Abnormal corticosteroid signalling in airway smooth muscle: mechanisms and perspectives for the treatment of severe asthma

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Summary

Growing in vivo evidence supports the concept that airway smooth muscle produces various immunomodulatory factors that could contribute to asthma pathogenesis via the regulation of airway inflammation, airway narrowing and remodelling. Targeting ASM using bronchial thermoplasty has provided undeniable clinical benefits for patients with uncontrolled severe asthma who are refractory to glucocorticoid therapy. The present review will explain why the failure of glucocorticoids to adequately manage patients with severe asthma could derive from their inability to affect the immunomodulatory potential of ASM. We will support the view that ASM sensitivity to glucocorticoid therapy can be blunted in severe asthma and will describe some of the factors and mechanisms that could be responsible for glucocorticoid insensitivity.

Introduction

There is a body of in vitro evidence suggesting that targeting the ‘pro-inflammatory responses’ in the airway smooth muscle (ASM), the main effector tissue regulating the bronchomotor tone, may lead to more effective therapies for asthma. ASM may play a role in the progression and/or perpetuation of asthma symptoms not only by regulating airway narrowing, but more importantly via its extraordinary ability to secrete an array of inflammatory mediators which have the potential to regulate key asthmatic features including airway remodelling, airway inflammation and bronchial hyper-responsiveness [1–3]. It is therefore not surprising to see that bronchial thermoplasty (BT), a recent bronchoscopy-based procedure that ablates ASM mass, is considered as a promising therapeutic option for the treatment of severe, uncontrolled asthma. Indeed, the clinical benefits of BT treatment include improvement in the quality of life and exacerbation rate [4–6]. The clinical efficacy provided by BT points to two major conclusions. First, it does suggest that directly targeting ASM can offer a novel and effective way of treating patients with severe asthma. Second, it is possible that the failure of current pharmacological therapies to appropriately control severe asthma lies, at least in part, in their inability to modulate the immunomodulatory function of ASM. In the present review, we will summarize the key in vitro and in vivo reports showing that the anti-inflammatory actions of glucocorticoids (GCs) in ASM may be blunted in patients with severe asthma, thus contributing to their reduced efficacy seen in these refractory patients.

In vivo evidence showing the immunomodulatory potential of ASM in asthma

The present section will not cover the numerous reports that have used cultured ASM cells to support the concept that ASM has the capacity to produce different ‘pro-asthmatic’ mediators in asthma because this interesting area of research has been extensively discussed in excellent reviews (see [3, 7–9]). Rather, we have decided to focus on the emerging in vivo evidence in patients with asthma demonstrating that ASM can produce a variety of immunomodulatory factors that are
capable of participating in the pathogenesis of the disease (summarized in Table 1). To address this question, several groups have relied on immunohistochemistry technique for staining structural tissues in endobronchial tissue sections. These groups have convincingly shown an increased expression of inflammatory mediators in ASM bundles of patients with asthma when compared to tissues from non-asthmatic subjects. Expression of chemokines such as CCL5 [10], CCL11 [11], CCL19 [12] and CX3CL1 [13] was reported to be elevated in asthmatic ASM tissues. The functional relevance of these chemokines was confirmed by in vitro studies using chemotaxis assays showing their chemotactic activity towards different inflammatory cells including mast cells (CX3CL1) and eosinophils (CCL11 and CCL5). In addition to recruiting inflammatory cells, other mediators produced by ASM could act on these infiltrated immune cells and orchestrate the allergic responses within the lungs. Thus, ASM tissues from asthmatic subjects express high levels of thymic stromal lymphopoietin (TSLP) [14], a cytokine that has been shown to regulate Th2 response during in allergic diseases [15]. TSLP has also been associated with the induction of GC insensitivity in an experimental model of allergic asthma [16]. In ragweed-allergic patients with asthma, segmental allergen challenge significantly increased protein expression of TNF-related apoptosis-inducing ligand (TRAIL) in the bronchoalveolar lavage (BAL) fluids as well as in various airway tissues including ASM. Neutralizing TRAIL function using blocking antibodies reveals its role in promoting eosinophil survival [17]. IL-33 is another key cytokine potentially involved in the initiation and maintenance of local Th2 responses via its action on mast cells, T cells and eosinophils [18]. Interestingly, IL-33 was also demonstrated to be increased in the ASM tissues of patients with asthma [19]. In addition, another study showed that IL-33 expression in the airways correlates with features of airway remodelling in paediatric patients with severe steroid-resistant asthma [20]. The chemokine receptor CCR1 was found to be expressed in ASM tissues of asthmatics, although its role in asthma remains to be further investigated [21]. CCL15, a ligand for CCR1, was recently reported to be highly expressed in asthmatic biopsies and produced by ASM cells [22]. Similar to other chemokines, some have reported that CCL15 can promote the recruitment of monocytes and eosinophils in the airways in asthma [23].

