



Glucocorticosteroid subsensitivity and asthma severity

Manali Mukherjee, Sarah Svenningsen, and Parameswaran Nair

Purpose of review

Glucocorticosteroids (GCSs) remain the cornerstone of therapy for treating the inflammatory component of asthma. Clinical response to GCS is heterogeneous, varying both within asthma 'endotypes', as well as the same individual. Different factors and micro-environment can alter the canonical GCS-induced signalling pathways leading to reduced efficacy, collectively termed as GCS subsensitivity, which includes the entire spectrum of steroid insensitivity and steroid resistance.

Recent findings

In the past, steroid subsensitivity has been associated with dysregulated expression of glucocorticoid-receptor isoforms, neutrophilic inflammation and Th17 cytokines, oxidative stress-inducing factors and their downstream effect on histone deacetylase activities and gene expression. The review highlights recent observations, such as GCS-induced dysregulation of key transcription factors involved in host defence, role of airway infections altering expression of critical regulatory elements like the noncoding microRNAs, and the importance of interleukin (IL)-10 in reinstating steroid response in key immune cells. Further, emerging concepts of autoimmunity triggered because of delayed resolution of eosinophilic inflammation (due to GCS subsensitivity) and observed lymphopenia (plausibly a side-effect of continued GCS use) are discussed.

Summary

This review bridges concepts that have been known, and those under current investigation, providing both molecular and clinical insights to aid therapeutic strategies for optimal management of asthmatics with varying degree of steroid subsensitivity and disease severity, with particular emphasis on the PI3 kinase pathways.

Keywords

airway microbiome, microRNA, severe asthma, sputum eosinophils, steroid insensitivity

INTRODUCTION

As early as 1949, it was evident that asthma is responsive to glucocorticosteroids (GCS) [1]. Since then, corticosteroids have remained the mainstay treatment for asthma. However, there is considerable patient-to-patient variability in clinical response to both inhaled (ICS) and oral (OCS) GCS therapy. The earliest description of steroid-resistance in asthma was in 1968 in a small group of six patients [2], and thereafter in 1981 in 58 patients [3]. Since then, the definition of steroid response in asthma has been revised several times. The nominalist definition identifies 'asthma' as a disease characterized by abnormal airway function with episodic variable airway flow limitations [4]. The eosinophilic bronchitis component, contributing to airway dysfunction is targeted by GCS therapy, includes approximately 40–60% of the asthmatic population [4,5]. Approximately, 10% of this population requires high doses of ICS

(≥ 2000 mcg of fluticasone propionate or equivalent) and approximately 1% needs daily treatment with OCS to maintain adequate asthma control [6]. A subset of this glucocorticoid-dependent population who remain poorly controlled despite maximal treatment or experience worsening of asthma control during corticosteroid-discontinuation/dose-reduction are considered 'corticosteroid insensitive'. These patients experience sporadic exacerbations that require prednisone 'bursts' or

Department of Medicine, McMaster University & St Joseph's Healthcare, Hamilton, Ontario, Canada

Correspondence to Dr Professor Parameswaran Nair, MD, PhD, FRCP, FRCP, Firestone Institute for Respiratory Health, St Joseph's Healthcare, 50 Charlton Avenue East, Hamilton, Ontario L8N 4A6, Canada. Tel: +1 905 522 1155 x 35044; fax: +1 905 521 6183; e-mail: parames@mcmaster.ca

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KEY POINTS

- GCS 'subsensitivity' collectively encompasses patients who are steroid resistant and steroid insensitive.
- Patients with poor clinical response to GCS therapy are no longer restricted to the noneosinophilic asthma subset but includes diverse asthma endotypes, viz., eosinophilic or Th2 dominant or those with a predominant neutrophilic inflammation (Th17 endotype), or those with a dual Th2^{low}/Th17^{high} signature.
- Different underlying mechanisms contribute collectively to the heterogeneity observed in the clinical response to GCS in different endotypes based on the micro-environment and stimuli/factor, ranging from lifestyle influences like smoking, obesity; disease-associated factors like recurrent infections, delayed resolution of Th2 inflammation; GCS-induced changes in molecular machinery; or a combination of them.
- Novel molecular pathways underlying corticosteroid subsensitivity have been elucidated resulting in novel therapeutic options such as inhibiting PI3 kinase activity and specific microRNA antagomirs are being considered in preclinical models that hinder the downstream regulation of infection-induced dysregulated microRNAs.
- Via the identification of underlying aberrant mechanisms, emerging therapeutic strategies have been outlined that can overcome corticosteroid subsensitivity (steroid-sparing anti-Th2 cytokine mAbs), restore corticosteroid sensitivity (e.g., IL-10 immunotherapy, phosphodiesterase inhibitors) or intervene within impaired pathways (e.g. inhibitors for PI3k α , p38MAPK, or anti-IL23 mAb for inhibiting Th17 pathway).

