

Local and Systemic Inflammation in Patients with Chronic Obstructive Pulmonary Disease

Soluble Tumor Necrosis Factor Receptors Are Increased in Sputum

Juanita H. Vernooy, Mehmet Küçükaycan, Jan A. Jacobs, Niels H. Chavannes, Wim A. Buurman, Mieke A. Dentener, and Emiel F. Wouters

Nutrition and Toxicology Research Institute Maastricht, Departments of Pulmonology and General Surgery, Maastricht University; and Departments of Medical Microbiology and General Practice, University Hospital Maastricht, Maastricht, The Netherlands

Chronic obstructive pulmonary disease (COPD) is characterized by significant chronic inflammation in the pulmonary compartment as well as in the circulation. This study aimed to elucidate the relationship between local and systemic inflammation in smoking-induced COPD by assessing levels of soluble (s) tumor necrosis factor (TNF) receptors, TNF- α , and interleukin-8 (IL-8) in induced sputum and in plasma. Sputum induction was performed in 18 subjects with COPD (FEV₁ 56% predicted) and 17 healthy smokers (FEV₁ 99% predicted). Patients with COPD showed significantly higher percentages of neutrophils and levels of sTNF-R55 and IL-8 in sputum as compared with control subjects, whereas sputum sTNF-R75 levels tended to be higher in COPD. Sputum TNF- α levels were similar in both groups. When comparing sTNF receptors in sputum and plasma, no direct correlations were found despite elevation of circulating sTNF-R75 levels in patients with COPD. In addition, sputum sTNF receptors were inversely related to the FEV₁ in patients with COPD, whereas circulating sTNF receptors were not, suggesting different regulation of inflammation in the pulmonary and systemic compartment. When subjects were divided according to their current smoking status, levels of sTNF-R55, sTNF-R75, and IL-8 in sputum were significantly elevated in ex-smoking versus currently smoking patients with COPD, suggesting ongoing inflammation in airways and circulation of patients with COPD after smoking cessation.

Keywords: chronic obstructive pulmonary disease; pulmonary inflammation; systemic inflammation; smoking cessation; tumor necrosis factor receptors

Chronic obstructive pulmonary disease (COPD) is characterized by the progressive development of airflow limitation that is not fully reversible (reviewed in Reference 1). The main risk factor for COPD is cigarette smoking, and at present, cessation of smoking is the only intervention with a significant attenuation of the degree of lung function impairment (2–4). There is increasing evidence that COPD is associated with a chronic inflammatory response in both airways and lung parenchyma. Histopathologic studies have shown that patients with COPD have increased numbers of macrophages and CD8+ T cells in the peripheral airways and lung parenchyma (5–8), which may be associated with latent adenoviral infection (9, 10). On the other hand, a marked increase in neutrophils in sputum and bronchoalveolar lavage

fluid was demonstrated in patients with COPD compared with control subjects (11, 12), reflecting neutrophilia in the central airways (13). The extent of neutrophilia correlated with the degree of airflow limitation (12, 14, 15). Analysis of induced sputum revealed that levels of interleukin-8 (IL-8) and tumor necrosis factor- α (TNF- α), which are generally considered to be important mediators in neutrophil recruitment, are elevated in patients with COPD (14, 16, 17). In addition, elevated levels of neutrophil granule proteins were demonstrated in induced sputum and bronchoalveolar lavage fluid from patients with severe COPD and smoking subjects with subclinical emphysema, respectively, indicating activation of recruited neutrophils (18, 19).

TNF- α is a potent proinflammatory cytokine known to exert its activities by interaction with two structurally related, but functionally distinct, transmembrane receptors, referred to as TNF-R55 and TNF-R75 in accordance with their molecular weight (reviewed in Reference 20). Although the two receptors are independently coexpressed on the surface of most cell types, several studies have shown that TNF-R55 is mainly expressed on cells of epithelial origin, whereas TNF-R75 is primarily found on the cell surface of cells of myeloid origin (21, 22). A variety of inflammatory stimuli, including endogenous TNF- α formation (23), is known to induce proteolytic shedding of the extracellular cytokine-binding domains of the TNF receptors. Therefore, soluble TNF (sTNF) receptors are often considered as markers of a proinflammatory state.

