Characterization of cigarette smoke-induced inflammatory and mucus hypersecretory changes in rat lung and the role of CXCR2 ligands in mediating this effect

Christopher S. Stevenson, Kevin Coote, Ruth Webster, Helinor Johnston, Hazel C. Atherton, Andrew Nicholls, June Giddings, Rosemary Sugar, Alan Jackson, Neil J. Press, Zarin Brown, Keith Butler, and Henry Danahay
Novartis Institute of Biomedical Research, Respiratory Disease Area, Horsham, West Sussex, United Kingdom

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Smoking is the main etiologic factor for developing chronic obstructive pulmonary disease (COPD), the fourth most common cause of chronic morbidity and mortality in the United States (32). The disease is defined by the World Health Organization/National Heart, Lung, and Blood Institute Global Initiative for Chronic Obstructive Lung Disease as “a disease state characterized by airflow limitation not fully reversible . . . associated with an abnormal inflammatory response of the lungs to noxious particles and gases” (20). Cigarette smoke elicits a repetitive, acute inflammatory insult that leads to this chronic pathological condition. COPD is characterized by neutrophilia and mucus hypersecretion in the lung (described pathologically as chronic bronchitis) and in some cases the eventual loss of alveolar structures (described clinically as emphysema). Patients do not respond to steroid treatment, and there are few effective therapies available for treating COPD, none of which can reverse or halt its progression. This is due in large part to the poor understanding of the inflammatory mechanisms involved in its pathogenesis.

The COPD phenotype is typified by an accelerated rate of age-related decline in the forced expiratory volume in 1 s, increased numbers of CD8+ T cells and macrophages in the interstitium, increased levels of neutrophils in the air spaces, and a mucus hypersecretory phenotype associated with goblet cell metaplasia (12). It has been demonstrated that greater levels of both neutrophil chemokines, such as IL-8 and growth-related oncogene (GRO)-α, and neutrophils can be found in the induced sputum and bronchoalveolar lavage (BAL) fluid of patients with COPD compared with healthy smokers (14, 29, 30). In addition, there is an inverse correlation between the number of neutrophils present in BAL fluid and the lung function of smokers (6). However, these are all clinical observations from a single time point that, while they may infer potential mechanisms, cannot distinguish between causative and consequential factors of the disease process.

COPD is often diagnosed in the later stages of the disease when lung function is already significantly reduced. Consequently, there are no clinical reports of early inflammatory events associated with smoke inhalation that likely initiate the chronic inflammatory response in the lungs. Several animal models have been designed to try and understand these processes. Models using inhaled sulfur dioxide, ozone, nitric oxide, or lipopolysaccharide are widely used to mimic aspects of the “bronchitic-like” phenotype in laboratory species, although their relevance to CS-induced lung disease is questionable (13, 16, 8, 6). These stimuli will induce increases in lung neutrophilia and goblet cell metaplasia but may be more appropriate for modeling disease exacerbations.

Here, for the first time, we characterize the acute inflammatory changes in both the airways (by BAL) and lung tissue after...
an acute exposure cycle of CS in the rat. Several groups have also described the effects of CS inhalation on small laboratory animals. However, most of these studies have focused on assessing chronic changes such as the loss of alveolar integrity and lung function changes (9, 18, 31). Although these studies demonstrate CS can induce airway neutrophilia, goblet cell metaplasia, and in many cases emphysema, none characterize the kinetics of CS-induced inflammation after an acute exposure. As such, it remains unclear what mechanisms are involved in the development of CS-induced inflammation and mucus hypersecretion.

The aims of the present study were, therefore, to describe the acute inflammatory changes initiated by CS, identify potential mechanisms driving airway/lung inflammation, and find endpoints that may have clinical relevance and could serve as markers for subsequent pharmacological profiling. Here, we demonstrate for the first time elevated levels of the rat GRO-α homologs, cytokine-induced neutrophil chemoattractant (CINC)-1–3, in response to acute CS exposure and inhibition of CS-induced inflammation by a specific CXCR2 antagonist.

MATERIALS AND METHODS

Statement on animal welfare. Studies described herein were performed under a Project License issued by the United Kingdom Home Office, and protocols were approved by the Local Ethical Review Process at Novartis Institute of Biomedical Research, Horsham.