intravenous steroid, and often suffer from associated systemic side-effects. On the extreme end of this spectrum lies a distinct population of eosinophilic asthmatics who do not respond to GCS therapy (steroid resistant), clinically defined as <15% improvement in forced expiratory volume in 1 s (FEV₁) from baseline following 14 days of OCS (40 mg/daily) therapy, while demonstrating marked bronchodilator response to β_2 -agonists. And finally, the steroid nonresponsive patients who do not chart any clinical improvement on ICS/OCS, since their disease severity is driven by elements that are beyond the pharmacologic realm of GCS therapy, for example, asthmatics without an airway inflammatory component that may not be associated with a cellular response composed of either eosinophils, lymphocytes, basophils or mast cells. Thus, treatment response to steroid is heterogeneous, as is the disease itself.

Suboptimal response to GCS has been long considered as an abnormal GCS pharmacokinetic profile, that is changes in the absorption, distribution, metabolism or rate of clearance of prednisone [7,8]. Factors ranging from abnormal methylation of the drug to patient-specific factors such as obesity has been linked with defective GCS-PK and suboptimal GCS response [9[•]]. Early studies show the use of depot methyl prednisolone to be effective in steroid-insensitive patients labelled as 'fatal asthma' [10]. On the contrary, no detectable differences in prednisone clearance rate or the number/binding affinity of the cellular receptors between GCS-sensitive and -resistant asthmatics have been reported [11]. Clinical investigations with sensitive mass spectrometry assays of serum and airway glucocorticoid levels are necessary to fully understand the role of pharmacokinetics to steroid insensitivity in severe asthma and this is beyond the scope of this review.

Since the presence of eosinophils was considered to be an ideal predictor of GCS response in asthma [12], steroid insensitivity was associated with noneosinophilic or 'neutrophilic' asthma [13]. However, heterogeneity in GCS response within the eosinophilic asthmatic population suggests disease-associated mechanisms that could induce/sustain/worsen steroid sensitivity within the population and in the same individual over the course of time. This is particularly evident in those who remain 'uncontrolled' on high dose OCS, in addition to marked reduction in GCS response during infective exacerbations, and/or incremental increase in maintenance GCS dose for maintaining adequate asthma control. Indeed, increased GCS requirement is an index of asthma severity, and increase in maintenance dose marks disease progression. Taken together, asthmatics demonstrating steroid 'subsensitivity', a term used to represent 'reduced GCS response than the expected maximal', is difficult to manage clinically and contributes to approximately 80% of the costs associated with asthma [14–16].

HETEROGENEITY OF GLUCOCORTICOSTEROID RESPONSE WITHIN THE ASTHMA POPULATION

The underlying mechanisms that determine 'subsensitivity' vary between different cell types (reviewed in [17^{••}]) and logically, remains heterogeneous within the 'endotypes' of asthma, stratified by the dominant immune cell type (inflammatory mechanism) [18]. The eosinophilic or T-helper type 2 cell dominant (Th2^{high}) asthma endotype characterized by eosinophilia and increased Th2