Besides the presence of chronic local inflammation in the respiratory organ, there is increasing evidence of systemic inflammation in patients with stable disease as well as during episodes of acute exacerbations. Recent studies demonstrated that levels of sTNF-R55 and sTNF-R75 were significantly increased in the circulation of patients with COPD (24–28). The presence of a systemic inflammatory response has an important influence on the quality of life as weight loss and muscle wasting are linked to systemic inflammation (24, 28–30). The main cause for the presence of systemic inflammation in patients with COPD still remains to be elucidated. Systemic hypoxia is suggested to be a good candidate, as systemic hypoxia is associated with activation of the TNF- α system in patients with COPD (27). However, no information is yet available comparing the local inflammatory involvement in the respiratory organ with the presence of a systemic inflammatory response in COPD. In addition, except for elevation of TNF- α in sputum of patients with COPD, no information is currently available about sTNF receptors in sputum of patients with COPD. Therefore, this study is directed at unraveling the relationship between local and systemic inflammation in COPD. To this end, levels of sTNF receptors, TNF- α , and IL-8 in induced sputum and plasma were compared. Furthermore, persistence of the local and systemic inflammatory response after smoking cessation was

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Correspondence and requests for reprints should be addressed to Juanita H. J. Vernooy, M.Sc., Department of Pulmonology, University Hospital Maastricht, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands. E-mail: j.vernooy@pul.unimaas.nl

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assessed. To this end, subjects were divided according to their current smoking status, and levels of inflammatory mediators in induced sputum and plasma were compared between current smokers and ex-smoking individuals who completely abstained for at least 1 year.

METHODS

Subjects

Eighteen patients with smoking-related, clinically stable COPD followed up by general practitioners participated in the study. All of the patients met American Thoracic Society criteria for the diagnosis of COPD (31). Inclusion criteria for patients with COPD included stable airflow limitation with an FEV₁ of less than 70% predicted with reversibility of less than 11% predicted FEV₁ or less than 200 ml after inhaled β -agonist administration and a previous history of at least 20 pack-years of smoking. Six subjects with COPD were ex-smokers who had quit smoking for at least 1 year before the start of the study. Regular use of inhaled steroids was considered as an exclusion criterion for participation to the study: none of the studied patients with COPD therefore used inhaled steroids. Thirteen patients were prescribed combination therapy (fenoterol/ipratropiumbromide) on a regular basis. Two patients were on long-acting β_2 -agonists, and all other patients were prescribed bronchodilating agents on demand. Furthermore, a history of respiratory diseases other than COPD as well as increased respiratory complaints or respiratory tract infection during 4 weeks preceding the study was considered a criterion for exclusion. A control group of so-called healthy smokers was recruited that consisted of 17 subjects with a normal FEV₁ and no medical history of lung disease. A smoking history of at least 15 pack-years was used as criterion for inclusion. Seven control subjects were ex-smokers. The control subjects lived in the same geographic area as the patient population. The study was approved by the medical ethics committee of the University Hospital of Maastricht. Written informed consent was obtained from all subjects.

Pulmonary Function Test and Blood Sampling

FEV₁ was measured by trained lung function technicians using a spirometer (Masterlab; Jaeger, Würzburg, Germany) before and 15 minutes after inhalation of β -agonist via a metered-dose inhaler. Blood samples were collected in evacuated blood collecting tubes containing ethylenediaminetetraacetic acid (Sherwood Medical, St. Louis, MO) before sputum induction (8:00 to 10:00 A.M.). Plasma samples were stored at -80°C until analyzed.

Sputum Induction and Processing

Sputum was induced after a procedure as reported elsewhere (14). Briefly, subjects inhaled 3% hypertonic saline, nebulized via an ultrasonic nebulizer (NEB2000; TEFA-Portanje, Woerden, The Netherlands) during three 7-minute periods. Before expectoration, subjects were instructed to mouthwash thoroughly with saline solution to minimize saliva contamination. The collected sputum was pooled and kept at 4°C for not more than 2 hours before further processing.

The volume of the pooled sputum (without selection of sputum plugs) was recorded, and an equal volume of 0.2% dithiothreitol (DTT; Sputolysin; Calbiochem, La Jolla, CA) was added. The samples were then mixed gently by a vortex mixer and incubated for 20 minutes at room temperature to ensure complete homogenization. Cell-free supernatants were frozen at -80°C until subsequent analysis. The total cell count and cell viability were assessed using a standard hemocytometer (Coulter Z1; Coulter Electronics, Mijdrecht, The Netherlands) and by trypan blue exclusion, respectively. Cytospins were stained according to the May-Grünwald Giemsa method. Differential cell counts were performed by one observer blinded to the clinical characteristics, counting 500 nucleated cells. The numbers of squamous epithelial cells were subtracted, and the differential cell counts were expressed as corrected percentages. A sample was considered adequate if the slides contained 15% or less squamous epithelial cells.

