Defective macrophage phagocytosis of bacteria in COPD

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ABSTRACT (200 words)

Exacerbations of chronic obstructive pulmonary disease (COPD) are an increasing cause of hospitalizations and are associated with accelerated progression of airflow obstruction. Approximately half of COPD exacerbations are associated with bacteria and many patients have lower airways colonization. This suggests that bacterial infection in COPD could be due to reduced pathogen removal. This study investigated whether bacterial clearance by macrophages is defective in COPD.

Phagocytosis of fluorescently labelled polystyrene beads and *Haemophillus influenzae* and *Streptococcus pneumoniae* by alveolar macrophages and monocyte-derived macrophages (MDM) were assessed by fluorimetry and flow cytometry. Receptor expression was measured by flow cytometry.

Alveolar macrophages and MDM phagocytosed polystyrene beads similarly. There was no difference in phagocytosis of beads by MDM from COPD patients compared with cells from smokers and non-smokers. MDM from COPD patients showed reduced phagocytic responses to *S.pneumoniae* and *H. influenzae* compared with non-smokers and smokers. This was not associated with alterations in cell surface receptor expression of TLR2, TLR4, MARCO, CD163, CD36 or the mannose receptor. Budesonide, formoterol, or azithromycin did not suppress phagocytosis suggesting that reduced responses in COPD MDM were not due to medications.

COPD macrophage innate responses are suppressed and may lead to bacterial colonisation and increased exacerbation frequency.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease comprising small airways disease and emphysema [1]. It is currently the 6th most common cause of death globally and is predicted to become the 3rd most common cause of death by 2020 [2]. This may be due to the increased incidence of cigarette smoking as this is the most common risk factor for COPD and contributes to approximately 85% of all cases. Approximately, 15% of smokers will develop COPD whereas the incidence in non-smokers is 1.6% [3]. In healthy individuals and smokers without lung obstruction, the lower airways are sterile, but in COPD patients there is often colonization of the lower respiratory tract, with *Streptococcus pneumoniae* and *Haemophilus influenzae* being the most common bacterial pathogens [4]. Exacerbations of COPD are an increasing cause of hospitalizations in the UK [5], are associated with accelerated disease progression [6] and account for much of the healthcare costs associated with COPD [7,8]. The causes of exacerbations vary, but ~50% of infective exacerbations are bacterial in origin. Alveolar macrophages contribute 90-95% of cells found in bronchoalveolar lavage fluid and are highly phagocytic, producing multiple inflammatory mediators [9]. Moreover, their role in removal of potentially pathogenic micro-organisms via phagocytosis is essential in maintaining the normally sterile environment within the lung. One reason for the increased incidence of bacterial infections in the respiratory tract of COPD patients might be failure of macrophages to clear pathogens because of reduced phagocytosis due to chronic activation [10,11].

Alveolar macrophages from COPD patients phagocytose fewer apoptotic epithelial cells [12] and *Escherichia coli* [13] compared with non-smokers and less *H. influenzae* compared with smokers without COPD [14]. Presently, there are no animal models of this aspect of COPD limiting study to primary human cells. Therefore, we compared the phagocytic responses of alveolar macrophages and monocyte-derived macrophages (MDM) from COPD patients with cells from non-smokers and smokers without lung obstruction. The use of MDM examined whether reduced phagocytic responses of alveolar macrophages in COPD was due to these cells becoming replete or whether differentiation in a specific, pro-inflammatory, lung environment was required to establish this defect.

METHODS

Subject selection

Healthy subjects and smokers were recruited from the NHLI, Royal Brompton Hospital, London. COPD subjects were recruited from the Royal Free Hospital, London or Wexham Park Hospital, Slough. All subjects gave written informed consent as approved by the Royal Brompton and Harefield NHS Trust Ethics Committee. Bronchoalveolar lavage (BAL) fluid was obtained from consenting patients at St. Mary's Hospital, London or Wexham Park Hospital, Slough. Demographic data are presented in Table 1. COPD patients were significantly older than the control groups but there were no differences in smoking history with the smoking controls (Table 1).

Cell culture

Monocytes were isolated from PBMC using a Monocyte Isolation kit II (Miltenyi Biotec, Surrey, UK) and cultured in the presence of 2ng/ml GM-CSF for 12d to generate MDM as described previously [15]. Alveolar macrophages were isolated from BAL fluid as described previously [16].

Phagocytosis assays

Non-typeable *H. influenzae* strain 1479 and *S. pneumoniae* serotype 9V, strain 10692 were cultured and heat killed at 60ºC for 2h. Bacteria were fluorescently labelled using Alexafluor 488 dye (2mg/ml in DMSO) in the dark, at room temperature overnight. The labelled bacteria were washed repeatedly in PBS to remove unbound label and resuspended in PBS. For alternative experiments, live bacteria were resuspended in broth containing CellTracker

Red CMPTX dye (12.5μM) (Molecular Probes, Invitrogen, UK) and incubated in the dark at 37°C for 45 min. The labelled bacteria were washed in D-PBS until the free dye was removed and stored at -20 ^{o}C. Fluorescently labelled polystyrene beads or bacteria were added to cells and incubated for the times indicated. Cells were washed with D-PBS and fluorescence of extracellular particles was quenched by adding Trypan blue (2%$^{v}/_{v}$) for 1 min. Excess fluid was removed and fluorescence determined for beads and heat killed bacteria using an excitation λ 480nm and emission λ 520nm. For experiments using live bacteria, MDM were exposed for 4h prior to measurement in a platereader using an excitation λ 570 nm and emission λ 610 nm. Initial experiments and pharmacology experiments with *E.coli* were performed using the Vybrant Phagocytosis kit according to the manufacturer's instructions (Invitrogen Ltd, Paisley, UK). Data are presented as phagocytosis relative to the fluorescence of each specific bacterium to account for differences in labelling. To confirm the labelling procedure was not altering the ability of cells to phagocytose, *E.coli* (strain K12, Sigma, Dorset, UK) were labelled under identical conditions and used in comparative experiments. Alternatively, following exposure of cells to bacteria as described above, cells were removed from the plate by agitation and fluorescence measured using a flow cytometer.

