Relative corticosteroid insensitivity of alveolar macrophages in severe asthma compared to non-severe asthma

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Summary

Objectives

About 5-10% of patients with asthma suffer from poorly-controlled disease despite corticosteroid (CS) therapy, which may indicate the presence of CS insensitivity. We determined whether relative CS insensitivity is present in alveolar macrophages (AMs) from severe asthma patients and its association with p38 mitogen-activated protein kinase (MAPK) activation and MAPK phosphatase-1 (MKP-1).

Methods

Fiberoptic bronchoscopy and bronchoalveolar lavage (BAL) were performed in 19 severe and 20 non-severe asthma patients, and for comparison, in 14 normal volunteers. AMs were exposed to lipopolysaccharide (LPS, 10 μg/ml) and dexamethasone (10^{-8} and 10^{-6}M). Supernatants were assayed for cytokines using an ELISA-based method. p38 MAPK activity and MKP-1 messenger RNA (mRNA) expression were assayed in cell extracts.

Results

The inhibition of LPS-induced IL-1β, IL-6, IL-8, MCP-1 and MIP-1α release by dexamethasone (10^{-6}M) was significantly less in AMs from severe asthma compared to AMs from non-severe asthma. There was increased p38 MAPK activation in AMs from severe asthma patients. MKP-1 expression induced by dexamethasone and LPS, expressed as a ratio of LPS-induced expression, was reduced in severe asthma.

Conclusion

AMs from patients with severe asthma demonstrate corticosteroid insensitivity associated with increased p38 MAPK activation that may result from impaired inducibility of MKP-1.

Key words

Alveolar macrophages, corticosteroid-dependent asthma, MAPK phosphatase-1, p38 mitogen-activated protein kinase, severe asthma.

INTRODUCTION

Although most asthma patients suffer from mild-to-moderate disease that responds to inhaled corticosteroids (CS), a minority of patients have severe disease characterised by asthma symptoms and exacerbations that are largely unresponsive to treatment including systemic CS (1). Despite the advent and use of efficacious inhaled CS and long-acting β-agonists as combination therapy, many patients with asthma do not achieve reasonable control of their asthma (2). These patients consume a more significant proportion of medical resources in terms of use of drugs, admissions to hospital or use of emergency services, and time off work or school (3;4). Definitions of severe asthma or therapy-resistant asthma have been proposed by working groups of the European Respiratory and American Thoracic Societies (5) (6), and the clinical features of recently-described cohorts (7-9) attest to the persistent loss of control of asthma despite the optimal use of asthma medication.

An important aspect of severe asthma is the relative reduction in effectiveness of CS in controlling asthma. In asthmatics, who have been defined as being corticosteroid-resistant as defined by a response of <15% of baseline FEV_1 after taking prednisolone (30-40 mg/day) over 14 days while demonstrating marked bronchodilator response to inhaled β_2-agonists (10), a reduction in the suppressive effect of dexamethasone on the proliferative response of or release of a neutrophil activating factor from peripheral blood mononuclear cells (PBMCs) has been observed compared to cells from CS-responsive asthmatics who have a >25% improvement in FEV_1 after prednisolone (11;12). However, these CS-resistant asthmatics are not similar in clinical presentation or in asthma severity as patients with severe asthma.

The macrophage is an important immune and inflammatory effector cell in asthma, and is a potential source of many pro-inflammatory mediators. The alveolar or airway macrophage is in a greater state of activation in patients with asthma compared to that from non-asthmatic individuals (13-15). The alveolar macrophage (AM) may also be an important site of action of CS since inhaled CS therapy causes a reduction in the ability of AMs of patients with mild-to-moderate asthma to release proinflammatory cytokines (14). In a recent investigation, we showed that PBMCs obtained from severe asthma patients demonstrated diminished CS suppression of IL-1β, IL-8 and MIP-1α release relative to PBMCs from non-severe asthma patients(16).

In the current work, we determined whether AMs also show similar corticosteroid insensitivity in severe asthma. In order to determine the activation status of AMs, we assayed the activity of p38 mitogen-activated protein kinase (MAPK) which is responsive to environmental stresses including heat, osmotic shock, inflammatory cytokines and lipopolysaccharide (LPS) (17). In addition, p38 MAPK is known to regulate the production of many cytokine proinflammatory cytokines such as IL-8 and GM-CSF from AMs (18;19), and overreactivity of p38 MAPK has been associated with CS resistance in PBMCs induced by a combination of IL-4 and IL-13 (20). MAPK phosphatase-1 (MKP-1) dephosphorylates and inactivates MAPKs, including p38 MAPK (21), and we therefore also measured the expression of MKP-1 in the macrophages from patients with severe asthma.