Animal maintenance conditions. Male Sprague-Dawley rats (350–400 g; Charles River, Margate, UK) were housed in rooms maintained at constant temperature (21 ± 2°C) and humidity (55 ± 15%) with a 12-h light cycle and 15–20 air changes per hour. Three animals were housed per cage containing two nest packs filled with grade 6 sawdust (Datesand, Manchester, UK), nesting material (Enviro-Dri, Lillico, UK), maxi fun tunnels, and Aspen chew blocks (Lillico, UK) to provide environmental enrichment. Animals were allowed food, RM1 Pellets (SDS UK), and water ad libitum.

CS exposures. Animals were placed in 7-liter Perspex chambers (three rats/chamber) and exposed to 50 ml of smoke every 30 s with fresh air being pumped in for the remaining time. The smoke was generated from 2R4F Research Cigarettes (University of Kentucky, Louisville, KY) and was drawn into the chambers via a peristaltic pump. Sham control animals were exposed to air only in the same manner for the same duration of time (~32 min per exposure period). Animals were killed with an overdose of terminal anesthetic (200 mg of pentobarbital sodium ip) followed by exsanguination.

Preparation of BAL fluid and lung tissue leukocytes. We lavaged the lungs via a butterfly cannula inserted into the trachea and instilled them with 3 × 4-ml aliquots of sterile PBS. All aliquots were combined for individual rats. For lung tissue cell analysis, the lower right lobe of the lung was removed, and the inflammatory cells were extracted by enzyme digestion based on a method described by Holt and colleagues (10). Lung tissue was diced into 500-μm slices with a McIlwain tissue chopper, and 300 mg of chopped tissue were added to a 10-ml solution containing RPMI 1640 (GIBCO, Paisley, UK), 10% FCS (GIBCO), penicillin (100 U/ml) (GIBCO), streptomycin (100 U/ml) (GIBCO), collagenase D (1 mg/ml) (Roche Diagnostics, Hertfordshire, UK), and DNase I (Roche Diagnostics). The homogenate suspension was incubated at 37°C in a water bath and shaken at 50 rpm for 1.5 h. The resulting suspension was filtered through a 70-μm cell sieve (Marathon Lab Supplies), pelleted by centrifugation, and then washed twice with 10 ml of RPMI 1640 containing 10% FCS, penicillin (100 U/ml), and streptomycin (1,000 U/ml). Blood was not perfused out of the lung tissues as previous experiments showed no significant difference between perfused and nonperfused lungs (data not shown). Total cell counts for both BAL and tissue cells were performed on an automated cell counter (Sysmex UK, Milton Keynes, UK). Differential cell counts were performed according to standard morphological criteria on Hema-Gurr-stained cytospins (300 cells/sample; Merck, Poole, UK). We determined leukocyte numbers by multiplying the percentage of each leukocyte subpopulation with the total number of cells for each sample and expressing this as cells/ml for BAL cells and cells/mg lung tissue for cells from tissue digest.

Fig. 1. Time-dependent effects of cigarette smoke (CS) exposure on neutrophil infiltration and mucus hypersecretion. Bronchoalveolar lavage (BAL) fluid neutrophil infiltration (A), goblet cell density (B), and BAL fluid mucin levels (C) were measured 24 h after exposure to five 2R4F research cigarettes for 2, 3, or 4 days. This is 1 representative study of 2 with group sizes of n = 9 per treatment. Data are presented as mean values ± SE; *P < 0.05 compared with sham control.
BAL fluid and tissue cytokine analysis. After collection, aliquots of BAL fluid and lung tissue for cytokine analysis were frozen in liquid N2 and stored at −80°C. We further processed tissues before cytokine analysis by homogenizing 250 mg of tissue in 2 ml of ice-cold saline. Homogenates were spun at 800 g, and supernatants were used to measure cytokine levels by ELISA. CINC-1/GRO was measured by ELISA following the manufacturer’s protocol (Amersham Biosciences, Amersham, UK). CINC-2, CINC-3, IL-1β, TNF-α, IFN-γ, and IL-6 were all measured using ELISA Duo-Sets from R&D Systems (Abingdon, UK). Tissue homogenate protein levels were measured with the Bio-Rad protein assay (Bio-Rad Laboratories, Hertfordshire, UK), and cytokine values were normalized against protein levels for individual homogenate samples. BAL protein levels were below the level of detection of the assay.

