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Original Paper

Monocyte chemoattractant protein 1, interleukin 8, and chronic airways inflammation in COPD

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Abstract

Chronic obstructive pulmonary disease (COPD) is one of the most common causes of death, with cigarette smoking among the main risk factors. Hallmarks of COPD include chronic airflow obstruction and chronic inflammation in the airway walls or alveolar septa. An earlier study reported elevated numbers of macrophages and mast cells within the bronchiolar epithelium in smokers with COPD, compared with smokers without. Since specific chemokines may be involved in this influx, the *in situ* protein and mRNA expression of monocyte chemoattractant protein 1 (MCP-1) and of interleukin 8 (IL-8) were studied in tumour-free peripheral lung tissue resected for lung cancer of current or ex-smokers with COPD ($FEV_1 < 75\%$; $n = 14$) and without COPD ($FEV_1 > 84$; $n = 14$). MCP-1 was expressed by macrophages, T cells, and endothelial and epithelial cells. Its receptor, CCR2, is expressed by macrophages, mast cells, and epithelial cells. IL-8 was found in neutrophils, epithelial cells, and macrophages. In subjects with COPD, semi-quantitative analysis revealed 1.5-fold higher levels of MCP-1 mRNA and IL-8 mRNA and protein in bronchiolar epithelium ($p < 0.01$) and 1.4-fold higher levels of CCR2 in macrophages ($p = 0.014$) than in subjects without COPD. The bronchiolar epithelial MCP-1 mRNA expression correlated with both CCR2 expression on macrophages and mast cells ($p < 0.05$) and the numbers of intra-epithelial macrophages and mast cells ($p < 0.04$). The epithelial IL-8 expression did not correlate with the numbers of neutrophils, macrophages, CD45RO+, CD8+, or mast cells. These data suggest that MCP-1 and CCR2 are involved in the recruitment of macrophages and mast cells into the airway epithelium in COPD.

Keywords: human; lung; COPD; MCP-1; IL-8; CCR2; immunohistochemistry; *in situ* hybridization

Introduction

Chronic obstructive pulmonary disease (COPD), characterized by a reduced forced expiratory flow, is a major health problem [1]. COPD comprises lung emphysema and chronic bronchitis with chronic airflow obstruction. Cigarette smoking is a main risk factor, but for unknown reasons, only 20% of smokers develop COPD [1]. Pathological characteristics of COPD include thickened bronchiolar walls accompanied by an influx of specific inflammatory cells [1]. Several studies have demonstrated increased numbers of macrophages, mast cells, neutrophils, or CD8+ T lymphocytes in the airway walls associated with COPD [2–6], but little is yet known about the molecular mechanisms underlying the pathogenesis of this chronic airway inflammation.

Monocyte chemoattractant protein 1 (MCP-1) is a beta chemokine which induces chemotaxis of human monocytes/macrophages, murine mast cells, and a subset of human T lymphocytes [7–10]. MCP-1 is expressed in various tissues including human lungs, where it occurs in macrophages, endothelial, bronchial epithelial, and smooth muscle cells [11–13]. Increased expression of MCP-1 was shown in interstitial pulmonary fibrosis (IPF) and sarcoidosis [11–13], diseases also characterized by infiltrates of macrophages. Capelli *et al.* [14] reported increased concentrations of MCP-1 in the bronchoalveolar lavage (BAL) fluid of smokers with or without chronic bronchitis, compared with healthy non-smoking subjects. Apart from the recently described C–C chemokine receptor CCR10, the specific receptor for MCP-1 is CCR2, which is mainly expressed by monocytes, macrophages, and T cells [15–18].

Interleukin 8 (IL-8) is an alpha chemokine known to be chemotactic for neutrophils and CD8+ T cells *in vitro*, and to stimulate degranulation of neutrophils [19,20]. IL-8 expression by airway epithelial cells can be stimulated by cigarette smoke [21]. Keatings *et al.* [22] reported increased levels of IL-8 in induced sputum from smokers with COPD, compared with smokers without COPD or non-smokers, suggesting a role for IL-8 in recruiting neutrophils and CD8+ T cells in COPD.

As yet, little is known about the pulmonary tissue

expression pattern of MCP-1, IL-8, or CCR2, or their relationship with the chronic airways inflammation in smokers with COPD. We have now examined the distribution of MCP-1 and IL-8 mRNA and protein, as well as CCR2 protein, in relation to the airways inflammation, in current or ex-smokers with and without COPD. In addition, the number of CD8+ T cells was examined within the bronchial and bronchiolar walls. The expression data were correlated with the numbers of inflammatory cells.

Materials and methods

Antibodies

Antibodies were purchased as follows: a polyclonal goat anti-human CCR2 antibody raised against a synthetic peptide corresponding to amino acids 341–360 of CCR2b (Santa Cruz Biotechnology, Santa Cruz, CA, USA); monoclonal antibodies against CD68 (clone KP1), neutrophil-derived elastase (clone NP57), and tryptase (clone AA1); the rabbit anti-human CD3 antibody, as well as secondary antibodies (Dako, Glostrup, Denmark); mouse monoclonal antibody against CD8 (clone 4B11) (Novocastra, Newcastle upon Tyne, UK); and mouse monoclonal anti-human MCP-1 and IL-8 antibodies (R&D Systems, Minneapolis, MN, USA).