METHODS

Study participants

Patients with severe and non-severe asthma were recruited amongst the cohorts of patients referred to and attending our Asthma Clinic. Asthma patients demonstrated either an improvement in FEV_1 after inhaling 400 μg of salbutamol from a metered-dose inhaler of ≥12% of baseline forced expiratory volume in one sec (FEV_1) or bronchial hyperresponsiveness with a PC_{20} of <16 mg/ml. Current and ex-smokers of >5 pack-years were excluded. *Severe asthmatics* (n=20) were defined according to the American Thoracic Society major criteria of either continuous or near-continuous oral CS or high dose inhaled CS or both in order to achieve a level of mild-moderate persistent asthma, and by the presence of 2 or more minor criteria of asthma control (6). These patients underwent a protocol during which the diagnosis of asthma was confirmed, the severity assessed and therapeutic issues were optimised including adherence to therapy, potential aggravating factors assessed and co-morbidity issues evaluated and treated as required (7).

Non-severe asthmatic patients (n=19) were those that did not fall into the severe asthma category and who used 0-2,000 μg of inhaled beclomethasone or equivalent dosage per day with perfect control of their asthma. In order to see how the responses of these asthmatic patients compared to those of non-asthmatic subjects, healthy volunteers (n=14; 2 female; 37.6±2.4 years; FEV_1 %predicted= 98% ±5.0) with no history of asthma, on no medication and who had never smoked were recruited.

The study protocol was approved by the National Heart and Lung Institute and Royal Brompton Hospital Ethics Committee. All volunteers gave written informed consent to participate in the study.

Fiberoptic bronchoscopy

The entry criteria for performing bronchoscopies in patients with severe asthma comprised of (i) an FEV_1 ≥ 45% predicted prior to and FEV1 ≥ 55 % predicted following bronchodilators (ii) less than 6 asthma exacerbations in the previous 6 months to bronchoscopy (iii) no asthma hospitalization within 6 months; (iv) no endotracheal intubation for asthma within 1 year; and (v) regular daily oral dose of < 20 mg of prednisolone.

Fibreoptic bronchoscopy was performed using topical anaesthesia with lignocaine to the upper and lower airways and with intravenous midazolam (3-6 mg) and alfentanyl (125 μg). Warmed 0.9% NaCl solution was instilled (50 ml x 4) in right middle lobe and broncho-alveolar lavage (BAL) fluid was recovered by gentle suction.

Alveolar Macrophage Isolation

Washed BAL cells were suspended in culture media (RPMI with 0.5% fetal calf serum, antibiotics and L-glutamine) and counted on a haemocytometer. Cytospins were stained with Diff Quick stain (Harleco, Gibbstown, NJ) for differential cell counts. Macrophages were purified by adhesion to the plastic well for 4 hours and then exposed for 18 hours to LPS (10 μg/ml) in the presence or absence of dexamethasone (10^{-8} or 10^{-6} M). LPS at 10 μg/ml was the submaximal stimulatory concentration in terms of release of IL-8.

Cytokine release

Macrophage supernatants were mixed with microsphere beads (Beadlyte®, Upstate Technology, NY) coated with capture antibodies to ten cytokines: MCP-1, MIP-1α, RANTES, TNFα, IL-1β, IL-8, INF-γ, IL-6, IL-10 and GM-CSF. Biotinylated reporter antibodies were added to bind the microsphere bead-cytokine complexes. Finally, a

fluorophore, streptavidin-phycoerythrin was added to bind the biotinylated reporter thus emitting a fluorescent signal, that is measured in a Luminex® 100 laser spectrophotometer (Luminex Corporation, Austin, TX)**.** Microsphere beads for each cytokine emitted a unique ratio of two other fluorophores. Cytokine concentrations were derived from mean fluorescence intensity standard curves.

p38 MAPK phosphorylation

To determine p38 MAPK activity, additional macrophages from a subset of 10 non-severe and 10 severe patients were stimulated with LPS 10 μg/ml for 30 minutes in the presence or absence of dexamethasone (10^{-6} M) and were scraped. These studies were possible in patients in whom the recovery of macrophages was sufficient. The contents of each well were stored in radioimmunoprecipitation lysis buffer at -70ºC for later assay. Maximal p38 MAPK activation occurred at 30 min. Samples were assayed for phosphorylated and total p38 MAK kinase by enzyme immunoassay (Titerzyme, Cambridge Bioscience, UK), using rabbit polyclonal antibodies to phospho-p38 and to total p38.

Real-time reverse transcription-polymerase chain reaction (RT-PCR) for MKP-1

Macrophages after 24 hours of incubation with LPS or with LPS and dexamethasone from the last 7 non-severe and 6 severe asthma patients enrolled were used. Total RNA extracts were prepared using RNAqueous-Micro kit (Ambion). Contaminating genomic DNA was digested with DNase I treatment, DNAse was removed by vortexing with DNAse inactivation resin. The RNA was transferred to an RNAse-free tube and stored at – 20^{o}C. Complementary DNA (cDNA) for real-time quantitative PCR analysis was synthesised from 500 ng total RNA using random hexamer primers and AMV reverse transcriptase (Promega, Madison, WI). Real-time PCR were performed using the Rotor Gene 3000 (Corbett Research) with PCR amplifications performed in a 20 μl reaction volume using the SYBR Green Master Mix Reagent Kit (Promega). Cycling parameters were 95°C for 15 min, followed by annealing and extension at 45 cycles of 94°C for 15s, 60°C for 25s and 72°C for 25s. To control for variation within the procedure, a reference mRNA, 18s rRNA, was also measured. Oligonucleotide primer sequences, MKP-1: forward, 5'-GTACATCAAGTCCATCTGAC-3'; reverse, 5'-GGTTCTTCTAGGAGTAGACA-3'; 18s rRNA: forward, 5'-CTTAGAGGGACAAGTGGCG-3'; reverse, 5'-ACGCTGAGCCAGTCAGTGTA-3'.