Confocal microscopy

Macrophages were cultured on Lab-tek Permanox chamber slides and fixed with 4%$^{w}/_{v}$ paraformaldehyde. Nuclei were stained with DAPI and cell cytoplasm by incubation with Evans blue dye (0.1%$^{w}/_{v}$). Slides were viewed on a Leica TCS 4D Confocal microscope with a Krypton-Argon laser to detect

fluorescence of the FITC/yellow-green/Alexafluor 488 fluorochromes. Images of the three stains (DAPI (blue), Evans Blue (red) and the fluorochromes (green)) were overlaid.

Electron microscopy

MDM were incubated with *H. influenzae* (1mg/ml, 1h) and fixed by addition of glutaraldehyde. Secondary fixation was performed with osmium tetroxide. Samples were then suspended in molten 2% ($^{w}/_{v}$) agar and dehydrated by incubation with increasing concentrations of methanol. Cells were infiltrated with propylene oxide and Araldite resin, followed by incubation and embedding in Araldite alone. Ultrathin sections were then cut, mounted on copper support grids and stained using uranyl acetate and Sato's lead citrate. Grids were transferred to the TEM for visualization.

Flow cytometric analysis of macrophage receptor expression

MDM were removed from the cell culture plates using non-enzymatic cell dissociation solution (Sigma, Dorset, UK), washed in PBS containing bovine serum albumin (BSA) (0.5%) and sodium azide (PAB) (0.1%) and then resuspended at a concentration of 1×10^6 ml. MDM suspension (180 µl) was incubated for 1 h on ice with 20 µl of either the appropriate mouse isotype control antibody, or mouse monoclonal antibody against CD14, CD163, CD36, mannose receptor, MARCO, PS receptor, HLA-DR, TLR2 and TLR4, all at 50 µg/ml. The cells were washed twice with PAB and a $F(ab)'_2$ fragment of a PE-labelled goat anti-mouse IgG (20 µg/ml) (Dako Ltd, Cambridgeshire, UK) was incubated with the cells for 30 min on ice. The cells were washed with

PAB and finally resuspended in 300 µL of FACSFlow™ containing 0.5% (v/v) formaldehyde prior to analysis on a FACScan cytometer (Becton Dickinson, Oxon, UK). Five thousand events were acquired and the fluorescence staining of the MDM was assessed at 575 nm. The specific mean fluorescence (SMF) values were calculated as the fold difference of the mean fluorescence intensity (MFI) the isotype control to the MFI of the test antibody.

Statistical Analysis

Data are presented as mean ± SEM for 'n' observations. Comparisons between subject groups or cell types were performed using Kruskal-Wallis analysis using GraphPad Prism software followed by Dunn's multiple comparison test (GraphPad Software Inc., San Diego, CA) or a Mann-Whitney test where appropriate. Differences were considered significant where $p<0.05$.

RESULTS

Comparison of alveolar macrophage and MDM phagocytosis

Initial experiments demonstrated that phagocytosis of polystyrene beads by both alveolar macrophages and MDM were similar (Fig. 1a). Moreover, MDM from non-smokers, smokers and COPD patients phagocytosed beads equally (Fig. 1b) which was confirmed as internalization by confocal microscopy (Fig. 1c). This would indicate that the MDM model reflects alveolar macrophage phagocytosis and that inert particle removal is not altered in COPD. In order to ascertain whether similar responses were observed when macrophages were exposed to bacteria, a series of experiments were devised. Alveolar macrophages from COPD patients phagocytosed less *E. coli* compared with cells from non-smokers and smokers (Fig. 2a). By contrast, monocytes from each of the subject groups had low phagocytic responses that did not differ with either smoking or disease status (Fig. 2b). However, MDM from COPD patients exhibited a reduced capacity to ingest bacteria similar to that of alveolar macrophages (Fig.2c). We confirmed this observation using FACS analysis. Phagocytosis by MDM from non-smokers and smokers were similar (Figs. 2d and e) but cells from COPD patients exhibited a significant curve shift to the left (Fig. 2f), indicating that fewer cells had engulfed bacteria. These comparative phagocytosis experiments suggest that in COPD, defective macrophage phagocytosis is acquired during differentiation and that MDM may be useful for studying the underlying defective mechanism.

MDM phagocytosis of *H.influenzae* and *S.pneumoniae*

To investigate whether this reduced phagocytic response to bacteria was restricted to *E.coli* the phagocytic responses of MDM to fluorescently-labelled *H. influenzae, S. pneumoniae* and *E. coli* were evaluated. All MDM phagocytosed the three bacterial strains in a concentration-dependent manner (Fig. 3a-c) but the response of COPD MDM was attenuated, not only towards *E. coli* (Fig 3a) but also for *S. pneumoniae* (Fig. 3b) and *H. influenzae* (Fig. 2c). To investigate as to whether defective phagocytosis in COPD MDM was an artefact of using heat killed bacteria, experiments were devised using labelled, live bacteria. Under these conditions, MDM from control subjects phagocytosed increased *H. influenzae* and *S. pneumoniae* ($p<0.05$) compared with cells from COPD patients (*H. influenzae*: control – 3587 ± 390 *vs*. COPD – 2170 ± 166 fluorescence units, n=9; *S. pneumoniae*: control – 3885 ± 344 *vs*. COPD – 2514 ± 288 fluorescence units, n=9). Confocal microscopy confirmed internalization of *S.pneumoniae* (Fig. 3d) and *H. influenzae* (Fig. 3e) and electron microscopy confirmed bacterial ingestion and formation of phagosomes (Fig. 4) indicating that MDM from COPD patients have the capacity to mount correct phagocytic responses, albeit attenuated.