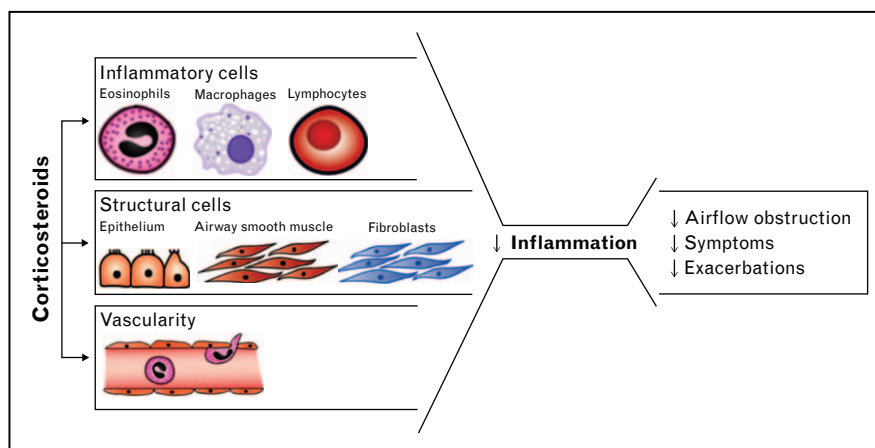


FIGURE 1. Effect of corticosteroids. Glucocorticosteroids act on diverse cell types as depicted in the figure and cause an overall reduction in inflammation. Reduced inflammation results in reduced clinical symptoms that are usually measured as three clinical outcomes – reduction in rate of exacerbation, reduction in symptoms and improvement in airflow obstruction. These outcomes are traditionally used to define ‘steroid sensitivity’ or ‘insensitivity’.

inflammatory cytokines (IL-4, IL-5, IL-13) is widely regarded as corticosteroid responsive [19]. However, 10–20% of Th2^{high} asthmatics demonstrate steroid insensitivity. GCS treatment has been established as relatively ineffective in noneosinophilic asthma [20], typically characterized by low eosinophil sputum counts [13], high airway neutrophil counts and Th17 cytokines (usually indicative of recurrent airway infections) [21]. Recently, two mutually exclusive subsets of Th17^{high} and Th2^{low}/Th17^{high} populations were identified within a moderate-to-severe predominantly ‘eosinophilic’ population without any significant difference in GCS use. The Th17 signature in absence of potent neutrophilic inflammation was reported to be induced as a result of therapeutic blockade of Th2 inflammation [22[■]]. In fact, poor GCS response is predominantly associated with Th17 asthma [23,24]. It is not known if the Th17 inflammation directly contributes to the steroid insensitivity in clinical scenarios. In addition to inflammatory cells, the response of structural cells and the vasculature to corticosteroids affect the clinical outcomes that determine ‘subsensitivity’, and are schematically represented in Fig. 1. Therefore, this review will focus on our current understanding of asthma-related microenvironments that alter downstream mechanisms and lead to GCS ‘subsensitivity’ in different endotypes, and the therapeutic strategies available or under development to overcome subsensitivity.

GLUCOCORTICOSTEROID MECHANISM OF ACTION

Before delving into the altered molecular signalling that leads to GCS subsensitivity, it is important to

review the diverse mechanisms of action by which GCS exert their downstream effect (reviewed extensively in [6,17[■],25]). Briefly, exogenously administered glucocorticoid diffuses into the cytoplasm and binds to the α -subunit of glucocorticoid receptor. The binding breaks off complexed chaperone proteins, and results in subsequent homodimerization and nuclear translocation of the GCS–GR complex via nuclear import proteins (importins). The homodimers bind to glucocorticoid responsive elements (GREs) in the 3' UTR promoter region of responsive genes resulting in ‘transactivation’ (increased expression) of anti-inflammatory genes such as mitogen-activated protein kinase [MAPK] phosphatase-1 (MKP-1), I κ B α (NFKBIA), glucocorticoid-induced leucine zipper (GILZ), cytokine IL-10 and annexin A1 (ANXA1) (reviewed in [26]). Alternately, homomeric ligand-bound glucocorticoid receptor inhibit the expression of proinflammatory genes via protein–protein interaction of DNA-bound transcription factors like nuclear-factor kappaB (NF- κ B) and activator protein-1 (AP-1), a phenomenon termed as ‘transrepression’. Upon ligand binding, glucocorticoid receptors become acetylated which helps in histone deacetylase (HDAC) recruitment. The ligand-bound glucocorticoid receptor binds to GREs (in the NF- κ B activated inflammatory gene complex), and thereby directs deacetylation of the target genes by recruited HDAC2 leading to repression of the inflammatory genes [27,28]. Downstream effects of glucocorticoid receptor activity can also produce secondary post-transcriptional and translation effects. For instance, glucocorticoid receptor transactivation can increase gene expression of proteins like tristetraprolin (TTP) that destabilizes mRNAs of inflammatory proteins

such as IL-2, granulocyte/macrophage colony-stimulating factor (GM-CSF) and tumour-necrosis factor- α (TNF α) [29,30]. The nongenomic mechanisms of GCS action include rapid activation of cellular pathways including extracellular signal-regulated kinases (ERK), p38MAPK, SRC kinase, and phosphoinositide-3 (PI3) kinase, in diverse cell types, both inflammatory and structural.