In addition to attracting and activating infiltrated cells, these chemokines produced in vivo in ASM tissues may participate in asthma via altering the function of ASM itself. In vitro studies found that CCL19 and CCL11 act as potent chemoattractants for ASM cells by activating the specific cell surface receptors CCR7 [12] and CCR3 [21], respectively. ASM migration can be induced by nerve growth factor (NGF) [24], which was also found to be significantly increased in ASM tissues of patients with asthma [25]. A recent report found that expression of pentraxin 3 (PTX3), an acute phase protein used as a serological biomarker for chronic inflammatory conditions, was significantly elevated in ASM bundles of severe asthmatics [26]. The role of PTX3 in asthma has not yet been examined, but the authors provided the first evidence that PTX3 can stimulate CCL11 production and inhibit mitogenic responses of ASM cells. Expression of heparin-binding EGF (HB-EGF) was also elevated in ASM in asthma, was correlated with disease severity and was upregulated in proliferating ASM [27]. HB-EGF represents therefore a new marker of ASM hyperplasia in severe asthma. Finally, ADAM metallopeptidase domain 33 (ADAM33) was reported by two independent studies to be increased in ASM tissues in severe asthmatics [28, 29]. Increased expression of ADAM33 was found to correlate with asthma severity (decreased FEV₁), which could be explained by the ability of ADAM33 to directly alter ASM contractile properties [30].

| Author       | Immuno-modulatory mediators | Role in asthma                              |
|--------------|-----------------------------|---------------------------------------------|
| Berkman 1996 | CCL5                        | Chemotaxis                                  |
| Ghaffar 1999 | CCL11                       | Chemotaxis                                  |
| Kaur 2006    | CCL19*                      | Chemotaxis                                  |
| El-Shazly 2006 | CX3CL1                     | Chemotaxis                                  |
| Kaur 2012    | Thymic stromal lymphopoietin (TSLP)* | TH2 allergic response Corticosteroid Insensitivity |
| Robertson 2002 | TNF-related apoptosis-inducing ligand (TRAIL) | Eosinophil Survival |
| Prefontaine 2009 | IL-33*                    | Chemotaxis Th2 allergic response Airway remodelling Chemotaxis |
| Joubert 2012 | CCL15                       | Chemotaxis                                  |
| Olgart Hoglund 2002 | Nerve growth factor (NGF) | ASM migration Mast cell activation and survival |
| Zhang 2012   | Pentraxin (PTX3)*           | Chemokine secretion (ASM)                   |
| Foley 2007; Lee 2006 | ADAM metallopeptidase domain 33 (ADAM33)* | Impaired lung function ASM contractile function |
| Hassan 2010  | Heparin-binding EGF*        | ASM remodelling                              |

*Denotes mediators still expressed in patients with severe asthma.
Together, these reports provide undeniable evidence that ASM is capable of producing an array of immunomodulatory factors in vivo in patients with asthma. The role of these ASM-derived mediators in the pathogenesis of asthma could be concluded from various in vitro studies showing their dual chemotactic and activating potentials towards infiltrated key inflammatory cells (mast cells, T cells and eosinophils). These ASM-derived mediators were also shown to affect ASM function such as cell proliferation and mechanical properties (summarized in Fig. 1).