Data analysis

Results were expressed as mean ± SEM. The main statistical analysis pertained to examination of differences between non-severe and severe asthma patients. Differences in concentrations for each cytokine released from AMs caused by LPS and by LPS plus dexamethasone were calculated and ranked, and a multivariate analysis of variance was performed to determine differences between severe and non-severe asthma. Baseline data between severe asthma and non-severe asthma patients were compared using the Mann-Whitney U test. Correlations were determined using Spearman rank correlation coefficient. A p value of < 0.05 was taken as significant.

RESULTS
Patient characteristics

	Non-severe asthma	Severe asthma
Gender (F:M)	8:12	14:6
Age (years)	40.0± 2.4	41.4 ±2.6
Duration of asthma (years)	27.4± 3.2	27.3± 2.6
Atopy[1]	19/20	18/20
FEV_1 (% predicted)	84.9± 3.0	59.9± 4.7***
FVC (% predicted)	93.9± 3.7	72.9± 4.9***
FEV_1/FVC ratio	74.6 ±2.1	66.9± 2.4*
Bronchodilator response[2]	9.5 ±1.8	20.9 ±3.9**
Log PC_{20} (mg/ml)	0.19± 0.14	-0.48± 0.17**
Prednisolone dose (mg/day)	0	11.8 ± 13.7***
BDP equivalent (μg/day)	526± 167	2530± 292***
BAL cells		
Total cell count (x 10^6)	7.32 ± 0.87	5.76 ± 0.68
Macrophage (%)	97.1 ± 0.84	93.2 ± 1.11**
Lymphocyte (%)	1.27 ± 0.34	2.56 ± 0.63
Neutrophils (%)	0.98 ± 0.29	2.58 ± 0.5**
Eosinophils (%)	0.63 ± 0.29	1.73 ± 0.65

Table 1. Characteristics of non-severe and severe asthma

Abbreviations: BAL: bronchoalveolar lavage; F = female; M =male; eNO= exhaled nitric oxide levels in parts per billion (ppb); FEV_1=forced expiratory volume in one second; FVC= forced vital capacity; PC_{20}= provocative concentration of methacholine causing a 20% fall in FEV_1; BDP: beclomethasone dipropionate; ND not determined. Values represent mean ± SEM.

[1]Atopy defined as positive skin prick tests to one or more common aeroallergens.

[2]Measured as per cent increase in FEV_1 after 400 μg salbutamol aerosol.

*p<0.05; **p≤0.01; ***p≤0.001 compared to non-severe

Ten severe asthma patients were on oral prednisolone (5-40 mg/day) and 9 of the non-severe asthma patients were not on ICS. Severe asthmatics had more airflow obstruction with a lower FEV_1 (p<0.001), greater bronchial hyperresponsiveness (p<0.05) and a greater bronchodilator reversibility (p<0.01) (Table 1). BAL fluid from severe asthmatics showed a greater percentage of neutrophils (p<0.009) and a smaller percentage of macrophages (p<0.008) than that from non-severe asthmatics (Table 1).

The bronchoscopic procedure was well-tolerated by all subjects. Two of the 20 severe asthmatics who underwent bronchoscopy developed an asthma exacerbation requiring systemic corticosteroids and extended hospital stay beyond 24 hours. Both responded fully to standard asthma therapy.

LPS-stimulated cytokine release

Baseline release of GM-CSF, IFN-γ, IL-10, and IL-1β was close to the limit of detection while for the other six cytokines, levels did not differ between severe and non-severe asthmatics (Fig 1A). Baseline release of the cytokines was higher than the release observed in each of the 2 asthmatic groups. LPS-stimulated cytokine release was also similar between severe and non-severe asthmatics, except for IL-8 release which was greater from macrophages of non-severe asthmatics (Fig 1B).

Corticosteroid suppression of cytokine release

To quantify the degree of sensitivity of AM to dexamethasone, the release of each cytokine following dexamethasone together with LPS-stimulation was expressed as a percentage of the cytokine release following LPS-stimulation (Fig 2). In general, there was lesser suppression of cytokine release by dexamethasone 10^{-6} M (Fig 2A) in macrophages from severe asthmatics compared to non-severe asthmatics. Using a multivariate analysis of variance to analyse the suppressed level of each cytokine by dexamethasone (10^{-6} M), statistical significance was reached for IL-1β ($p=0.05$), IL-6 ($p<0.01$), IL-8 ($p<0.01$), MCP-1 ($p<0.05$) and MIP-1α ($p=0.01$). In general, the degree of suppression of cytokine release from macrophages of normal subjects appeared similar to that of the non-severe asthmatic group, apart from IL-8. There was no difference in dexamethasone sensitivity between AMs of severe and non-severe asthmatics at 10^{-8} M (Fig 2B), apart from IFNγ which was less suppressible in AMs from patients with severe asthma ($p<0.05$).