MECHANISMS OF STEROID SUBSENSITIVITY

Mechanisms of steroid resistance/insensitivity have been extensively reviewed elsewhere [6,17²²,25,31,32]. It is to be noted that steroid subsensitivity is considered after at least 2 weeks of a reasonable dose of corticosteroids that the patient has adhered to. Optimal dose is arbitrary and varies from 15 to 40 mg daily.

Genetic factors

There is uncertainty around the evidence of the association between genetic variants and corticosteroid response. Although genetic abnormalities in the glucocorticoid receptor of GCS-resistant asthmatics were not evident in a thorough mutational analysis of the glucocorticoid receptor complementary DNA [33], a particular polymorphism in the glucocorticoid receptor (GR β isoform) has been linked with reduced transrepressional response which could explain the loss in therapeutic efficacy [34]. Over the years, single nucleotide polymorphisms (SNPs) with altered response to GCS therapy have been reported in asthma (reviewed in [32]). Polymorphisms in the importin 13 gene, involved in nuclear translocation of the glucocorticoid receptor-GCS complex was associated with improved airway hyper-responsiveness (AHR) in asthmatic children [35]. However, another paediatric asthma study failed to show an association with reported SNPs and dose of GCS required to achieve asthma control [36]. Importantly, the largest and the most recent analysis showed no association of genetic variants or reported SNPs with clinical response (changes in FEV₁) following 8–12 weeks of ICS therapy [37²³].

Increased expression of GR β isoforms

GR β isoform is nonligand (GCS) binding and is considered to be a dominant-negative inhibitor of GR α that competitively binds GRE and influences transactivation. Increased expression of GR β in airway immune cells and concurrent reduced nuclear translocation of GR α (and in response to steroids, *in vitro*) were documented in asthmatics with marked

GCS insensitivity. GR α nuclear translocation and subsequent transactivation were proportionately reduced by the level of viral transduction of GR β gene, while transient silencing of GR β *ex vivo* resulted in enhanced steroid-induced GR α activity [38]. Further clinical trials are required to establish the clinical relevance of these ex-vivo observations.

Factors altering receptor biology

Clinically relevant factors such as allergen exposure [39], lifestyle influences like obesity [40] and smoking [41], can directly influence clinical response to GCS by altering receptor biology. We will briefly discuss the different asthma-related stimuli/micro-environment that can modulate GCS activity and plausible mitigating strategies (schematically represented in Fig. 2).

Nitric oxide and glucocorticoid receptor nitrosylation

High levels of nitric oxide generated *in situ* as a result of eosinophilic inflammation can cause nitrosylation of the glucocorticoid receptor at HSP90 (chaperone) binding site, and decrease the affinity for chaperone proteins that protect it from cytoplasmic degradation. Nitrosylation also reduces the binding affinity to GCS (ligand) in structural cells like fibroblasts [42]. Asthmatics with persistent airway eosinophilia with increased localized nitric oxide production and possibly increased remodelling may become steroid insensitive through repeated nitrosylation of glucocorticoid receptor.

Th2 cytokines and induction of alternate pathways

Allergen-exposure to ragweed causes heightened IL-2/IL-4 expression which affects GCR-binding

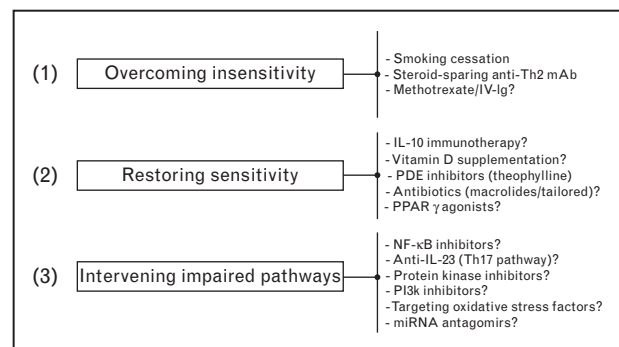


FIGURE 2. Therapeutic strategies to overcome corticosteroid insensitivity, restore corticosteroid sensitivity and to improve corticosteroid subsensitivity in asthma. IV-Ig: intravenous immunoglobulin; NF- κ B: nuclear factor kappaB; PDE: phosphodiesterase; PI3k: phosphoinositide 3-kinase; PPAR γ : peroxisome proliferator-activated receptor- γ .