**Impaired sensitivity of ASM to GCs in severe asthma**

It is yet unknown whether the clinical benefits provided by GC therapy in the vast majority of well-controlled patients with asthma could result, at least in part, from their direct action on ASM. Only one double-blind intervention study on steroid-free patients has examined whether a 14-day course with oral prednisolone (0.5 mg/kg/day) was associated with changes in gene expression in ASM isolated by laser capture microdissection and analysed using the RNA-seq strategy. The authors made the interesting finding that oral prednisolone induced significant changes in ASM of 15 genes, 2 of which (called FAM129A and SYNPO2) were associated with bronchial hyper-responsiveness [31]. The clinical importance of this study is twofold. First, it does reveal that oral GC therapy has a direct modulatory action on ASM in vivo which could contribute to their therapeutic actions in steroid responsive asthmatics. Second, the striking observation that different pro-asthmatic factors such as IL-33 [32], ADAM33 [28], CCL19 [12], TSLP [14] and pentraxin 3 [26] were still being expressed in ASM tissues of severe patients who were taking oral and/or high doses of inhaled GCs strongly suggests that the ability of GC therapy to suppress inflammatory gene expression in ASM is impaired in severe asthma. This possibility was initially suggested back in 2004 when Roth and colleagues discovered for the first time that a dysfunctional GC signalling (interaction with C/EBP-α) mediates the reduced antiproliferative action of dexamethasone in ASM cells from asthma when compared to non-asthma subjects [33]. Three elegant studies performed by Chung’s group comparing severe and non-severe asthmatics have since provided additional proof for a reduced sensitivity of cultured ASM cells to GC in patients with severe asthma [34–36]. The authors found that dexamethasone was less efficient (both in potency and maximum responses) in suppressing the production of CCL11 and CXCL8 induced by TNF-α in severe asthmatic ASM cells. The mechanisms have not been fully characterized, but the authors found an impaired

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![Fig. 1. Role of the immunomodulatory factors produced in vivo by ASM in asthma pathogenesis.](image-url)
nuclear translocation of GC receptor GR-α in response to dexamethasone. Previous studies from the same group have demonstrated a role of a p38 MAPK in mediating GC sensitivity in alveolar macrophages from patients with severe asthma [37, 38]. We have recently shown a critical role of p38MAPK in regulating GR-α phosphorylation in healthy ASM cells [39]. These studies show that GC insensitivity seen in both structural cells and immune cells could involve common mechanisms affecting GR-α function through changes in receptor phosphorylation. Because GR-α can be phosphorylated on multiple residues including serine 211, 203 and 226 [40], with different impacts on GR-α function, it would be valuable to determine which phosphorylation sites (if any) are affected in severe asthma and the underlying mechanisms. We have unpublished evidence to suggest that abnormal GR-α phosphorylation can explain the inability of dexamethasone to block the abnormal proliferative responses seen in ASM cells from severe asthmatics. The impaired in vitro GC sensitivity seen in ASM cells does not seem to be a specificity of patients with severe asthma. ASM cells derived from patients with chronic obstructive pulmonary disease, another condition characterized by poor sensitivity to GC therapy, have a reduced sensitivity to dexamethasone when compared to cells from non-smokers. Production of both CXCL8 and GM-CSF induced by TNF-α was less sensitive to dexamethasone in ASM cells from COPD [41]. Interestingly, GC insensitivity seen in ASM cells from both severe asthmatics [34, 35] and COPD patients [41] appears to be complex with the degree of GC impairment varying greatly according to the gene type studied. For example, while the inhibitory effect of dexamethasone towards CXCL8 and CCL11 was blunted in severe asthma, the enhancing effect of dexamethasone on cytokine-induced CX3CL1 production was not affected [34]. Similarly, there was no difference in the inhibitory action of dexamethasone on the production of VEGF by TNF-α in cells from COPD and non-COPD subjects [41]. These studies raise the important conclusion that not all anti-inflammatory pathways activated by GC in ASM are dysfunctional in lung diseases characterized by GC refractoriness (severe asthma and COPD).

