Neutrophil Chemotaxis Caused by Chronic Obstructive Pulmonary Disease Alveolar Macrophages: The Role of CXCL8 and the Receptors CXCR1/CXCR2

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ABSTRACT

Alveolar macrophages produce neutrophil chemoattractants; this cellular cross-talk contributes to neutrophilic airway inflammation in chronic obstructive pulmonary disease (COPD). We have investigated the chemokine cross-talk mechanisms between these cells using COPD alveolar macrophages. Using conditioned media from stimulated COPD alveolar macrophages, we investigated the relative contributions of growth-related oncogene (CXCL1), interleukin-8 (CXCL8), and regulated on activation normal T cell expressed and secreted (CCL5) to neutrophil chemotaxis and evaluated the effect of blocking the chemokine receptors CXCR1 and CXCR2 on chemotaxis caused by macrophage-conditioned media. Furthermore, we evaluated whether corticosteroid treatment of stimulated alveolar macrophages inhibited the chemotaxis ability of conditioned media. Alveolar macrophages isolated from COPD (n = 8) and smoker (S) (n = 8) lungs were treated with ultra-pure lipopolysaccharide in the presence and absence of dexamethasone (1 μM). Supernatants were used for neutrophil chemotaxis assays. SB656933 [2-hydroxy-N,N-dimethyl-3-(2-[[[(R)-1-(5-methyl-furan-2-yl)-1-propyl]amino]-3,4-dioxo-cyclobut-1-eny]amino]-3,4-dioxo-cyclobut-1-enylamino}-benzamide] (CXCR2 antagonist) and Sch527123 [1-(2-chloro-3-fluorophenyl)-3-(4-chloro-2-hydroxy-3-piperazin-1-ylsulfonylphenyl)urea, 3-(2-chloro-3-fluoro-phenyl)]-1-(4-chloro-2-hydroxy-3-piperazin-1-ylsulfonylphenyl)urea] (dual CXCR1 and CXCR2 antagonist) and blocking antibodies for CXCL8, CXCL1, and CCL5 were assessed. Conditioned media caused neutrophil chemotaxis in COPD and smokers (60.5 and 79.9% of total cells, respectively). Dexamethasone did not significantly reduce neutrophil chemotaxis in COPD or S. SB656933 and Sch527123 inhibited chemotaxis in a concentration-dependent manner, with the dual antagonist Sch527123 causing greater inhibition of chemotaxis. CXCL8 antibody inhibited neutrophil chemotaxis to basal levels, although there was no significant effect of blocking either CXCL1 or CCL5 (P > 0.05). CXCL8 plays a major role in neutrophil chemotaxis caused by alveolar macrophage-derived conditioned media, and this is most effectively inhibited by dual antagonism of CXCR1 and CXCR2. Corticosteroids do not inhibit chemotaxis caused by macrophage-derived chemokines.

Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory disorder of the airways (Hogg et al., 2004). COPD is characterized by an abnormal innate immune response with increased numbers of macrophages and neutrophils in the lungs. The chemokines interleukin-8 (CXCL8), growth-related oncogene (CXCL1), and regulated on activation normal T cell expressed and secreted (CCL5) are potent neutrophil chemoattractants (de Boer et al., 2000) and are released by a variety of lung cells, including macrophages and epithelial cells as well as neutrophils themselves (Scapini et al., 2000). CXCL8 binds to G protein–coupled chemokine receptors CXCR1 and CXCR2 (Ludwig et al., 1997), whereas CXCL1 acts solely through CXCR2 to promote the influx of neutrophils into tissue sites of inflammation.

Antagonists targeted against CXCR2 alone and both CXCR1 and CXCR2 have been developed for the treatment of COPD (Holz et al., 2010; Lazaar et al., 2011). Dual antagonists against CXCR1/2 may inhibit chemotaxis to a greater extent than CXCR2 alone. Furthermore, dual antagonists may also inhibit CXCR1-mediated neutrophil activation such as the release of reactive oxygen species (Jones et al., 1996).