affinity in peripheral blood mononuclear cells (PBMCs) [39]. This can be explained by the IL-2/IL-4-induced suppression of GR α (and not GR β) expression in PBMCs [43]. Again, IL-2 and IL-4 synergistically reduce nuclear translocation and binding affinity in T-cells, orchestrated via the p38MAPK pathway; this effect can be reversed by a p38 inhibitor [44]. p38MAPK activity was demonstrated to be higher in alveolar macrophage from patients with poor response to GCS compared to 'responders'. The expression of MKP-1 (DUSP1 gene), an endogenous inhibitor of the MAPK pathway, was significantly reduced in alveolar macrophages after GCS exposure, leading to increase in p38MAPK activity [45]. The study also showed that dexamethasone could not suppress lipopolysaccharide (LPS)-induced release of pro-inflammatory cytokines. Indeed, IL-2 and IL-4 overexpression were reported in the airways of asthmatics with poor response to GCS, the levels of which could not be attenuated with 1 week of 40 mg daily prednisone therapy [46]. Another Th2 cytokine, IL-13, alone diminished GCR binding affinity in the non-T-cell fraction of PBMCs. Further, the effect of hydrocortisone in suppressing LPS-induced IL-6 production by monocytes was demonstrated to be significantly hindered when the cells were primed by IL-13 [47]. IL-5-primed eosinophils were protected against GCS-induced apoptosis via synergistic upregulation of nuclear-factor IL-3 (NFIL3) due to a cross-talk between GCS-induced trans-activation signalling and IL-5 antiapoptotic pathway, thereby posing as a possible explanation of steroid insensitivity observed in severe eosinophilic asthma with increased prevalence of IL-5 activated eosinophils [48^{***}].

Th1 cytokines and glucocorticoid receptor phosphorylation

Phosphorylation of glucocorticoid receptor can reduce its ligand-binding ability and subsequent nuclear translocation. Th1 cytokines like TNF α and interferon-gamma (IFN γ) cause protein phosphatase 5 (PP5) induction in airway smooth muscles, thereby impairing glucocorticoid receptor phosphorylation. Blockade of calcium channels (K_{Ca}3.1) suppresses cytokine-induced PP5 expression and restores fluticasone-induced GR α phosphorylation at serine 211 [49]. In fact, phosphorylation at serine 211 regulates one of the most important glucocorticoid receptor-induced anti-inflammatory proteins GILZ-1 (reviewed in [50]). TNF α was also shown to activate the c-Jun N-terminal kinase (JNK), that directly phosphorylated GR α at Ser226, which inhibited GRE-binding [51].

Altered microbiome and microRNA regulation

In a mouse model of steroid-resistant asthma, concomitant administration of LPS+IFN γ (that mimicked airway infection) attenuated PP2A activity (that induced JNK), led to phosphorylation of GR α at Ser226, thereby disrupting glucocorticoid receptor nuclear translocation in pulmonary macrophages. It was established that the attenuation of PP2A activity was orchestrated by LPS+IFN γ -induced increase in expression of a small noncoding miR9 which regulates the regulatory unit of the enzyme (PPP2R5D) [52^{***}]. In fact, a localized increase in miR9 was further confirmed in the sputum of neutrophilic asthmatics with high total cell count (indicative of infection) compared with eosinophilic asthmatics [52^{***}]. As further evidence, increased sputum endotoxin levels were recently reported to be directly associated with impaired lung-function response to OCS therapy [53]. A steroid-insensitive allergic mouse model was engineered by serial infections with *Chlamydia*, *Haemophilus*, influenza and respiratory syncytial virus postovalbumin challenge [54]. Unlike the LPS+IFN γ g mice [52^{***}], this model charted an increase in miR21 that was associated with increased AHR, and steroid-insensitive airway inflammation with exaggerated Th1/Th17 response, mediated by PI3k. A recent study reports Proteobacteria species in the airway microbiome to be associated with Th17 genes, worsening asthma scores and high sputum total leukocyte count [55^{*}].

Th17 cytokines and increased GR β

IL-17 and IL-23 cytokines significantly upregulate GR β , which can compete with the functional GR α (as discussed above). In combination, they antagonize dexamethasone-induced transrepression gene activity. Th17 cells produce IL-17A, IL-17F, IL-21, and IL-22. Among these cytokines, IL-17A and IL-17F have critical roles in host defense and infection [56]. Of interest, in a chronic allergic model, eosinophilia predominated in acute inflammation, while neutrophilia and IL-17 increased in chronic disease [57], which agreed with the Th2^{low}/Th17^{high} endotype reported by Choy *et al.* [22^{***}]. The former showed abrogation of IL-10/T-regulatory cells in a Th17 micro-environment. Chambers *et al.* [58^{***}] demonstrated reduced IL-17 production by lymphocytes isolated from steroid-insensitive asthmatics by exogenous addition of IL-10, *in-vitro*. Another study showed that anti-IL-17 therapy can augment dexamethasone anti-inflammatory action in diesel-exhaust particle-induced neutrophilic steroid insensitivity [59].