Collectively, these in vivo observations strongly support the current view that ASM may be a key player in severe asthma by being a source of different immunomodulatory mediators. More importantly, the anti-inflammatory actions of GC therapy appear to be somewhat altered in ASM of severe asthmatics both in vivo in native tissues and in vitro in cultured cells. Future studies are required to fully understand the ‘pro-asthmatic’ pathways that interfere with GC function in ASM in severe asthma. The following section will discuss some of the potential molecular mechanisms susceptible of blunting GC responsiveness in ASM cells.

Pathways regulating GC sensitivity in ASM: lessons from our in vitro model

The aforementioned evidence shows that the anti-inflammatory actions of GC are dramatically reduced in ASM in severe asthma as suggested by the in vitro observation that the synergistic induction of the ecto-enzyme CD38 in healthy ASM cells treated with TNF-α/IFN-γ combination is resistant to the inhibitory action of fluticasone [42]. The role of CD38 in asthma has been attributed to its ability to regulate the major allergic responses including bronchial hyper-responsiveness and airway inflammation [43]. This initial observation led us to postulate that under inflammatory conditions, GC responsiveness could be altered in ASM cells. A number of subsequent studies have confirmed that the synergistic induction of 'pro-asthmatic' mediators by both TNF-α and IFN-γ in ASM cells was indeed insensitive to GC treatment (dexamethasone and fluticasone) (summarized in Table 2). GC-insensitive proteins include IL-33 [19], CXCL10 [44, 45], CX3CL1 [46], CCL5 [45, 47], CD38 [42] and CCL11 [45]. Because the promoter region of the genes encoding these proteins contains binding elements for the transcription factor NF-κB, it was thought that GC-insensitive state induced by TNF-α/IFN-γ was linked to the failure of GC to block NF-κB pathways. However, this seems be an unlikely possibility as the synergistic expression of another NF-κB-inducible gene called CCL15 by TNF-α/IFN-γ was found to be completely inhibited by dexamethasone [22]. These data raise the prospect that GC insensitivity induced by TNF-α/IFN-γ in ASM cells is highly complex and rather gene specific as reported previously in ASM cells from severe asthmatics.

In subsequent studies, we have uncovered some of the different mechanisms potentially explaining the loss of GC activity in ASM cells treated with TNF-α/IFN-γ. We demonstrated that the impaired cellular sensitivity to GCs induced by IFN-γ and TNF-α was directly related to the modulation of GR-α transactivation properties. Indeed, IFN-γ and TNF-α simulated the expression of the dominant negative isoform GR-β (seen after 12 hour poststimulation) which is thought to interfere with GR-α function through the formation of GR-α/GR-β heterodimers [48]. Although the role of GR-β in
modulating GC signalling in lung diseases is still controversial [49], we found that fluticasone failed to induce GRE-dependent gene transcription and to suppress TNF-α-induced CD38 expression in ASM cells transfected with constitutively active GR-β [42].

We also demonstrated that GR-β-independent mechanisms were involved in mediating cytokine-induced GC insensitivity. We found that at shorter incubation times (<6 hour when GR-β was not induced), TNF-α/IFN-γ treatment was still capable of blunting ligand-induced GR-α transactivation activity when assessed using a GRE reporter vector [50]. Silencing strategies combined with overexpressing approaches confirmed that the transcription factor interferon regulatory factor-1 (IRF-1) is playing a key role in blunting fluticasone-induced GR-α transactivation by competing for a common coactivator called glucocorticoid receptor interacting protein 1 (GRIP-1). GRIP-1 belongs to nuclear receptor coactivators for a number of nuclear receptors. Three homologous members have been described, namely NCOA1/SCR1, NCOA2 (TIF2/GRIP-1/SRC2) and NCOA3 (RAC3/ACTR/pCIP/AIB-1/SRC3). These multifunctional coactivators interact with GC-bound GR-α to recruit histone acetyltransferases and methyltransferases which facilitate the access of transcription factors to target gene promoters via the unpacking of the condensed chromatin network. These NCOAs also interact and regulate the activity of a number of transcription factors including members of the IRF family [51]. We made the novel observation that in ASM cells, GRIP1 was critical for driving the transcrip-
tional activity by depleting GRIP-1 from GR-α complex thus leading to GC-insensitive state. There is some evidence to suggest a role of IRF-1 in asthma. Studies performed in patients found an association of polymorphisms in IRF-1 and a risk of atopy [53] and childhood atopic asthma [54]. IRF-1 has the capacity to regulate the expression of a number of different inflammatory genes that are involved in asthma [55], and its levels are significantly increased in airway epithelium of patients asthma [56]. The above studies show that IRF-1 has the potential to regulate airway inflammation and to diminish GC efficacy by blunting GR-α transactiva-
tion activity. An elegant review has provided strong evidence for a role of transactivation in the anti-
inflammatory actions of GC [57].