This reduced clearance of bacterial pathogens by macrophages may account for increased infections and the concomitant decline in lung function observed in COPD. Therefore, we next examined the relationship between lung function parameters and phagocytosis. Phagocytic responses of MDM to both *E. coli*, *H. influenzae* or *S.pneumoniae* did not correlate with FEV_1% predicted (r=0.07, r=-0.05, and r=0.12 respectively). Similarly, FEV_1/FVC ratio also did not correlate, with phagocytosis of *E. coli, H. influenzae* or

S.pneumoniae (r=-0.12, r=-0.10, and r=0.32 respectively). In addition, there was no relationship between phagocytic response and current smoking status. Despite COPD patients having smoked more cigarettes than smokers and being older (see Table 1) there were no correlations between either age or number of cigarettes smoked (pack-years) and phagocytosis of any bacteria examined.

Cell surface expression of receptors involved in bacterial recognition

There are numerous receptors involved in bacterial recognition by macrophages [17,18] thus reduced receptor expression in COPD could account for attenuated phagocytosis. However, there were no differences in the expression of TLR2, CD14, TLR4, or CD163 on MDM from COPD patients compared with cells from the other subject groups (Table 2). There were also no differences in expression of non-specific scavenger receptors including the mannose receptor, macrophage receptor with collagenous structure (MARCO) or CD36 (Table 2). Nor were there any differences in expression of the phosphatidylserine (PS) receptor or HLA-DR on these cells (Table 2).

MDM from COPD patients phagocytosed beads normally (Fig. 1b) limiting defective phagocytosis to engulfment of pathogenic bacteria. Since there were no differences in expression of a number of receptors considered important in recognition of non-opsonized particles (Table 2), the downstream mechanisms emanating from receptor ligation that regulate phagocytic responses were examined. Pharmacological modulation of these pathways was utlilized to investigate whether altered signal transduction could account for these observations. The phosphoinositol-3-kinase (PI3K) inhibitor, LY-

294002 and the p38 inhbitior, SB 203580, inhibited MDM phagocytosis of *E. coli* from all subjects similarly (Figs. S1a and S1c). By contrast, the Rho kinase inhibitor, Y-27632, had no effect on phagocytosis at any of the concentrations tested (Fig. S1b).

Effect of pharmacological agents on MDM phagocytosis

COPD patients are currently taking a variety of medicaments to ameliorate their symptoms. These include glucocorticosteroids and bronchodilators. Therefore, it was possible that systemic effects of these drugs could alter the responses of blood-derived macrophages. To test this possibility, MDM from non-smokers, smokers and patients with COPD were pre-treated with various pharmacological agents prior to phagocytosis assay (Fig. 5).

Exposure of MDM to budesonide improved the phagocytic responses of cells from COPD patients and smokers towards both *H.influenzae* and *S.pneumoniae* (Figs. 5a and 5d). Agents that elevate cAMP are considered to be inhibitory for phagocytosis [19], however formoterol did not inhibit phagocytosis of the bacteria in this system (Figs. 5b and 5e) but stimulated the response of cells from smokers. There was no effect on the responses of cells derived from patients with COPD. The macrolide, azithromycin, is reported to restore the phagocytic response of alveolar macrophages from COPD patients and enable removal of apoptotic cells [20], however in this system azithromycin had no effect on the response of cells from patients with COPD but improved the responses of cells from smokers (Figs. 5c and 5f). Other drugs (tiotropium bromide (10^{-9}-10^{-6} M) or theophylline (10^{-8}-10^{-5}M))

prescribed commonly to patients with COPD had no effect on the phagocytic response (data not shown).

DISCUSSION

Alveolar macrophages are the sentinel cell of the lung, patrolling the airways to remove any inhaled particles or pathogens. Failure of this innate response could lead to pulmonary damage and persistent infection. These are features of COPD and contribute to the worsening of disease. Therefore, this study examined, in detail, the phagocytic responses of macrophages to physiological lung pathogens in COPD.

Using a MDM model, we showed no difference in the capacity of cells from patients with COPD to remove inert particles. This confirms observations in alveolar macrophages from COPD patients where phagocytosis of inert beads occurs to the same extent as cells from control subjects [12,21]. However, we observed a very clear defect in the phagocytic response of MDM and alveolar macrophages from COPD patients to bacteria. This was not an artefact of using heat-killed bacteria, since we observed qualitatively similar data with live bacteria. Recently, alveolar macrophages from cigarette smokers have been shown to have a reduced phagocytic response for apoptotic epithelial cells that was associated with suppression of CD31, CD91, CD44 and CD71 [22]. The present study did not show any effect of smoking on the phagocytic response of MDM or alveolar macrophages for bacteria indicating that the mechanisms for apoptotic cell recognition and bacterial pathogens are quite distinct. Initial experiments using *E.coli* did not show a clear difference in the phagocytic response of MDM from smokers and COPD patients. However, a more detailed analysis using *E.coli* labelled 'in-house' showed reduced phagocytosis of this bacterium between cells from smokers and COPD patients. This discrepancy might reflect sensitivity of labelling

between commercially obtained phagocytic prey and bacteria labelled 'in-house'. Another study has suggested previously that alveolar macrophages from COPD patients phagocytose less *H. influenzae* compared with cells from smokers but not non-smokers [14]. In contrast to our data, they reported no differences in responses of COPD MDM [14]. The reason for this discrepancy with our data is unclear but may reflect differences in the methods used to differentiate monocytes to MDM. Our methodology employed the use of GM-CSF which drives monocytes towards a more alveolar macrophage-like phenotype [23] and we also further validated our model by comparison with alveolar macrophages with respect to phagocytosis of polystyrene beads and *E. coli*.