Corticosteroids are the most widely used anti-inflammatory therapy for the treatment of COPD. However, the clinical benefit of these drugs is limited and probably restricted to
a subset of patients (Brightling et al., 2005). Corticosteroids incompletely suppress the production of neutrophil chemokines, such as CXCL1 and CXCL8, by alveolar macrophages (Standiford et al., 1992; Armstrong et al., 2009, 2011). There is a need for novel therapies that inhibit the actions of neutrophil chemokines.

Neutrophil chemotaxis in vitro can be studied using single chemokines (Yoshikawa et al., 2007; Corhay et al., 2009). However, this method does not resemble the natural environment in the lungs where a range of chemokines exist. Induced sputum supernatants have been used to investigate neutrophil chemotaxis (Richman-Eisenstat et al., 1993; Mikami et al., 1998; Beeh et al., 2003; Babusyte et al., 2010; Aul et al., 2012); this method is physiologically relevant as a number of chemokines are present in the media. We have recently shown that induced sputum supernatants from healthy subjects, obtained after lipopolysaccharide (LPS) inhalation, cause a greater degree of chemotaxis compared with the same concentrations of individual chemokines found in the sputum (Aul et al., 2012). Furthermore, dual antagonists blocking CXCR1/2 had a greater inhibitory effect on sputum supernatant–induced neutrophil chemotaxis compared with CXCR2 blockade alone.

Induced sputum samples are derived from the proximal airways, and the chemokines within the supernatant are secreted by inflammatory cells within the lumen, such as neutrophils and macrophages as well as the bronchial epithelium. We have specifically investigated neutrophil chemotaxis caused by alveolar macrophages alone, by using conditioned media obtained from stimulated COPD alveolar macrophages. These experiments provide insights into the macrophage-neutrophil interactions in the distal lung. The novel aspects of this study are that we focused on the effects of corticosteroids on macrophage-induced neutrophil chemotaxis, and the effects of CXCR1 and CXCR2 antagonism on this interaction. The roles of CXCR1 and CXCR2 in this system were investigated using the CXCR2 antagonist SB656933 (2-hydroxy-N,N-dimethyl-3-[2-[[R]-1-(5-methylfuran-2-yl)-propyl]amino]-3,4-dioxocyclobut-1-ene) (benzamido) and the dual CXCR1/2 antagonist Sch527123 (1-(2-chloro-3-fluorophenyl)-3-(2-chloro-3-fluoro-phenyl)-3-piperazin-1-ylsulfonylphenyl)urea, 3-(2-chloro-3-fluoro-phenyl)-1-(4-chloro-2-hydroxy-3-piperazin-1-ylsulfonylphenyl)urea, 3-(2-chloro-3-fluoro-phenyl)-1-(4-chloro-2-hydroxy-3-piperazin-1-ylsulfonyl-phenyl)urea, and the roles of the chemokines CXCL1 and CXCL8 were investigated using blocking antibodies. We demonstrate that corticosteroid treatment of COPD alveolar macrophages fails to inhibit neutrophil chemotaxis, which is principally dependent on CXCL8 and can be inhibited by blocking CXCR1 and CXCR2.

**Materials and Methods**

**Patients.** Patients undergoing lung surgery for known or suspected lung cancer were recruited and categorized according to previous physician diagnosis of COPD according to current guidelines; eight patients with COPD and eight patients with smoker inflammation, immunopharmacology, and asthma (S) but without COPD provided alveolar macrophages for experiments. Two healthy never smokers provided blood samples for neutrophil isolation for chemotaxis experiments. Patient demographics are provided in Table 1. All patients gave written informed consent. This study was approved by the local research ethics committee (South Manchester Research Ethics Committee).

