



Effect of p38 MAPK inhibition on corticosteroid suppression of cytokine release in severe asthma

P. Bhavsar*, N. Khorasani*, M. Hew*, M. Johnson[#] and K.F. Chung*

ABSTRACT: Patients with severe asthma respond less well to corticosteroids than those with non-severe asthma. Increased p38 mitogen-activated protein kinase (MAPK) activation in alveolar macrophages (AMs) from severe asthma patients has been associated with a reduced inhibition of cytokine release by dexamethasone.

We determined whether p38 MAPK inhibitors would modulate corticosteroid suppression of cytokine release from AMs and peripheral blood mononuclear cells (PBMCs).

PBMCs were isolated from venous blood and AMs by bronchoalveolar lavage in severe and non-severe asthma patients. PBMCs and AMs were exposed to lipopolysaccharide (LPS) with and without the p38 MAPK inhibitor, SD282, or dexamethasone. We determined the concentration-dependent effects of another p38 MAPK inhibitor, GW-A, on dexamethasone-induced inhibition of interleukin (IL)-8 release from PBMCs. Cytokines were assayed using an ELISA-based method.

SD282 (10^{-7} M), with dexamethasone (10^{-6} M), caused a greater inhibition of release of IL-1 β , IL-6, macrophage inflammatory protein-1 α and IL-10, than with dexamethasone alone in AMs from severe and non-severe asthma. At 10^{-9} and 10^{-10} M, GW-A, that had no direct effects, increased the inhibitory activity of dexamethasone (10^{-8} and 10^{-6} M) on LPS-induced IL-8 release in PBMCs from severe asthma.

Corticosteroid insensitivity in severe asthma patients may be improved by inhibitors of p38 MAPK.

KEYWORDS: Alveolar macrophages, corticosteroid-resistant asthma, cytokines, p38 mitogen-activated protein kinase, severe asthma

Most asthma patients are well controlled on inhaled corticosteroid therapy, but a small proportion experience continuing symptoms. These patients, labelled as having severe asthma, consume a more significant proportion of medical resources in terms of drugs, admissions to hospital or use of emergency services, and time off work or school [1]. Definitions and the clinical features of recently described cohorts attest to the persistent loss of control of asthma despite the optimal use of asthma medication. Such patients demonstrate a poor response to the therapeutic effects of corticosteroids. Peripheral blood mononuclear cells (PBMCs) and alveolar macrophages (AMs) from patients with severe asthma are less sensitive to inhibition by dexamethasone in terms of the stimulated release of pro-inflammatory cytokines, when compared with cells from well-controlled non-severe asthma patients [2, 3].

The mechanisms underlying this poor suppressive response to corticosteroids in severe asthma are unclear but many have been proposed from *in*

vitro studies in cells [4]. We observed that AMs from patients with severe asthma demonstrated a greater degree of activation of p38 mitogen-activated protein kinase (MAPK) [2]. p38 MAPK is a family of serine-threonine kinase that act on a variety of substrates including transcription factors, such as nuclear factor (NF)- κ B and activator protein-1 and has been implicated in inflammation, cell proliferation and cell death relevant to asthma pathophysiology [5]. We hypothesised that activation of p38 MAPK could be linked to the reduced inhibition of cytokine release by corticosteroids since part of the action of corticosteroids may relate to inhibition of MAPK activation. In addition, p38 MAPK activation has been previously shown to induce corticosteroid insensitivity in PBMCs through phosphorylation of the glucocorticoid receptor [6], indicating one potential mechanism of corticosteroid insensitivity.

Selective inhibitors of p38 MAPK, mainly the α isoform, have been developed. In seeking to implicate a role for p38 MAPK activation in

AFFILIATIONS

*Section of Airways Disease, National Heart & Lung Institute, Imperial College & Royal Brompton and Harefield NHS Trust Hospital, London, and
[#]GlaxoSmithKline, Uxbridge, UK.

CORRESPONDENCE

K.F. Chung
National Heart & Lung Institute
Dovehouse St
London SW3 6LY
UK
E-mail: f.chung@imperial.ac.uk

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corticosteroid sensitivity, we examined whether inhibitors of p38 MAPK could improve the ability of dexamethasone in suppressing cytokine release in AMs and PBMCs from patients with severe asthma.