Subjects and clinical data

Lung tissue specimens, including peripheral airways, of subjects with or without COPD were retrospectively obtained from the pathology tissue bank and used as previously described [4]. Tissue specimens were selected from current or ex-smokers who had undergone lobectomy or pneumonectomy for lung cancer. Fourteen subjects with COPD were included [FEV_1 $<75\%$ of the predicted (pred.) value before bronchodilatation; reversibility in FEV_1 $\leq 13\%$ pred. after 400 μg inhaled salbutamol], as well as 14 subjects without COPD (FEV_1 before bronchodilatation $>84\%$ pred.). The total lung capacities (TLC) were at normal levels (TLC $\geq 80\%$ pred.). Tissue was excluded if diffuse pulmonary inflammation or fibrotic disorders were present, if no tumour-free or post-stenotic pneumonia-free lung tissue specimen was found, and if the central bronchus was obstructed due to the tumour. Lung function data were described previously [4] and are partly given in Table 1. All patients lacked upper respiratory tract infection and did not receive antibiotics peri-operatively. None of the patients had received glucocorticosteroids in a period of 3 months before resection. Four patients received glucocorticosteroids peri-operatively (Table 1). Based on the available information, subjects with COPD could not be subdivided into patients with predominantly either chronic bronchitis or emphysema.

Immunohistochemistry

Serial paraffin-embedded tissue sections (3 μm) were alternately used for mRNA *in situ* hybridization (RISH) and immunohistochemistry (IHC) for MCP-1, IHC for CCR2, or RISH and IHC for IL-8. Detection of cell-specific markers on adjacent sections was performed in order to confirm the cell type. IHC was performed essentially as previously described [4,23]. After deparaffinization, endogenous peroxidase was inactivated with 0.3% hydrogen peroxide. Sections to be stained with anti-CD68 were treated with proteinase K. Sections to be stained for MCP-1 and CD8 were pretreated by microwave antigen retrieval in 0.01 M citrate, pH 6.0 and 1.0 mM EDTA, pH 8.0, respectively. Subsequently, sections were preincubated with 1% (w/v) bovine serum albumin (BSA). Antigen expression was demonstrated with appropriate dilutions of the primary antibodies in conjugated immunoenzyme assays using a secondary biotin conjugated antibody and a tertiary complex of streptavidin–biotin conjugated to horseradish peroxidase, and 3-amino-9-ethyl-carbazole as the chromogen. Sections were counterstained with Mayer's haematoxylin. Incubation with phosphate-buffered saline (PBS) with 1% BSA instead of the primary antibody served as a negative control. Negative controls for immunostaining of CCR2 included isotype-matched goat immunoglobulins and anti-CCR2 antibody preincubated with a synthetic peptide corresponding to amino acids 341–360 of the CCR2b protein, instead of the primary antibody. IHC using negative controls did not reveal any staining.

Chemokine and receptor expression was assessed by a semi-quantitative method of analysis using an arbitrary visual scale [23,24]. The expression was examined throughout the entire section. A subset was analysed twice to assess intra-observer variability (Pearson correlation coefficient $r=0.75$; $p<0.05$). The staining intensity in each of the following cell types was scored in a blinded manner: epithelial cells, smooth muscle cells, macrophages (CD68+), and endothelial cells of larger blood vessels. The staining intensity was graded and expressed as follows: 0 = absence of staining; 1 = moderate staining; 2 = intense staining; 3 = very intense staining [23].

Cell counting of CD8+ T cells was done using the KS400 version 2 digital image acquisition and analysis system (Zeiss, Oberkochen, Germany). Cells were counted within the bronchial and bronchiolar epithelium and in the lamina propria in the widest possible area, extending up to 100 μm deep beneath the epithelial basement membrane. Smooth muscle and bronchial glands were excluded from the lamina propria area. Cell numbers were expressed within the epithelium per mm of basement membrane and within the lamina propria per 0.1 mm^2 of lamina propria.

mRNA *in situ* hybridization

Full-length human MCP-1 cDNA cloned into pGEM-7Zf(+) (Promega, Madison, USA) was obtained from

Table 1. Characteristics of subjects without (non-COPD) and with COPD including numbers of males (M), females (F), peri-operative steroid users (peri), and current versus ex-smokers

	Sex	Age (years)	py	FEV_1	FEV_1/FVC	Steroid	Smokers
Non-COPD	3 F 11 M					None	10 ex 4 current
Range		28–79	1–110	86–133	0.62–0.86		
Mean ± SEM		64 ± 3.7	42 ± 7.7	101 ± 3.3	0.72 ± 0.02		
COPD	14 M					4 peri 10 no	8 ex 6 current
Range		48–76	19–68	48–72	0.38–0.66		
Mean ± SEM		64 ± 2.3	44 ± 0.8	63 ± 2	0.54 ± 0.02		
p-value		0.84	0.82	5×10^{-9}	5×10^{-6}		

FEV_1 and FVC are given as percentages of the predicted values before bronchodilatation. py = number of pack-years.

the American Type Culture Collection (ATCC), and full-length human IL-8 cDNA cloned into pBluescript II SK(−) was kindly provided by Dr C. Power (formerly of Glaxo Institute for Molecular Biology, Geneva, Switzerland). The specific cRNA probes were labelled with digoxigenin following the manufacturer's protocol (Boehringer, Mannheim, Germany). RISH was performed essentially as previously described, on sections adjacent to those on which MCP-1 or IL-8 immunoreactivity was assessed [23,24]. After pretreatment, sections were hybridized with 50 ng of riboprobe per slide for 16 h at 42°C. Subsequently, sections were washed in 2X SSC with 50% formamide at 37°C, in 0.1X SSC with 20 mM β-mercaptoethanol at 42°C, and finally treated with 2U/ml RNAse T1 (Boehringer, Mannheim, Germany) in 2X SSC plus 1 mM EDTA at 37°C. Immunodetection of digoxigenin-labelled hybrids was done using nitro blue tetrazolium as the chromogen and bicholylindolyl phosphate as the coupling agent (Boehringer, Mannheim, Germany). Sense riboprobes included as negative controls did not show any staining. The staining intensity was expressed as described for IHC.