**Alveolar Macrophage Isolation.** Alveolar macrophages were isolated as previously described (Kent et al., 2009). Lung tissue distant from the tumor site was removed before perfusing with 0.1 M NaCl for macrophage isolation. The retrieved fluid was centrifuged at 400g for 10 minutes, and the cell pellet was resuspended in RPMI 1640 (Sigma-Aldrich, Poole, UK). Cell suspension was frozen over a Ficoll-Paque gradient (GE Healthcare, Buckinghamshire, UK) and centrifuged at 400g for 30 minutes. The mononuclear cells at the Ficoll interface were extracted and resuspended in phenol red-free growth media. Viable macrophages were isolated and counted using trypsin blue exclusion. We have shown that this method results in >90% cells confirmed as macrophages expressing CD68 (Kent et al., 2009; Southworth et al., 2012).

**Cell Culture.** Alveolar macrophages in phenol red-free RPMI 1640 supplemented with 1% L-glutamine (Invitrogen, Paisley, UK), 1% penicillin/streptomycin (Sigma-Aldrich), and 10% fetal bovine serum (Invitrogen) were seeded in 24-well plates at 400,000 cells per well. Cells were either treated or not treated with dexamethasone (1 μM) for 2 hours prior to the addition of ultrapure lipopolysaccharide (UP-LPS; 10 μg/ml) for 24 hours. Supernatants were harvested and stored at −20°C for future analysis.

**Blood Neutrophil Isolation.** Polymorphonuclear leukocytes were isolated from peripheral blood of healthy never smoker donors as previously described (Plumb et al., 2012). In brief, venous blood (up to 7 ml) was layered onto 3 ml of Mono-Poly Resolving medium (MP Biomedicals, Cambridge, UK) and centrifuged at 800g for 45 minutes at 18°C. Polymorphonuclear leukocytes were washed and resuspended in phenol red-free RPMI 1640 medium (Sigma-Aldrich). Cell counts were determined using a Neubauer hemocytometer. We have previously shown that this method results in >90% cells confirmed as neutrophils (Plumb et al., 2012).

**Chemokine Analysis.** Release of CXCL8, CCL5, and CXCL1 was measured on diluted samples and determined by enzyme-linked immunosorbent assay duo sets (R&D Systems, Cambridge, MA), according to manufacturer guidelines. The lower limit of detection was 31.25 pg/ml for CXCL8 and CCL5 and 15.6 pg/ml for CXCL1.

**Neutrophil Chemotaxis.** Chemotaxis assay was carried out in a 96-well plate format using Millipore MultiScreen-MIC plates (Millipore, Billerica, MA). These plates comprise an insert with a 96-well plate format using Millipore MultiScreen-MIC plates housed within a polyethylene terephthalate tear drop receiver plate (bottom chamber). The active surface area of the membrane was 0.3 cm² with a nominal pore density of 2 × 10⁶/cm². Neutrophils at 1 × 10⁶/ml were placed into the top chamber of the chemotaxis plates (80,000/well). In the bottom chamber, 150 μl of conditioned macrophage media (diluted 1:60) was added. This dilution was chosen based on preliminary experiments in which the effects of dexamethasone on chemotaxis using different dilutions were investigated; dilutions of 1:15 and 1:30 were not able to demonstrate any effect of dexamethasone (see Supplemental Fig. 2). Chambers were incubated separately for 1 hour at 37°C prior to sandwiching together for 1 hour to allow chemotaxis.
to occur. This time point was selected based on previously published studies which used 1 hour as the optimal migration time (Harvath et al., 1980; Yoshikawa et al., 2007; Corhay et al., 2009; Vacca et al., 2011; Blidberg et al., 2012). The nonmigrated cells in the top chamber were removed after an hour. The migrated cells were incubated with lysis buffer (0.4% Triton X-100, 30 mM sodium hydroxide, 2 M sodium chloride, 4 mM EDTA, 4 mM phosphate buffer) containing PicoGreen nucleic acid dye (Invitrogen) overnight at room temperature in the dark. The fluorescence of the migrated cells was read in a multiwell fluorescence plate reader (BMG Optima, Aylesbury Bucks, UK; excitation 485 nm, emission 535 nm). Cell migration was expressed as a percentage of maximum cell migration (cells added directly to the bottom wells). There was no effect of the conditioned media on chemokine release from neutrophils (Supplemental Fig. 1), indicating that observed chemotaxis was due to the chemokines released by alveolar macrophages.