METHODS

Study participants

Asthma patients with either bronchodilator responses to albuterol of $\geq 12\%$ of baseline forced expiratory volume in 1 s (FEV₁) or with a provocative concentration of methacholine causing a 20% fall in FEV₁ (PC₂₀) of $<16 \text{ mg}\cdot\text{mL}^{-1}$ (table 1) were recruited from the Royal Brompton Severe Asthma Clinic (London, UK). Current and ex-smokers of >5 pack-yrs were excluded. "Severe" asthmatics were defined according to the American Thoracic Society criteria [7]. "Non-severe" asthmatic patients used 0–2,000 μg of inhaled beclomethasone-equivalent per day with control of their asthma. The protocols were approved by the local ethics committee (the Royal Brompton, Harefield and National Heart and Lung Institute Ethics Committee, London UK). All volunteers gave written informed consent.

Isolation of alveolar macrophages

Fibreoptic bronchoscopy was performed with intravenous midazolam and alfentanil. Bronchoalveolar lavage was performed in the right middle lobe with 0.9% NaCl solution. Macrophages ($5 \times 10^5 \cdot \text{well}^{-1}$) were purified by adhesion to plastic wells for 4 h and then exposed for 18 h to lipopolysaccharide (LPS; $10 \mu\text{g}\cdot\text{mL}^{-1}$) in the presence or absence of dexamethasone (10^{-6} M) or of a selective p38 MAPK inhibitor, SD282 (10^{-7} M ; Scios Inc, Freemont, CA, USA) [8, 9] or both.

Isolation of PBMCs

Venous blood (80 mL) was diluted 1:1 with Hank's buffered saline solution and layered on Ficoll-Hypaque-Plus. Following

centrifugation, PBMCs were re-suspended in culture media, plated ($7.5 \times 10^5 \text{ cells}\cdot\text{well}^{-1}$) and stimulated with LPS ($10 \mu\text{g}\cdot\text{mL}^{-1}$) with or without dexamethasone (10^{-6} M) or SD282 (10^{-7} M) or both. Supernatants at 18 h were analysed for macrophage inflammatory protein (MIP)-1 α , interleukin (IL)-1 β , IL-6 and IL-10.

LPS-stimulated PBMCs were also used to examine the effect of GW-A (10^{-12} – 10^{-6} M), a p38 MAPK inhibitor from GlaxoSmithKline (Stevenage, UK), or dexamethasone (10^{-12} – 10^{-6} M) or of the combination of GW-A and dexamethasone on IL-8 release.

p38 MAPK phosphorylation

To determine p38 MAPK activity, PBMCs were stimulated with LPS overnight in the presence or absence of dexamethasone (10^{-8} M) and/or GW-A (10^{-9} M). The contents of each well were stored in Beadlyte cell lysis buffer B (Beadlyte®, Upstate Technology, NY, USA) at -70°C for later assay for phosphorylated and total p38 MAPK using microsphere beads coated with antibodies to P-p38 and total p38 using the Beadlyte® protocol.

Measurement of cytokine release

The cytokines MIP-1 α , IL-1 β , IL-6 and IL-10 were assayed simultaneously using microsphere beads (Beadlyte®) coated with capture antibodies. Biotinylated reporter antibodies were used to bind the microsphere bead-cytokine complexes. Finally, a fluorophore, streptavidin-phycoerythrin was added to bind the biotinylated reporter, and the fluorescent signal measured in a laser spectrophotometer (Luminex Corporation, Austin, TX, USA). Microsphere beads for each cytokine emitted a unique ratio of two other fluorophores. IL-8 was measured using ELISA.

TABLE 1 Characteristics of non-severe and severe asthma subjects in the SD282 and GW-A study

	SD282				GW-A
	BAL		PBMC		PBMC
	Non-severe asthma	Severe asthma	Non-severe asthma	Severe asthma	Severe asthma
Sex F:M n	3:3	5:1	2:4	5:1	6:2
Age yrs	32.8 ± 3.88	47 ± 2.67	36.7 ± 3.61	43.2 ± 5.71	43.2 ± 5.71
FEV ₁ % pred	89.8 ± 3.28	$52.8 \pm 7.73^{***}$	88.2 ± 3.32	$55.2 \pm 8.19^{***}$	68.8 ± 8.19
Bronchodilator response [#] %	9.1 ± 2.24	23.3 ± 3.97	8.7 ± 2.0	21.3 ± 9.4	20.1 ± 8.4
Log PC ₂₀ $\text{mg}\cdot\text{mL}^{-1}$	0.52 ± 0.15	-0.60 ± 0.18	0.0 ± 0.28	0.1 ± 0.09	-1.67 ± 0.21
Prednisolone $\text{mg}\cdot\text{day}^{-1}$	0	$12.5 \pm 4.33^{**}$	0	$8.3 \pm 4.01^*$	16.25 ± 8
BDP equivalent $\mu\text{g}\cdot\text{day}^{-1}$	33.3 ± 33.3	$2800 \pm 744^{***}$	333.3 ± 151	$3200 \pm 722^{***}$	1725 ± 146
Cell counts					
Total count $\times 10^6$	5.85 ± 0.81	5.05 ± 0.49			
Macrophage %	94.55 ± 1.37	92.9 ± 1.63			
Neutrophils %	1.8 ± 0.46	$3.32 \pm 1.57^*$			
Eosinophils %	1.13 ± 0.51	1.25 ± 0.82			
Lymphocytes %	2.54 ± 0.43	2.53 ± 1.12			