GCS subsensitivity and reduced host defense

Many innate and host defense factors are spared from the repressive activity of GCS. Importantly, GCS can aid host defense by increasing scavenging activity, expression of antimicrobial and antiviral proteins, complement factors, etc., in monocytes [60] and PBMCs [61]. In fact, increased expression of MKP-1, and upregulation of IRF-1 transcription factor is considered to be GCS induced [62]. IRF-1 regulates CXCL10 and IL-12 expression for combating viral infections [63[■]]. In fact, reduced MKP-1 expression, with increased MAPK activity was reported in AVMs isolated from severe asthmatics with steroid subsensitivity [45]. Hence, an altered GCS pathway can lead to repression of genes involved in host defense, and could explain the poor response to steroids in asthma exacerbations. Indeed, GCS-insensitive eosinophilic asthmatics with recurrent infections was associated with poor lung function and higher severity indices [64[■]].

Oxidative stress, PI3 kinase activity and reduced HDAC2

Oxidative stress from smoking leading to downstream reduction of HDAC2 activity has been well described as a mechanism of steroid resistance in chronic obstructive pulmonary disease (COPD), reviewed extensively elsewhere [28]. PBMCs isolated from prednisone-dependent asthmatics showed increased phosphorylation of AKT (increased PI3k-delta activity) and reduced total HDAC activity compared to ICS-maintained moderate asthmatics and healthy volunteers [65[■]]. Phospho-Akt leads to phosphorylation of HDAC which then goes through proteasome-mediated degradation [31]. HDAC is also reported to be involved in the regulation of macrophage scavenger receptors [66]. Of interest, amongst eosinophilic asthmatics, patients with recurrent infections had reduced expression of scavenging receptor macrophage receptor with collagenous structure (MARCO), with an associated low HDAC, compared to those who did not have a history of recurrent infective bronchitis [65[■]]. MARCO was reported to be paramount in macrophage clearance of bacteria in conjunction with TLR2 and NOD2 [67], reduced expression of which makes the host susceptible to infection. As discussed before, infection can cause dysregulation in the miR, which inhibits GCS pathway via increased PI3k activity.

IL-10, regulatory T-cells and autoimmune mechanisms

IL-10, a key anti-inflammatory cytokine, is secreted by regulatory cells in response to GCS [68] which

further suppresses cytokine production by Th2 lymphocytes [69]. Peripheral and sputum IL-10 levels were seen to be significantly lower in asthmatics compared with healthy controls [70,71], indicative of low levels of regulatory lymphocytes [71,72]. One of the well-known side-effects of prolonged corticosteroid use is lymphopenia, which is associated with reduced regulatory lymphocyte population and development of autoimmunity [73]. Animal models with lymphopenia have been shown to spontaneously develop autoimmune diseases due to compensatory homeostatic expansion of autoreactive T-cells [74,75]. In fact, a lower incidence of CD25⁺ CTLA4⁺ Foxp3⁺ T-regulatory cells was observed in the sputum of allergic asthmatics [76]. In addition to steroid-induced lymphopenia, delayed resolution of inflammation can trigger autoimmune mechanisms by skewing the ratio of regulatory and effector cells [77]. These regulatory cells are known to limit self-reactive lymphocytes via IL-10-dependent mechanism [78,79]. Although asthma is not typically considered an immunogenic disorder, there is evidence of circulating immune complexes [80] and antinuclear antibodies (ANAs) in 55% asthmatics with aspirin sensitivity [81,82]. More recently, 'granulomas' in the lung parenchyma of prednisone-dependent severe asthmatics were described. The steroid-sparing response to drugs like methotrexate and azathioprine, classically prescribed for autoimmune diseases, indicates a plausible autoimmune-type anomaly [26]. Finally, the presence of IgG antibodies specific to eosinophil peroxidase, a marker for asthma severity [83], and ANAs in the sputum of severe asthmatics was reported [84,85[■]], with reduced sputum IL-10 (Mukherjee *et al.*, unpublished). In fact, IL-10 is one of the key anti-inflammatory genes transactivated by glucocorticoid receptor–GCS complex [68]. Therefore, reduced IL-10 production due to altered response to GCS in steroid-insensitive asthmatics, or IL-10⁺ regulatory lymphocytes as a result of GCS-induced lymphopenia, is of concern owing to its immunoregulatory role of limiting self-reactive lymphocytes [86,87] as well as attenuating allergic inflammation [68].

