions, *Mol. Endocrinol.* 21 (10) (2007) 2311–2319.
- [41] I.M. Adcock, S.J. Lane, C.R. Brown, T.H. Lee, P.J. Barnes, Abnormal glucocorticoid receptor-activator protein 1 interaction in steroid-resistant asthma, *J. Exp. Med.* 182 (6) (1995) 1951–1958.
- [42] I.M. Adcock, Glucocorticoid-regulated transcription factors, *Pulm. Pharmacol. Ther.* 14 (3) (2001) 211–219.
- [43] E. Iruen, J.G. Matthews, A. Takahashi, P.J. Barnes, K.F. Chung, I.M. Adcock, p38 Mitogen-activated protein kinase-induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid-insensitive asthma, *J. Allergy Clin. Immunol.* 109 (4) (2002) 649–657.
- [44] D.Y. Leung, R.J. Martin, S.J. Szefer, E.R. Sher, S. Ying, A.B. Kay, Q. Hamid, Dysregulation of interleukin 4, interleukin 5, and interferon gamma gene expression in steroid-resistant asthma, *J. Exp. Med.* 181 (1) (1995) 33–40.
- [45] D. Gurgone, L. McShane, C. McSharry, T.J. Guzik, P. Maffia, Cytokines at the interplay between asthma and atherosclerosis? *Front. Pharmacol.* 11 (2020) 166.
- [46] M.C. Peters, S.E. Wenzel, Intersection of biology and therapeutics: type 2 targeted therapeutics for adult asthma, *Lancet* 395 (10221) (2020) 371–383.
- [47] K. Ito, S. Yamamura, S. Essilfie-Quaye, B. Cosio, M. Ito, P.J. Barnes, I.M. Adcock, Histone deacetylase 2-mediated deacetylation of the glucocorticoid receptor enables NF-kappaB suppression, *J. Exp. Med.* 203 (1) (2006) 7–13.
- [48] J.J. Li, H.L. Tay, S. Maltby, Y. Xiang, F. Eyers, L. Hatchwell, H. Zhou, H.D. Toop, J.C. Morris, P. Nair, J. Mattes, P.S. Foster, M. Yang, MicroRNA-9 regulates steroid-resistant airway hyperresponsiveness by reducing protein phosphatase 2A activity, *J. Allergy Clin. Immunol.* 136 (2) (2015) 462–473.
- [49] R.D. Britt Jr, M.A. Thompson, S. Sasse, C.M. Pabelick, A.N. Gerber, Y.S. Prakash, Th1 cytokines TNF- α and IFN- γ promote corticosteroid resistance in developing human airway smooth muscle, *Am. J. Physiol. Lung Cell Mol. Physiol.* 316 (1) (2019) L71–L81.
- [50] R.Y. Kim, J.C. Horvat, J.W. Pinkerton, M.R. Starkey, A.T. Essilfie, J.R. Mayall, P. M. Nair, N.G. Hansbro, B. Jones, T.J. Haw, K.P. Sunkara, T.H. Nguyen, A. G. Jarnicki, S. Keely, J. Mattes, I.M. Adcock, P.S. Foster, P.M. Hansbro, MicroRNA-21 drives severe, steroid-insensitive experimental asthma by amplifying phosphoinositide 3-kinase-mediated suppression of histone deacetylase 2, *J. Allergy Clin. Immunol.* 139 (2) (2017) 519–532.
- [51] Y. Chung, S.H. Chang, G.J. Martinez, X.O. Yang, R. Nurieva, H.S. Kang, L. Ma, S. S. Watowich, A.M. Jetten, Q. Tian, C. Dong, Critical regulation of early Th17 cell differentiation by interleukin-1 signaling, *Immunity* 30 (4) (2009) 576–587.
- [52] J.F. Alcorn, C.R. Crowe, J.K. Kolls, TH17 cells in asthma and COPD, *Annu. Rev. Physiol.* 72 (2010) 495–516.
- [53] L. McKinley, J.F. Alcorn, A. Peterson, R.B. Dupont, S. Kapadia, A. Logar, A. Henry, C.G. Irvin, J.D. Piganelli, A. Ray, J.K. Kolls, TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice, *J. Immunol.* 181 (6) (2008) 4089–4097.