LPS-induced p38 phosphorylation

p38 MAPK phosphorylation, measured as the ratio of phosphorylated p38 to total p38, was increased in AMs of non-severe and asevere asthma patients by LPS, but not in Ams from normal subjects (Fig 3A). the baseline p38 MAPK activity of unstimulated AMs were not significantly different between the groups. When expressed as a ratio of p38 phosphorylation after LPS to unstimulated levels, there was significantly higher activation in AMs from non-severe asthmatics and severe asthmatics compared to those from normal subjects ($p<0.05$ and $p<0.01$ respectively; Fig 3B); however, there was greater activation in the severe asthmatics compared to non-severe asthmatics ($p<0.05$).

When the data from the 2 asthmatic groups were combined, LPS-induced p38 MAPK phosphorylation correlated directly with the degree of steroid insensitivity of cytokine release for IL-1β ($p<0.05$), IL-6 ($p<0.05$), IL-8 ($p<0.05$) and MIP-1α ($p<0.01$) (Fig 4). There was no significant correlation for any of the 4 cytokines within the non-severe asthma group; however, within the severe group, there was significant correlation for IL-1β (rho= 0.76; $p=0.037$) and IL-6 (rho=0.7; $p=0.043$).

Induction of MAPK phosphatase-1 (MKP-1) by LPS and dexamethasone

Induction of MKP-1 gene expression, as measured by mRNA abundance, in AMs stimulated with dexamethasone and LPS was higher than in cells stimulated with LPS alone in normal and in non-severe asthmatic subjects ($p<0.01$ and $p<0.05$

respectively). However, such an increase in MKP-1 mRNA abundance was not observed in AMs from severe asthmatics. Baseline transcript levels of MKP-1 were not significantly different between the 3 groups. MKP-1 mRNA expression following co-treatment with dexamethasone and LPS, compared to that induced by LPS alone, was significantly higher in non-severe asthmatics than in severe asthmatics ($p<0.05$; Fig 5).

DISCUSSION

AMs from patients with severe asthma patients demonstrate impaired CS suppression of release of several inflammatory cytokines (IL-1β, IL-6, IL-8, MCP-1 and MIP-1α) stimulated by LPS, compared to AMs from non-severe asthmatics. CS sensitivity of AMs from non-severe asthmatics paralleled that of AMs from non-asthmatic subjects. The differences between severe and non-severe asthmatics were present at dexamethasone 10^{-6}M and not at 10^{-8}M indicating a reduction in maximal CS response of the former group. Our results are therefore similar to those we previously reported in peripheral blood mononuclear cells obtained from patients with severe asthma(16). In the current study, we examined the possibility that the decrease in CS sensitivity of the AMs in severe asthma was related to an increased activation of p38 MAPK phosphorylation. We found that p38 MAPK activation was increased in the severe asthma group compared to the non-severe asthma and normal groups. Furthermore, the degree of CS insensitivity was correlated with the degree of p38 MAPK activation, providing support for p38 MAPK activation being a potential determinant of CS responsiveness.

Although there was increased activation of p38 MAPK in AMs from patients with severe asthma, we did not find any increase in cytokine release induced by LPS in these patients. In addition, there was only a relatively small difference in inflammatory markers between the 2 groups, with a significant increase in neutrophils in bronchoalveolar lavage fluid from patients with severe asthma, despite these patients being treated with higher doses of inhaled CS and despite 50% of these patients also taking a daily dose of prednisolone. This may be a reflection of the relative reduction of CS sensitivity observed in patients with severe asthma. It was the effect of dexamethasone in inhibiting cytokine release that was impaired, an observation that was associated with relative MKP-1 underexpression induced by dexamethasone. This indicates that there may be a defect in MKP-1 expression, which is known to be induced by CS (22), that may allow for excessive p38 MAPK activity. Macrophage cell lines transfected with MKP-1 show an accelerated inactivation of p38 MAPK (23) and murine alveolar macrophages treated with the MKP-1 inhibitor, triptolide, show prolonged p38 activity (24). Similarly, dexamethasone-mediated suppression of the proinflammatory cytokines, TNFα and IL-1β, was impaired in macrophages from MKP-1 knock-out mice, indicating that the expression of MKP-1 was required for inhibition of these cytokines by dexamethasone(25). The inability of CS to upregulate MKP-1 activity in severe asthma could be a mechanism by which CS insensitivity occurs since some of the biological effects of CS may occur through inactivation of MAPK kinases (26). However, the non-asthmatic macrophages also demonstrated a reduction in MKP-1 expression in the presence of dexamethasone, although to a lesser extent when compared to macrophages from non-asthmatic subjects, indicating that the asthmatic state is also associated with an inability of MKP-1 upregulation. However, given the small number of asthmatic samples examined, the data on MKP-1 should be considered preliminary and needs to be confirmed in larger studies.