Statistics

IHC and RISH data showed a normal distribution and were expressed as mean ± SEM. Significance levels were obtained using the unpaired, two-tailed Student's *t*-test. Pearson correlation analysis and statistics between expression levels and numbers of intra-epithelial mast cells and macrophages were done using Stata Statistical Software 5.0 (Stata Corp., College Station, USA). At $p < 0.05$, differences were considered to be statistically significant.

Results

Expression patterns

MCP-1 mRNA and protein were localized mainly in inflammatory cells, including CD3+ T cells and CD68+ cells (regarded as macrophages), and, to a lesser extent, in alveolar epithelial cells and in the apical parts of airway epithelial cells (Figures 1 and 2 and Table 2). Relatively high levels of MCP-1 transcripts, but low MCP-1 immunoreactivity, were also found in endothelial cells (Table 2). In subjects with COPD, MCP-1 mRNA (1.5 times) levels were significantly higher ($p < 0.0005$) in airway and alveolar epithelial cells than in subjects without COPD (Figure 2 and Table 2). The MCP-1 protein expression levels did not differ significantly between subjects with and without COPD ($p = 0.25$).

CCR2 expression is predominantly found in macrophages and mast cells, and, unexpectedly, in airway and alveolar epithelium (Figures 1 and 2 and Table 3). Neutrophils were negative for CCR2. In the airways of subjects with COPD, CCR2 expression was significantly higher in macrophages (1.4 times, $p = 0.014$) and epithelial cells (1.5 times, $p = 0.01$), and tended to be higher in mast cells (1.4 times, $p = 0.05$), than in subjects without COPD (Figure 2 and Table 3).

As shown in Figure 1 and Table 2, IL-8 mRNA or protein was mainly localized in neutrophils, airway and alveolar epithelial cells, and macrophages. In subjects with COPD, approximately 1.5 times higher IL-8 mRNA and protein levels were found in bronchiolar and alveolar epithelium ($p < 0.05$) than in subjects without COPD. IL-8 protein levels were also two times higher in neutrophils in subjects with COPD ($p = 0.0006$).

The smoking status of the patient (ex-smoker versus current smoker) did not affect the expression data of either chemokine or of CCR2.

CD8+ T-cell numbers

Complementary to our previous findings on cell type-specific numbers [4], we assessed the number of CD8+ T cells within the bronchial and the bronchiolar airways of the same patients in the epithelium and the lamina propria. Within the epithelial layer, CD8+ T cells were predominantly localized near the basement membrane. We did not find significant differences in CD8+ T cell numbers between subjects with or without COPD (Table 4).