Analysis of sTNF Receptors, TNF- α , and IL-8 Levels in Sputum and Plasma

sTNF-R55 and sTNF-R75 assays. sTNF-R were measured in sputum supernatant and plasma using specific sandwich enzyme-linked immunosorbent assay (ELISA) as described (32, 33). Rabbit anti-human sTNF-R55 polyclonal antibody and murine anti-human sTNF-R75 monoclonal antibody (MR2-2) were used as coating antibodies. Assays for sTNF-R were not affected by presence of TNF (33), indicating that total amounts of sTNF-R55 and sTNF-R75 (free and bound to TNF- α) were measured. The lower detection limit was 60 pg/ml for sTNF-R55 and 30 pg/ml for sTNF-R75.

TNF- α assays. Levels of TNF- α were measured using (1) a specific sandwich ELISA as described previously (34, 35) that recognizes bioactive TNF- α with a lower detection limit of 15 pg/ml as well as by (2) a commercially available ELISA (HyCult Biotechnology BV, Uden, The Netherlands) that recognizes free TNF- α as well as TNF- α bound to sTNF receptors (referred to as total TNF- α fraction; personal communication HyCult Biotechnology BV) with a lower limit of detection of 20 pg/ml.

IL-8 assay. IL-8 levels were determined using specific sandwich ELISA as described by Bouma and colleagues (36). The lower detection limit was 8 pg/ml.

DTT effect. To study the effect of DTT on ELISA determination of the inflammatory mediators assessed, standard curves of appropriate recombinant proteins were incubated with 0.1% DTT (Sputolysin) under the same conditions as sputum samples during processing (20 minutes at room temperature). The presence of DTT resulted in less than 5% inhibition of the detection of sTNF-R55 and IL-8 by ELISA and less than 10% inhibition in case of sTNF-R75, bioactive TNF- α , and total TNF- α fraction, indicating that DTT has little or no effect on the assays used in this study (data not shown).

Microvascular Leakage

Microvascular leakage was determined in each individual as follows: sputum albumin (mg/L)/serum albumin (g/L). The albumin concentration in sputum and plasma was measured using a newly developed ELISA. Immunomaxisorp plates (Nunc, Roskilde, Denmark) were coated overnight with albumin-specific monoclonal antibody 4D5 (HM17). Diluted plasma samples and a standard dilution series with human albumin (Sigma, St. Louis, MO) were added to the plate. Albumin was detected with a horseradish peroxidase—labeled polyclonal rabbit anti-human albumin immunoglobulin G (Cappel; Organon Teknika Corporation, West Chester, PA) followed by 3',5'-tetramethylbenzidine (Kirkegaard and Perry Lab, Gaithersburg, MD) as substrate. The lower detection limit was 10 ng/ml. The presence of DTT resulted in a less than 5% inhibition of the detection of albumin by ELISA.

Statistical Analysis

Results are presented as mean \pm SD for normally distributed variables and median (range) otherwise. Groups were compared by analysis of covariance adjusted for age, sex, or pack-years where appropriate. Nonparametric data were compared by the Mann-Whitney U test. The chi-square test was used to compare categorical variables. Correlations between parameters were evaluated using Pearson's rank correlation analysis (Statistical Package for the Social Sciences, version 10.0 for Windows; SPSS Inc., Chicago, IL). A p value of less than 0.05 denotes the presence of a significant statistical difference.

RESULTS

Clinical Characteristics

Clinical characteristics of patients with COPD and healthy smokers are shown in Table 1. Patients with COPD had significant airflow limitation, whereas control subjects had normal FEV₁ values (percentage predicted) according to the selection criteria.

Sputum Characteristics

All subjects included in this study tolerated well the procedure of sputum induction, and an adequate specimen of sputum was

TABLE 1. CHARACTERISTICS OF THE SUBJECT GROUPS INVESTIGATED*

	COPD (n = 18)	Healthy Smokers (n = 17)	p Value†
Mean age, yr	60.9 ± 8.1	54.6 ± 6.9	0.017
Sex, M/F	16/2	6/11	0.001
Mean FEV ₁ , % predicted	55.5 ± 14.3	98.7 ± 16.2	< 0.001
Pack-years	47.9 ± 28.0	32.3 ± 13.2	0.045
Current smoker	12	10	NS‡
Ex-smoker	6	7	NS‡

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; NS = not significant; pack-year = smoking one pack per day for 1 year.

* Values are means ± SD.

† Student t test.