To assess the role of CXCR receptors, neutrophils in the top chamber were incubated with increasing concentrations of either SB656933 (0.1–10 μM) or Sch527123 (0.1–10 μM) for 1 hour prior to sandwiching the top and bottom chambers together. To investigate the involvement of the chemokines, UP-LPS (10 μg/ml)-treated macrophage-conditioned medium was incubated with the specific neutralizing antibodies for 1 hour prior to allowing chemotaxis to occur. The concentrations of antibodies used were as follows: CXCL8 monoclonal antibody (mAb; 0.01–100 μg/ml), CCL5 mAb (0.1–100 μg/ml), and CXCL1 mAb (0.01–10 ng/ml). Recombinant proteins for CCL5 and CXCL1 were used at 10 nM. Recombinant proteins and antibodies were purchased from R&D Systems.

Terminal Deoxynucleotidyl Transferase-Mediated Digoxigenin-Deoxyuridine Nick-End Labeling Assay. Neutrophil cytoslides were fixed in 4% paraformaldehyde (Sigma-Aldrich) in phosphate-buffered saline (PBS) at room temperature for 20 minutes. Terminal deoxynucleotidyl transferase-mediated digoxigenin-deoxyuridine-nick end labeling (TUNEL) assay (Roche Diagnostics, Hertfordshire, UK) was performed following manufacturer guidelines. In brief, cells were washed 3 × 3 in PBS and incubated in permeabilization buffer (0.1% Triton X-100 in 0.1% sodium citrate) for 2 minutes on ice. After washing, cells were incubated in TUNEL reaction mixture at 37°C for 1 hour. Cells were then rinsed again in PBS. Cells were counterstained in 4',6-diamidino-2-phenylindole (Invitrogen). A total of 400 cells per slide were examined and identified as positive or negative for apoptosis.

Rapi-Diff Staining. Blood neutrophils were fixed in methanol for 30 minutes at room temperature prior to staining in Rapi-Diff. Apoptosis was assessed by examining the disappearance of chromatin bridges between nuclear lobes (early apoptosis) and shrinkage or fragmentation of the nucleus (late apoptosis) in a total of 400 cells per cytoslide.

Statistical Analysis. All data were normally distributed, thus comparisons were performed using a parametric one-way analysis of variance (ANOVA). If the ANOVA was significant (P < 0.05), subsequent paired t tests were performed. All analyses were carried out using GraphPad Prism version 5 (GraphPad Software, Inc., San Diego, CA).

Results
Chemokine Production. UP-LPS significantly increased CXCL8, CXCL1, and CCL5 production (P < 0.01 for all comparisons of UP-LPS stimulation against baseline in patients with COPD and S; see Fig. 1). There were no significant differences between patients with COPD and S in the stimulated chemokine levels (P > 0.05 for each chemokine). Dexamethasone (1 μM) significantly inhibited CXCL8 production by 68.4% (P = 0.007) and 59.5% (P = 0.015) in patients with both COPD and S, respectively. CCL5 production was suppressed significantly by dexamethasone in patients with both COPD and S by 77.2% (P = 0.008) and 58.1% (P = 0.008), respectively. CXCL1 production in patients with both COPD and S was also inhibited by 80.7 and 76.1% (P = 0.005 and 0.001), respectively.

