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Characterization of the inflammatory response to inhaled lipopolysaccharide in mild to moderate chronic obstructive pulmonary disease

Vandana Gupta,[1] Antonia Banyard,[1] Aoibheann Mullan,[1] Srividya Sriskantharajah,[2] Thomas Southworth[1] & Dave Singh[1]

[1]Medicines Evaluation Unit, University Hospital of South Manchester Foundation Trust, University of Manchester, Southmoor Rd, Manchester and [2]Refractory Respiratory Inflammation Discovery Performance Unit, GlaxoSmithKline, Stevenage, UK

Correspondence
Dr Vandana Gupta MRCP, BMBS, BMedSci (Hons), Medicines Evaluation Unit, University Hospital South Manchester, University of Manchester, Manchester, M23 9LT, UK.
Tel.: 0161 946 4050
Fax: 0161 946 1459
E-mail: vgupta@meu.org.uk

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Inhaled lipopolysaccharide (LPS) has been used safely in healthy subjects, asthmatics and smokers to induce increased neutrophilic inflammation and to assess the effects of novel drugs.
- Chronic obstructive pulmonary disease (COPD) exacerbations are characterized by increased neutrophilic inflammation.

WHAT THIS STUDY ADDS

- We performed LPS inhalation safely in 12 COPD patients.
- Inhaled LPS in COPD patients increased airway and systemic inflammation; this appears to be a model that resembles COPD exacerbations and could be used to evaluate novel COPD therapies.

AIMS

Lipopolysaccharide (LPS) inhalation causes increased airway and systemic inflammation. We investigated LPS inhalation in patients with chronic obstructive pulmonary disease (COPD) as a model of bacterial exacerbations. We studied safety, changes in sputum and systemic biomarkers. We have also investigated interleukin (IL)-17 concentrations in this model.

METHODS

Twelve COPD patients inhaled 5 μg LPS. Safety was monitored over 24 h. Sputum was induced at baseline, 6 and 24 h for cells and IL-8, IL-17, neutrophil elastase, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1β (MIP-1β) in supernatants. Serum was collected at baseline, 4, 8 and 24 h for IL-6, C-reactive protein (CRP) and Clara cell protein (CC-16) concentrations. Peripheral blood mononuclear cells (PBMCs) were isolated at baseline and 4 h for systemic IL-17 analysis.

RESULTS

LPS 5 μg was well tolerated. The greatest FEV_1 change was 11.7% (mean) at 1 h (95% CI 5.1–18.2%). There was a large range in maximal fall (2.5–37.7%). Total sputum cell count and neutrophil count significantly increased 6 and 24 h post-LPS. There was no change in sputum supernatant mediators. IL-6, CRP and CC-16 increased post-inhalation, with different temporal patterns. CD4+ and CD8+ cell associated IL-17 significantly increased at 4 h.

CONCLUSIONS

Inhaled LPS in COPD patients safely causes increased airway and systemic inflammation. This may be a model for studying COPD exacerbations.

Introduction

Chronic obstructive pulmonary disease (COPD) patients suffer with progressive airflow obstruction [1]. A hallmark feature of this condition is increased airway inflammation, involving neutrophils, macrophages and lymphocytes [2]. A subgroup of COPD patients also have significant systemic inflammation [1]. Exacerbations are defined as acute worsenings of COPD beyond the normal day to day variation [3]. COPD patients who frequently exacerbate have increased mortality, accelerated decline in lung function and worse quality of life [3]. Exacerbations may be caused by viral or bacterial infections, and are characterized by increased airway and systemic inflammation [4]. There is a need to develop novel therapies to prevent exacerbations.

Lipopolysaccharide (LPS), also known as endotoxin, is a constituent of the outer cell membrane of gram negative bacteria and triggers an innate immune response after binding to Toll like receptor 4 (TLR4) [5]. Inhaled LPS has been used experimentally in healthy subjects and patients with asthma to cause acute neutrophilic airway inflammation [6, 7], which is accompanied by a short lived systemic immune response involving an increase in C-reactive protein (CRP) and interleukin 6 (IL-6) concentrations [6, 8, 9]. This model has been used safely in healthy subjects in order to investigate the effects of anti-inflammatory drugs on neutrophilic airway inflammation [10–12]. Novel drugs in early clinical development for the treatment of COPD can be tested using this healthy volunteer model before proceeding to larger studies involving COPD patients.