- [54] A. Vazquez-Tello, A. Semaili, J. Chakir, J.-G. Martin, D.Y. Leung, D.H. Eidelman, Q. Hamid, Induction of glucocorticoid receptor-beta expression in epithelial cells of asthmatic airways by T-helper type 17 cytokines, *Clin. Exp. Allergy* 40 (9) (2010) 1312–1322.
- [55] S. Ouyang, C. Liu, J. Xiao, X. Chen, A.C. Lui, X. Li, Targeting IL-17A/ glucocorticoid synergy to CSF3 expression in neutrophilic airway diseases, *JCI Insight* 5 (3) (2020).

- [56] H.Y. Kim, H.J. Lee, Y.J. Chang, M. Pichavant, S.A. Shore, K.A. Fitzgerald, Y. Iwakura, E. Israel, K. Bolger, J. Faul, R.H. DeKruyff, D.T. Umetsu, Interleukin-17-producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity, *Nat. Med.* 20 (1) (2014) 54–61.
- [57] C. Hawrylowicz, D. Richards, T.K. Loke, C. Corrigan, T. Lee, A defect in corticosteroid-induced IL-10 production in T lymphocytes from corticosteroid-resistant asthmatic patients, *J. Allergy Clin. Immunol.* 109 (2) (2002) 369–370.
- [58] E.J. Peek, D.F. Richards, A. Faith, P. Lavender, T.H. Lee, C.J. Corrigan, C. M. Hawrylowicz, Interleukin-10-secreting "regulatory" T cells induced by glucocorticoids and beta2-agonists, *Am. J. Respir. Cell Mol. Biol.* 33 (1) (2005) 105–111.
- [59] E. Xystrakis, S. Kusumakar, S. Boswell, E. Peek, Z. Urry, D.F. Richards, T. Adikibi, C. Pridgeon, M. Dallman, T.K. Loke, D.S. Robinson, F.J. Barrat, A. O'Garra, P. Lavender, T.H. Lee, C. Corrigan, C.M. Hawrylowicz, Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients, *J. Clin. Invest.* 116 (1) (2006) 146–155.
- [60] E. Livingston, C.E. Darroch, R. Chaudhuri, I. McPhee, A.D. McMahon, S. J. Mackenzie, N.C. Thomson, Glucocorticoid receptor alpha:beta ratio in blood mononuclear cells is reduced in cigarette smokers, *J. Allergy Clin. Immunol.* 114 (6) (2004) 1475–1478.
- [61] K. Ito, S. Lim, G. Caramori, K.F. Chung, P.J. Barnes, I.M. Adcock, Cigarette smoking reduces histone deacetylase 2 expression, enhances cytokine expression, and inhibits glucocorticoid actions in alveolar macrophages, *FASEB J.* 15 (6) (2001) 1110–1112.
- [62] B.G. Cosío, B. Mann, K. Ito, E. Jazrawi, P.J. Barnes, K.F. Chung, I.M. Adcock, Histone acetylase and deacetylase activity in alveolar macrophages and blood monocytes in asthma, *Am. J. Respir. Crit. Care Med.* 170 (2) (2004) 141–147.
- [63] S. Rogers, A.R. de Souza, M. Zago, M. Iu, N. Guerrina, A. Gomez, J. Matthews, C. J. Baglolo, Aryl hydrocarbon receptor (AhR)-dependent regulation of pulmonary miRNA by chronic cigarette smoke exposure, *Sci. Rep.* 7 (2017) 40539.
- [64] C. McSharry, M. Spears, R. Chaudhuri, E.J. Cameron, H. Husi, N.C. Thomson, Increased sputum endotoxin levels are associated with an impaired lung function response to oral steroids in asthmatic patients, *J. Allergy Clin. Immunol.* 134 (5) (2014) 1068–1075.
- [65] E.H. Bel, Smoking: a neglected cause of glucocorticoid resistance in asthma, *Am. J. Respir. Crit. Care Med.* 168 (11) (2003) 1265–1266.
- [66] N.G. Hansbro, J.C. Horvat, P.A. Wark, P.M. Hansbro, Understanding the mechanisms of viral induced asthma: new therapeutic directions, *Pharmacol. Ther.* 117 (3) (2008) 313–353.
- [67] A.L. Durham, G. Caramori, K.F. Chung, I.M. Adcock, Targeted anti-inflammatory therapeutics in asthma and chronic obstructive lung disease, *Transl. Res.* 167 (1) (2016) 192–203.