Data are presented as mean \pm SEM, unless otherwise stated. BAL: bronchoalveolar lavage; PBMC: peripheral blood mononuclear cells; F: female; M: male; FEV₁: forced expiratory volume in 1 s; % pred: % predicted; PC₂₀: provocative concentration of methacholine causing a 20% fall in FEV₁; BDP: beclomethasone dipropionate. #: measured as percentage increase over baseline FEV₁ after 400 μg albuterol aerosol; *: $p < 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$ compared with non-severe asthma.

Data analysis

Results were expressed as mean \pm SEM. Cytokine release induced by LPS with or without dexamethasone or p38 MAPK inhibitor was calculated by subtraction of baseline release. The release of each cytokine following dexamethasone and LPS combined was calculated as a percentage of cytokine release following LPS stimulation alone. Corticosteroid sensitivity between severe asthma and non-severe asthma patients was compared using the Mann–Whitney U-test. The effect of SD282 on the suppressive effect of dexamethasone was analysed by Wilcoxon paired t-test. Concentration-dependent responses were examined using one-way ANOVA (Kruskal–Wallis test) followed by a Dunn's Multiple Comparison test. A p-value of <0.05 was considered significant.

RESULTS

Corticosteroid insensitivity in AMs and PBMCs

Baseline and LPS-stimulated release of IL-1 β , IL-6, MIP-1 α and IL-10 from both AMs and PBMCs did not significantly differ between patients with severe asthma and patients with non-severe asthma. There was less inhibition of cytokine release from AMs in the severe asthma group compared with that in the non-severe group for IL-1 β , IL-6 and MIP-1 α , with the percentage of cytokine release after LPS and dexamethasone to that after LPS alone being 71 ± 8 versus 26 ± 7 ($p < 0.01$), 52 ± 9 versus 20 ± 5 ($p < 0.05$), and 70 ± 9 versus 28 ± 7 ($p < 0.01$), respectively. There was no significant difference regarding IL-10 (12 ± 4 versus 30 ± 10) (fig. 1a).

For PBMCs, there was a non-significant trend for less suppression by dexamethasone in patients with severe asthma compared with non-severe asthma: IL-1 β (46 ± 19 versus 27 ± 8); IL-6 (35 ± 8 versus 22 ± 6); MIP-1 α (48 ± 14 versus 45 ± 19); IL-10 (68 ± 10 versus 42 ± 9) (fig. 1b).

Effect of SD282 on dexamethasone inhibition of cytokine release

In AMs from non-severe asthma, the suppressive effect of dexamethasone was relatively greater than that of SD282 and there was more inhibition of IL-6 and MIP-1 α with the combination, with a trend towards greater suppression for IL-1 β (fig. 2a). In AMs from severe asthma, SD282 alone caused only a small degree of suppression of LPS-induced IL-1 β , IL-6, MIP-1 α and IL-10 release, being similar in magnitude to that seen with dexamethasone alone (fig. 2b). Combined SD282 and dexamethasone caused a greater inhibition of cytokine release compared with dexamethasone alone for the four cytokines.

In PBMCs, the inhibitory effect of SD282 was greater than that observed in AMs. Although there were significant differences between the effect of dexamethasone and that of dexamethasone and SD282 for the release of all four cytokines, with less effect on the release of MIP-1 α from PBMCs of non-severe asthma patients, there was no evidence of additivity in the presence of both dexamethasone and SD282 (fig. 3a). Similar observations were made in PBMCs from severe asthma patients (fig. 3b).

Effect of dexamethasone and GW-A on p38 activation and IL-8 release from PBMCs

To determine whether GW-A (10^{-9} M) demonstrates inhibitory effects on p38 MAPK activity, the ratio of phosphorylated p38

to total p38 was measured at 24 h. GW-A (10^{-9} M) alone or in combination with dexamethasone (10^{-8} M) attenuated p38 activity induced by LPS stimulation (fig. 4).