The fact that patients with severe asthma still have uncontrolled asthma despite taking high doses of inhaled CS, sometimes together with oral CS, has led to the hypothesis that patients with severe asthma are relatively resistant to the therapeutic effect of CS. Such patients are not absolutely resistant to the effects of CS since stopping CS therapy usually leads to a worsening of asthma in these patients. Rather, such

patients, often labelled as *corticosteroid-dependent asthma,* have a partial impaired response to CS. This current work on AMs activation studied ex-vivo provides some support for this concept. Recent cohorts of patients with severe asthma have revealed that these patients show daily symptoms and increased use of reliever medication, increased urgent health care utilisation with recurrent exacerbations of asthma, and a substantial proportion of patients on a regular oral dose of prednisolone (27;28). Patients with severe asthma recruited to the current study showed a greater degree of airflow obstruction, greater bronchodilator response to salbutamol, and had a greater degree of methacholine airway responsiveness compared to non-severe asthma patients, all indicators of severity of asthma. However, in terms of inflammation, there was only a small increase of 2.5-fold in BAL neutrophils. Airway neutrophilia has been recognised as a feature of severe asthma (29;30), although this could be a reflection of oral corticosteroid therapy(31). One might indeed impute the reduced CS sensitivity in patients with severe asthma to the continuous use of CS, particularly oral CS, that could lead to down-regulation of the CS receptor expression and translocation. However, we found no differences in dexamethasone suppression of AM function from severe asthmatics on inhaled CS alone and those on inhaled and oral CS therapy. Similarly, there were no differences in AM sensitivity from non-severe asthma patients on inhaled CS, and those not taking inhaled CS.

We have previously shown that IL-2 and IL-4 exposure of blood monocytes caused a reduction in CS ligand binding affinity due to phosphorylation of the glucocorticoid receptor (GR), that could be reversed by a p38 MAPK inhibitor (20), or by an indirect effect on the ligand binding domain of GR (32). p38 MAPK activation may also lead to phosphorylation and phosphoacetylation of histones at the promoter regions of NF-κB dependent genes such as those activated by LPS resulting in enhanced recruitment of the transcription factor NF-κB (19;33). This is consistent with our previous observation that histone deacetylase (HDAC) activity is reduced in PBMCs and alveolar macrophages of asthmatic patients(16;34). 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 (35), particularly of. IL-1β, IL-6, IL-8, and MIP-1α (36-38). These potential downstream effects of p38 MAPK may lead to a reduction in the effectiveness of dexamethasone.

In summary, alveolar macrophages from severe asthma patients demonstrate relative CS insensitivity compared to those of non-severe asthma patients. Increased p38 MAPK activation may underlie the insensitivity but further work will be needed to directly implicate p38 MAPK activation in the CS insensitivity of severe asthma.

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Competing interests: None declared.

LEGEND TO FIGURES

Figure 1. Concentrations of ten cytokines measured in cell culture supernatants of unstimulated alveolar macrophages (Panel A) and of alveolar macrophages stimulated with lipopolysaccharide 10 μg/ml (Panel B) from normal volunteers, non-severe asthmatics and severe asthmatics. Data shown as median and interquartile range.

Figure 2. Effect of dexamethasone 10^{-6} M (panel A) and 10^{-8} M (panel B) on the suppression of release of ten cytokines from alveolar macrophages stimulated by lipopolysaccharide (10μg/ml). Data is expressed as cytokine release after dexamethasone and lipopolysaccharide (LPS) as a % of release after LPS alone. Data shown as median and interquartile range.

Figure 3. Panel A. p38 MAPK phosphorylation measured as the ratio of phosphorylated p38 to total p38 at baseline (unstim) and following stimulation by lipopolysaccharide (LPS; 10 μg/ml) in alveolar macrophages from normal subjects and severe and non-severe asthmatics. Panel B. Similar data expressed as fold-increase in p38 MAPK phosphorylation after LPS compared to baseline. Horizontal bars are the median values.

Figure 4. Correlation between relative steroid suppression of IL-1β, IL-6, IL-8 and MIP-1α release and the increase of p38 phosphorylation induced by LPS in alveolar macrophages of patients with asthma. ●: severe asthma, ○: non-severe asthma.

Figure 5. Induction of MAPK phosphatase-1 (MKP-1) mRNA expression measured by quantitative RT-PCR. Panel A shows effect of LPS and of combination of lipopolysaccharide (LPS) and dexamethasone (Dex, 10^{-6}M), and panel B the ratio of expression after LPS and dexamethasone to that after LPS alone in alveolar macrophages from normal volunteers, and non-severe and severe asthma patients. Horizontal bars are median values.