A potential criticism of the LPS model in healthy non-smokers is that acute inflammation in healthy lungs does not resemble COPD lung inflammation. The numbers of airway neutrophils are increased in healthy smokers compared with healthy non-smokers [13] and are further increased in COPD patients [14]. We have recently demonstrated that inhaled LPS causes an acute increase in airway neutrophil numbers in healthy smokers [15]. This might more closely resemble the acute increase in airway neutrophil numbers that occurs on a background of chronic neutrophilic airway disease during COPD exacerbations [16]. We were interested to develop this model further by characterizing the immune response to LPS in COPD patients. This could serve as a model of bacterial exacerbations in COPD patients, in order to test the efficacy of novel pharmacotherapies.

Recently, there has been much interest in COPD biomarkers that are secreted by the lung and measurable systemically. An example is CC-16 (Clara cell protein), which is produced by lung Clara cells and has anti-inflammatory actions [17]. CC-16 concentrations are reduced in serum and sputum of COPD subjects compared with controls [17]. There is also an increase in CC-16 concentrations in healthy non-smokers after inhalation of LPS and ozone [18, 19] and healthy smokers after LPS inhalation [15]. These findings suggest that CC-16 concentrations are increased by acute lung inflammation but decreased during the chronic inflammation that occurs in COPD.

LPS inhalation in COPD patients may serve as a model of COPD exacerbations that could be used to investigate the effects of novel pharmacotherapies in early phase clinical trials. The aims of this study were to examine the safety of LPS inhalation in mild to moderate COPD patients and to characterize changes in airway and systemic inflammation. We measured changes in sputum cellular counts, sputum supernatant IL-8, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1β (MIP-1β) concentrations, peripheral blood IL-6, CRP and CC-16 concentrations and IL-17 expression in the sputum and peripheral blood lymphocytes.

Methods

Patients

Twelve COPD patients with a forced expiratory volume in 1 s (FEV_1) ≥70% and <100% predicted and a FEV_1 : forced vital capacity (FVC) ratio <70% were recruited. Demographic details are shown in Table 1. Eight patients were taking inhaled corticosteroids. Patients were excluded if they had any other significant respiratory disease including asthma, any other inflammatory disease or an exacerbation within 6 weeks of screening. All patients were previously diagnosed with COPD by their primary care

Table 1

A summary of baseline demographics of the 12 subjects

Age (years)	62.2 ± 4.6
M/F	10/2
FEV_1 (l)	2.6 ± 0.7
FEV_1 % predicted	85.3 ± 9.3
FEV_1 : FVC	60.4 ± 6.3
BMI (kg m^{-2})	27.0 ± 3.2
Pack years	38.8 (11.4–122.2)*
ICS (Y/N)	8/4
LAMA (Y/N)	4/8
LABA (Y/N)	8/4
Current/Ex smoker	5/7
Chronic bronchitis (Y/N)	8/4
Number of exacerbations in previous 12 months	0 (0–2)*
FEV_1 reversibility (ml)	219 ± 49.9
FEV_1 reversibility (%)	9.6 ± 2.5
CAT score (n = 7)	17.9 ± 2.3
MMRC score (n = 7)	1 ± 0.2

All values are expressed as mean ± SD, apart from *, expressed as median (range). BMI, body mass index; CAT, COPD assessment tool; FEV_1, forced expiratory volume in 1 s post 200 μg inhaled salbutamol; FVC forced vital capacity; ICS inhaled corticosteroids; LABA long acting β-adrenoceptor agonist; LAMA long acting muscarinic antagonist; mMRC score Modified Medical Research Council score.

physician and the clinical history of COPD was checked by a physician at the research unit. In order to be eligible for the study patients had to produce an adequate sputum sample at the screening visit. All 12 patients produced adequate samples at subsequent visits. Written informed consent was obtained from all subjects and the study was approved by the Greater Manchester South ethics committee (reference 11/H1003/1).

Study design

The protocol specified that the safety and effects of 5 μg and 30 μg LPS inhalation on systemic and airway inflammation in COPD patients would be assessed, with all patients completing the lower dose before proceeding to the higher dose. The patients underwent baseline sputum induction. After an interval of at least 48 h, an inhaled LPS challenge (*Escherichia coli* serotype 026:B6, Sigma-Aldrich, Gillingham, UK) was performed. LPS was reconstituted in 0.9% saline and delivered via a Mefar dosimeter (Markos Mefar, Bresica, Italy). Sputum induction was repeated at 6 and 24 h post-LPS inhalation. Pulse, blood pressure, oxygen saturations, temperature and spirometry (using Vitalograph, Buckinghamshire, UK) were performed pre-challenge, 5 and 30 min and then every hour for 8 h post-challenge, with the exception of spirometry, which stopped after sputum induction at 6 h when patients were administered salbutamol. Spirometry was also measured pre-sputum induction at 24 h. Patients were asked to report any new symptoms up to 24 h after LPS inhalation. Blood samples for biomarkers were collected pre-challenge and at 4, 8 and 24 h post-LPS challenge. Subjects were asked to refrain from smoking for at least 2 h prior to LPS inhalation.