Immunohistochemical staining of mucin and quantification of goblet cell density. Both tracheal and primary bronchus mucin were detected by a two-stage immunoperoxidase method using Ulex europaeus agglutinin-1 (UEA-1; Sigma, Dorset, UK) validated by Jackson and colleagues (11). The trachea and lungs were excised from rats and inflated with 5 ml of 10% neutral buffered formalin (NBF) and immersed in NBF for 24 h. The trachea and the left lobe were processed into paraffin blocks. The left lobe was cut into six slices with the central four slices put into the block. Sections (3 μm) were dried overnight at 37°C, dewaxed in xylene, taken to 70% ethanol, and treated with 0.5% hydrogen peroxidase in methanol to block endogenous peroxidase. After pretreating sections with 0.1% trypsin, we reduced unwanted background staining with 0.1% BSA in Tris-buffered saline (TBS), and the sections were incubated overnight with FITC-conjugated UEA-1 (Vector Laboratories, Peterborough, UK). Sections were brought to ambient temperature, washed in TBS, and incubated with peroxidase-conjugated anti-FITC (Dakocytomation, Ely, UK) for 30 min. Sites of reaction were visualized with diaminobenzidene (Sigma), and the nuclei were counterstained with hematoxylin. The area of UEA-1 staining was assessed under a Zeiss Axioplan microscope (×10 magnification) with an Imaging Associates KS-400 image analyzer (Imaging Associates, Bicester, UK). Tracheal sections were used for initial kinetic and dose response studies. Subsequent studies used the second slice of the four central left lobe sections, and the two major airways were scored for UEA-1-positive stained area. Between 15 and 25 fields were scored for each animal. Data are presented as mean values ± SE; *P < 0.05 compared with sham control.
presented as goblet cell density (GCD), which is defined as the ratio of stained area (μm²) to length (μm) of epithelium scored. Previous studies by Jackson and colleagues (11) have demonstrated a significant correlation between the UEA-1-stained area in rat lung and intrapulmonary goblet cell score.

Determination of BAL fluid mucin concentrations. Samples were assayed by a mucin enzyme-linked lectin assay as previously described by Jackson and colleagues (2, 11). Briefly, 96-well plates were coated with UEA-1 (Sigma) at 1.25 μg/ml overnight, blocked for 1 h at 37°C, followed by the addition of purified human mucin standard (24 units/ml) and BAL fluid samples in duplicate, and incubated for 1 h at 37°C, followed by the addition of substrate (0.05% orthophenylenediamine dihydrochloride, Sigma). The reaction was stopped by addition of 4 M H₂SO₄. Data are presented as units of mucin/ml.

Effect of a CXCR2 antagonist on CS-induced inflammation. A specific CXCR2 antagonist, SB-332235 [N-(2-hydroxy-3-sulfamyl-4-chlorophenyl)-N-(2,3-dichlorophenyl)urea], was used to determine whether CINC were central to CS-induced neutrophilia and mucin production (21, 30). CS exposures were performed as described above for 3 days using 5× 1R3F University of Kentucky research cigarettes (40 ml smoke/min). Sham control animals were exposed to air only in the same manner for the same duration of time (∼50 min per exposure period). Animals were dosed orally with 0.5 ml, 0.3, 1, or 3 mg/kg SB-332235 in 25% PEG 200 75% saline or vehicle 1 h before and 4 h after each CS exposure. Animals (n = 7–9 per group) were killed with an overdose of terminal anesthetic (200 mg pentobarbital sodium ip) followed by exsanguination. BAL was performed, lung tissue was taken for cytokine analysis and cell digestion, and the right lobe was fixed in formalin for assessment of GCD. Endpoints were measured as described in above experiments.

Statistical analysis. All data are presented as means ± SE. Statistical significance of data was determined by a two-tailed t-test with a Bonferroni correction. A result was considered significant if the P value was <0.05.