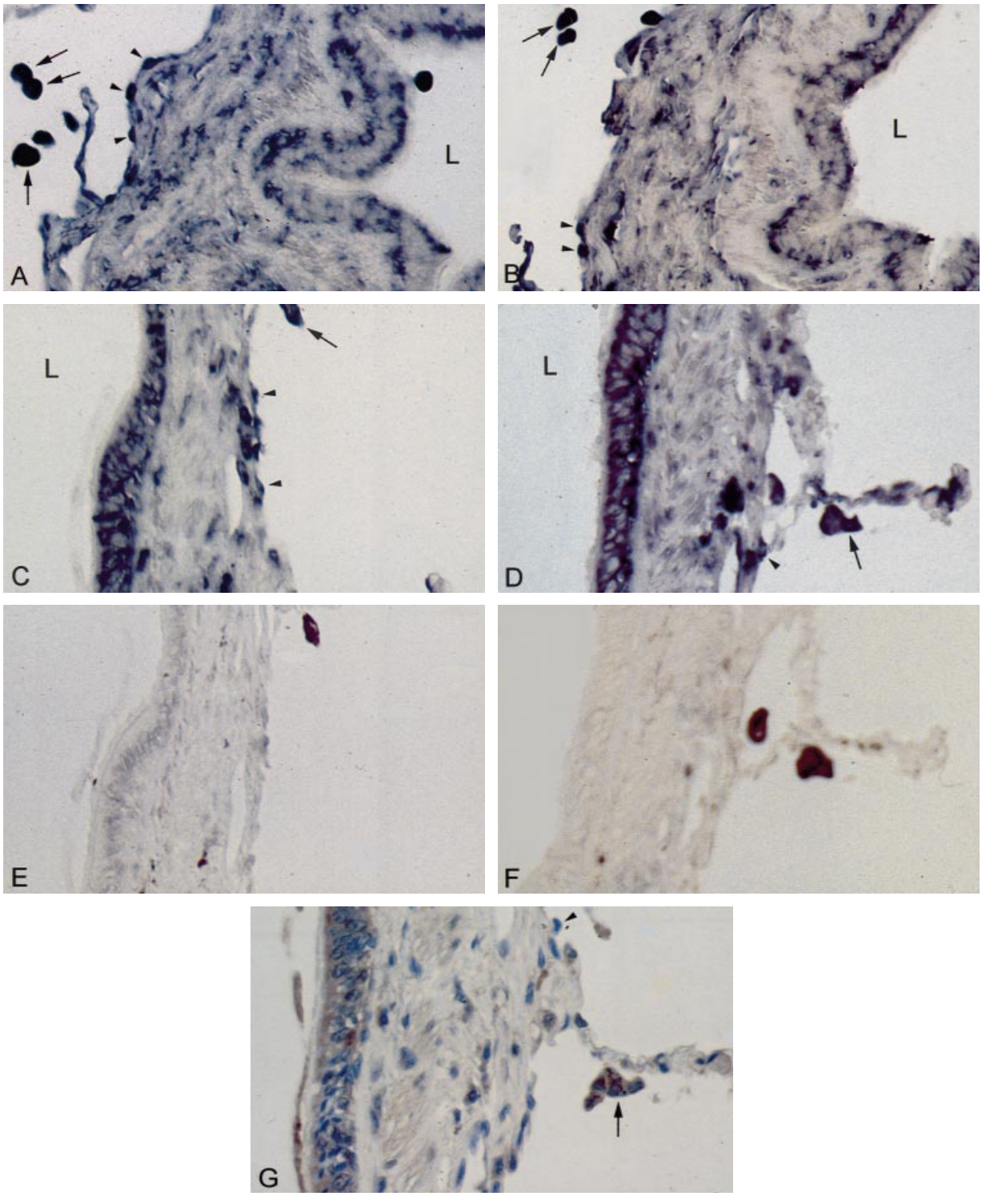

Figure 1. Micrographs of lung tissue sections from subjects without COPD (A, B) and with COPD (C–G). The respective photographs show MCP-1 mRNA (A, C) and IL-8 mRNA (B, D) localization in bronchioles, using the antisense cRNA probes, and adjacent sections after incubation with the sense MCP-1 (E) or IL-8 (F) cRNA probe and anti-CD68 antibody (red). CCR2 immunoreactivity (red) in a bronchiole is shown in G. Large arrows indicate macrophages, and arrow-heads pneumocytes; L = bronchiolar lumen. The sections in A–F are not counterstained

Statistical analysis

MCP-1 mRNA levels in bronchiolar epithelial cells correlated with the number of intra-epithelial macrophages ($r = 0.50$, $p = 0.01$), the number of intra-epithelial mast cells ($r = 0.42$, $p = 0.04$), and also with CCR2 levels in airway macrophages ($r = 0.44$, $p = 0.048$) and mast cells ($r = 0.58$, $p = 0.01$) (Figure 3). The MCP-1 mRNA expression in airway endothelial cells also correlated with CCR2 expression in airway macrophages ($r = 0.58$, $p = 0.007$) and mast cells ($r = 0.61$, $p = 0.008$). Bronchiolar epithelial IL-8

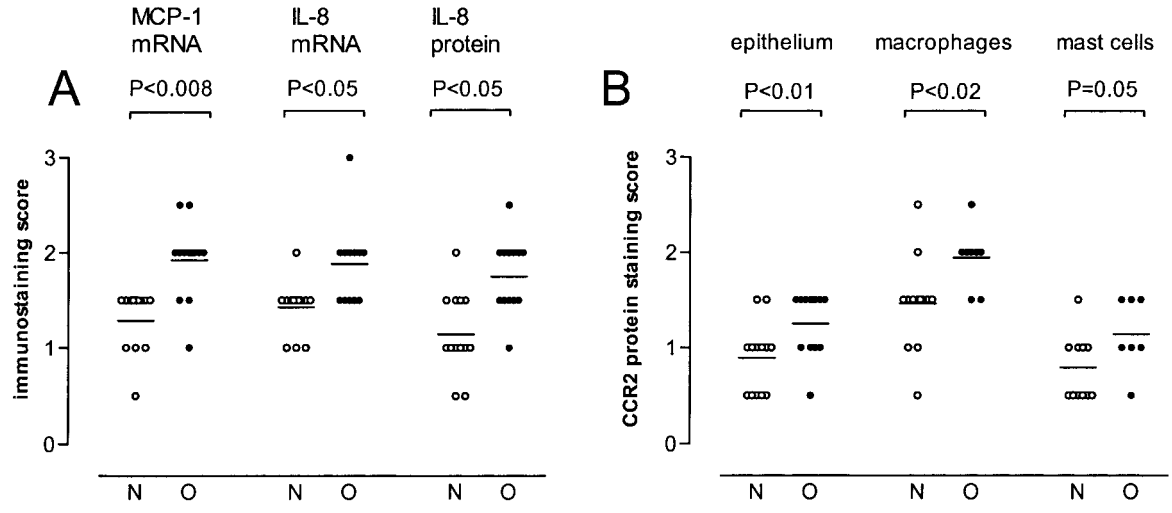

Figure 2. Semi-quantitative analysis of the MCP-1 and IL-8 mRNA and IL-8 protein in bronchiolar epithelial cells (A) and CCR2 protein expression per indicated cell type in bronchiolar airways (B). The immunostaining score is given on the *Y*-axis, ranging from 0 (no staining) to 3 (very intense staining). Open and closed circles represent the individual data from subjects without COPD (N) and with COPD (O), respectively. Means are indicated by a horizontal line

Table 2. Pulmonary expression levels of MCP-1 and IL-8 mRNA and protein per cell type in patients without (non-COPD) and with COPD. Scores are indicated separately for bronchiolar and for alveolar tissue