- [68] C. Pelaia, A. Vatrella, A. Sciacqua, R. Terracciano, G. Pelaia, Role of p38-mitogen-activated protein kinase in COPD: pathobiological implications and therapeutic perspectives, *Expert Rev. Respir. Med.* 14 (5) (2020) 485–491.
- [69] P.J. Barnes, Inflammatory mechanisms in patients with chronic obstructive pulmonary disease, *J. Allergy Clin. Immunol.* 138 (1) (2016) 16–27.
- [70] S. Al Heialy, M. Gaudet, R.K. Ramakrishnan, A. Mogas, L. Salameh, B. Mahboub, Q. Hamid, Contribution of IL-17 in steroid hyporesponsiveness in obese asthmatics through dysregulation of glucocorticoid receptors α and β , *Front. Immunol.* 11 (2020) 1724.
- [71] Z. Wang, H. Liu, F. Wang, Y. Yang, X. Wang, B. Chen, M.R. Stampfli, H. Zhou, W. Shu, C.E. Brightling, Z. Liang, R. Chen, A refined view of airway microbiome in chronic obstructive pulmonary disease at species and strain-levels, *Front. Microbiol.* 11 (2020) 1758.
- [72] A.T. Essilfie, J.L. Simpson, J.C. Horvat, J.A. Preston, M.L. Dunkley, P.S. Foster, P. G. Gibson, P.M. Hansbro, Haemophilus influenzae infection drives IL-17-mediated neutrophilic allergic airways disease, *PLoS Pathog.* 7 (10) (2011), e1002244.
- [73] J. Beale, A. Jayaraman, D.J. Jackson, J.D.R. Macintyre, M.R. Edwards, R. P. Walton, J. Zhu, Y. Man Ching, B. Shamji, M. Edwards, J. Westwick, D. J. Cousins, Y. Yi Hwang, A. McKenzie, S.L. Johnston, N.W. Bartlett, Rhinovirus-induced IL-25 in asthma exacerbation drives type 2 immunity and allergic pulmonary inflammation, *Sci. Transl. Med.* 6 (256) (2014), 256ra134.
- [74] X. Yang, Y. Wang, S. Zhao, R. Wang, C. Wang, Long-term exposure to low-dose Haemophilus influenzae during allergic airway disease drives a steroid-resistant neutrophilic inflammation and promotes airway remodeling, *Oncotarget* 9 (38) (2018) 24898–24913.
- [75] D. Paróczai, T. Mosolygó, D. Kócai, V. Endrész, D.P. Virok, A. Somfay, K. Burián, Chlamydia pneumoniae influence on cytokine production in steroid-resistant and steroid-sensitive asthmatics, *Pathogens* 9 (2) (2020).
- [76] A. Papi, M. Contoli, I.M. Adcock, C. Bellettato, A. Padovani, P. Casolari, L. A. Stanciu, P.J. Barnes, S.L. Johnston, K. Ito, G. Caramori, Rhinovirus infection causes steroid resistance in airway epithelium through nuclear factor κ B and c-Jun N-terminal kinase activation, *J. Allergy Clin. Immunol.* 132 (5) (2013), 1075–1085.e6.
- [77] J. Reidl, E. Monsó, Glucocorticoids and antibiotics, how do they get together? *EMBO Mol. Med.* 7 (8) (2015) 992–993.
- [78] S. Fakhri, M. Tulic, P. Christodoulouopoulos, M. Fukakusa, S. Frenkiel, D.Y. Leung, Q.A. Hamid, Microbial superantigens induce glucocorticoid receptor beta and steroid resistance in a nasal explant model, *Laryngoscope* 114 (5) (2004) 887–892.
- [79] R.Y. Kim, J.W. Pinkerton, A.T. Essilfie, A.G.B. Robertson, K.J. Baines, A.C. Brown, J.R. Mayall, M.K. Ali, M.R. Starkey, N.G. Hansbro, J.A. Hirota, L.G. Wood, J. L. Simpson, D.A. Knight, P.A. Wark, P.G. Gibson, L.A.J. O'Neill, M.A. Cooper, J. C. Horvat, P.M. Hansbro, Role for NLRP3 inflammasome-mediated, IL-1 β -Dependent responses in severe, steroid-resistant asthma, *Am. J. Respir. Crit. Care Med.* 196 (3) (2017) 283–297.