In order to determine whether there was additivity or synergy between dexamethasone and a p38 MAPK inhibitor, we measured the inhibitory effect of dexamethasone and GW-A alone on LPS-induced IL-8 release from PBMCs from severe asthmatics. A maximal inhibition of $51.2 \pm 4.01\%$ release was achieved at 10^{-6} M dexamethasone with a median inhibitory concentration (IC₅₀) of 3.9×10^{-7} M, while GW-A caused maximal suppression of $51.6 \pm 7.1\%$ at 10^{-6} M with an IC₅₀ of 3.7×10^{-7} M.

The effect of dexamethasone at 10^{-6} M (fig. 5a) in suppressing IL-8 release was improved in the presence of GW-A (10^{-10} – 10^{-6} M) ($p < 0.0001$, one-way ANOVA) with a maximal suppression of $89.6 \pm 2.6\%$ with GW-A at 10^{-6} and 10^{-7} M, compared with $51.9 \pm 4.0\%$ suppression with dexamethasone alone ($p < 0.001$). With GW-A 10^{-9} M and 10^{-10} M, which alone had an inhibitory effect of $<3\%$, IL-8 suppression by dexamethasone (10^{-6} M) was increased to $76.8 \pm 3.5\%$ ($p < 0.05$) and $70.1 \pm 2.0\%$ ($p < 0.01$), respectively (fig. 5b).

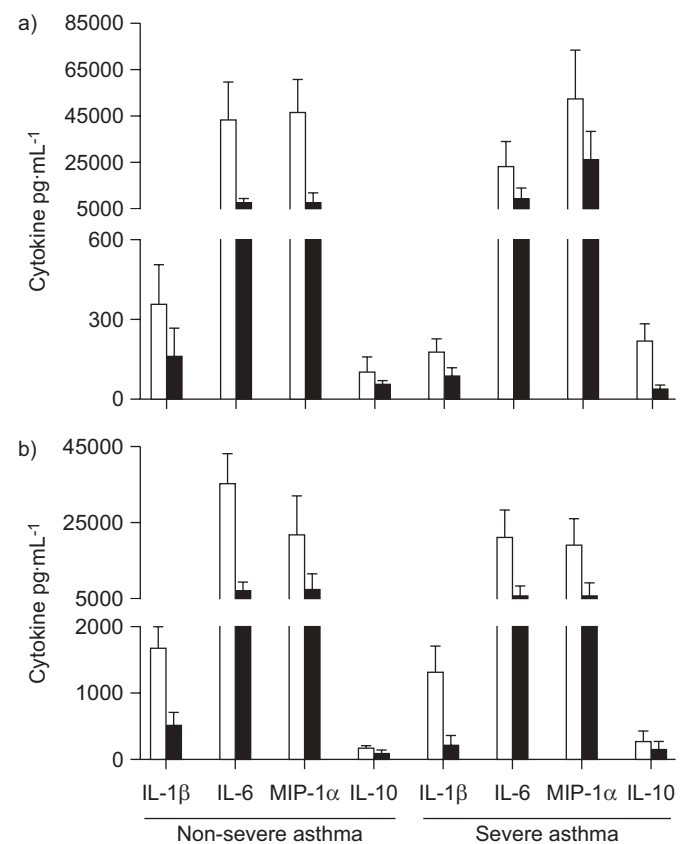


FIGURE 1. Concentrations of cytokines in cell culture supernatants of a) alveolar macrophage and b) peripheral blood mononuclear cells from patients with non-severe and severe asthma, stimulated with lipopolysaccharide ($10 \mu\text{g}\cdot\text{mL}^{-1}$) in the presence (■) and absence (□) of dexamethasone (10^{-6} M). There were no significant differences in stimulated levels of interleukin (IL)-1 β , IL-6, macrophage inflammatory protein (MIP)-1 α and IL-10 between patients with severe and non-severe asthma. There was a significant decrease in release of cytokines in the presence of dexamethasone for both cell types and both patient groups.