‡ Chi-square test.

collected in every participant. Induced sputum was analyzed for total and differential cell counts, as shown in Table 2. The total cell counts and cell viability in sputum were not different in patients with COPD versus healthy smokers. Neutrophils (percentage) were significantly higher in patients with COPD in comparison with control subjects, whereas macrophages (percentage) were significantly lower.

In addition, levels of the neutrophil chemoattractants IL-8 and TNF-α were determined in the sputum supernatant. IL-8 levels were significantly elevated in patients with COPD compared with healthy smokers (Table 3) and were strongly correlated with the number of neutrophils in the COPD group, but not in the control group (Table 4). Concerning TNF-α, both bioactive TNF-α and the total TNF-α fraction (free TNF-α and TNF-α bound to sTNF receptors) were determined (Table 3). Bioactive TNF-α was not detected in sputum samples of either patients with COPD or healthy smokers. Using the assay for the total TNF-α fraction, low concentrations of TNF-α were detected, but total TNF-α levels in sputum were not significantly different between the two groups.

To characterize further the local inflammation in COPD, sputum levels of sTNF-R55 and sTNF-R75 were determined. As shown in Table 3, both sTNF receptors were detectable in induced sputum of patients with COPD and healthy smokers. Sputum sTNF-R55 levels in patients with COPD patients showed a twofold increase as compared with healthy smokers. Sputum sTNF-R75 levels showed a tendency to increase in patients with COPD, but did not reach the level of statistical significance (p = 0.08). As shown in Table 4, sputum levels of sTNF-R55 and sTNF-R75 correlated highly with each other, and with IL-8 levels

TABLE 2. TOTAL AND DIFFERENTIAL CELL COUNTS IN PATIENTS WITH COPD AND HEALTHY SMOKERS*

	COPD (n = 18)	Healthy Smokers (n = 17)	p Value†
Total cell count, 10 ⁶ cells/ml	4.0 ± 3.3	4.0 ± 4.1	NS
Viability, %	90.3 ± 7.8	90.3 ± 10.0	NS
AM, %	17.1 ± 10.9	36.1 ± 15.9	< 0.001
PMN, %	80.5 ± 12.4	62.4 ± 16.4	0.001
Eos, %	1.8 ± 3.6	0.7 ± 1.0	NS
Lym, %	0.7 ± 1.2	0.9 ± 0.8	NS

Definition of abbreviations: AM = alveolar macrophages; COPD = chronic obstructive pulmonary disease; Eos = eosinophils; Lym = lymphocytes; NS = not significant; PMN = neutrophils.

* Data are presented as means ± SD.

† Analysis of variance adjusted for age, sex, and pack-years.

TABLE 3. INFLAMMATORY MEDIATORS IN SPUTUM OF PATIENTS WITH COPD AND HEALTHY SMOKERS*

	COPD (n = 18)	Healthy Smokers (n = 17)	p Value†
IL-8, pg/ml	3.7 (1.4–12.2)	2.3 (0.8–5.2)	0.008
Bioactive TNF-α, pg/ml	< 15‡	< 15‡	NS
Total TNF-α fraction, pg/ml	59 (20–143)	57 (20–206)	NS
sTNF-R55, pg/ml	237 (60–2,501)	109 (60–435)	0.02
sTNF-R75, pg/ml	70 (30–911)	77 (30–256)	NS

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; IL-8 = interleukin-8; NS = not significant; sTNF-R55 = soluble TNF-receptor 55; sTNF-R75 = soluble TNF-receptor 75; TNF-α = tumor necrosis factor-α.

* Data are presented as median (range).

† Mann-Whitney U test.

‡ Below the detection limit.

in both study groups. In contrast, sputum levels of the sTNF-receptors strongly correlated with the percentage of neutrophils in patients with COPD, but this was not the case in healthy smokers. To address discrepancy, study groups were split according to the level of sputum neutrophils using the mean + 1 × SD of sputum neutrophils in healthy controls as cutoff point. As shown in Figure 1, neutrophils (percentage) in sputum of 11 patients with COPD and 2 healthy smokers were out of the range of those found in healthy smokers. Furthermore, there was a large variance in sputum levels of sTNF-R55 and sTNF-R75 in patients with COPD with high neutrophil levels, whereas this was not the case in patients with COPD with low neutrophil levels.

Next, we investigated whether the pulmonary inflammation was related to the degree of airflow limitation. To this end, we looked for correlations between the FEV₁ (percentage predicted) and sputum levels of IL-8, sTNF receptors, and TNF-α. As shown in Figure 2, sputum levels of IL-8 and sTNF-R55 strongly correlated with airflow limitation in patients with COPD but not in healthy smokers. Sputum sTNF-R75 levels tended to correlate with the FEV₁ in patients with COPD, but this did not reach the level of statistical significance (sTNF-R75, r = -0.444, p = 0.08). The total TNF-α fraction in sputum was not related to airflow limitation in patients with COPD (r = -0.258, p = 0.317).