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Figure 1

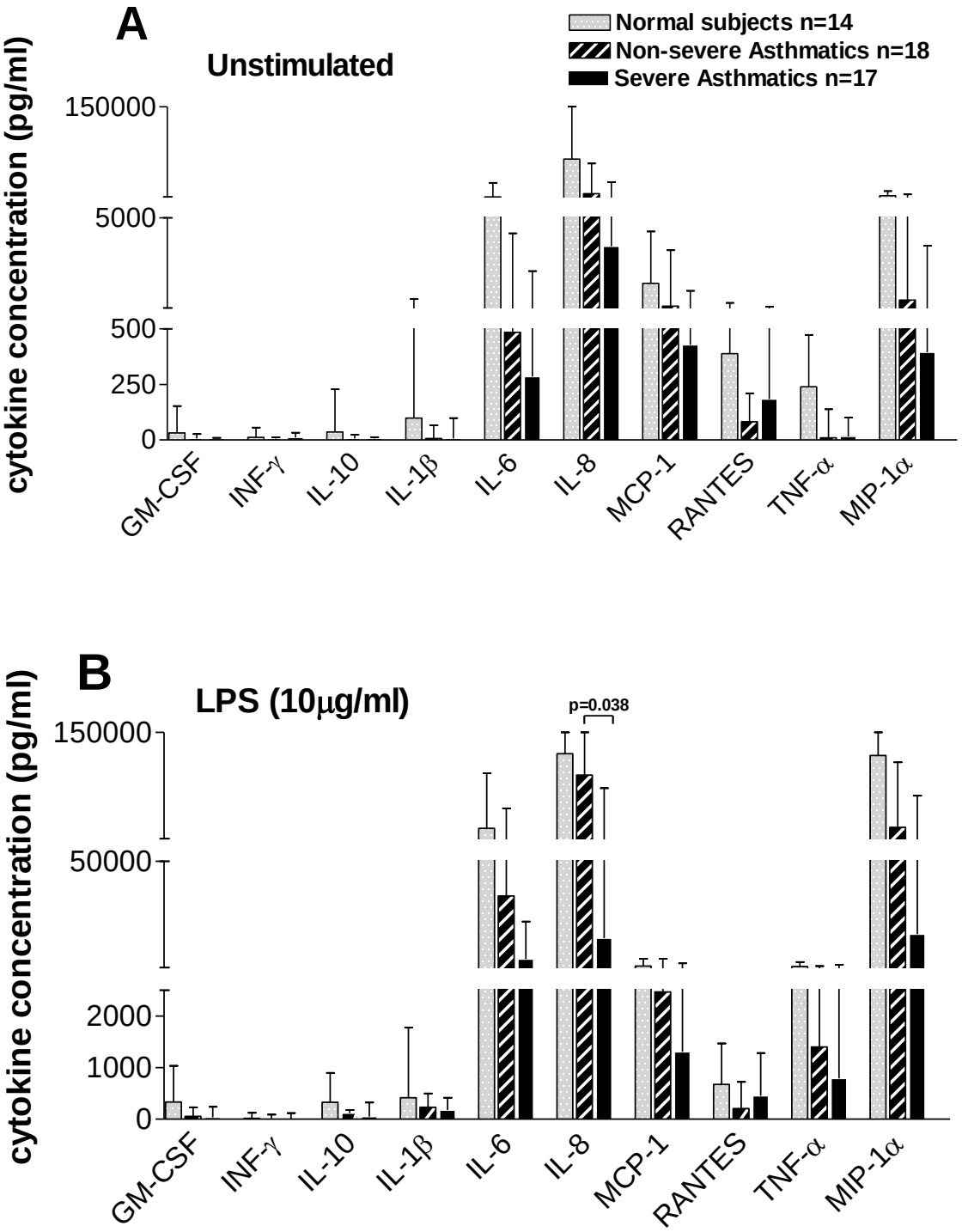

A
Unstimulated
Normal subjects n=14
Non-severe Asthmatics n=18
Severe Asthmatics n=17
cytokine concentration (pg/ml)
150000
5000
500
250
0
GM-CSF
INF-γ
IL-10
IL-1β
IL-6
IL-8
MCP-1
RANTES
TNF-α
MIP-1α
B
LPS (10μg/ml)
p=0.038
cytokine concentration (pg/ml)
150000
50000
2000
1000
0
GM-CSF
INF-γ
IL-10
IL-1β
IL-6
IL-8
MCP-1
RANTES
TNF-α
MIP-1α