RESULTS

Time and dose response to CS. To determine the peak acute inflammatory response to CS exposure, we conducted a time course whereby the animals were exposed to five cigarettes daily for up to 4 days and culled 24 h after the completion of the last exposure. Exposure to CS induced a time-dependent increase in the number of BAL fluid neutrophils. Neutrophil numbers increased from 4.2 ± 2.0 × 10⁵ cells/ml in sham controls to their peak level of 36.9 ± 4.5 × 10³ cells/ml (P < 0.001) after 3 days of exposures (Fig. 1A). Tracheal GCD and BAL fluid mucin levels were significantly elevated above sham control at all time points (P < 0.01; Fig. 1, B and C). In dose-response studies, animals were exposed to two to five 2R4F research cigarettes or air for 3 consecutive days and culled 24 h after the end of the last exposure. CS induced dose-dependent increases in the BAL fluid neutrophil chemokine levels (Fig. 2, A and B) and the number of neutrophils infiltrating the airways (Fig. 2C, P < 0.05 compared with sham control). These changes were significant at each of the doses of CS, with the five-cigarette dose increasing neutrophil chemokines (rat GRO-α homologs), CINC-1/GRO-α levels from 82.2 ± 9.2 to 341.5 ± 58.8 pg/ml (P < 0.01), and CINC-2α/β levels from 422.3 ± 31.2 to 957.2 ± 87.4 pg/ml (P < 0.01; Fig. 2, A and B), and neutrophil numbers from 2.3 ± 0.8 × 10³ to 98.5 ± 8.0 × 10³ cells/ml (P < 0.001, Fig. 2C). Conversely, BAL macrophage numbers decreased in a dose-dependent fashion, reducing from 132.7 ± 19.3 × 10³ to 66.5 ± 9.6 × 10³ cells/ml after exposure to five cigarettes. CS also generated dose-dependent increases in tracheal GCD and the mucin content of BAL fluid. The five-cigarette dose induced the greatest changes causing tracheal GCD to increase from 0.8 ± 0.1 to 6.6 ± 0.8 units/μm (P < 0.001) and BAL mucin to rise from 0.5 ± 0.1 to 5.6 ± 2.2 units/ml (P < 0.05; Fig. 2, D and E).

Twenty-four-hour time course of CS-induced inflammation. Next we evaluated the kinetics of the inflammatory changes that occurred over the 24-h period immediately after an acute exposure to CS. Initial studies indicated that there were few significant or consistent time-dependent changes after a single exposure (data not shown). The preceding studies demonstrated that CS induced changes in the lung after as little as two exposures; therefore, subsequent investigations into CS-mediated changes focused on this double exposure regimen. For these time course studies, animals were exposed to smoke from four 2R4F research cigarettes either once and culled 24 h later...
or twice (15 h between exposures) and culled 1, 3, 6, 12, or 24 h from the end of the last exposure. The four-cigarette dose was chosen based on previous data demonstrating four cigarettes was the minimum level of exposure to ensure a robust inflammatory response (Fig. 2). Neutrophil numbers increased from 2.7 ± 0.9 × 10^3 to a maximum of 37.7 ± 6.7 × 10^3 cells/ml (P < 0.0001) 24 h after 2 CS exposures (Fig. 3A). BAL macrophage levels tended to decrease after smoke exposure, but this was not significant at any time point (data not shown). Tissue neutrophil numbers also increased from 2.8 ± 0.5 × 10^3 in sham control animals to 6.0 ± 1.2 × 10^3 cells/mg lung tissue (P < 0.05) at 24 h after a double exposure (Fig. 3B). There was a trend toward an increase in tissue neutrophil numbers after a single exposure; however, it was not statistically significant (Fig. 3B). There were no significant changes to tissue macrophage numbers (data not shown).

Twenty-four-hour time course of CS-induced inflammatory mediator production. To elucidate putative mechanisms that may be involved in the inflammatory response elicited by CS, we performed ELISAs on BAL fluid and lung tissue homogenates from the 24-h time course studies described above. In whole tissue homogenate, IL-6, IFN-γ, monocyte chemotactic protein (MCP)-1, and TNF-α could not be detected. CINC-1/GRO-α levels were significantly elevated in lung tissue at all time points examined and reached a maximal level 24 h after the second exposure, increasing from 67.5 ± 9.0 to 195.6 ± 10.7 pg/mg tissue homogenate (P < 0.001, Fig. 4A). CINC-2αβ levels in lung tissue increased in a biphasic pattern, peaking 3 h and then 24 h after the second exposure. The maximum increase in CINC-2αβ was detected 24 h after the second exposure, increasing from 81.9 ± 8.5 to 166.9 ± 12.6 pg/mg tissue homogenate (P < 0.001, Fig. 4B). CINC-3 was detected at significantly elevated levels in the tissue at most time points examined (Fig. 4C). IL-1β was detected at significantly elevated levels only 24 h after the second exposure, where it increased from 78.3 ± 9.8 to 139.1 ± 20.7 pg/mg tissue homogenate (P < 0.01, Fig. 4D).