Plasma Levels of sTNF Receptors, TNF-α, and IL-8

Systemic inflammation was assessed by determination of plasma levels of sTNF-R55, sTNF-R75, TNF-α, and IL-8 by specific

TABLE 4. CORRELATIONS BETWEEN SPUTUM MARKERS IN PATIENTS WITH COPD AND HEALTHY SMOKERS*

	COPD (n = 18)		Healthy Smokers (n = 17)	
	R Value	p Value	R Value	p Value
IL-8/neutrophils	0.704	0.001	0.345	NS
sTNF-R55/sTNF-R75	0.973	< 0.001	0.750	0.001
sTNF-R55/neutrophils	0.677	0.002	0.414	NS
sTNF-R75/neutrophils	0.607	0.008	0.362	NS
sTNF-R55/IL-8	0.699	0.001	0.742	< 0.001
sTNF-R75/IL-8	0.562	0.015	0.780	< 0.001
sTNF-R55/total TNF-α	0.177	NS	-0.105	NS
sTNF-R75/total TNF-α	0.171	NS	0.032	NS

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; IL-8 = interleukin-8; NS = not significant; sTNF-R55 = soluble TNF receptor 55; sTNF-R75 = soluble TNF receptor 75; TNF-α = tumor necrosis factor-α.

* Pearson's rank correlation.

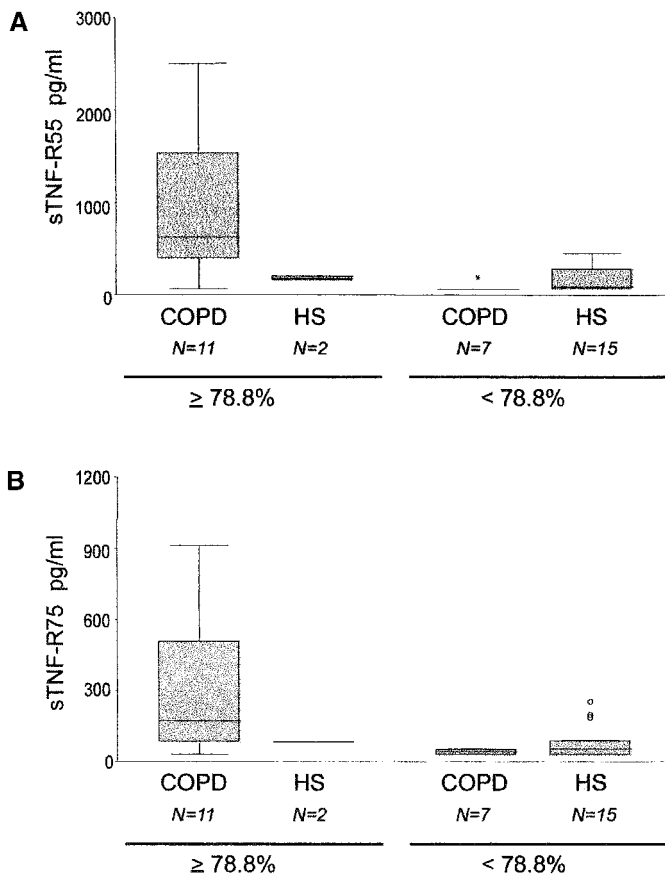


Figure 1. Levels of (A) sTNF-R55 and (B) sTNF-R75 in induced sputum obtained from patients with COPD and healthy smokers (HS). Study groups were split according to the level of sputum neutrophils using the mean + 1 × SD of sputum neutrophils in healthy controls as cut-off point (78.8%). The open circle symbolizes outliers.

ELISA. As shown in Table 5, no significant difference was seen in circulating sTNF-R55 levels of patients with COPD and healthy control subjects. In contrast, plasma levels of sTNF-R75 were significantly increased in patients with COPD. Like in sputum, plasma levels of sTNF-R55 and sTNF-R75 correlated highly with each other in both patients with COPD ($r = 0.797$, $p < 0.001$) and healthy smokers ($r = 0.732$, $p = 0.001$). IL-8 was detectable in 4 out of 18 patients with COPD in comparison with none of the 17 control subjects, resulting in significantly increased levels