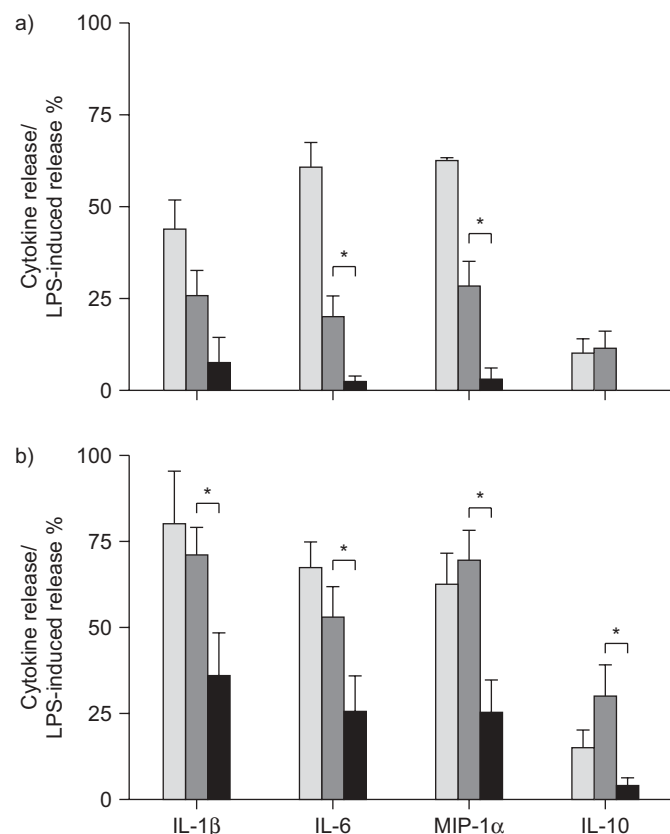


FIGURE 2. Inhibition of lipopolysaccharide (LPS)-induced cytokine (interleukin (IL)-1 β , IL-6, macrophage inflammatory protein (MIP)-1 α , IL-10) release from alveolar macrophages of a group of a) six non-severe and b) six severe asthmatics by p38 mitogen-activated protein kinase inhibitor (SD282; 10^{-7} M; ■), dexamethasone (10^{-6} M; ■), or both (■). *: $p < 0.05$.

With dexamethasone (10^{-8} M), the inhibitory effect was also improved in the presence of GW-A ($p < 0.001$, one-way ANOVA), with maximal suppression with GW-A (10^{-6} M) being $73.4 \pm 4.5\%$ compared with $31.3 \pm 3.8\%$ with dexamethasone alone. Similar effects were observed with dexamethasone at a concentration of 10^{-9} M, in combination with GW-A (fig. 5a). We obtained similar results when examining the release of IL-6 (fig. 6).

DISCUSSION

We have previously shown impaired corticosteroid sensitivity in AMs and PBMCs from patients with severe asthma and increased activation of p38 MAPK in AMs of severe asthmatics [2, 3]. We now demonstrate that a low concentration of a p38 MAPK inhibitor, SD282, that had little effect on IL-1 β , IL-6 and MIP-1 α release, improved the inhibitory activity of dexamethasone in AMs from severe asthma patients. This effect was not observed in PBMCs from patients with severe asthma because the concentration of SD282 chosen had a major suppressive effect on cytokine release. To investigate more closely any interaction between p38 MAPK inhibition and corticosteroids, we studied a wider range of concentrations of another p38 MAPK inhibitor, GW-A, and showed an enhancement of the suppressive effects of dexamethasone in PBMCs from patients with severe asthma at concentrations of GW-A that had no

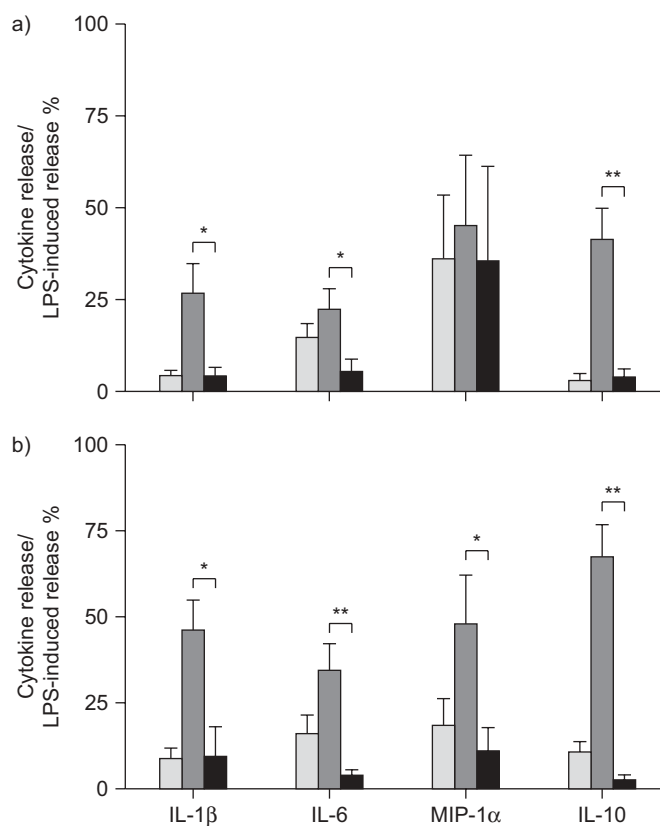


FIGURE 3. Inhibition of lipopolysaccharide (LPS)-induced cytokine (interleukin (IL)-1 β , IL-6, macrophage inflammatory protein (MIP)-1 α) release from peripheral blood mononuclear cells of a group of a) six non-severe and b) six severe asthmatics by p38 mitogen-activated protein kinase inhibitor (SD282; 10^{-7} M; ■), dexamethasone (10^{-6} M; ■), or both (■). *: $p < 0.05$; **: $p < 0.01$.