Sputum supernatant and serum biomarkers

Sputum was induced and processed with dithiothreitol (DTT) using established methods [20]. Further details are in the online supplement. Sputum supernatants were analyzed for IL-17A and IL-8 by ELISA, along with MCP-1 and MIP-1β by Luminex assay. Limits of detection are stated in the online supplement. The effect of DTT on IL-17, MCP-1 and MIP-1β recovery by immunoassay was assessed by reconstituting standards with and without 0.05% DTT. It is already known that DTT does not affect IL-8 recovery [21]. Neutrophil elastase was quantified using Rhodamine. Full details are in the online supplement. Serum concentrations of IL-6 and CC-16 were measured by ELISA, while CRP was measured using an immunoturbidimetric technique. Full details are in the online supplement. All immunoassays were performed in triplicate. All values were required to be within 10% of each other. The mean of these triplicates was used.

Flow cytometry

Analysis of IL-17 positive lymphocytes from blood samples obtained from 11 patients before and 4 h post-LPS inhalation was carried out by flow cytometry. Further details are in the online supplement. Gating strategy is represented in Figure S1.

Statistical analysis

No formal sample size calculation was performed, as there are no previous LPS challenge data in COPD patients to perform such a calculation. A sample size of 12 was chosen based on previous studies with similar sample sizes that were able to [7, 15] detect changes in FEV_1 and sputum inflammatory markers.

Safety data were analyzed using one way ANOVA with Bonferroni correction. Neutrophil and macrophage differential counts and serum CC-16 concentrations were normally distributed, with LPS effects analyzed by Student's paired *t*-tests. Other sputum cell counts, sputum supernatant measurements, and serum IL-6 and CRP were non-parametric, so were log transformed and then compared using Student's paired *t*-tests. Flow cytometry IL-17 concentrations were compared using a Wilcoxon matched paired test. $P < 0.05$ was considered significant. Two sputum slides obtained after LPS challenge from 10 patients were counted, in order to assess within sample repeatability; Bland–Altman analysis and intraclass correlation coefficients (ICC) were performed.

Results

Safety

Following inhalation of 5 μg LPS, five patients experienced minor symptoms of headache ($n = 4$) and mild cough ($n = 1$), which resolved at 24 h. Figure 1A shows there was a significant fall in FEV_1 from 0.5 to 6 h after LPS inhalation, which returned to normal at 24 h. The greatest mean change in FEV_1 was 11.7% at 1 h (95% CI 5.1, 18.2%). The maximal fall in FEV_1 varied between patients (2.5% to 37.7%; Figure 1B), and occurred between 5 min and 5 h post-LPS inhalation (mean 155.4 ± 30.7 min). Given this large fall in FEV_1 observed in some patients after inhalation of 5 μg LPS, the investigators decided that it was unsafe to proceed to inhalation of 30 μg. There were no significant relationships between smoking status or inhaled corticosteroid use and maximal fall in FEV_1.

There were no significant changes in the mean temperature and pulse after LPS inhalation (Figure 1C, D), or oxygen saturations and blood pressure (see Figure S2 for oxygen saturations; blood pressure data not shown). There was, however, a large range in observed changes in temperature and pulse post-LPS. Maximal increase in temperature and pulse varied between 0 and 1.1°C and 1 and 28 beats min^{-1}, respectively.

Airway inflammation

Sputum cell counts There were significant increases in total cell count g^{-1} of sputum at 6 and 24 h (2.5 and 2.7 fold