Examination of the mechanism of reduced bacterial clearance by COPD macrophages led to investigation of cell surface molecules that could be responsible for recognition of bacteria leading to phagocytosis. However, these analyses indicated that various receptors were expressed similarly on MDM from all subject groups. This contrasts to reports that have shown that TLR2 is decreased on the surface of alveolar macrophages in smokers and patients with COPD [24]. Although, TLR may modulate the phagocytic response [25], the observation that removal of both Gram positive and Gram negative bacteria is reduced in COPD would suggest that these receptors are not pivotal in this response. Furthermore, the concept that reduced alveolar macrophage phagocytosis in COPD is due to cells becoming replete in the lung environment is unlikely as MDM, differentiated *in vitro* show the same reduced response.

Investigation into the cell signalling pathways showed that phagocytosis was mediated by a PI-3-kinase dependent mechanism but that Rho kinase is unlikely to be involved. Rho kinase is critical in complement receptor-mediated but not FcγR-mediated phagocytosis [26] and is inhibitory during phagocytosis of apoptotic cells [27]. As Y-27632 had no effect on phagocytosis of *E. coli* by MDM, the defective mechanism in COPD cells is likely to be distinct from that of apoptotic cells and complement opsonised particles. The inhibition of the phagocytic response by a non-selective PI-3-kinase inhibitor and a p38 inhibitor could limit the benefit of these agents as putative anti-inflammatory therapies in diseases such as COPD where the phagocytic response in already suppressed.

Since this study utilised circulating cells that were subsequently differentiated, systemic levels of pharmacological agents used for the treatment of COPD may be responsible for the suppression of the phagocytic response observed in this study. However, none of the pharmaceutical agents examined suppressed phagocytosis of either *H. influenzae* or *S.pneumoniae* in cells from any of subject groups. Therefore, it is unlikely that differences in treatment regimes between the COPD patients and the control groups could account for the suppressed phagocytic response. It is of note that budesonide stimulated the phagocytic response in MDM obtained from patients with COPD. Although glucocorticosteroids do not improve the long term decline in lung function observed in COPD patients, they have been shown to reduce exacerbation frequency [28]. This may be due to improved phagocytic responses of macrophages in the lungs of these patients. However, the concentrations of steroid required to significantly improve phagocytosis *in vitro*

are high (10^{-7}-10^{-6}M) and unlike alveolar macrophages from COPD patients, MDM do respond to steroids (data not shown).

In summary, MDM from COPD patients demonstrate reduced phagocytosis for common airway pathogens. This defect is specific to pathogenic bacteria. MDM from COPD patients are not replete, nor has differentiation occurred in a chronically inflamed lung, suggesting that lack of pathogen removal is an inherent defect in circulating monocytes from COPD patients that unmasks during maturation into macrophages. This defect in phagocytosis of bacteria that most frequently cause acute exacerbations of COPD is likely to be an important factor leading to colonization of the lower airways and the propensity for bacterial exacerbations. Furthermore, the persistence of bacteria in the lower airways may act as a chronic antigenic drive for pulmonary inflammation and could contribute to the increased numbers of T- and B-lymphocytes in the airways of COPD patients [29]. Defining the molecular basis of this defect may lead to identification of susceptibility markers for airway obstruction in asymptomatic smokers and to development of novel therapies that stimulate phagocytic functions, leading to sterilisation of the respiratory tract and a reduction in the bacterial load that may drive chronic inflammation in COPD patients.

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FIGURE LEGENDS

Figure 1. Comparison of alveolar macrophage and MDM phagocytosis of beads

Panel a) alveolar macrophages (open bars), n=7 and MDM (hatched bars) n=7, were exposed to fluorescent beads for 6 h. Phagocytosis was measured using a fluorometric plate reader. Panel b) comparison of phagocytic capacity of MDM from non-smokers (NS) (open bars), n=9, smokers (S) (hatched bars), n=13 and COPD patients (solid bars), n=10 following exposure to 50 x 10^6 beads/ml for 6h. Data are presented as mean $\pm$ SEM. Panel c) Confocal micrograph of MDM engulfing fluorescent beads (50 x 10^6 beads/ml)

Figure 2. Phagocytosis of *E.* coli by alveolar macrophages, monocytes and MDM.

Panel a) Phagocytic response of alveolar macrophages from non-smokers (NS) (open bars), n=5, smokers (S) (hatched bars), n=5 and COPD patients (solid bars), n=4 following exposure to 1 mg/ml FITC-*E. coli* for 1h. Panel b) Phagocytic response of monocytes from non-smokers (NS) (open bars), n=4, smokers (S) (hatched bars), n=4 and COPD patients (solid bars), n=6 following exposure to 1 mg/ml FITC-*E.coli* for 1h. Panel c) Phagocytic response of MDM from non-smokers (NS) (open bars), n=7, smokers (S) (hatched bars), n=6 and COPD patients (solid bars), n=8 following exposure to 1 mg/ml FITC-*E. coli* for 1h. Data are presented as median ± interquartile range, where * represents $p<0.05$ for differences from non-smokers. The phagocytic response of MDM to fluorescently labelled *E. coli* was also determined by flow cytometry. Panel d) represents non-smokers, panel e)

smokers and panel f) COPD patients. Data is presented of a representative FACS histogram where the grey lines are cells in the absence of bacteria and the purple lines are cells in the presence of bacteria for at least n=4 independent experiments.