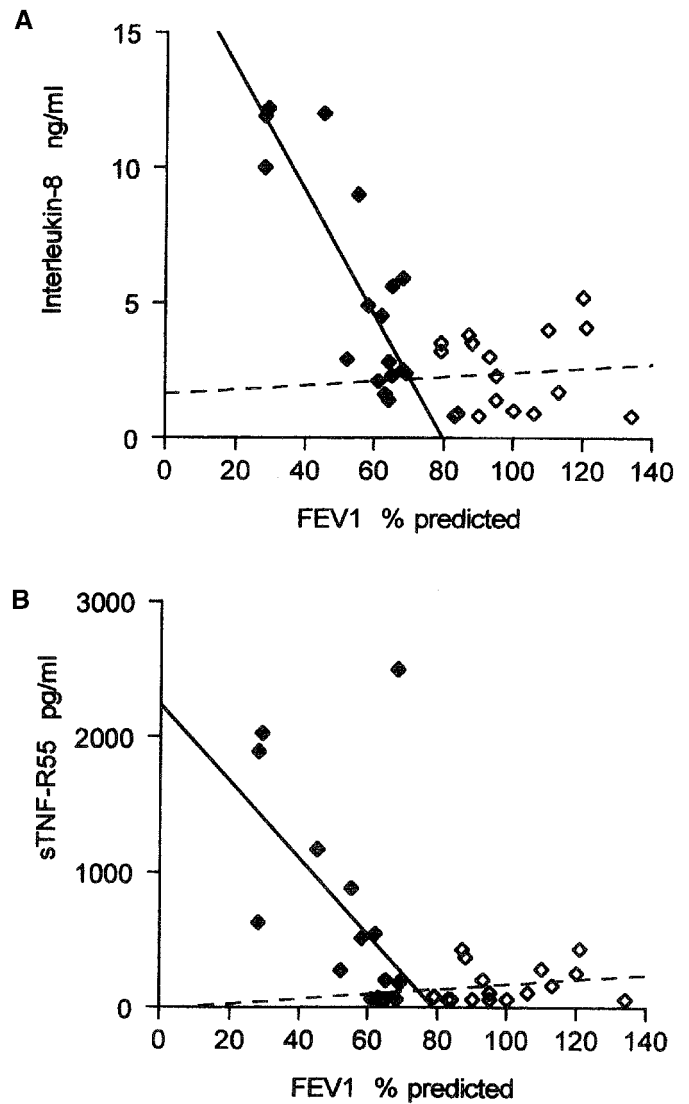


Figure 2. Correlation between the FEV₁ and sputum levels of (A) IL-8 (COPD, $r = -0.836$, $p < 0.001$; control, $r = 0.093$, $p = 0.723$) or (B) sTNF-R55 (COPD, $r = 0.519$, $p = 0.033$; control, $r = 0.213$, $p = 0.411$) in patients with COPD (closed diamond) and healthy smokers (open diamond).

of IL-8 in the COPD group. Circulating TNF- α was measured using the assay for total TNF- α . Plasma TNF- α levels of all individuals except two were below the lower detection limit of the assay.

TABLE 5. INFLAMMATORY MEDIATORS IN PLASMA OF PATIENTS WITH COPD AND HEALTHY SMOKERS*

		COPD (n = 18)		Healthy Smokers (n = 16)	p Value†
sTNF-R55, ng/ml		2.0 (1.4–3.3)		1.7 (1.2–2.6)	NS
sTNF-R75, ng/ml		1.9 (1.3–2.9)		1.4 (1.1–2.7)	0.004
Total TNF- α , pg/ml	n = 17	< 20‡	n = 15	< 20‡	NS
	n = 1	36.6	n = 1	34.9	
IL-8, pg/ml	n = 14	< 8.0‡	n = 16	< 8.0‡	0.049
	n = 4	9.9 (8.4–10.6)			

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; IL-8 = interleukin-8; NS = not significant; sTNF-R55 = soluble TNF receptor 55; sTNF-R75 = soluble TNF receptor 75; TNF- α = tumor necrosis factor- α .

* Data are presented as median (range).

† Mann-Whitney U test.

‡ Below the detection limit.

TABLE 6. CORRELATIONS BETWEEN SPUTUM AND PLASMA LEVELS OF INFLAMMATORY MEDIATORS IN PATIENTS WITH COPD AND HEALTHY SMOKERS*

Sputum to plasma	COPD (n = 18)		Healthy Smokers (n = 16)	
	R Value	p Value	R Value	p Value
sTNF-R55	0.155	0.539	0.359	0.172
sTNF-R75	0.068	0.788	0.377	0.150
IL-8	0.292	0.240	—	—

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; IL-8 = interleukin-8; NS=not significant; sTNF-R55 = soluble TNF receptor 55; sTNF-R75 = soluble TNF receptor 75; TNF = tumor necrosis factor.