The BAL fluid CINC-1/GRO levels remained significantly elevated at all time points examined and reached a maximal level 24 h after the second exposure, increasing from 103.4 ± 13.7 to 295.5 ± 47.5 pg/ml (P < 0.02, Fig. 5A). CINC-2αβ levels in BAL fluid followed a biphasic pattern similar to that observed in the tissue, peaking in the airways at 3–6 h and then again 24 h after each exposure. The maximum level detected was 24 h after the second exposure, where it increased from 583.6 ± 45.5 to 1,086.2 ± 87.5 pg/ml (P < 0.001, Fig. 5B). In BAL fluid, no IL-6, IFN-γ, MCP-1, TNF-α, IL-1β, or CINC-3 could be detected.

CS-induced goblet cell metaplasia in the double exposure system. This acute exposure system (two CS exposures 15 h apart with four 2R4F research cigarettes) clearly induced goblet cell metaplasia in the epithelial layers of the large, medium, and smaller airways of CS-exposed animals. This is illustrated by Fig. 6, A and B, which shows the epithelial layers of the primary bronchus from a sham- and CS-exposed animal, respectively. GCD in the primary bronchus was significantly increased from 0.19 ± 0.03 to 0.64 ± 0.13 units/µm (P < 0.001, Fig. 6C).

Effect of SB-332235, a CXCR2 antagonist, on CS-induced changes in the lung. CS increased neutrophils from 1.3 × 10^5 to 6.3 × 10^5 cells/ml in the BAL fluid (P < 0.001) and from 3.0 × 10^3 to 5.6 × 10^3 cells/mg in the lung tissue. SB-332235 dose dependently inhibited neutrophil numbers in the BAL fluid by 40, 53, and 60% at 0.3, 1, and 3 mg/kg, respectively, with significant levels of inhibition at 1 and 3 mg/kg (P < 0.05 compared with smoke-exposed vehicle controls, Fig. 7A).
compound also inhibited tissue neutrophilia; however, significant inhibition was achieved only at 1 mg/kg (P < 0.05 compared with smoke-exposed vehicle controls, Fig. 7B). GCD increased from 0.5 ± 0.2 to 2.3 ± 0.5 units/μm (P < 0.005) and was significantly attenuated by SB-332235 at 0.3 mg/kg by 54 ± 11% (P < 0.05 compared with smoke-exposed vehicle controls). Interestingly, GCD in the primary bronchus of rats treated with SB-332235 at 1 and 3 mg/kg were apparently not reduced compared with smoke-exposed vehicle controls (2.2 ± 0.5 units/μm at 1 mg/kg and 2.56 ± 0.5 units/μm at 3 mg/kg, Fig. 7C). SB-332235 also dose dependently inhibited CINC-2 production in both tissue and BAL fluid but had no effect on IL-1β levels in the tissue (data not shown).

**DISCUSSION**

Herein, we have characterized the kinetics of the inflammatory changes in an acute model of CS-induced lung injury. This study is very different from previous laboratory animal models of CS-induced lung injury, which have primarily focused on chronic exposures to develop changes in lung function and airway morphology, i.e., emphysema (9, 18, 31). Although the chronic changes that occur as the disease progresses are central to the impaired lung function, it is likely the repetitive, acute inflammatory insults generated by CS help drive these processes. Evidence lies in studies looking at the effect smoking cessation has on early/mild cases of COPD. In these cases, the accelerated rate of age-related lung function decline returns to the normal rate after smoking cessation (5). This suggests that the acute insult generated after CS inhalation is an integral factor in the health decline associated with COPD. As such, we focused on establishing some of the acute changes which occur immediately after exposure to CS.