UP-LPS Stimulation of Alveolar Macrophage Induces Neutrophil Chemotaxis. Conditioned media from unstimulated alveolar macrophages caused neutrophil chemotaxis in patients with both COPD and S; the mean percentages of cells migrating were 12.7 and 28.9%, respectively (Fig. 2). Conditioned media from UP-LPS–stimulated alveolar macrophages caused an increase in chemotaxis for patients with both COPD and S; the mean percentages of cells migrating were 60.5 and 79.9%, respectively (P < 0.001). The
conditioned media did not alter CXCR1 or CXCR2 expression (see Supplemental Fig. 3).

The addition of dexamethasone (1 μM) to alveolar macrophages prior to UP-LPS stimulation caused a reduction in chemotaxis that was not statistically significant (P > 0.05 in both groups). These data show that neutrophil chemotaxis induced by conditioned media from alveolar macrophages is corticosteroid-insensitive in patients with both COPD and S. There were no differences in chemotaxis between groups in any of the conditions (P > 0.05 for all comparisons).

**A Role for CXCL8 in Neutrophil Chemotaxis.** To determine the extent to which the chemokines examined play a role in neutrophil chemotaxis, we used neutralizing antibodies to inhibit CXCL8, CCL5, and CXCL1. As there were no significant differences between COPD and S chemotaxis responses (see Fig. 1), we conducted further experiments using only the conditioned media from COPD alveolar macrophages (n = 8). Figure 3 shows that new experiments using the UP-LPS–stimulated macrophage-conditioned media robustly induced neutrophil chemotaxis compared with conditioned macrophage media without UP-LPS stimulation (61.9 versus 12.0%, 5.1-fold increase). CXCL8 mAb significantly inhibited neutrophil chemotaxis in LPS-stimulated media in a concentration-dependent manner (ANOVA P < 0.0001); complete inhibition back to basal levels using media from unstimulated cells was observed at 0.1 μg/ml (Fig. 3). In contrast, there was no significant effect on neutrophil chemotaxis when using either CCL5 mAb (ANOVA P = 0.63) or CXCL1 mAb (ANOVA P = 0.82).

To rule out any nonspecific effects of the CXCL8 mAb, N-formyl-methionyl-leucyl-phenylalanine (fMLP) was used to induce neutrophil chemotaxis; fMLP robustly induced neutrophil chemotaxis to 78.7%. CXCL8 mAb (100–0.01 μg/ml) did not change fMLP-induced neutrophil chemotaxis (P = 0.31) (Fig. 4). We observed no effect of the CCL5 and CXCL1 mAb on chemotaxis caused by conditioned media; we verified the ability of these antibodies to block chemotaxis using recombinant proteins for both CXCL1 and CCL5 to induce neutrophil chemotaxis. CXCL1 and CCL5 induced neutrophil chemotaxis 17 and 19%, respectively, which was inhibited by the respective mAb at the concentrations used in the conditioned media experiments (P = 0.0001 and 0.05, respectively) (Fig. 5). We also verified the specific effect of CXCL8 mAb. Recombinant CXCL8 caused neutrophil chemotaxis 60.6% which was significantly reduced to 20.3% at 100 μg/ml of CXCL8 mAb (P = 0.0002) (Fig. 5).

**The Role of CXCR1/2 in Neutrophil Chemotaxis.** New experiments using UP-LPS–stimulated conditioned COPD macrophage media (n = 8) increased chemotaxis to 46.8% compared with 8.8% using conditioned macrophage media without UP-LPS (5.3-fold increase). Both SB656933 and Sch527123 inhibited neutrophil chemotaxis in a concentration-dependent manner (Fig. 6; ANOVA P, 0.0002 and P, 0.0001, respectively). The dual CXCR1/2 antagonist Sch527123 had a greater effect than the CXCR2 antagonist SB656933 at both SB656933 and Sch527123 inhibited neutrophil chemotaxis in a concentration-dependent manner (Fig. 6; ANOVA P, 0.0002 and P, 0.0001, respectively). The dual CXCR1/2 antagonist Sch527123 had a greater effect than the CXCR2 antagonist SB656933 at...
the highest concentration of 10 μM; the absolute reduction of the number of migrating cells was 38.9 versus 29.1% \((P = 0.008)\), with only Sch527123 causing complete inhibition back to basal levels using media from unstimulated cells. Sch527123 inhibited chemotaxis at 1 μM, whereas SB656933 had no effect at this concentration; both drugs inhibited chemotaxis at the higher concentrations of 3 and 10 μM. There was no effect on neutrophil cell viability when examined by TUNEL assay and Rapi-Diff staining (Fig. 7).