Eosinophilic inflammation, degranulation and steroid subsensitivity

Nonspecific IgG, as well as ragweed-specific IgG isolated from the sera of allergic asthmatics, induced eosinophil degranulation that could not be inhibited by physiologically relevant doses of dexamethasone [88,89]. Indeed, ligation of IgG with IgG receptor (FcγRII) on eosinophils has been shown to inhibit GCS-induced eosinophil apoptosis [90]. Therefore, an increase in IgG levels due to

allergic/autoimmune mechanisms (as discussed in the previous section) could result in eosinophil degranulation with release of effector molecules leading to exacerbation of disease, a mechanism evidently unresponsive to GCS therapy.

Therapeutic strategies to improve corticosteroid subsensitivity in asthma

It is important to first consider poor adherence to prescribed therapy, comorbidities like chronic rhinosinusitis and polyposis, and alternate diagnoses (such as eosinophilic granulomatosis and polyangiitis), in all patients in whom 'steroid subsensitivity' is suspected [91]. Thereafter, as an adjunct therapy for increasing ICS response in the mild-to-moderate population, LABAs are often used since they, in ex-vivo studies, potentiate the GCS-induced anti-inflammatory action (reviewed in [25,32]). However, there is very little in-vivo evidence of this in asthma [92].

One could adopt a number of strategies to overcome corticosteroid insensitivity in severe asthmatics (Fig. 2). For patients with persistent eosinophilia, novel anti-Th2 biologics, currently under development, can be used as adjunct steroid-sparing therapy [18]. Severe eosinophilic asthma with IL-5 driven disease can be targeted by mepolizumab/reslizumab (FDA-approved), or potentially by benralizumab (currently in Phase III trials) [93,94[■],95[■]]. Dupulimab, a monoclonal antibody (mAb) targeting the common IL-4/IL-13 receptor in a recent Phase III trial showed improved lung function [96[■]], and is currently being assessed for its prednisone-sparing effect (NCT02528214). On similar note, omalizumab, an anti-IgE mAb, with proven ICS-sparing effect [97], is being evaluated for its prednisone-sparing ability in severe allergic asthmatics (NCT02049294). Fevipiprant, a prostaglandin D₂ receptor 2 antagonist, was recently trialed in moderate-to-severe eosinophilic asthma and reduced sputum eosinophil percentage was noted in the treatment group despite inhaled corticosteroid use [98]. Recently, methotrexate and azathioprine, commonly used for suppressing an exaggerated inflammatory response as seen in autoimmune diseases, were successfully used as steroid-sparing agents to treat prednisone-dependent asthmatics with biopsy-proven granulomas [99].

A second approach is restoring corticosteroid sensitivity by replenishing/recruiting the affected molecule. In this regard, IL-10 immunotherapy is under consideration. In a placebo-controlled trial, 4 weeks of vitamin D3 (calcitriol) caused significant improvement in corticosteroid responsiveness in severe asthmatics with a Th17 signature by

restoring dexamethasone-induced IL-10 production [58[■],100]. Two more trials are currently ongoing (NCT00712205, NCT01248065). In neutrophilic asthmatics, an infective exacerbation due to altered microbiome has been found to be associated with poor GCS response [55[■]]. Currently, antibiotics such as azithromycin and solithromycin are being assessed for restoring GCS sensitivity (Table 1). A recent case report revealed obligate anaerobes (*Haemophilus*, *Prevotella*) in the sputum of an asthmatic who suffered three exacerbations over 6 months with poor GCS response. 16S deep sequencing allowed identification of these infrequent bacterial species that would not be otherwise detected on normal routine cultures [119[■]]. This strategy allows tailoring of antibiotic therapy and limiting GCS dose. Theophylline, a phosphodiesterase inhibitor (PDE4), efficiently recruits HDAC [120], increases PP2A activity, and represses TNF α -induced IL-8 release [121[■]]. In asthmatic smokers with compromised HDAC activity, low-dose theophylline had no clinical improvement [122], while as an adjunct to beclomethasone it improved lung function and symptoms [106].