The peak of the acute inflammatory response occurred after just 3 days of CS exposures. CS induced dose-dependent effects on neutrophil chemokine production and neutrophil infiltration into the lung and airway lumen. This could suggest that CS induces the local production of CXCR2 ligands (IL-8 and GRO-α homologs) from epithelial cells and/or alveolar macrophages, which would then serve to recruit neutrophils into the airways (4, 23). CS also induced dose-dependent increases in goblet cell formation and mucin secretory capacity of rat airways. These inflammatory, mucus, and mechanistic changes are consistent with those observed after chronic exposure to cigarette smoke for up to 8 mo, which eventually leads to emphysematous changes in the lung (26). These acute changes are three major phenotypic characteristics also observed in chronic smokers and in COPD patients, albeit to different degrees. It is generally accepted that in response to CS, resident lung cells produce neutrophil chemokines, which serve to recruit circulating neutrophils into the lung from the bloodstream. Neutrophils, in turn, become activated and release a number of mediators and proteases that perpetuate the inflammatory response and contribute to the destruction of the small airways and alveoli. In addition, mucus hypersecretion is believed to be an important factor for increasing patients’ susceptibility to lung bacterial/viral infections and correlates to increases in morbidity and mortality in COPD (22).

It was also of note that the number of macrophages retrieved in the BAL fluid decreased after smoke exposure. The reason for this is presently unknown, although it has been reported that acute CS exposure can induce alveolar macrophages to undergo apoptosis (1). However, our preferred hypothesis is that CS can enhance adhesion of macrophages to the alveolar walls, possibly through a mechanism involving the covalent modification of matrix proteins (15). In support of this mechanism, we have observed that after more chronic exposures to CS, large number of macrophages remained adhered to the alveolar walls even after BAL was performed.

Having observed acute changes in inflammatory indexes, we next examined the temporal changes occurring after a single set of CS exposures to identify and characterize mechanisms that may be involved in driving airway neutrophil infiltration. Initial experiments indicated that a single exposure was insufficient to elicit consistent and significant changes from which...
conclusions could be drawn. As such, we focused on using a double exposure system to look for temporal changes that occurred immediately after CS exposure. Neutrophil infiltration into the airways generally appeared to progress steadily with time. The strength of the response was more robust after a second exposure as both BAL and tissue neutrophil numbers were significantly elevated. The fold increase in tissue neutrophils was not as great as in the airway, most likely due to the fact there was already a large population of resident neutrophils in the lung, presumably to enable a rapid migration into the air spaces after exposure to a stimulus like CS. Neutrophil infiltration into the airways paralleled changes in the levels of the rat neutrophil chemokines, CINC-1/GRO-α and CINC-2α/β, in both the airways and the tissue. CINC-1 and CINC-2, homologs of human GRO-α, are potent neutrophil chemokines, and this study represents their first characterization in a model of CS-induced lung inflammation in rat. CINC-1 levels mirrored changes in neutrophil infiltration, suggesting that this mediator may be produced by neutrophils themselves and could be important for recruiting additional inflammatory cells to the lung from the circulation. Another possibility is that CINC-1 may be a prosurvival signal that prolongs the ability of activated neutrophils to release their contents into the lung microenvironment before undergoing apoptosis. It has been demonstrated that IL-8 and GRO-α have similar antiapoptotic effects on human neutrophils (7). Interestingly, CINC-2 displayed a biphasic increase in both tissue and BAL fluid after CS exposure. This suggests that CINC-2 may be an initiating factor released from epithelial cells and/or resident macrophages immediately after CS exposure to recruit neutrophils into the lung. The second wave of CINC-2 production, which coincides with the greatest levels of CINC-1 and neutrophils in the lung, may act in a comparable fashion to that hypothesized for CINC-1. These results are similar to clinical observations indicating the excessive neutrophilia observed in the airways of COPD patients may be due in part to the overproduction of neutrophil chemoattractants, like IL-8 and GRO-α, by resident tissue cells like alveolar macrophages or epithelial cells (4, 23). IL-8 and GRO-α are found at elevated levels in the induced sputum of COPD patients compared with healthy smokers (14, 29). In addition, these cytokines are also detected at higher levels in smokers compared with nonsmokers. These data can suggest the production of these chemokines is part of the normal host defense response to CS and may contribute to neutrophil recruitment from earliest stages of the disease [i.e., the normal host defense response somehow becomes unregulated (amplified) in COPD patients]. This hypothesis was further supported by the inhibition of CS-induced neutrophilia with a specific CXCR2 antagonist, SB-3323235. Although the inhibition was only partial, it points to these GRO-α homologs