		Bronchioles		Alveoli	
		Non-COPD	COPD	Non-COPD	COPD
MCP-1					
Epithelium	mRNA	1.3±0.1	1.9±0.1*	2.1±0.1	2.8±0.1*
	Protein	1.2±0.1	1.4±0.1	0.8±0.2	1.0±0.2
Vascular SMC	mRNA	0.9±0.1	0.9±0.1	1.0±0.1	1.2±0.1
	Protein	0.3±0.1	0.2±0.1	0.1±0.1	0.04±0.04
Airway SMC	mRNA	1.0±0.1	1.1±0.1	1.3±0.1	1.3±0.1
	Protein	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.1
Endothelium	mRNA	1.9±0.1	2.3±0.2**	2.1±0.1	2.2±0.2
	Protein	0.3±0.1	0.4±0.1	0.3±0.1	0.4±0.1
Macrophages	mRNA	2.5±0.1	2.6±0.2	2.9±0.1	2.7±0.2
	Protein	0.9±0.1	1.2±0.1	0.8±0.1	1.0±0.1
IL-8					
Epithelium	mRNA	1.4±0.1	1.9±0.1*	1.7±0.1	2.0±0.1**
	Protein	1.1±0.1	1.8±0.1*	1.1±0.1	1.8±0.1*
Vascular SMC	mRNA	0.9±0.1	1.0±0.1	1.0±0.1	0.7±0.1
	Protein	0.8±0.1	0.8±0.1	0.7±0.1	0.8±0.1
Airway SMC	mRNA	1.1±0.1	1.0±0.1	1.2±0.1	0.8±0.2
	Protein	0.8±0.1	0.8±0.1	ND	ND
Endothelium	mRNA	1.8±0.1	1.9±0.1	1.9±0.1	1.6±0.2
	Protein	0.9±0.1	0.8±0.1	0.5±0.1	0.9±0.1*
Macrophages	mRNA	1.8±0.1	1.8±0.1	2.3±0.2	2.1±0.1
	Protein	1.0±0.1	0.9±0.1	1.4±0.1	1.4±0.1
Neutrophils	mRNA	ND	ND	ND	ND
	Protein	1.0±0.1	2.0±0.1*	1.0±0.1	1.8±0.1*

Scores range from 0 (no staining) to 3 (very intense staining) and are given as mean±SEM. Asterisks indicate significance levels of differences between subjects with and without COPD:
*$p<0.005$.
**$p\leq0.05$.
SMC = smooth muscle cells.
ND = not determined.

mRNA or protein levels did not correlate with the numbers of macrophages, mast cells, neutrophils, CD45RO+ or CD8+ cells within the lamina propria or epithelium of the bronchioles.

Taking all subjects together reveals a significant inverse correlation between FEV_1 and the bronchiolar epithelial MCP-1 and IL-8 mRNA expression ($r=-0.51$, $p=0.01$) as well as IL-8 protein expression in bronchiolar epithelial cells ($r=-0.60$, $p=0.001$). CD8+ T-cell numbers did not correlate with FEV_1.

Discussion

In the present study, expression patterns of MCP-1, IL-8, and CCR2 were examined in the airways and

Table 3. Expression levels per cell type of CCR2 protein in bronchiolar and alveolar tissue of patients without (non-COPD) and with COPD

	Bronchioles		Alveoli	
	Non-COPD	COPD	Non-COPD	COPD
Epithelium	0.9±0.1*	1.3±0.1**	0.8±0.1	1.0±0.1
Vascular SMC	0.1±0.1	0.3±0.1	0.5±0.1	0.3±0.1
Airway SMC	0.3±0.1	0.5±0.1	0.5±0.1	0.4±0.1
Endothelium	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Macrophages	1.5±0.1	1.9±0.1**	1.9±0.1	1.9±0.1
Mast cells	0.8±0.3	1.1±0.1***	0.7±0.1	0.9±0.2

Immunostaining scores range from 0 (no staining) to 3 (very intense staining) and are given as mean±SEM. Significance levels of differences between subjects with and without COPD are indicated by asterisks:
*$p<0.01$.
**$p<0.02$.
***$p=0.05$.
SMC = smooth muscle cells.

Table 4. Numbers of CD8+ cells in subjects without (non-COPD) and with COPD

	Epithelium		Lamina propria	
	Bronchi	**Bronchioles**	**Bronchi**	**Bronchioles**
Non-COPD				
Range	2.2–74.7	5.1–32.6	30.0–143.2	24.5–106.5
Median	8.7	25.0	8.7	53.6
COPD				
Range	2.7–23.0	0.6–47.0	2.7–23.0	18.5–350.5
Median	5.1	7.9	5.1	56.7
p value	0.20	0.19	0.14	0.63

Data are given as median cell numbers and ranges in the epithelium (per mm basement membrane) and in the lamina propria (per 0.1 mm^2 area), both in the bronchi and in the bronchioles. Statistical significance levels are given as the *p* value.

alveoli of subjects with and without COPD. The results were correlated with inflammatory cell numbers in the bronchiolar walls of these subjects. Only the higher expression of MCP-1 in bronchiolar epithelial cells in COPD correlated with the CCR2 expression and the increased numbers of macrophages and mast cells in the bronchiolar epithelium in COPD [4]. These data suggest the involvement of MCP-1 and CCR2 in the influx of macrophages and mast cells into the bronchiolar epithelium in COPD. Several studies have suggested the implication of MCP-1, IL-8 or macrophage inflammatory protein (MIP-1) in chronic inflammatory processes in lung diseases of different aetiology, including sarcoidosis, interstitial pulmonary fibrosis (IPF), and COPD [12,13,22,25,26]. However, these studies examined either BAL fluid or sputum [12,13,22], or plasma [25]. Although it was suggested that enhanced MCP-1 and IL-8 levels affect macrophage recruitment in these lung diseases, no correlation was made with macrophage numbers in most of these studies. One study presented a correlation between the numbers of MIP-1α-positive bronchial epithelial cells and the numbers of subepithelial macrophages in COPD [26].