Figure 2

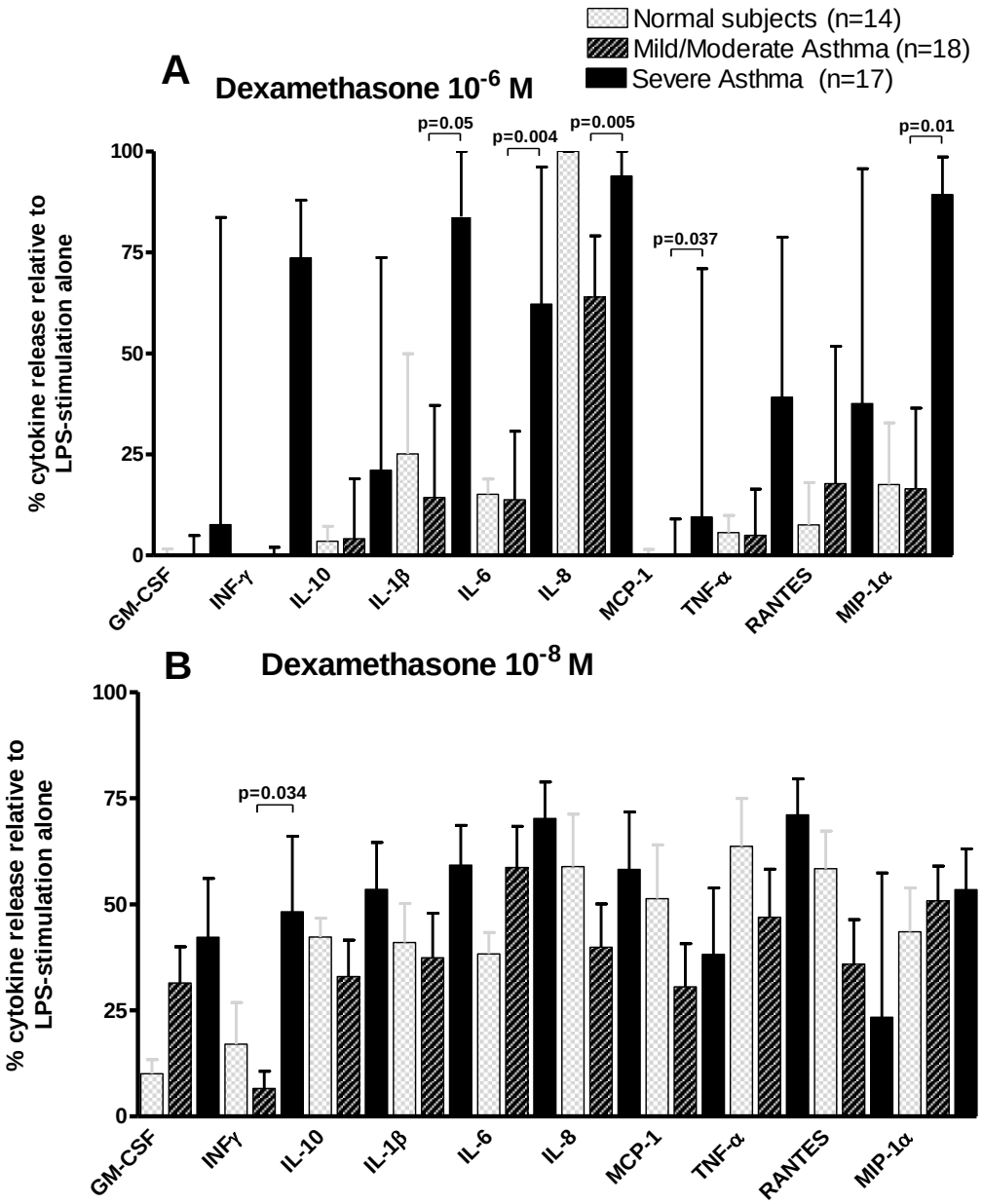

Normal subjects (n=14)
Mild/Moderate Asthma (n=18)
Severe Asthma (n=17)
A
Dexamethasone 10-6 M
% cytokine release relative to LPS-stimulation alone
p=0.05
p=0.004
p=0.005
p=0.037
p=0.01
GM-CSF
INF-γ
IL-10
IL-1β
IL-6
IL-8
MCP-1
TNF-α
RANTES
MIP-1α
B
Dexamethasone 10-8 M
p=0.034

Figure 3

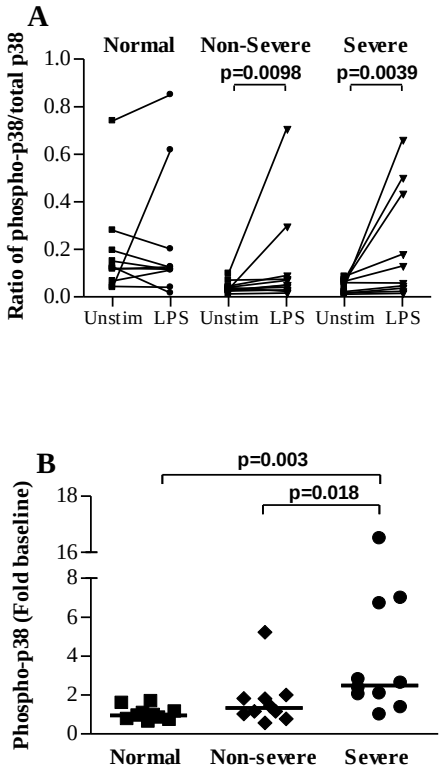

A
Normal
Non-Severe
Severe
p=0.0098
p=0.0039
Ratio of phospho-p38/total p38
1.0
0.8
0.6
0.4
0.2
0.0
Unstim LPS Unstim LPS Unstim LPS
B
p=0.003
p=0.018
Phospho-p38 (Fold baseline)
18
16
8
6
4
2
0
Normal
Non-severe
Severe