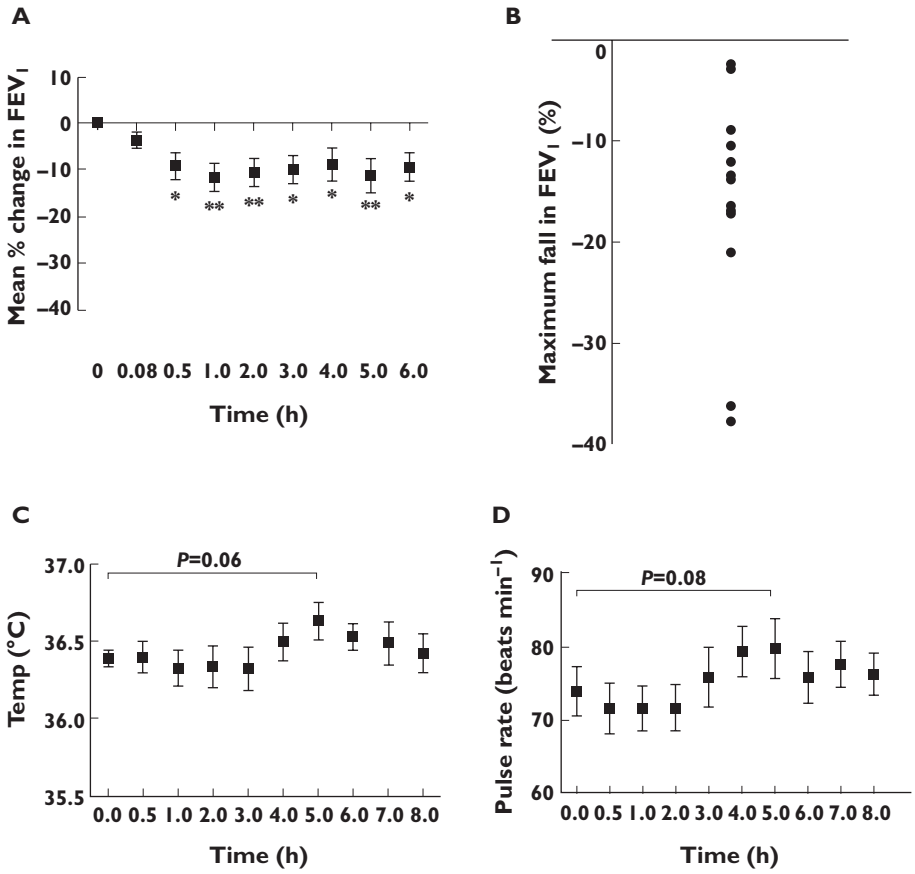

Figure 1

Safety assessments following LPS inhalation. (A) percentage change in FEV_1, (B) maximal fall in FEV_1 for each individual subject, (C) change in temperature and (D) change in pulse rate. All data points in (A), (C) and (D) represent mean and error bars SEM, (all $n = 12$). * indicates $P \leq 0.05$ and ** indicates $P \leq 0.001$ compared with baseline

Table 2

Sputum cell counts at baseline, 6 h and 24 h post-LPS inhalation

	Baseline	6 h	24 h
Neutrophil %	72.0 (± 14.6)	78.5 (± 11.6)	76.6 (± 10.8)
Macrophage %	22.3 (± 11.0)	18.6 (± 10.9)	18.6 (± 10.4)
Eosinophil %†	0.8 (0.3–13.5)	0.8 (0–3.3)	0.8 (0–11)
Lymphocyte %†	0.0 (0–0.8)	0.0 (0.0–1.0)	0.0 (0–1.0)
Epithelial cell %†	1.8 (0–9.8)	1.4 (0–4.8)	2.4 (0–5.7)
Total cell count ($\times 10^6$) per gram†	2.1 (0.3–13.9)	5.2 (2.1–25.2)*	5.6 (1.9–17.3)*
Neutrophil cell count ($\times 10^6$) per gram†	1.6 (0.3–11.3)	4.1 (1.1–22.4)*	3.7 (1.4–15.5)*
Macrophage cell count ($\times 10^6$) per gram†	0.4 (0.1–4.4)	0.9 (0.2–2.7)	0.1 (0.0–1.8)
Eosinophil cell count ($\times 10^6$) per gram†	0.04 (0.00–0.16)	0.04 (0.00–0.15)	0.04 (0–0.75)
Lymphocyte cell count ($\times 10^6$) per gram†	0.00 (0.00–0.01)	0.00 (0.00–0.02)	0.00 (0.00–0.05)
Epithelial cell count ($\times 10^6$) per gram†	0.06 (0.00–0.46)	0.07 (0.00–0.13)	0.11 (0.00–0.69)

Neutrophil and macrophage % are presented as mean (± SD) and results compared with baseline using Student's paired *t*-test. †Data are presented as median (range), ($n = 12$). These results have been natural log transformed and then compared using Student's paired *t*-test. * indicates $P \leq 0.05$.

respectively), and also total neutrophil count g^{-1} of sputum at 6 and 24 h (2.5 and 2.3 fold respectively; Table 2). There were no significant changes in sputum neutrophil % or the percentage or absolute number g^{-1} of any other cell type after LPS inhalation. There were few squamous cells present in the cytospins (mean 0.6%). There were no significant relationships between maximal fall in FEV_1 and total neutrophil count g^{-1} sputum. There were also no sig-