The functional consequences of the increased IL-8 expression in COPD observed in the present study are not clear. Neutrophils, CD8+ T cells, and even mast cells may respond to IL-8 *in vitro* [19,20,27]. However, the numbers of neutrophils, CD45RO+, and CD8+ T cells in the airway walls did not differ between

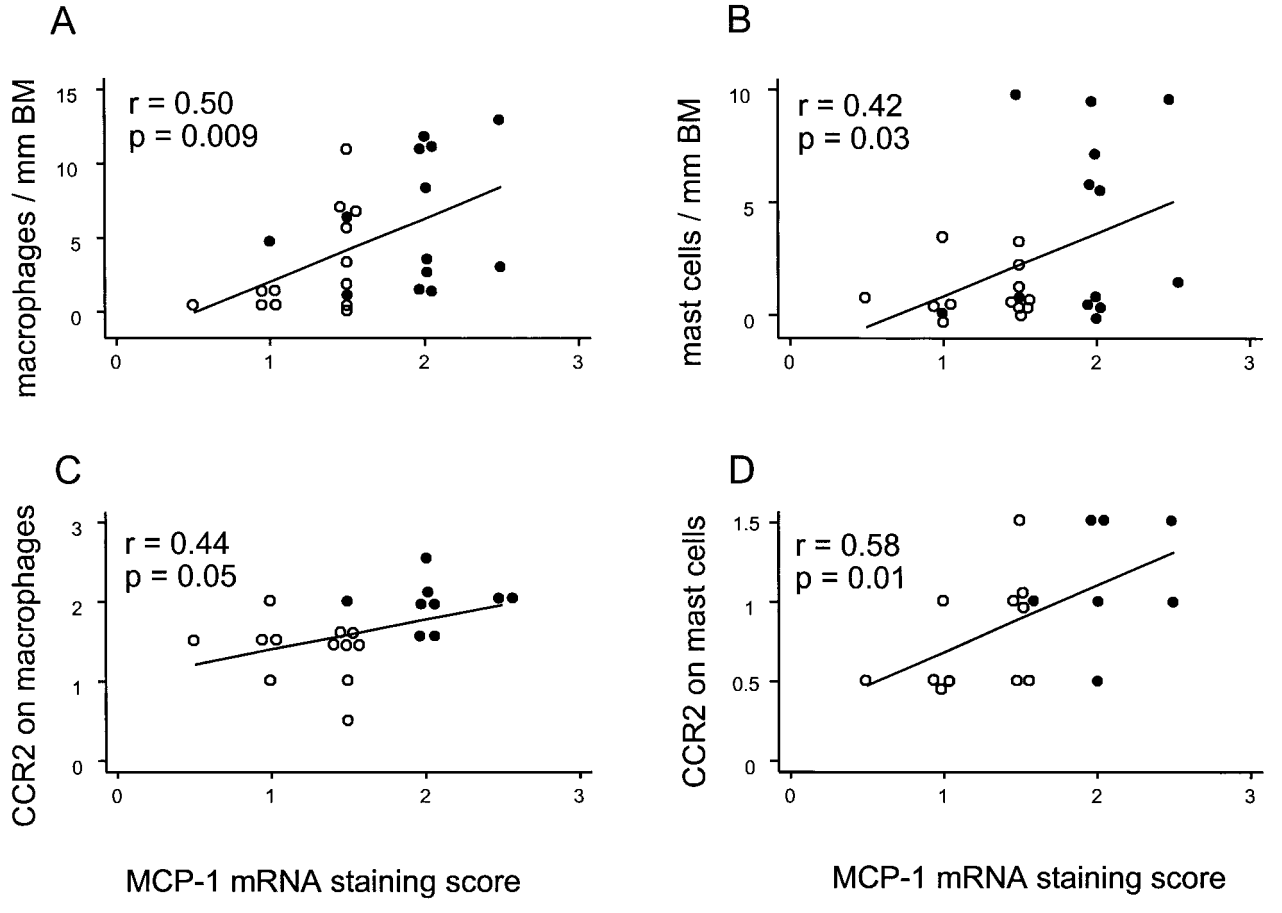

Figure 3. Correlations between MCP-1 mRNA expression in bronchiolar epithelium, intra-epithelial inflammatory cell numbers, and CCR2 protein expression on inflammatory cells. (A) Macrophage numbers and MCP-1 mRNA levels; (B) mast cell numbers and MCP-1 mRNA levels; (C) CCR2 expression on macrophages and MCP-1 mRNA expression; (D) CCR2 expression on mast cells and MCP-1 mRNA expression. Cell numbers are given per mm of basement membrane (BM). Open circles represent data from subjects without COPD; closed circles data from subjects with COPD. Data were obtained by linear regression analysis. Correlation (*r*) and significance level (*p* value) are given

subjects with or without COPD [4] (this study). In addition, the IL-8 expression in bronchiolar epithelial cells did not correlate with the numbers of neutrophils, T cells, or mast cells within either the epithelial or the subepithelial layer in the present study [4]. Hence, the higher epithelial IL-8 expression in COPD may not be sufficient to increase the numbers of neutrophils or T cells. Lacoste *et al.* [28] showed that although neutrophil numbers in the BAL fluid from smokers with COPD are higher than in smokers without COPD, the neutrophil numbers within the airway walls do not differ. Thus, as an alternative explanation, the higher epithelial IL-8 expression in COPD reported here may lead to a faster transit of neutrophils and/or CD8+ T cells from the airway wall into the lumen.

Only a few reports have mentioned significant differences in CD8+ cell numbers within the epithelium or lamina propria between smokers with normal FEV_1 and smokers with COPD [2,6], whereas others could not confirm this [3,5,26] (this study). It should be noted that in all of these studies the subject subgroups showed different (clinical) characteristics. Nevertheless, this indicates that the role of CD8+ T cells in the pathogenesis of COPD remains to be elucidated.