- [80] Z. Zhang, J.M. Biagini Myers, E.B. Brandt, P.H. Ryan, M. Lindsey, R.A. Mintz-Cole, T. Reponen, S.J. Vesper, F. Forde, B. Ruff, S.A. Bass, G.K. LeMasters, D. I. Bernstein, J. Lockey, A.L. Budelsky, G.K. Khurana Hershey, β -Glucan exacerbates allergic asthma independent of fungal sensitization and promotes steroid-resistant T(H)2/T(H)17 responses, *J. Allergy Clin. Immunol.* 139 (1) (2017), 54–65.e8.
- [81] S. Castanhinha, R. Sherburn, S. Walker, A. Gupta, C.J. Bossley, J. Buckley, N. Ullmann, R. Grychtol, G. Campbell, M. Maglione, S. Koo, L. Fleming, L. Gregory, R.J. Snelgrove, A. Bush, C.M. Lloyd, S. Saglani, Pediatric severe asthma with fungal sensitization is mediated by steroid-resistant IL-33, *J. Allergy Clin. Immunol.* 136 (2) (2015), 312–22.e7.
- [82] K. Hirahara, N. Mato, K. Hagiwara, T. Nakayama, The pathogenicity of IL-33 on steroid-resistant eosinophilic inflammation via the activation of memory-type ST2(+) CD4(+) T cells, *J. Leukoc. Biol.* 104 (5) (2018) 895–901.
- [83] P.J. Bray, R.G. Cotton, Variations of the human glucocorticoid receptor gene (NR3C1): pathological and in vitro mutations and polymorphisms, *Hum. Mutat.* 21 (6) (2003) 557–568.
- [84] J. Zhou, D.F. Liu, C. Liu, Z.M. Kang, X.H. Shen, Y.Z. Chen, T. Xu, C.L. Jiang, Glucocorticoids inhibit degranulation of mast cells in allergic asthma via nongenomic mechanism, *Allergy* 63 (9) (2008) 1177–1185.
- [85] E. Ayroldi, L. Cannarile, G. Migliorati, G. Nocentini, D.V. Delfino, C. Riccardi, Mechanisms of the anti-inflammatory effects of glucocorticoids: genomic and nongenomic interference with MAPK signaling pathways, *FASEB J.* 26 (12) (2012) 4805–4820.
- [86] E. Goleva, L.B. Li, P.T. Eves, M.J. Strand, R.J. Martin, D.Y. Leung, Increased glucocorticoid receptor beta alters steroid response in glucocorticoid-insensitive asthma, *Am. J. Respir. Crit. Care Med.* 173 (6) (2006) 607–616.
- [87] N. MacRitchie, M. Frleta-Gilchrist, A. Sugiyama, T. Lawton, I.B. McInnes, P. Maffia, Molecular imaging of inflammation - current and emerging technologies for diagnosis and treatment, *Pharmacol. Ther.* 211 (2020), 107550.
- [88] S.D. Harding, E. Faccenda, C. Southan, P. Maffia, J.A. Davies, A new guide to immunopharmacology, *Nat. Rev. Immunol.* 18 (12) (2018) 729.
- [89] S.D. Harding, E. Faccenda, C. Southan, A.J. Pawson, P. Maffia, S.P.H. Alexander, A.P. Davenport, D. Fabbro, F. Levi-Schaffer, M. Spedding, J.A. Davies, The IUPHAR Guide to immunopharmacology: connecting immunology and pharmacology, *Immunology* 160 (1) (2020) 10–23.
- [90] R.M. Elbehidy, D.M. Youssef, A.S. El-Shal, S.M. Shalaby, H.S. Sherbiny, L. M. Sherief, N.E. Akeel, MicroRNA-21 as a novel biomarker in diagnosis and response to therapy in asthmatic children, *Mol. Immunol.* 71 (2016) 107–114.
- [91] H. Jiang, X. Chi, X. Zhang, J. Wang, Increased serum VDBP as a risk predictor for steroid resistance in asthma patients, *Respir. Med.* 114 (2016) 111–116.
- [92] Y.H. Park, A.M. Fitzpatrick, C.A. Medriano, D.P. Jones, High-resolution metabolomics to identify urine biomarkers in corticosteroid-resistant asthmatic children, *J. Allergy Clin. Immunol.* 139 (5) (2017), 1518–1524.e4.
- [93] S.L. Ma, L. Zhang, Elevated serum OX40L is a biomarker for identifying corticosteroid resistance in pediatric asthmatic patients, *BMC Pulm. Med.* 19 (1) (2019) 66.