Phosphorylation of GR-α can occur on three major residues located on its N terminus (ser203, ser211 and ser226) and regulates key functions including receptor turnover, subcellular trafficking and activity. However, it is GR-α phosphorylation on ser211 that is critical for its optimal transcriptional activity [58]. We found that in addition to depleting GRIP-1 from GR-α, TNF-α/IFN-γ treatment impaired GC transactivation in ASM cells by inhibiting fluticasone-induced GR-α phosphorylation specifically at ser211, while no effect was seen at ser226. This dephosphorylating action could be prevented by silencing the protein phosphatase PP5 [59]. A direct link between PP5 and inhibition of GR-α function has been previously reported by Goleva and colleagues who showed that PP5 knockdown restored GC responsiveness in oestrogen-treated breast cancer cells [60]. Our study was the first to suggest a similar role in ASM cells that was dependent on the potassium channel KCa3.1. Although little is known about the role of PP5 in asthma, there are growing reports to support a

Table 2. Glucocorticoid-insensitive proteins induced by TNFα/IFNγ in ASM cells

| Author                | Immuno-modulatory mediators                                                                 | Role in asthma                                      |
|-----------------------|---------------------------------------------------------------------------------------------|----------------------------------------------------|
| Clarke 2010; Chachi 2013 | CXCL10                                                                 | Chemotaxis                                         |
| Chachi 2013           | CCL5                                                                                     | Chemotaxis                                         |
| Sukkar 2004; Chachi 2013 | CX3CL1                                                                                   | Chemotaxis                                         |
| Prefontaine 2009      | IL-33*                                                                                    | chemotaxis, Th2 allergic response, Airway remodelling |
| Joubert 2012          | CCL15                                                                                     | Chemotaxis                                         |
| Tliba 2008            | IRF-1                                                                                     | Transcription of inflammatory genes                |
|                       |                                                                                           | Inhibition of GRα transactivation via GRIP-1 depletion |
| Chachi 2013           | CCL11                                                                                     | Chemotaxis                                         |
| Tliba 2006            | CD38                                                                                      | Airway inflammation                                |
|                       |                                                                                           | Airway hyperresponsiveness                          |
| Tliba 2006            | GRβ                                                                                       | Inhibition of GRα transactivation via dominant negative action |
| Bouazza 2012; Chachi 2013 | PP5                                                                                      | Inhibition of GRα transactivation via ser211 dephosphorylation |

*Denotes mediators still expressed in native ASM tissues in severe asthmatics.
role of KCa3.1 channels in the pathogenesis of allergic asthma. *In vitro* studies showed that KCa3.1 channels regulate mast cell degranulation and migration [61–63], fibrocyte migration [64] and ASM proliferation [65]. KCa3.1 channels also regulate migration of lung dendritic cells to different chemokines such as CCL19 and CCL21 [66]. More convincing evidence comes from preclinical reports in animal models of asthma showing that specific KCa3.1 blockers can block the key allergic features of asthma including airway inflammation, remodelling and airway hyper-responsiveness [67, 68]. We now show that KCa3.1 channels drive GC insensitivity in ASM cells [45], although it remains to be examined how cytokines actually activate KCa3.1 channels.