Few human studies have reported a correlation between higher MCP-1 expression and increased macrophage numbers. Marra *et al.* [29] demonstrated a correlation between enhanced hepatic epithelial expression of MCP-1 in human chronic liver disease and infiltration of monocytes/macrophages into the bile ducts. Engelhardt *et al.* [30] reported an influx of macrophages in regenerating human skin paralleled by an increased epithelial MCP-1, but not MIP-1, mRNA expression. Functional *in vivo* studies indicated that targeted overexpression of MCP-1 in murine lungs causes recruitment of macrophages, in particular [31]. Evidence for a role of MCP-1 in a mouse model of emphysema was found by Hautamaki *et al.* [10], who showed that intratracheal instillation of MCP-1 in mice resulted in pulmonary recruitment of macrophages and enhancement of cigarette smoke-induced emphysema. Studies on mice lacking CCR2 indicated that CCR2 is important in the recruitment of macrophages [32,33]. Taken together, our data support the hypothesis that MCP-1 causes increased numbers of macrophages within the bronchiolar walls in subjects with COPD.

Whereas the MCP-1 mRNA levels differed between subjects with and without COPD, the MCP-1 protein expression did not differ significantly and did not correlate with mast cell or macrophage numbers. The apical localization of MCP-1 in bronchial epithelial cells [12] (this study), the predominant apical secretion by alveolar epithelial cells *in vitro* [34], and the fact that MCP-1 is not stored within epithelial cells support the hypothesis that lack of differences between cell-associated MCP-1 protein levels in patients with COPD versus patients without COPD may be due to a predominant direct apical secretion of MCP-1 by epithelial cells into the epithelial lining fluid. This point could not be addressed in this retrospective study, since BAL fluid was not available. This does not preclude baso-lateral secretion of MCP-1 proteins by airway epithelial cells. Both immunohistochemistry on tissue sections and mRNA analysis of primary bronchial epithelial cells (data not shown) showed the presence of CCR2 in epithelial cells, suggesting an autocrine role.

We have previously shown a correlation between levels of transforming growth factor β_1 ($TGF\beta_1$) protein and mRNA in bronchiolar epithelial cells and the numbers of intra-epithelial macrophages and mast cells in COPD, using adjacent tissue sections [23]. We now observe that the $TGF\beta_1$ mRNA and protein expression also correlates with the MCP-1 mRNA levels in the bronchiolar epithelium ($r=0.65$ and $r=0.59$; $p<0.002$, respectively, by Spearman rank test). Since $TGF\beta_1$ can induce the expression of MCP-1 in fibroblasts [35], this may suggest functional relationships between the epithelial $TGF\beta_1$ and MCP-1 expression.

Our data show that chemokine expression partly correlates with cell influx, and also correlates with FEV_1 if all subjects are taken together. Within the group of subjects with COPD, there was no significant correlation, which may be due to the lack of subjects with mild or severe COPD in our study. The correlation of the higher MCP-1 mRNA expression in bronchiolar epithelial cells with the elevated CCR2 expression on bronchiolar macrophages and mast cells, and with higher numbers of intra-epithelial macrophages and mast cells in COPD, suggests the involvement of MCP-1 and CCR2 in the recruitment of macrophages and mast cells into the bronchiolar epithelium in COPD. Apart from MCP-1, $TGF\beta_1$ [23] and MIP-1α [26] may also be involved in the influx of macrophages and mast cells into the bronchiolar epithelium in COPD. This also points to future studies on MCP-1 antagonists in therapeutic strategies for COPD.

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References

1. Jeffery PK. Structural and inflammatory changes in COPD: a comparison with asthma. *Thorax* 1998; **53**: 129–136.
2. Fournier M, Lebargy F, Le Roy Ladurie F, Lenormand E, Pariente R. Intra-epithelial T-lymphocyte subsets in the airways of normal subjects and of patients with chronic bronchitis. *Am Rev Respir Dis* 1989; **140**: 737–742.
3. Saetta M, Turato G, Facchini FM, *et al.* Inflammatory cells in the bronchial glands of smokers with chronic bronchitis. *Am J Respir Crit Care Med* 1997; **156**: 1633–1639.
4. Grashoff WFH, Sont JK, Sterk PJ, *et al.* Chronic obstructive pulmonary disease: the role of bronchiolar mast cells and macrophages. *Am J Pathol* 1997; **151**: 1785–1790.