Figure 3. Phagocytic responses of MDM from non-smokers, smokers and COPD patients to pathogenic bacteria.

MDM were generated from non-smokers (open bars) n=15-16, smokers (hatched bars), n=13-14 and COPD patients (solid bars), n=16-17 and exposed to increasing concentrations of fluorescently labelled *E. coli* (panel a), *S. pneumoniae* (panel b) and *H. influenzae* (panel c) for 1h. Phagocytosis was measured using a fluorometric plate reader. Data are presented as median ± interquartile range where * indicates $p<0.05$ and ** $p<0.01$. Internalization of particles was confirmed using confocal microscopy. Panels d and e show representative orthogonal Z-stack views of phagocytosed *S. pneumoniae* and *H. influenzae* respectively.

Figure 4. Electron microscopy images of MDM phagocytosis of *H. influenzae.*

MDM were generated from COPD patients and incubated with 1mg/ml *H. influenzae* for 1h. Cells were then fixed, dehydrated and embedded in Araldite resin. Ultrathin slices were cut and stained, then viewed on a transmission electron microscope. Arrows indicate bacteria. Image a shows a bacterium on the surface of a MDM, images b and c show formation of phagocytic cups around bacteria, this is proceeded by complete encapsulation by pseudopodia

as indicated in images d and e. The bacteria are then transported into the cell cytoplasm within a phagosome (image f).

Figure 5. Effects of pharmacological agents on phagocytosis of bacteria by MDM.

MDM from non-smokers (■), smokers (▲) and COPD patients (●) were pre-treated with budesonide (panels a and d) or formoterol (panels b and e) for 1h or azithromycin (panels c and f) for 24h prior to exposure of the cells to either 1 mg/ml *H.influenzae* (panels a-c) or *S.pneumoniae* (panels d-f) for 1h. Data were normalised to the phagocytosis response of each cell type in the absence of drug (100%). Data are presented as mean ± SEM for n=5-6.

Table 1. Study participant demographics

	Non-smokers (n=20)	Smokers (n=17)	COPD (n=19)
Age (years)	48 ± 3	53 ± 2	70 ± 2*+
Sex (M:F)	11:9	10:7	11:8
Smoking history (pack years)	0.0±0.0+	33.3 ± 4.6	45.0 ± 4.9*
FEV_1 (l)	3.3 ± 0.15	2.9 ± 0.3	1.2 ± 0.1*+
FEV_1 % predicted	102.7 ± 2.6	93.8 ± 3.8	50.6 ± 4.0*+
FVC (l)	4.2 ± 0.2	3.8 ± 0.3	2.4 ± 0.3*+
FEV_1:FVC	0.8 ± 0.03	0.8 ± 0.02	0.5 ± 0.03*+

Data are presented as mean ± SEM. 1 pack year represent 20 cigarettes per day for 1 year. * represents $p<0.001$ *vs*. non smokers, + represents $p<0.001$ *vs*. smokers.

Table 2. Receptor expression on monocyte-derived macrophages

		Non smokers	Smokers	COPD
HLA-DR	**% expression**	92.4±2.9 n=5	96.7±1.7 n=6	93.6±1.7 n=12
	SMF	9.8±4.3 n=5	16.6±2.0 n=7	12.8±2.2 n=12
CD14	**% expression**	92.8±3.0 n=8	97.5±1.3 n=5	97.7±0.5 n=12
	SMF	18.2±6.3 n=8	20.2±2.4 n=5	15.8±2.2 n=12
TLR2	**% expression**	5.5±0.6 n=5	7.7±4.6 n=8	19.7±16.1 n=6
	SMF	1.2±0.1 n=5	1.4±0.3 n=8	2.8±1.6 n=6
TLR4	**% expression**	4.1±0.8 n=5	2.1±0.4 n=8	10.4±6.9 n=6
	SFI	1.1±0.1 n=5	1.0±0.1 n=8	1.3±0.2 n=6
CD163	**% expression**	21.1±4.1 n=7	22.9±6.1 n=9	18.8±2.2 n=5
	SMF	6.0±0.7 n=7	6.2±0.9 n=9	4.6±0.6 n=5
CD36	**% expression**	82.2±7.2 n=7	87.6±3.0 n=9	83.3±5.2 n=5
	SMF	8.1±1.2 n=7	7.4±1.1 n=9	7.2±0.8 n=5
Mannose receptor	**% expression**	43.5±9.8 n=4	51.2±14.0 n=8	32.7±3.2 n=5
	SMF	2.3±0.2 n=4	3.6±0.6 n=8	2.3±0.3 n=5
MARCO	**% expression**	7.5±1.2 n=4	6.9±0.9 n=4	5.9±0.6 n=5
	SMF	2.3±0.2 n=4	2.1±0.1 n=4	1.7±0.3 n=5
PS receptor	**% expression**	58.2±4.6 n=7	64.6±9.8 n=6	47.9±0.8 n=5
	SMF	2.7±0.3 n=7	3.0±0.3 n=6	2.4±0.1 n=5

HLA-DR = human lymphocyte antigen - DR, MARCO = macrophage receptor with collagenous structure, PS = phosphatidylserine, SMF = specific mean fluorescence, TLR = Toll-like receptor

Data are presented as mean ± SEM for 'n' samples.