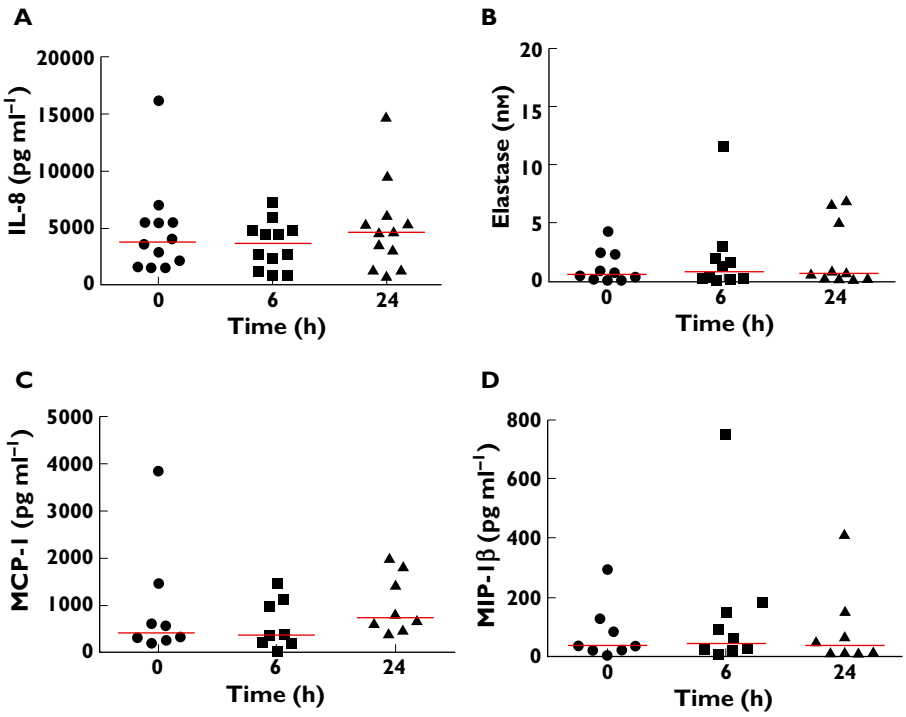

Figure 2

Changes in sputum supernatant IL-8 (A; $n = 12$), elastase activity (B; $n = 10$), MCP-1 (C; $n = 8$) and MIP-1β (D; $n = 8$) post- LPS inhalation. Individual data points are represented, and red lines represent median

nificant relationships between smoking status or inhaled corticosteroid use and total neutrophil count g^{-1} sputum. The mean difference between neutrophil percentage between slides from the same patient sample was −1.8%, with limits of agreement −8.1 to 4.5%. ICC was 0.95, indicating excellent repeatability.

Sputum supernatant There were no significant changes in NE activity, IL-8, MCP-1 or MIP-1β in sputum supernatants at 6 and 24 h post-LPS inhalation (Figure 2). IL-17 was not detected in sputum supernatants. The recovery rates of the standards after addition of 0.05% DTT (mean ± SD) were 83.6 ± 13.2% for MCP-1, 69.7 ± 4.3% for MIP-1β, and 24.8 ± 4.5% for IL-17.

Systemic inflammation

Serum biomarkers Figure 3 shows a significant increase in CC-16 at 4 h after LPS inhalation, which then returned to below baseline levels at 24 h. IL-6 concentrations were increased at 4 and 8 h after inhalation, with CRP increased at 24 h after challenge.

IL-17 concentrations The expression of IL-17 positive CD4 and CD8 blood lymphocytes increased significantly 4 h post-LPS inhalation compared with baseline (Figure 4), with $CD4^+$ $IL\text{-}17^+$ cells increasing from a median of 0.3% to 3.75% ($P = 0.004$) and $CD8^+$ $IL\text{-}17^+$ cells increasing from 0.3% to 2.75% ($P = 0.004$). The percentage of lymphocytes staining for CD3, CD4 and CD8 did not differ between baseline and 4 h post-LPS inhalation.

Discussion

LPS administered to COPD patients increased the total number of sputum neutrophils, with associated changes in serum levels of CC16, IL-6 and CRP. IL-17 expression in peripheral blood CD4 and CD8 cells was also increased, implicating TH17 cells in the acute systemic response after pulmonary TLR4 activation. We have safely administered LPS to COPD patients and suggest that this is a model of COPD bacterial exacerbations that can be used to investigate disease mechanisms or the effects of new pharmacotherapies.

The 5 µg inhaled LPS dose was symptomatically well tolerated, although there were large decreases in FEV_1 in some patients. We therefore felt it unsafe to proceed to the higher LPS dose stated in the protocol. Previous studies involving healthy volunteers have administered inhaled LPS doses up to 300 µg [6, 8, 22, 23], which have been associated with an increased incidence of side effects, such as decreases in lung function and flu-like symptoms. We used the same LPS serotype that we studied in healthy smokers, and observed that 5 µg caused greater decreases