effect on IL-8 release. For example, the maximal suppression of IL-8 release obtained with the highest concentration of dexamethasone (10^{-6} M), was also achieved with a 100-fold reduction in dexamethasone concentration (10^{-8} M) with addition of GW-A (10^{-9} M). The enhancement in the inhibitory effects of dexamethasone appears to be synergistic because the suppression by dexamethasone (10^{-8} M) and by GW-A (10^{-9} M) alone were 28 and 3%, respectively, whereas the suppression observed when added in combination was increased to 59%. We also found similar effects with IL-6 release.

It is of interest that there was also additivity in the effects of SD282 and dexamethasone in AMs from patients with non-severe asthma in suppressing the release of IL-1 β , IL-6 and MIP-1 α , albeit to a lesser extent, even though the degree of inhibition by dexamethasone alone was substantial. However, this was not seen with IL-10 release in AMs from non-severe asthma patients, but was present in AMs from severe asthma patients. Therefore, overcoming corticosteroid insensitivity by using p38 MAPK inhibitors can be demonstrated in severe asthmatics. Our data are supported by a recent study that reported that a p38 MAPK inhibitor in combination with dexamethasone caused a greater suppression of gene expression induced by LPS in monocyte-derived macrophages or AMs [10].

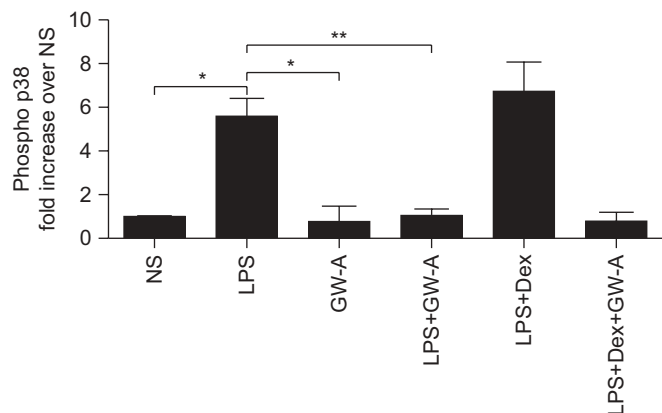


FIGURE 4. Inhibition of lipopolysaccharide (LPS)-induced p38 phosphorylation in peripheral blood mononuclear cells from severe asthmatic patients ($n=3$) by the p38 inhibitor, GW-A ($553; 10^{-9}$ M) in the presence or absence of dexamethasone (Dex; 10^{-8} M). NS: not stimulated. *: $p<0.05$; **: $p<0.01$.

The p38 MAPK inhibitor, SD282, is an indole-5-carboxamide selective p38 α MAPK inhibitor demonstrating a 14.3-fold greater potency for p38 α compared with p38 β [8, 9]. No detectable effect on other closely related kinases such as p38 δ , p38 γ , jun-N-terminal kinase and p38 activating kinases at concentrations up to 50 mM in human PBMCs has been observed [8] and, therefore, these effects are likely to result from the selective inhibition of the p38 α MAPK isoform. GW-A is another p38 MAPK inhibitor that is currently in clinical development [11]. We have shown that concentrations of GW-A (10^{-9} and 10^{-10} M) that had no effect on cytokine release, still demonstrated significant inhibition of p38 MAPK activity in peripheral blood monocytes exposed to LPS. This suggests that p38 MAPK activation is associated with corticosteroid insensitivity. Phosphorylation of p38 MAPK was measured as an indicator of the efficacy of GW-A, rather than one of its downstream targets, based on studies which have demonstrated that p38 inhibitors can prevent phosphorylation of p38 α *in vitro* [12, 13]. Additional studies have demonstrated that p38 α can *auto*-phosphorylate [14] and *trans*-phosphorylate [19].

Finally, SB203508, another p38 MAPK inhibitor related to GW-A, inhibits the enzymatic activity of both activated and unactivated forms of p38 α [15].