- [94] P.J. Barnes, I.M. Adcock, Glucocorticoid resistance in inflammatory diseases, *Lancet* 373 (9678) (2009) 1905–1917.
- [95] J.M. Rodriguez, M. Monsalves-Alvarez, S. Henriquez, M.N. Llanos, R. Troncoso, Glucocorticoid resistance in chronic diseases, *Steroids* 115 (2016) 182–192.
- [96] M. Spears, I. Donnelly, L. Jolly, M. Brannigan, K. Ito, C. McSharry, J. Lafferty, R. Chaudhuri, G. Braganza, I.M. Adcock, P.J. Barnes, S. Wood, N.C. Thomson, Effect of low-dose theophylline plus bclomethasone on lung function in smokers with asthma: a pilot study, *Eur. Respir. J.* 33 (5) (2009) 1010–1017.
- [97] N. Mercado, A. Hakim, Y. Kobayashi, S. Meah, O.S. Usmani, K.F. Chung, P. J. Barnes, K. Ito, Restoration of corticosteroid sensitivity by p38 mitogen activated protein kinase inhibition in peripheral blood mononuclear cells from severe asthma, *PLoS One* 7 (7) (2012), e41582.
- [98] J. Bi, Z. Min, H. Yuan, Z. Jiang, R. Mao, T. Zhu, C. Liu, Y. Zeng, J. Song, C. Du, Z. Chen, PI3K inhibitor ameliorates the glucocorticoid insensitivity of PBMCs in severe asthma, *Clin. Transl. Med.* 9 (1) (2020) 22.
- [99] O.S. Usmani, K. Ito, K. Maneechotesuwan, M. Ito, M. Johnson, P.J. Barnes, I. M. Adcock, Glucocorticoid receptor nuclear translocation in airway cells after inhaled combination therapy, *Am. J. Respir. Crit. Care Med.* 172 (6) (2005) 704–712.
- [100] N. Mercado, Y. To, Y. Kobayashi, I.M. Adcock, P.J. Barnes, K. Ito, p38 mitogen-activated protein kinase- γ inhibition by long-acting β 2 adrenergic agonists reversed steroid insensitivity in severe asthma, *Mol. Pharmacol.* 80 (6) (2011) 1128–1135.
- [101] J.D. Spahn, D.A. Foster, R. Covar, R.J. Martin, E.E. Brown, S.J. Szefer, D.Y. Leung, Clarithromycin potentiates glucocorticoid responsiveness in patients with asthma: results of a pilot study, *Ann. Allergy Asthma Immunol.* 87 (6) (2001) 501–505.
- [102] P.G. Gibson, I.A. Yang, J.W. Upham, P.N. Reynolds, S. Hodge, A.L. James, C. Jenkins, M.J. Peters, G.B. Marks, M. Baraket, H. Powell, S.L. Taylor, L.E. X. Leong, G.B. Rogers, J.L. Simpson, Effect of azithromycin on asthma exacerbations and quality of life in adults with persistent uncontrolled asthma (AMAZES): a randomised, double-blind, placebo-controlled trial, *Lancet* 390 (10095) (2017) 659–668.
- [103] A.T. Essilfie, J.C. Horvat, R.Y. Kim, J.R. Mayall, J.W. Pinkerton, E.L. Beckett, M. R. Starkey, J.L. Simpson, P.S. Foster, P.G. Gibson, P.M. Hansbro, Macrolide

- therapy suppresses key features of experimental steroid-sensitive and steroid-insensitive asthma, *Thorax* 70 (5) (2015) 458–467.
- [104] K. Maneechotesuwan, W. Ekjitrakul, K. Kasetsinsombat, A. Wongkajornsilp, P. J. Barnes, Statins enhance the anti-inflammatory effects of inhaled corticosteroids in asthmatic patients through increased induction of indoleamine 2, 3-dioxygenase, *J. Allergy Clin. Immunol.* 126 (4) (2010), 754-762.e1.
- [105] Y. He, J. Shi, Q.T. Nguyen, E. You, H. Liu, X. Ren, Z. Wu, J. Li, W. Qiu, S.K. Khoo, T. Yang, W. Yi, F. Sun, Z. Xi, X. Huang, K. Melcher, B. Min, H.E. Xu, Development of highly potent glucocorticoids for steroid-resistant severe asthma, *Proc. Natl. Acad. Sci. U.S.A.* 116 (14) (2019) 6932–6937.