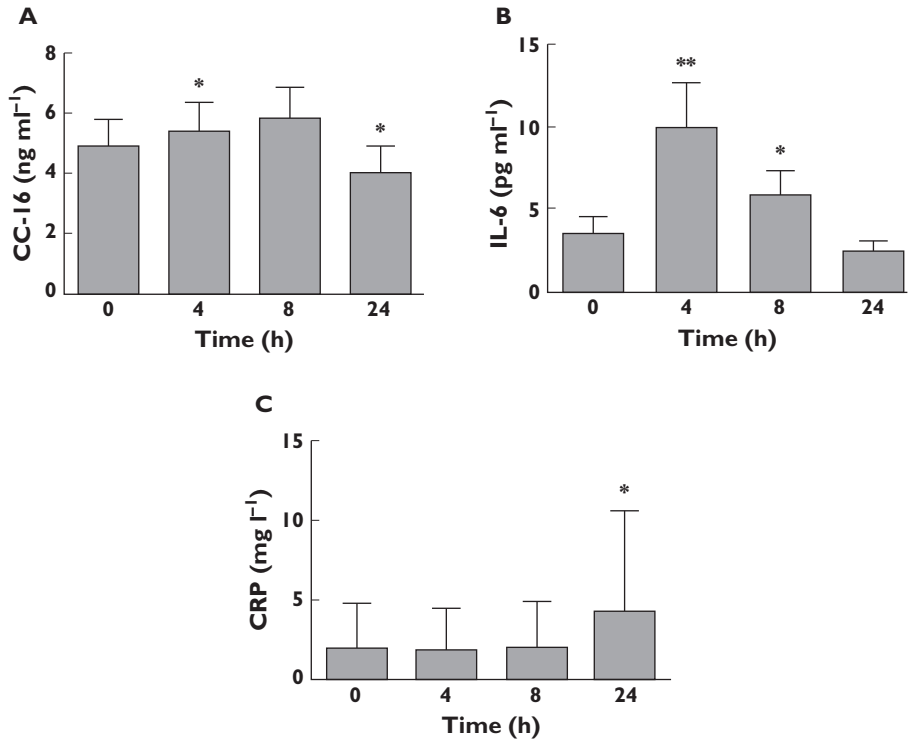

Figure 3

Changes in systemic biomarkers over time following LPS inhalation. (A) represents CC-16 concentrations post-challenge, (B) represents IL-6 concentrations post-challenge and (C) represents CRP concentrations post-challenge. (A) is presented as mean and error bars represent SEM. (B) and (C) are presented as median and error bars represent range, (all $n = 12$). * indicates $P \leq 0.05$, ** indicates $P \leq 0.001$

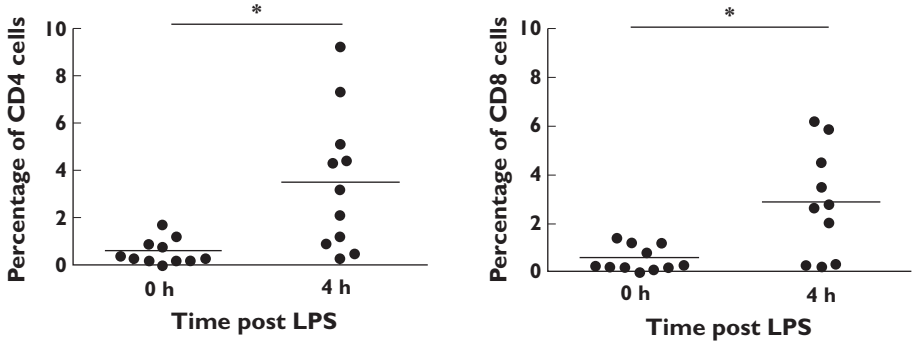

Figure 4

CD4 and CD8 IL-17 expression in peripheral blood mononuclear cells (PBMCs) 0 h and 4 h post-LPS inhalation expressed as a percentage of CD3 positive population ($n = 11$), * indicates $P \leq 0.05$

in FEV_1 in some of the COPD patients compared with healthy smokers [15] where there was a mean maximal decrease in FEV_1 of 8.2% (range 1.2–19.1%). This suggests that the presence of airflow obstruction in COPD predisposes to an increased tendency to bronchoconstriction after inhaled LPS. For this reason, we recommend that inhaled LPS challenges are only performed in patients with mild/moderate COPD. For safety reasons we included COPD patients with FEV_1 ≥70% predicted. As with FEV_1, there was a wide range in change in temperature (0–1.1°C) and pulse (1–28 beats min^{-1}) again indicating significant variation in systemic LPS responses.

The large variation in the FEV_1 response to inhaled LPS may be due to LPS tolerance [24], as the airways of some COPD patients are colonized with bacteria that stimulate TLR4 [25]. Current smoking status may also be important, as cigarette smoke contains LPS [26] and therefore could lead to tolerance. However, we did not find any relation-

ship between current smoking status and response to LPS, although the numbers involved in this sub-analysis were small. Alternatively, genetic polymorphisms in the TLR4 receptor complex may be responsible for this diversity [27]. Furthermore, although there was a standardized method of LPS inhalation, the delivered dose of LPS may have varied between patients.