A role for p38 MAPK activation in severe asthma has been suggested by the observation that macrophages from such patients demonstrate increased p38 MAPK activation when exposed to LPS [2]. Previous studies have indicated a role for p38 MAPK activation in murine asthma models. Thus, in a chronic allergen model, suppression of p38 α MAPK by an inhibitor or by antisense oligonucleotide-attenuated ovalbumin-induced bronchial hyperreactivity, eosinophilia, goblet cell hyperplasia, airway smooth muscle hypertrophy and bronchial hyperresponsiveness, through a reduction of T-helper cell type 2 cytokines associated with allergic inflammation [16, 17]. Another potential effect of p38 inhibition is the reversal of corticosteroid insensitivity that is present in severe asthma [3]. We have previously demonstrated that IL-2- and IL-4-mediated corticosteroid insensitivity of peripheral blood mononuclear cells can be reversed by inhibition of p38 MAPK activity [6]. This insensitivity was reflected by a reduction in corticosteroid ligand binding affinity due to phosphorylation of the glucocorticoid receptor, that could be reversed by a p38 MAPK inhibitor [6], or by an indirect effect on the ligand binding domain of glucocorticoid receptor [18]. Alternatively, p38 MAPK activation may also lead to phosphorylation and phosphoacetylation of histones in the promoter regions of NF- κ B dependent genes, such as those activated by LPS, resulting in enhanced recruitment of the transcription factor NF- κ B [19, 20]. This is consistent with previous observations that histone deacetylase activity is reduced in PBMCs and AMs of asthmatic patients [3, 21].

p38 MAPK activation may be involved in the stabilisation and increased translation of pro-inflammatory cytokine mRNA, dependent on the conserved AU-rich elements in the 3'-UTR region [22]. Of the pro-inflammatory cytokine mRNAs that can be stabilised by p38 MAPK activation in monocyte and macrophage cell lines, IL-1 β , IL-6, IL-8, and MIP-1 α [23–25] are the same cytokines that were less inhibited by dexamethasone in AMs from severe asthma patients, and where the extent

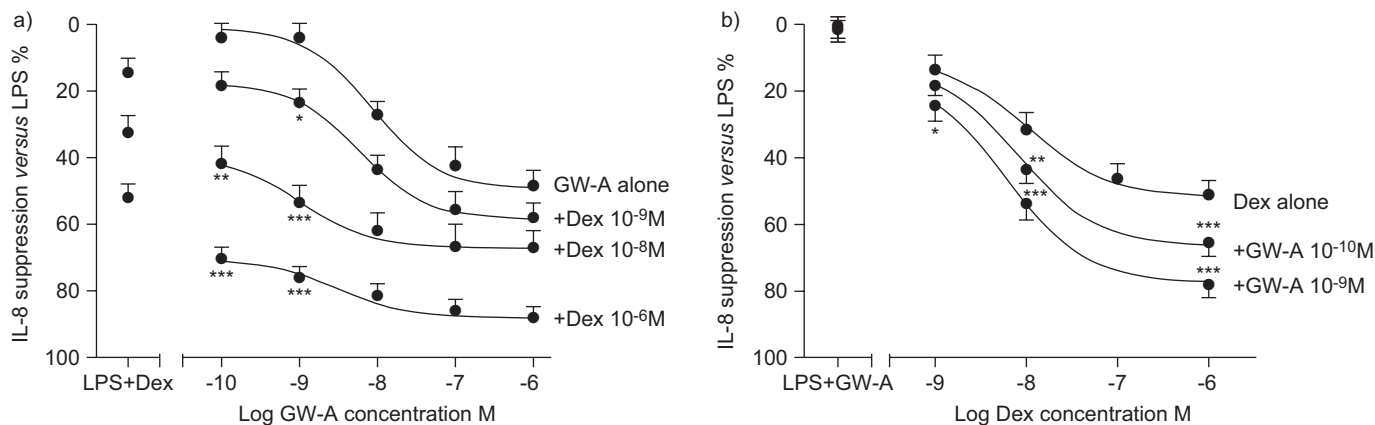


FIGURE 5. Inhibition of lipopolysaccharide (LPS)-induced interleukin (IL)-8 release from peripheral blood mononuclear cells by a) the p38 inhibitor, GW-A ($n=8$), in the presence of different concentrations of dexamethasone (Dex), and b) dexamethasone in the presence of different concentrations of GW-A. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$, compared with dexamethasone, at the given concentration, alone.