5. O'Shaughnessy TC, Ansari TW, Barnes NC, Jeffery PK. Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of CD8+ T lymphocytes with FEV_1. *Am J Respir Crit Care Med* 1997; **155**: 852–857.
6. Saetta M, Di Stefano A, Turato G, *et al*. CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998; **157**: 822–826.
7. Carr MW, Roth SJ, Luther E, Rose SS, Springer TA. Monocyte chemoattractant protein-1 acts as T-lymphocyte chemoattractant. *Proc Natl Acad Sci U S A* 1994; **91**: 3652–3656.
8. Taub DD, Proost P, Murphy WJ, *et al*. Monocyte chemotactic protein-1 (MCP-1), -2, and -3 are chemotactic for human T lymphocytes. *J Clin Invest* 1995; **95**: 1370–1376.
9. Conti P, Boucher W, Letourneau R, *et al*. Monocyte chemotactic protein-1 provokes mast cell aggregation and [^{3}H]5HT release. *Immunology* 1995; **86**: 434–440.
10. Hautamaki RD, Kobayashi DK, Senior RM, Shapiro SD. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. *Science* 1997; **277**: 2002–2004.
11. Antoniades HN, Neville-Golden J, Galanopoulos T, Kradin RL, Valente AJ, Graves DT. Expression of monocyte chemoattractant protein 1 mRNA in human idiopathic pulmonary fibrosis. *Proc Natl Acad Sci U S A* 1992; **89**: 5371–5375.
12. Iyonaga K, Takeya M, Saita N, *et al*. Monocyte chemoattractant protein-1 in idiopathic pulmonary fibrosis and other interstitial lung diseases. *Hum Pathol* 1994; **25**: 455–463.
13. Car BD, Meloni F, Luisetti M, Semenzato G, Gialdroni-Grassi G, Walz A. Elevated IL-8 and MCP-1 in the bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis and pulmonary sarcoidosis. *Am J Respir Crit Care Med* 1994; **149**: 655–659.
14. Capelli A, Di Stefano A, Lusuardi M, Gnemmi I, Balbi B, Donner CF. Increased MIP-1beta and MCP-1 in bronchoalveolar lavage fluid of chronic bronchitic patients. *Am J Respir Crit Care Med* 1998; **157** (Suppl 3): 796.
15. Combadiere C, Ahuja SK, Murphy PM. Cloning, chromosomal localization, and RNA expression of a human beta chemokine receptor-like gene. *DNA Cell Biol* 1995; **14**: 673–680.
16. Bonecchi R, Bianchi G, Bordignon PP, *et al*. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 1998; **187**: 129–134.
17. Nieto M, Frade JM, Sancho D, Mellado M, Martinez AC, Sanchez-Madrid F. Polarization of chemokine receptors to the leading edge during lymphocyte chemotaxis. *J Exp Med* 1997; **186**: 153–158.
18. Frade JM, Mellado M, Del Real G, Gutierrez-Ramos JC, Lind P, Martinez AC. Characterization of the CCR2 chemokine receptor: functional CCR2 receptor expression in B cells. *J Immunol* 1997; **159**: 5576–5584.
19. Oppenheim JJ, Zachariae COC, Mukaida N, Matsushima K. Properties of the novel proinflammatory supergene 'intercrine' cytokine family. *Annu Rev Immunol* 1991; **9**: 617–648.
20. Qin S, LaRosa G, Smith-Heath H, *et al*. Expression of monocyte chemoattractant protein-1 and interleukin-8 receptors on subsets of T cells: correlation with transendothelial chemotactic potential. *Eur J Immunol* 1996; **26**: 640–647.
21. Masubuchi T, Koyama S, Sato E, *et al*. Smoke extract stimulates lung epithelial cells to release neutrophil and monocyte chemotactic activity. *Am J Pathol* 1998; **153**: 1903–1912.
22. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor-α in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med* 1996; **153**: 530–534.
23. De Boer WI, Van Schadewijk A, Sont JK, *et al*. Transforming growth factor β_1 and recruitment of macrophages and mast cells in airways in COPD. *Am J Respir Crit Care Med* 1998; **158**: 1951–1957.
24. De Boer WI, Schuller AGP, Vermey M, Van der Kwast TH. Expression of growth factors and receptors during specific phases in regenerating urothelium after acute injury *in vivo*. *Am J Pathol* 1994; **145**: 1199–1207.
25. Hashimoto S, Nakayama T, Gon Y, *et al*. Correlation of plasma monocyte chemoattractant protein-1 (MCP-1) and monocyte inflammatory protein-1α (MIP-1α) levels with disease activity and clinical course of sarcoidosis. *Clin Exp Immunol* 1998; **111**: 604–610.
26. Di Stefano A, Capelli A, Lusuardi M, *et al*. Severity of airflow limitation is associated with severity of airway inflammation in smokers. *Am J Respir Crit Care Med* 1998; **158**: 1277–1285.
27. Lippert U, Artue M, Grützkau A, *et al*. Expression and functional activity of the IL-8 receptor type CXCR1 and CXCR2 on human mast cells. *J Immunol* 1998; **161**: 2600–2608.
28. Lacoste J-Y, Bousquet J, Chanez P, *et al*. Eosinophilic and neutrophilic inflammation in asthma, chronic bronchitis, and chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 1993; **92**: 537–548.
29. Marra F, DeFranco R, Grappone C, *et al*. Increased expression of monocyte chemotactic protein-1 during active hepatic fibrogenesis: correlation with monocyte infiltration. *Am J Pathol* 1998; **152**: 423–430.
30. Engelhardt E, Toksoy A, Goebeler M, Debus S, Bröcker E-B, Gillitzer R. Chemokines IL-8, GROα, MCP-1, IP-10, and Mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing. *Am J Pathol* 1998; **153**: 1849–1860.
31. Gunn MD, Nelken NA, Liao X, Williams LT. Monocyte chemoattractant protein-1 is sufficient for the chemotaxis of monocytes and lymphocytes in transgenic mice but requires an additional stimulus for inflammatory activation. *J Immunol* 1997; **158**: 376–383.
32. Boring L, Gosling J, Chensue SW, *et al*. Impaired monocyte migration and reduced type 1 (Th1) cytokine responses in C–C chemokine receptor 2 knockout mice. *J Clin Invest* 1997; **100**: 2552–2561.
33. Kurihara T, Warr G, Loy J, Bravo R. Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. *J Exp Med* 1997; **186**: 1757–1762.
34. Paine R 3rd, Rolfe MW, Standiford TJ, Burdick MD, Rollins BJ, Strieter RM. MCP-1 expression by rat type II alveolar epithelial cells in primary culture. *J Immunol* 1993; **150**: 4561–4570.
35. Slavin J, Unemri E, Hunt TK, Amento E. Monocyte chemotactic protein-1 (MCP-1) mRNA is down-regulated in human dermal fibroblasts by dexamethasone: differential regulation by TGF-beta. *Growth Factors* 1995; **12**: 151–157.