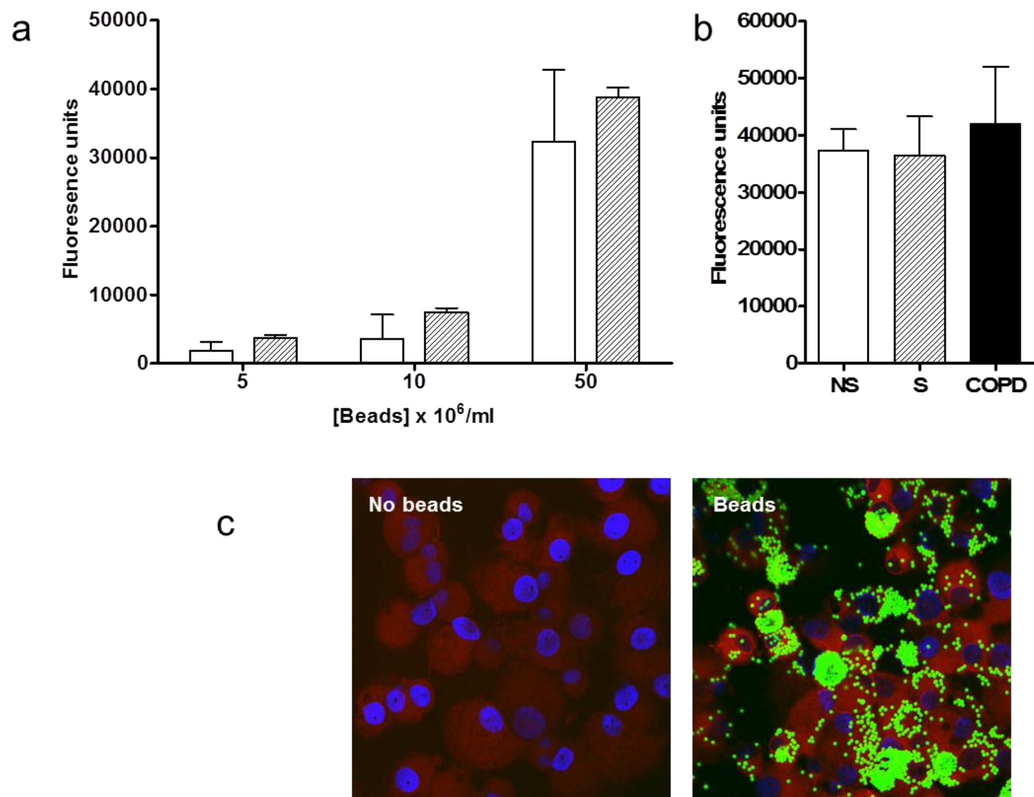

Figure 1

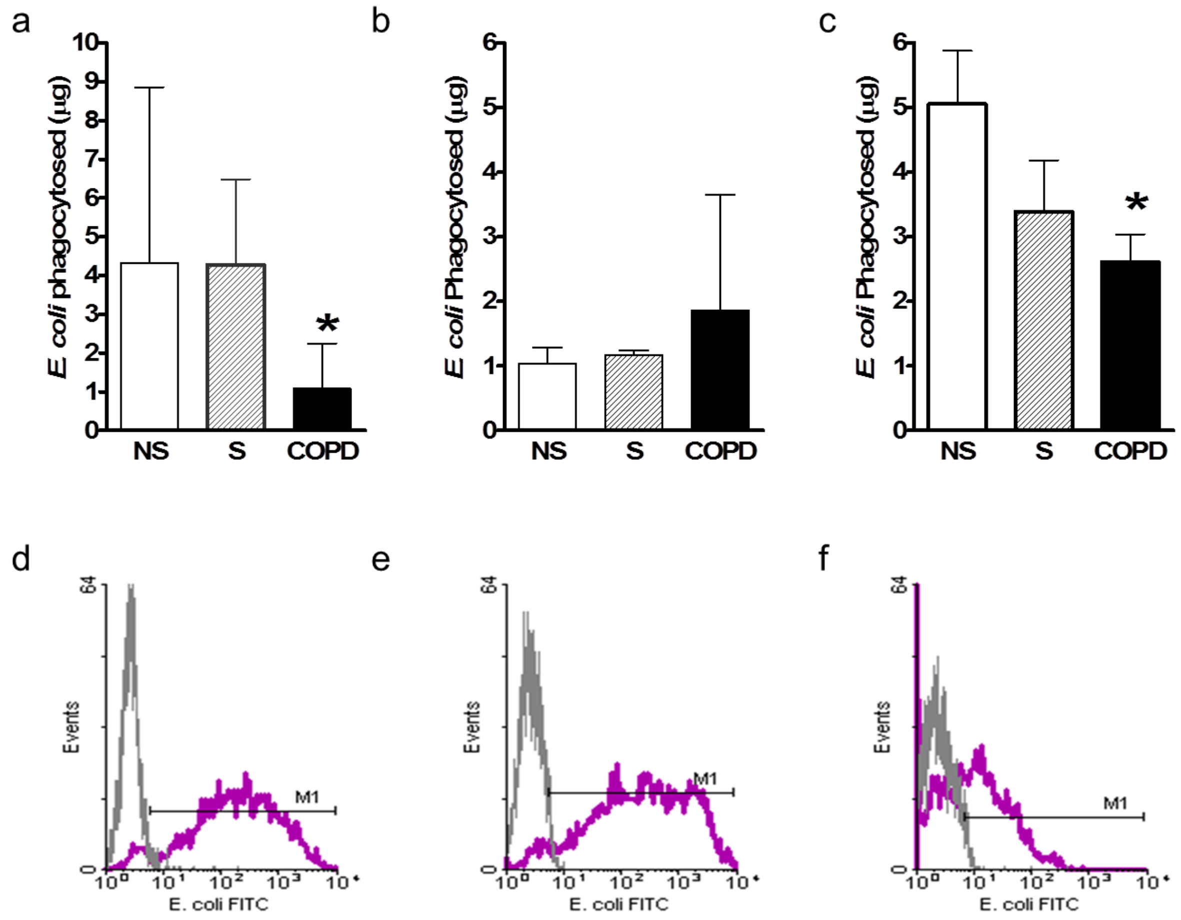

Figure 2

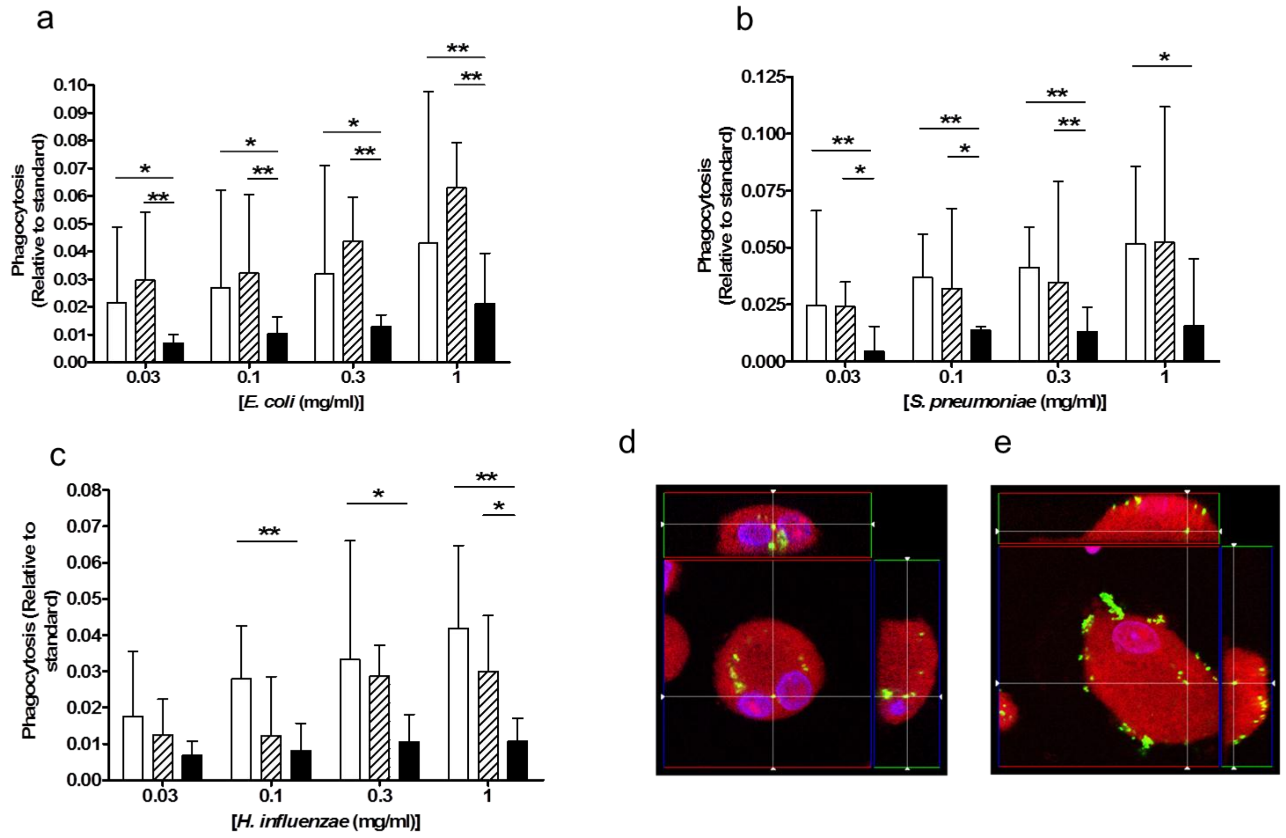

Figure 3

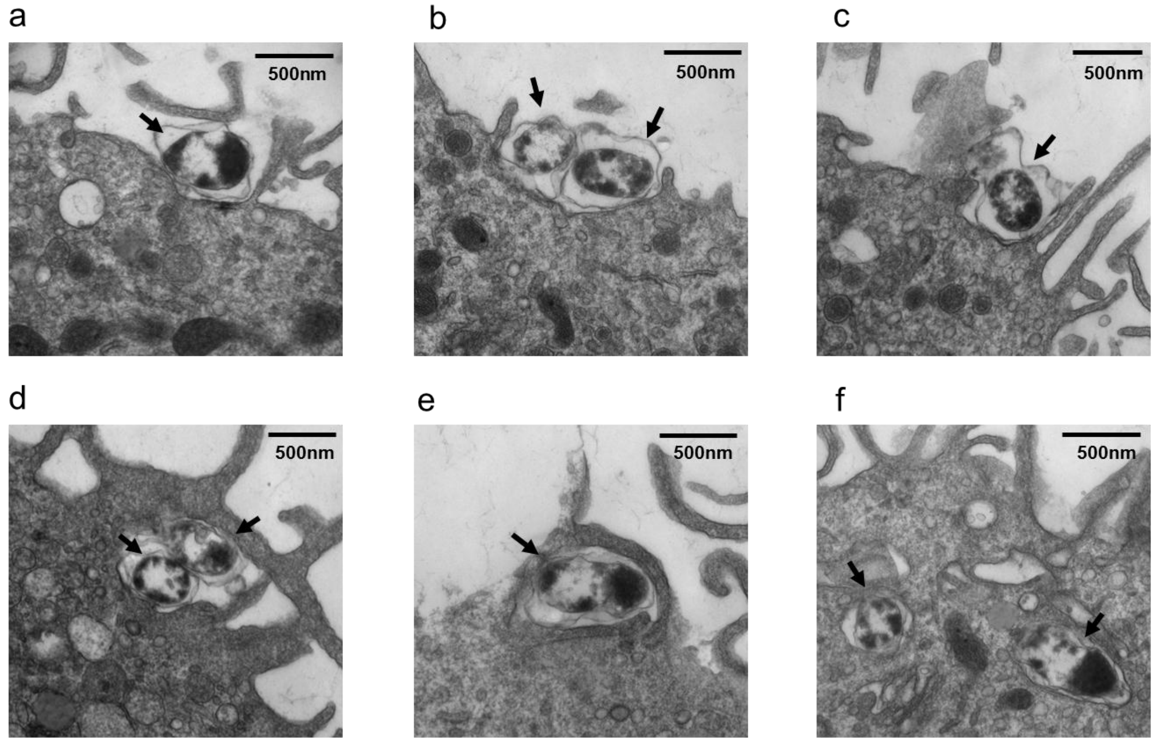

Figure 4

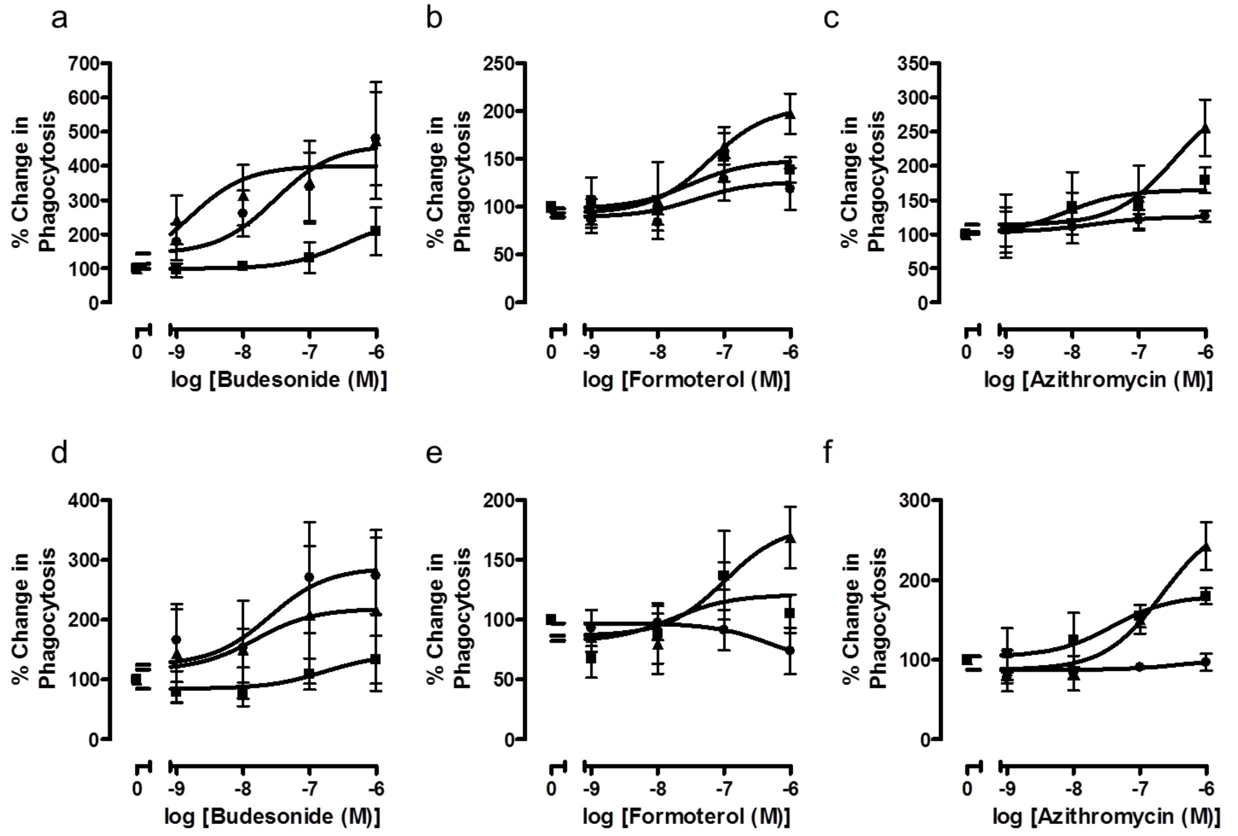

Figure 5

Supplementary Figure Legend

Figure S1. Effect of pharmacological inhibitors on MDM phagocytosis of *E.coli*.

MDM were generated from non-smokers (open bars), n=4-6, smokers (hatched bars), n=4-6 and COPD patients (solid bars), n=5-11, and exposed to the PI3 kinase inhibitor, LY-294001 (panel A) and the Rho kinase inhibitor, Y-27632 (panel B) or the p38 inhibitor, SB203580 (panel C) prior to exposure to FITC-labelled *E.coli*. Data are presented as % of the response of MDM in the absence of the inhibitor (100%) and as mean ± SEM.