The effects of both SB656933 and Sch527123 on chemotaxis caused by exogenously administered CXCL8 were also studied (Fig. 8). CXCL8 caused migration of 39.9% of neutrophils, which was significantly repressed to 21.5% by Sch527123 at 10 μM \((P = 0.009)\) and 32.6% at 3 μM \((P = 0.002)\) and repressed to 31.3% by SB656933 (10 μM; \(P = 0.03\)). These data confirm that these drugs block CXCL8-induced chemotaxis and show a greater effect of Sch527123. These data also suggest an important role for CXCR1 as well as CXCR2 in neutrophil chemotaxis.

Discussion

We show that corticosteroid treatment of COPD alveolar macrophages reduced the production of CXCL1, CXCL8, and CCL5 by approximately 70%. This incomplete suppression of chemokine production by dexamethasone was associated with no significant reduction in neutrophil chemotaxis caused by conditioned media. These in vitro experiments suggest that inhaled corticosteroids have little effect on neutrophil chemotaxis caused by alveolar macrophage–derived chemokines released within the lungs of patients with COPD. Furthermore, we show that CXCL8 plays a major role in neutrophil chemotaxis caused by alveolar macrophage–derived conditioned media, and that blockade of CXCR1 and CXCR2 inhibits this chemotaxis.

The incomplete suppression of UP-LPS–induced CXCL8, CXCL1, and CCL5 production from COPD alveolar macrophages in the current study was also observed in our previous work (Armstrong et al., 2009; Kent et al., 2009). We now show that the chemokine concentrations in conditioned media present after corticosteroid treatment cause neutrophil chemotaxis to be maintained at a similar level compared with no corticosteroid treatment. This suggests that chemokine concentrations present in conditioned media after UP-LPS stimulation are at the top of the concentration response curve for chemotaxis, as 70% suppression of these chemokines does not alter chemotaxis activity.

Lung neutrophils lack expression of the glucocorticoid receptor, unlike peripheral blood neutrophils (Plumb et al., 2012). Therefore, inhaled corticosteroids should have no direct effect on the activation of lung neutrophils. Alveolar macrophage–induced neutrophil chemotaxis and subsequent neutrophil activation is therefore corticosteroid-resistant.

CXCL8 levels are increased in induced sputum and bronchoalveolar lavage of patients with COPD compared with controls (Yamamoto et al., 1997; Mukaida, 2003). CXCL8 was identified as the principal chemokine present in the conditioned media that was responsible for neutrophil chemotaxis. CXCL8 has been identified as a major neutrophil chemotactant (Mukaida, 2003), and our data indicate a major role for this chemokine in neutrophilic lung inflammation in COPD. We also investigated CXCL1 and CCL5, as these are well known...
to be potent neutrophil chemoattractants (de Boer et al., 2000), and the levels of these proteins are increased in the lungs of patients with COPD compared with controls (Traves et al., 2002). However, our experiments indicate a dominant role for CXCL8 in macrophage-initiated neutrophil chemotaxis in COPD.

A number of other proteins are known to facilitate neutrophil chemotaxis, including interleukin-1β, tumor necrosis factor α, leukotriene B4, and Pro-Gly-Pro (Snelgrove et al., 2010; Afonso et al., 2012). However, we focused on the roles of CXCR1 and CXCR2 and their ligands, and therefore did not investigate these proteins. It would be of interest and importance to understand the roles of these proteins in alveolar macrophage-initiated neutrophil chemotaxis.