Almost nothing is known about the signalling pathways activated by KCa3.1 channels that regulate GC function. Most research so far has been focusing around the implication of KCa3.1 channels in the regulation of inflammatory gene expression in activated T cells, an effect that is likely due to changes in intracellular Ca2+ levels [69–71]. Because our conclusions were based on the use of two selective blockers of the KCa3.1 pore, it is likely that KCa3.1 channel activity is responsible for driving GC insensitivity, possibly via its capacity to regulate levels of intracellular Ca2+ [72]. This is a plausible mechanism as we found that cytokines such as TNF-α can alter Ca2+ handling in ASM cells in part via the increased expression of Ca2+ regulatory proteins such as ectoenzyme CD38 [73] or transient receptor potential C3 channels [74]. In addition, we showed that expression of some inflammatory genes in ASM cells clearly involved Ca2+-dependent pathways [75, 76]. We therefore speculate that KCa3.1 channels promote GC insensitivity in ASM cells via the activation of Ca2+-dependent pathways (e.g. transcription factors) that decreased GR-α function via changes in PP5 expression and/or activity. A report has indeed shown that PP5 function can be regulated by Ca2+-sensitive proteins called S100 which appear to regulate key cellular responses such as cell proliferation and gene expression [77]. Additional studies are needed to determine the nature of the KCa3.1-dependent Ca2+ signalling pathways in ASM cells.

This *in vitro* model of cytokine-induced GC insensitivity allowed us to uncover multiple key proteins that act independently to one another but in concert to suppress GR-α transactivation activity (see Fig. 2). This state of GC insensitivity induced by TNF-α/IFN-γ involved rapid inhibitory mechanisms (occurring within 6 hours) that include IRF-1-mediating dissociation of GR-α from its essential coactivator GRIP-1 and PP5-dependent upregulation and activation of the phosphatase PP5 which dephosphorylates GR-α specifically at serine 211 site that is essential for its transcriptional activity. The delayed inhibitory mechanisms (occurring 12 hours postcytokine stimulation) mediating GC insensitivity induced by TNF-α/IFN-γ involve the upregulation and action of the dominant negative GR-β isoform. Both rapid and delayed mechanisms contribute to the overall GC insensitivity induced by TNF-α/IFN-γ in healthy ASM cells. It remains to be determined how TNF-α/IFN-γ regulates the expression of these different proteins which interfere with GC signalling and if they are expressed both *in vivo* and *in vitro* in ASM cells in severe asthma.

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**Fig. 2.** Potential molecular mechanisms mediating cytokine-induced GC insensitivity in ASM cells. TNF-α/IFN-γ drives GC insensitivity in ASM via different inducible proteins that are capable of blunting GR-α function through 2 main mechanisms (defined as rapid and delayed). These mechanisms, independent from one another, act in concert to specifically suppress GC-induced GR-α transactivation activity. The rapid inhibitory mechanisms (that occur within 6 hours) mediating GC insensitivity induced by TNF-α/IFN-γ comprise i) the induction of the transcription factor IRF-1 which depletes GR-α from its indispensable transcriptional coactivator GRIP-1 and ii) the KCa3.1 channel-dependent upregulation and activation of the phosphatase PP5 which dephosphorylates GR-α specifically at serine 211 site that is essential for its transcriptional activity. The delayed inhibitory mechanisms (occurring 12 hours postcytokine stimulation) mediating GC insensitivity induced by TNF-α/IFN-γ involve the upregulation and action of the dominant negative GR-β isoform. Both rapid and delayed mechanisms contribute to the overall GC insensitivity induced by TNF-α/IFN-γ in healthy ASM cells. It remains to be determined how TNF-α/IFN-γ regulates the expression of these different proteins which interfere with GC signalling and if they are expressed both *in vivo* and *in vitro* in ASM cells in severe asthma.
dependent dephosphorylation of GR-α at ser211 via functional KCa3.1 channels. This state of GC insensitivity induced by TNF-α/IFN-γ also involved delayed inhibitory mechanisms (occurring after 12 hours) which impair GR-α transactivation via the dominant negative action of GR-β isoform.