Figure 4

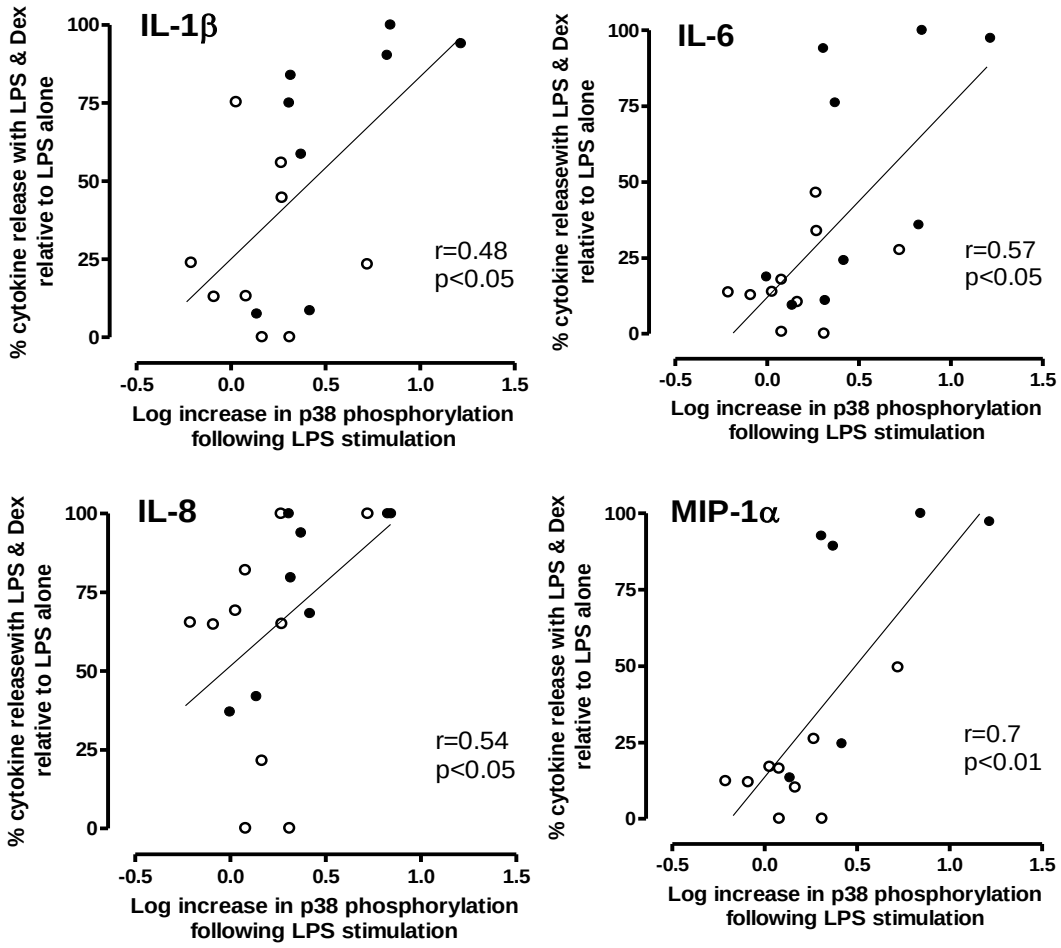
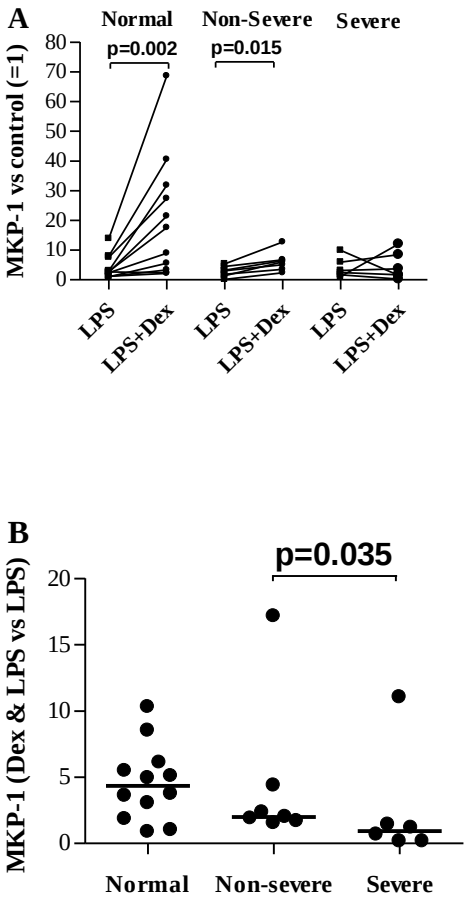

IL-1β
% cytokine release with LPS & Dex relative to LPS alone
Log increase in p38 phosphorylation following LPS stimulation
r=0.48
p<0.05
IL-6
% cytokine releasewith LPS & Dex relative to LPS alone
Log increase in p38 phosphorylation following LPS stimulation
r=0.57
p<0.05
IL-8
% cytokine releasewith LPS & Dex relative to LPS alone
Log increase in p38 phosphorylation following LPS stimulation
r=0.54
p<0.05
MIP-1α
% cytokine release with LPS & Dex relative to LPS alone
Log increase in p38 phosphorylation following LPS stimulation
r=0.7
p<0.01

Figure 5


A
Normal
Non-Severe
Severe
p=0.002
p=0.015
MKP-1 vs control (=1)
80
70
60
50
40
30
20
10
0
LPS
LPS+Dex
LPS
LPS+Dex
LPS
LPS+Dex
B
p=0.035
MKP-1 (Dex & LPS vs LPS)
20
15
10
5
0
Normal
Non-severe
Severe