Animal models have shown a role for CXCL1 in the development of neutrophilic airway inflammation (Reutershan et al., 2009; Arndt et al., 2011). However, we focused on the roles of CXCR1 and CXCR2 and their ligands, and therefore did not investigate these proteins. It would be of interest and importance to understand the roles of these proteins in alveolar macrophage-initiated neutrophil chemotaxis.

Clinical trials in healthy patients have shown that both SB656933 and Sch527123 inhibit ozone-induced airway neutrophilia (Holz et al., 2010; Lazaar et al., 2011). We previously compared these drugs using sputum supernatant obtained from healthy patients after inhaled LPS challenge; this model was used to simulate the mixture of cytokines present that cause neutrophilic lung disease (Aul et al., 2012). We observed a greater effect of Sch527123 compared with
Statistical analysis was assessed by one-way ANOVA and subsequent response to recombinant CXCL8 (0.1–10 nM) was incubated with either Sch527123 (0.1 μM) for 1 hour prior to assessing neutrophil migration in response to recombinant CXCL8 (n = 6). Data are expressed as the percentage of total migrated cells and presented as the mean ± S.E. Statistical analysis was assessed by one-way ANOVA and subsequent paired t test. *P < 0.05; **P < 0.001; ***P < 0.0001.

SB656933 on neutrophil chemotaxis, highlighting the potential advantage of targeting CXCR1 in addition to CXCR2 (Aul et al., 2012). The current study similarly showed a greater effect of Sch527123, which caused greater inhibition than SB656933 at the highest concentration, and showed significant inhibitory effects at lower concentrations (i.e., 1 μM). It should be noted that only Sch527123 completely inhibited chemotaxis down to levels observed using conditioned media from unstimulated cells. It appears that CXCR2 antagonism alone only causes partial inhibition of neutrophil chemotaxis caused by alveolar macrophages.

We confirmed that both SB656933 and Sch527123 inhibited chemotaxis caused by exogenous CXCL8. These experiments further confirmed that CXCR2 antagonism alone through SB656933 was less effective than dual antagonism of CXCR1 and CXCR2 using Sch527123 in this chemotaxis model.

There was no difference in the effect of dexamethasone between patients with COPD and smokers in the current study, in agreement with our previous work (Jones et al., 1996; Armstrong et al., 2011; Southworth et al., 2012). Alveolar macrophage numbers are increased in patients with COPD compared with controls (Di Stefano et al., 1998; Barnes, 2004; Hogg et al., 2004), contributing to an increased burden of inflammatory mediators found in the lungs of patients with COPD such as CXCL8 (Yamamoto et al., 1997). The production of CXCL8 by macrophages is only partially suppressed by corticosteroids in patients with both COPD and controls (Jones et al., 1996; Armstrong et al., 2011), but this limited effect is more clinically relevant in patients with COPD as the levels of CXCL8 after corticosteroid treatment still cause neutrophil chemotaxis. CXCL8 release is inhibited by 68% by dexamethasone (Fig. 1), yet neutrophil chemotaxis is highly CXCL8-dependent (Fig. 3), suggesting that the residual 32% is sufficient to cause neutrophil chemotaxis. Taken together, these data highlight the importance of using conditioned media in an in vitro system to assess chemokine involvement in neutrophil chemotaxis. Furthermore, the data provide evidence that neutrophil chemotaxis in response to alveolar macrophage-conditioned media is highly CXCL8-dependent yet CXCL1- and CCL5-independent. Finally, we demonstrate the importance of targeting CXCR1 as well as CXCR2 in the inhibition of airway neutrophilia.

Authorship Contributions
Participated in research design: Kaur, Singh.
Conducted experiments: Kaur.
Performed data analysis: Kaur.
Wrote or contributed to the writing of the manuscript: Kaur, Singh.

References