Pathways regulating GC sensitivity in ASM: alternative mechanisms from recent studies

Other potential mechanisms could also participate in reduced GC sensitivity to GCs seen in ASM cells from severe asthmatics especially with regard to the impaired GR-α nuclear translocation [35]. GCs exert their anti-inflammatory action in part via the recruitment of histone deacetylases (HDAC) to the inflammatory gene complex by the ligand activated GR-α [48]. A reduction in HDAC activity was found to correlate with the degree of GC insensitivity seen in peripheral blood mononuclear cells PBMCs in severe asthma [78], an observation that could result from the failure of GCs to stimulate HDAC protein expression [79]. Other studies found that GC insensitivity in PBMCs from refractory patients was associated with an impaired transactivation coupled with a reduced histone acetylation [80]. Oxidative and nitrative stress via the inactivation of HDAC function has been described as a leading mechanism for driving corticosteroid insensitivity in both COPD and severe asthma [48, 81]. One group recently demonstrated that cysteine oxidation can induce post-translational modification of GR-α and impair its nuclear translocation [82]. These are important observations as recent studies demonstrated that oxidative stress can affect a number of 'pro-asthmatic' responses in ASM. An excessive mitochondrial production of reactive oxygen species contributes to the increased mitogenic response seen in the ASM cells of COPD patients [83]. Our group also noted that NOX4-dependent oxidative stress was significantly increased in asthmatic ASM cells and was responsible for its hypercontractile phenotype [84]. It would be interesting to determine whether oxidative stress alters GC responsiveness in ASM cells by modulating GR-α signalling either via changes in HDAC activities or redox-related mechanisms.

There is also considerable evidence to suggest that serum levels of vitamin D can dramatically impact on the therapeutic responses of GCs in patients with asthma. Clinical studies both in children and in adults with asthma have indeed demonstrated that low serum levels of vitamin D (<30 ng/mL) were associated with either increased GC use [85] or reduced in vitro GC response [86]. A more recent proof-of-concept clinical trial found that calcitriol therapy significantly improved clinical response to prednisolone in GC-resistant asthmatics [87]. The use of PBMCs from GC-resistant patients revealed that vitamin D could restore the ability of GC to stimulate expression of the anti-inflammatory cytokine IL-10 [88] or even enhanced GC-induced GR-α activity as assessed by its recruitment to target gene promoter and levels of histone acetylation [89]. Similarly, vitamin D restored the ability of GC to induce GR-α nuclear translocation in the PBMCs of severe asthmatics [90]. It would therefore be valuable to examine whether vitamin D supplementation could reinstate the abnormal GC sensitivity seen in ASM cells from severe asthmatics.

Conclusive remarks

Considering the clinical benefits of removing ASM using BT therapy in severe asthma and the demonstration that ASM produces in vivo different pro-asthmatic factors, it is now clear that ASM should be considered as a central player in the pathogenesis of asthma. In addition to impairing lung function via the modulation of airway narrowing, ASM can contribute to other key features of asthma via its extraordinary capacity of secreting an array of immunomodulatory factors. These factors can participate in the chemotraction and activation of various inflammatory cells such as eosinophils, T lymphocytes and mast cells known to be infiltrated within the asthmatic airways. These ASM-derived factors can also contribute to other asthmatic features such as airway hyper-responsiveness and airway remodelling in part via an autocrine action on the proliferative and secretory functions of ASM.

There is also growing evidence to suggest that expression of pro-asthmatic factors by ASM specifically in severe asthmatics persists despite patients taking oral GC therapy. Our model of cytokine-induced GC insensitivity in healthy ASM cells has shed some light on the potential molecular players that are susceptible of altering GC function in ASM cells. These GC inhibitory signals that are capable of blunting GC transactivation properties include reduced access of the indispensable coactivator GRIP-1 for GR-α, dephosphorylation of GR-α via KCa3.1-dependent PP5 activity and impaired GR-α function via the dominant negative action of GR-β. Our ongoing studies show that levels of PP5 are increased in vivo in ASM of severe asthmatics pointing towards an impaired phosphorylation of GR-α (Chachi et al., manuscript in preparation). Recent elegant studies from Fan Chung’s laboratory demonstrated that ASM cells isolated from severe asthmatics retain their GC-insensitive state as seen in vivo in native tissues. This new model supports the current concept of abnormal GC sensitivity in severe asthmatic ASM and represents a unique opportunity to dissect potential factors that are interfering with GC signalling in severe asthma.
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Conflict of interest
The authors have no conflict of interest to declare.

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