Inhaled LPS increased the total sputum cell count and absolute number of neutrophils at 6 and 24 h post-inhalation. However, there was no change in sputum neutrophil percentage. This contrasts with studies in healthy non-smokers where inhaled LPS increased both the percentage and absolute number of sputum neutrophils [6, 7, 11, 22]. Healthy non-smokers have a neutrophil percentage that is generally <50%, affording an adequate 'window' for LPS to increase the neutrophil percentage [14]. It is well known that healthy smokers have an increased neutrophil percentage caused by cigarette smoking, often in the range 50–70% [15]. We have shown that inhaled LPS causes a further small increase in the neutrophil percentage in healthy smokers and also increases the total neutrophil cell count [15]. The high pre-LPS challenge sputum neutrophil percentage in COPD patients (71%) prevented a statistically significant increase in this measurement being observed after LPS challenge. However, we were still able to observe >2-fold increases in the total neutrophil count g^{-1} of sputum. In addition, previous studies investigating sputum neutrophils in COPD exacerbations compared with stable patients have also found increased sputum neutrophil numbers but not percentages [28–30]. Thus the sputum changes seen in our model resemble those seen during exacerbations. Within sample repeatability of sputum neutrophil percentage was excellent in this study, with an ICC of 0.95. Furthermore, there are multiple reports demonstrating both short term [31, 32] and long term [33] reproducibility of sputum cell counts. These results support the use of sputum neutrophils as a biomarker in COPD.

The majority of patients in this study (eight out of 12) were taking regular inhaled corticosteroids. A subgroup analysis showed no influence of inhaled corticosteroid use on the LPS response, although it is clear that the sample size for this analysis is small. It is unlikely that inhaled corticosteroids influenced the LPS response in this study, as oral corticosteroids do not affect LPS induced airway inflammation in healthy subjects [11].

Studies in healthy volunteers and patients with asthma have shown that inhaled LPS increases the concentrations of IL-8 and MCP-1 in induced sputum [7, 22], while in bronchoalveolar lavage there is an increase in IL-8 and MIP-1 concentrations after LPS inhalation [10, 34]. We did not observe any changes in the levels of these proteins in induced sputum after LPS challenge. COPD patients have higher levels of IL-8 and MCP-1 [14, 35] in induced sputum compared with healthy controls, and perhaps the reason for a lack of induction of IL-8 in COPD patients was that the levels were already elevated before LPS inhalation. Another possible explanation for our finding is that we terminated the study after a low dose of LPS, while a higher LPS dose might have increased the levels of these proteins. However, the increased sputum neutrophil cell count caused by the low dose of LPS in the current study suggests that LPS increased neutrophilic chemokine levels in the airways of COPD patients. Perhaps our study was underpowered to detect such changes in the sputum supernatant.

Sputum NE activity has been shown to be elevated in subjects with COPD compared with controls [36] and is further increased during exacerbations, in particular bacterial exacerbations [37]. There was no significant increase in NE activity post-LPS inhalation. Again, this may be due to a higher baseline in COPD subjects to begin with, and the low dose of LPS used.

LPS inhalation causes an acute increase in serum CC-16 concentrations in healthy smokers followed by a decrease to below pre-challenge levels by 24 h [15]. The same pattern was observed in COPD patients in this study. In the stable state, it is known that smokers and COPD patients have lower concentrations of CC16 in serum and sputum compared with healthy non-smokers [17]. This suggests that acute inflammation caused by TLR4 signalling in COPD causes an acute up-regulation of CC16 secretion, followed by chronic down-regulation.

Serum IL-6 and CRP concentrations are increased during exacerbations of COPD [4]. Inhaled LPS increased serum IL-6 and CRP in COPD patients. This has also been observed in healthy volunteers [6, 8, 9]. IL-6 regulates CRP production by the liver [38], explaining the temporal difference in the increase in these biomarkers. Although we detected an increase in CRP concentrations at 24 h only, this increase may have occurred at any time between the blood sampling times of 8 and 24 h. These changes in systemic inflammatory biomarkers indicate that the airway inflammatory changes caused by the low dose of LPS were associated with significant systemic inflammation. The airway and systemic inflammatory changes caused by LPS inhalation are similar to those observed during COPD exacerbations [4, 37], and suggest that this model could be used to test the effects of novel drugs designed to prevent the inflammatory response during COPD exacerbations.