* Pearson's rank correlation.

In addition, we investigated whether the systemic inflammation was related to the degree of airflow limitation by analyzing correlations between the FEV₁ (percentage predicted) and plasma levels of IL-8 and sTNF receptors. In contrast to inflammatory mediators in sputum, circulating levels of the sTNF-receptors and IL-8 did not show any correlation with the degree of airflow limitation (data not shown).

Relationship between Local and Systemic Compartment

To investigate the possible relationship between local and systemic inflammation in patients with COPD, we first determined to what extent the data are affected by microvascular leakage. To this end, we measured albumin levels in sputum and plasma of patients with COPD and healthy smokers and calculated the level of microvascular leakage in each individual as follows: sputum albumin (mg/L)/serum albumin (g/L). Sputum-to-plasma ratios of albumin were not significantly different between the two study groups (median [range] COPD, 1.68 [0.41–6.11]; control, 1.31 [0.29–4.00], p > 0.05), indicating a similar degree of microvascular leakage in patients with COPD and healthy smokers. Therefore, the values of the inflammatory mediators in sputum were not corrected for microvascular leakage.

Next, we looked for correlations between sputum and plasma levels of sTNF-R55, sTNF-R75, and IL-8 in patients with COPD and healthy smokers to examine the relationship between local and systemic inflammation. As shown in Table 6, no direct correlations were found between sputum and plasma concentrations of each inflammatory mediator analyzed in either patients with COPD or in healthy smokers.

Local and Systemic Inflammation in Current Smokers Versus Ex-smokers

To investigate the effect of smoking cessation on the local and systemic level of inflammation, subjects in both groups were divided according to their current smoking status, and levels of inflammatory mediators were compared between current and ex-smoking individuals. As shown in Table 7, sputum levels of sTNF-R55, sTNF-R75, and IL-8 were not different between current and ex-smokers in the control group. On the other hand, sputum levels of sTNF-R55, sTNF-R75, and IL-8 were significantly increased in patients with COPD who are ex-smokers compared with currently smoking patients with COPD. In addition, sTNF-R55 and IL-8 levels in sputum from ex-smoking patients with COPD were also significantly raised in comparison with ex-smoking healthy subjects, whereas sTNF-R75 levels showed a tendency to increase in ex-smoking patients with COPD (p = 0.09). In contrast, circulating levels of all inflammatory mediators assessed were not different in current smokers compared with ex-smokers in both study groups.

DISCUSSION

This study focused on the relationship between local and systemic inflammation in patients with mild-to-moderate smoking-related COPD. Analysis of induced sputum showed a significant increase in neutrophils (percentage) and IL-8 levels in patients with COPD versus healthy smokers, which correlated highly with each other and with the degree of airflow limitation. These findings are in line with previous observations in patients with COPD (11, 14, 16, 17). Concerning TNF-α, we found no significant differences in sputum TNF-α levels between mild-to-moderate patients with COPD and healthy smokers using either an ELISA that measures strictly intact TNF-α that is not bound by TNF-receptors or using an ELISA that detects the total TNF-α fraction, that is, free TNF-α and TNF-α bound to sTNF-R. In contrast, Keatings and colleagues reported a rise in TNF-α levels in induced sputum of patients with severe COPD (14). This discrepancy may be due to differences in severity of COPD in the two patient populations studied or to differences between the characteristics of the TNF-α assays used. In addition, study populations may differ in rates of bacterial colonization, which was demonstrated to result in higher TNF-α levels in spontaneously expectorated sputum from *Haemophilus influenzae*-colonized patients with COPD in comparison with noncolonized but otherwise similar patients with COPD (37). Finally, neutrophil

TABLE 7. LEVELS OF SOLUBLE TNF-R55, TNF-R75, AND IL-8 IN CURRENT SMOKERS VERSUS EX-SMOKERS*

	COPD		Controls	
	Ex-smokers (n = 6)	Current smokers (n = 12)	Ex-smokers (n = 7)	Current smokers (n = 10)
Sputum				
sTNF-R55, pg/ml	1,097 (60–2,026) ^{†‡}	389 (60–2,501)	213 (60–435)	139 (60–428)
sTNF-R75, pg/ml	365 (52–782) [†]	138 (30–911)	110 (30–256)	67 (30–190)
IL-8, ng/ml	8.3 (2.3–12.2) ^{†‡}	3.8 (1.4–11.9)	2.9 (1.0–4.1)	2.1 (0.8–5.2)
Plasma				
sTNF-R55, ng/ml	2.0 (1.6–2.4)	2.0 (1.4–3.3)	1.7 (1.2–2.4)	1.9 (1.2–2.6)
sTNF-R75, ng/ml	2.0 (1.3–2.7)	1.9 (1.4–2.9)	1.6 (1.1–2.7)	1.5 (1.1–2.0)
IL-8, pg/ml	n = 3, 9.7 (8.4–10.4)	n = 1, 10.6	ND	ND

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; IL-8 = Interleukin-8; ND = not detectable; sTNF-R55 = soluble TNF receptor 55; sTNF-R75 = soluble TNF receptor 75; TNF = tumor necrosis factor.