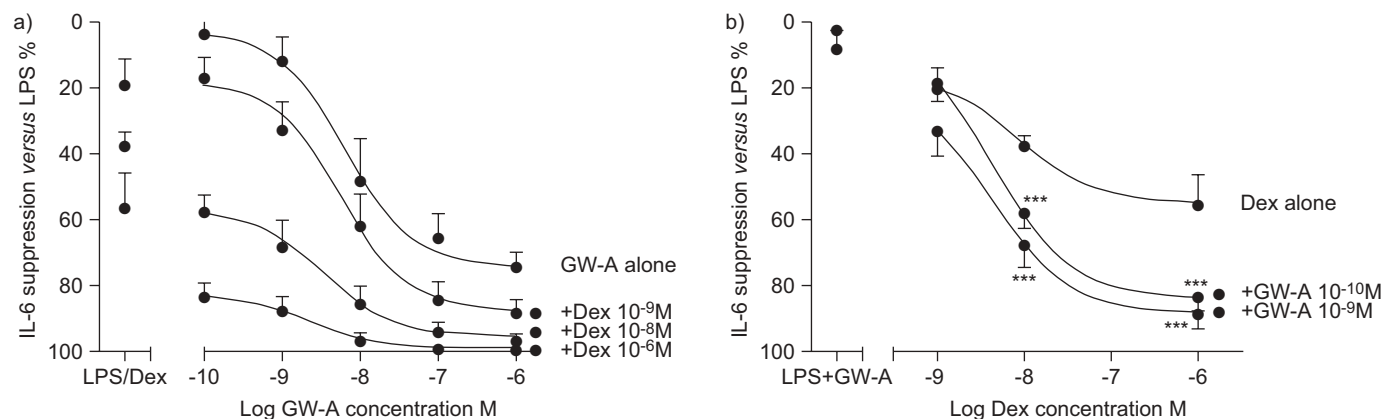


FIGURE 6. Inhibition of lipopolysaccharide (LPS)-induced interleukin (IL)-6 release from peripheral blood mononuclear cells by a) the p38 inhibitor, GW-A ($n=5$), in the presence of different concentrations of dexamethasone (Dex), and b) dexamethasone in the presence of different concentrations of GW-A. ***: $p<0.001$, compared with dexamethasone, at the given concentration, alone.

of inhibition by dexamethasone was correlated with the degree of p38 MAPK activation [3]. These potential downstream effects of p38 MAPK do not lead to enhanced release of cytokines from the AMs but to a reduction in the effectiveness of corticosteroids. Although this study has focused on p38 MAPK activity in this study, activation of other members of the MAPK family such as c-jun N-terminal kinase and extracellular signal-regulated kinase ERK [26, 27] have also been implicated in corticosteroid insensitivity. Whether there are interactions between the parallel downstream pathways, to influence corticosteroid sensitivity, deserves further investigation. In previous studies, we have also shown the reduced induction of MAPK phosphatase-1 by dexamethasone in AMs from patients with severe asthma [2]. This suggests another potential mechanism for increased activation of p38 MAPK activity.

The effect of dexamethasone in inhibiting IL-10 release induced by LPS from AMs and PBMCs may seem contradictory when induction of IL-10 release by corticosteroids has been shown in ex vivo studies of AMs or blood monocytes from patients who have been treated with either inhaled corticosteroids [30] or systemic methylprednisolone [31]. However, direct incubation of monocytes or AMs stimulated by LPS with dexamethasone led to an inhibition of IL-10 as we [2, 3] and others [31] have shown. p38 MAPK inhibition also reduced IL-10 release from PBMCs or AMs, as confirmed recently in monocytes [32]. We found that the combination of dexamethasone and GW-A caused an increase in inhibition of IL-10 release, particularly in PBMCs and AMs from patients with severe asthma.

Patients with severe asthma need effective new medications that will improve their asthma control. Our study points to a novel approach to the treatment of severe asthma. A p38 MAPK inhibitor may be used as an anti-inflammatory agent and has been shown to be effective in this way in asthma models [16, 17]. We have used this inhibitor to demonstrate its capacity to reverse corticosteroid insensitivity. Immunosuppressive drugs, such as cyclosporin A and methotrexate have been administered in patients with steroid-dependent asthma to lower maintenance oral corticosteroid dosage, while allowing the control of asthma to remain unchanged [28, 29], but these drugs have not proven to be useful. A p38 MAPK inhibitor could be effective in

reversing corticosteroid insensitivity at lower doses than those needed to inhibit inflammation with a lesser risk of side-effects, but will need to be used concomitantly with corticosteroids.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

Statements of interest for M. Johnson and K.F. Chung can be found at www.erj.ersjournals.com/misc/statements.dtl

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