The third approach involves intervening an impaired pathway that may contribute to steroid subsensitivity. One example would be inhibiting PI3k α [100], a molecule that has been repeatedly shown to be involved upstream of deviated GCS-response, in a micro-environment high in oxidative stress (smoke/recurrent infection). Again, BI655066 that targets IL-23 (upstream of Th17 pathway) is being assessed for its ability to reduce asthma exacerbations (NCT02443298) and can prove effective in Th17^{high} asthma [55[■]]. For suppressing the pro-inflammatory MAPK pathway, p38MAPK inhibitors like inhaled AZD7624 are currently being investigated in a corticosteroid-resistant asthmatic population (see Table 1). Imatinib, a tyrosine kinase inhibitor, is also under investigation to attenuate the increased kinase activities that could lead to irregular glucocorticoid receptor (phosphorylation) activity. All the molecules that are under consideration for managing steroid subsensitivity in asthmatics are tabulated in Table 1.

CONCLUSION

One of the major challenges of respiratory medicine is the management of patients with severe asthma who have poor clinical response to treatment with glucocorticosteroids. Clinical response to GCS is heterogeneous within the diverse patient population and the response varies within the same individual. While steroid pharmacokinetics and functional genomics may play a role, recent

Table 1. Emerging treatment targets to restore corticosteroid subsensitivity

Mechanism of action	Drug name	Highest development phase	Reference/NCT number
Protein kinase inhibitors			
p38 ^{MAPK} inhibitor	AZD7624 [inhaled]	2	NCT02753764
Tyrosine kinase inhibitor	Masitinib (AB1010)	2	[101]/NCT00842270
	Imatinib mesylate	2	NCT01097694
Phosphoinositide 3-kinase (PI3K) inhibitors			
PI3K δ inhibitor	GSK2269557 [inhaled]	2	NCT02567708
	Nortriptyline	<i>In vitro</i>	[102]
PI3K δ/γ inhibitor	RV1729	1	NCT02140320
	TG100–115 [inhaled]	Preclinical	[103]
Phosphodiesterase (PDE) inhibitors			
PDE4 inhibitor	Roflumilast	2	[104]/NCT01765192,NCT0136553
	GSK256066 [inhaled]	2	[105]/NCT00380354
	Theophylline	2/3	[106]/NCT00119496
Dual PDE ₃ and PDE ₄ inhibitors	RPL554 [inhaled]	2	[107]/NCT02427165
Peroxisome proliferator-activated receptor- γ (PPAR γ) agonists			
PPAR γ agonist	Rosiglitazone	2/3	[108]/NCT00119496
PPAR γ agonist analogue	ADC3277	Preclinical	[109,110]
NF- κ B intermediates			
NF- κ B inhibitor	Dehydroxymethylepoxyquinomicin (DHMEQ)	Preclinical	[111]
IKK- β inhibitor	S-1145, ML120B	<i>In vitro</i>	[112]
Combating recurrent infection macrolides			
	Azithromycin	3/4	[113,114]/NCT00760838,NCT00852579
	Solithromycin	Preclinical	[115,116]
Steroid sparing strategies			
IL-5	Mepolizumab	Approved	[117]
	Reslizumab	Approved	[118]
IL-5 receptor α	Benralizumab	3	[94 ^{***} ,95 ^{***}]/NCT02075255
IL-4/IL-13	Dupilumab	3	[96 [*]]/NCT02528214
IgE	Omalizumab	Approved	NCT02049294
IL-23	BI 655066	2	NCT02443298
Vitamin D3		3	[58 ^{***}]/NCT01248065

evidence suggests that ‘misbalanced’ airway microbiome and possible local autoimmune mechanisms, the latter triggered by delayed resolution of inflammation and observed peripheral lymphopenia, may contribute to steroid insensitivity. Novel findings on small noncoding RNAs that are differentially expressed by altered microbiome, have been shown to be master regulators that alter the GCS-induced signalling pathway by affecting key factors like protein phosphatases, endogenous kinases and HDAC activities. Emerging strategies

of endotyping that employ systems biology approaches may reveal novel pathways and targets to improve steroid sensitivity in patients with complex airway diseases.

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Conflicts of interest

P.N. has received consultancy fees from AstraZeneca, Boehringer Ingelheim, Sanofi, Teva, and Roche; research support from GlaxoSmithKline, AstraZeneca, and Novartis; and lecture fees from Roche, AstraZeneca, and Novartis.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

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