Fig. 6. Goblet cell metaplasia occurs after an acute exposure to CS. Representative sections from sham (A) and CS-exposed (B) were specifically stained for mucin epitopes to identify goblet cells in the primary bronchus. It is clear that CS induces goblet cell formation, and this increase has been quantified by imaging technology (C). This is 1 representative study of 2 with group sizes of n = 6–9 per treatment per study. Data are presented as mean values ± SE; *P < 0.05 compared with sham control.
as playing an essential role in CS-mediated inflammation. Additional mediators are also likely to contribute to the neutrophil infiltration such as leukotriene B4, matrix fragments, and C5a, thus inhibition of the CXCR2 receptor may serve to dampen the inflammation rather than completely abolish it.

We also observed that both CINC-3 and IL-1β levels were consistently elevated in this double exposure system. Both cytokines can be produced and secreted by a number of different cell types including neutrophils, macrophages, and epithelial cells. CINC-3 is most likely involved in neutrophil migration and possibly activation through its ability to potently induce calcium mobilization (24). IL-1β may also be involved in the activation of local cells, in particular macrophages, to sustain the inflammatory response.

Interestingly, we were unable to detect TNF-α at any endpoint examined, which is inconsistent with previous reports regarding the potentially important role this cytokine may play in CS-mediated inflammation in mice (3). This may be attributable to differences between species or the transient nature of this cytokine’s expression and subsequent rapid degradation. It may also be due to the mild nature of CS as an inflammatory stimulus, inducing a level of expression below the measurable range of the detection system used. As there were no real increases in macrophage or lymphocyte numbers it was not surprising MCP-1 and IFN-γ levels were not significantly changed.

We also demonstrated that a “bronchitic phenotype,” i.e., mucus hypersecretion, could be generated by this acute exposure schedule. There was a significant increase in the number of mucus-producing cells in the epithelial layer of both the trachea and primary bronchus. The mechanisms driving CS-induced mucus hypersecretion are not presently understood. Excessive neutrophilia and neutrophil chemokines, like IL-8 and GRO-α, have been linked to the development of the mucus hypersecretory phenotype present in COPD patients (19, 25). Our data also suggest there may be a role for neutrophils and CXCR2 ligands in the generation of the mucus phenotype. The reason for the inverse dose response observed with SB-332235 on GCD is presently not understood. One hypothesis is that both goblet cell formation and secretion may be attenuated by inhibiting CXCR2 (17, 25, and A. Jackson, unpublished observation). A lack of goblet cell exocytosis at the higher doses of the CXCR2 antagonist may result in an apparent increase in GCD. Interestingly, at the two highest doses of SB-332235, the doses where the GCD appears to be elevated, there was also a significant inhibition of neutrophil infiltration. Neutrophils are thought to contribute to mucus secretion through the release of neutrophil-specific products, like elastase, which can then act directly on the epithelium (27, 28). An attenuation of neutrophil numbers or activation status may therefore result in a reduction of both goblet cell exocytosis and goblet cell formation.

We have established that acute exposure of rats to CS induces a number of dose- and time-dependent changes in the lung that mimic some of the clinical observations of COPD. Furthermore, associated with these inflammatory and mucus hypersecretory changes are temporal alterations in mediators that serve as chemotactic and activating factors for both granulocytes and epithelial cells. We have demonstrated for the first time a role for the GRO-α homologs (CINCs 1–3) in mediating some of the inflammatory response to CS. In addition, this model generates a COPD-like phenotype by mechanisms that may be more relevant than those induced by other inflammatory stimuli, like LPS. It can be used as a rapid and straightforward method for further investigating the pathophysiological mechanisms involved in mediating the acute inflammatory changes induced by CS that, cumulatively, may contribute to the development of the chronic disease condition.

Fig. 7. SB-33235 inhibits CS-induced neutrophil infiltration and modifies CS-mediated mucin changes. This figure illustrates the effect of SB-33235, a CXCR2 antagonist, on BAL fluid neutrophils (A), tissue neutrophils (B), and goblet cell density (C) over 3 days of CS exposure to five 1R3F research cigarettes. This is 1 representative study of 2 with group sizes of n = 7–9 per treatment per study. Data are presented as mean values ± SE; *P < 0.05 compared with CS-exposed vehicle control and #P < 0.05 compared with sham vehicle control.
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