IL-17 is released by a distinct $CD4^+$ T-cell lineage called TH17 cells as well as $CD8^+$ T cells. The expression of IL-17 is increased in the airways of COPD patients compared with controls [39, 40]. TH17 cell numbers are also increased in the peripheral blood of subjects with COPD [41]. We observed that LPS inhalation increased the number of CD4+ and CD8+ cells expressing IL-17. Lymphocytes are usually activated by antigens processed by antigen presented cells and recognised by the T cell receptor (TCR) [42]. However, lymphocytes can also be activated by non-TCR mediated mechanisms such as cytokine activation or

through TLR receptors expressed on lymphocytes [43]. Although we delivered LPS locally to the lungs, it is possible that some was absorbed into the systemic circulation and caused TLR mediated IL-17 production. Alternatively, cytokine activation of these peripheral lymphocytes is possible.

There is evidence that bacteria can activate lymphocytes in COPD. Non-typeable *Haemophilus* influenza induces more IL-13, IL-17 and TNF-α release from COPD lung CD4 and CD8 cells than controls [44]. Our results further implicate bacterial antigens in the activation of lymphocytes in COPD.

The expression of TH17 cells during COPD exacerbations has not been studied. Our findings suggest that the fraction of IL-17 producing lymphocytes in the blood may be increased early during COPD exacerbations. IL-17 is known to promote neutrophilic inflammation [45] and, perhaps, pharmacological interventions designed to interfere with TH17 activity may reduce neutrophilic inflammation during COPD exacerbations.

IL-17 concentrations in sputum supernatants were all lower than the assay standards. A previous study also encountered low IL-17 concentrations in sputum supernatants, which were not detectable using ELISA, but could be measured using a high sensitivity immunoassay [46]. A further problem that we encountered with IL-17 detection was the low recovery rate (mean 24.8%). These technical issues mean that we cannot be sure whether there was an increase in IL-17 concentrations in sputum. In contrast, recovery rates of approximately 70% or above for MCP-1 and MIP-1β were observed, which meet the standards of acceptability set out in guidelines for sputum supernatant analysis [47]. We can conclude that there were no increases in the levels of these proteins.

There are other experimental human models of neutrophilic airway inflammation. Inhaled ozone challenge has been used to investigate the effects of novel anti-inflammatory drugs on neutrophilic airway inflammation in healthy volunteers [48–50]. In addition, inhaled platelet activating factor (PAF) [51] causes neutrophilic airway inflammation in asthmatics. Recently, experimental rhinovirus infection in COPD patients has been used as a model of COPD exacerbations, with increased sputum neutrophils observed [52].

A significant limitation of this study is that it was carried out in mild COPD patients (FEV >70%) and therefore the results may not be generalizable to patients with more severe disease. However, the FEV_1 reductions observed in some patients raises safety issues if this model were to be used in patients with more severe COPD. Our study design involved unblinded challenges, without a placebo challenge. An alternative design would have involved randomized, placebo controlled challenges. However, it should be noted that inhaled challenges in clinical trials, including LPS, are performed in an unblinded manner [10–12, 48–50]. We did not investigate reproducibility of challenges performed on different days, although a previous similar study demonstrated good reproducibility in healthy smokers [15].

We have demonstrated increased systemic and airway inflammation which resembles COPD exacerbations, but did not observe increased airway IL-8 or sputum neutrophil percentage which are both known to be associated with exacerbations [16, 37]. Our study demonstrates initial safety data for using LPS challenges in COPD and the changes in IL-8 and neutrophil percentage could be addressed in further larger studies.

In conclusion, we have safely conducted inhaled LPS challenges using a low dose (5 μg), which increased neutrophilic airway inflammation in COPD patients. This was associated with changes in systemic inflammation and overall the inflammatory changes observed resembled those seen in COPD exacerbations. Inhaled LPS challenge in COPD patients can therefore be used as a model to evaluate novel anti-inflammatory drugs designed to prevent COPD exacerbations.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare SK had support from GlaxoSmithKline for the submitted work and DS reports grants and personal fees from Almirall, grants and personal fees from AstraZeneca, grants and personal fees from Boehringer Ingleheim, grants and personal fees from Chiesi, grants and personal fees from GlaxoSmithKline, grants and personal fees from Merck, grants and personal fees from Novartis, grants and personal fees from Pfizer, grants and personal fees from Takeda, personal fees from CIPLA, personal fees from Forest, personal fees from Genetech outside the submitted work. There are no other relationships or activities that could appear to have influenced the submitted work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1
The gating strategy for the quantification of CD4 and CD8 IL-17 from a CD3 positive lymphocyte population basally (A) and 4 h post-LPS inhalation (B) with their corresponding isotype controls

Figure S2
Changes in oxygen saturations post-LPS inhalation. All data points represent mean ± SEM