* Data are presented as median (range).

[†] p < 0.05 compared with current smoking COPD patients (Mann-Whitney U test).

[‡] p < 0.05 compared with ex-smoking control subjects (Mann-Whitney U test).

elastase that is present in neutrophil microenvironment in induced sputum of patients with COPD is a potential parameter in the degradation of TNF- α into bioinactive TNF- α (38), which may hamper the detection of bioactive TNF- α in sputum.

Soluble receptors for TNF- α are considered to be markers of a proinflammatory state because of their shedding from the cell membrane in response to endogenous TNF- α and other inflammatory mediators. In this study, we showed that in particular sTNF-R55 was increased in induced sputum of patients with mild-to-moderate COPD compared with healthy smokers, which correlated with airflow limitation as well as other inflammatory markers (IL-8 and neutrophilia) in patients with COPD. Especially respiratory epithelial cells are reported to express TNF-R55 (22) and may therefore account significantly for the increased levels of sTNF-R55 in sputum. In addition, *in vitro* studies by Porteu and Nathan demonstrated that activation of neutrophils resulted in shedding of both sTNF-receptors from the cell surface, which suggests that sTNF-R55 and sTNF-R75 measured in sputum may also be derived from activated neutrophils that are extensively present in sputum of patients with COPD (39).

Previous studies from our group as well as other groups investigating systemic inflammation in patients with COPD demonstrated small but significant increases in plasma levels of sTNF-R55 and sTNF-R75 in patients with COPD in addition to enhanced levels of acute phase proteins (24–28). In line with these reports, we demonstrated that circulating sTNF-R75 levels were elevated in patients with COPD as compared with healthy smokers. The biologic significance in the sense of the role that such a difference plays in the biologic phenomenon is not clear from these studies. sTNF receptors are known to compete for TNF- α with the cell surface TNF receptors and may hereby play a role in regulating steady-state levels of TNF- α in the airways and circulation (40). Further studies using large study populations are necessary to elucidate the origin of sTNF receptors and their function—either proinflammatory or antiinflammatory—in chronic inflammation in COPD.

Although it is generally accepted that systemic inflammation contributes to the impaired health status in COPD, little is known about the origin of the systemic inflammation. Therefore, we assessed the possible relationship between the local and systemic inflammatory response. First, results of this study showed that patients with COPD had increased levels of sTNF-R55 levels in sputum as compared with healthy smokers, whereas the reverse situation occurred in plasma: levels of sTNF-R75 were higher in patients with COPD than in control subjects. Second, we found no correlation between the individual inflammatory mediators in sputum compared with plasma in both study groups. Furthermore, sputum levels of sTNF-R55 and sTNF-R75 were related with degree of airflow limitation in the COPD group, whereas plasma levels of sTNF receptors were not. Taken together, these results suggest that systemic inflammation in COPD is not due to an overflow of inflammatory mediators from the local compartment but that the inflammatory responses in the local and systemic compartment are differently regulated. In line with this hypothesis, Takabatake and colleagues recently reported that systemic hypoxia observed in patients with COPD due to deterioration of the lung function might contribute to enhanced levels of systemic inflammatory markers (27). Future studies are clearly required to elucidate the origin and regulation of the systemic inflammatory response in COPD.

Cigarette smoking is widely recognized as a primary risk factor for developing COPD in industrialized countries. The presence of harmful compounds in inhaled cigarette smoke, including endotoxin (41), is considered to be the starting point of the pathogenetic pathway in COPD. Previous studies have shown that smoking cessation resulted in a significant reduction

of the age-related decline in FEV₁ (2–4). However, a longitudinal follow-up study of smoking-induced lung density changes by high-resolution computed tomography demonstrated that the worsening airspace abnormality in the upper lung zones of smokers did not slow down with cessation of smoking (42). In addition, other studies investigating airway inflammation reported that smoking cessation did not influence the cellular profile and levels of IL-8 in central and peripheral airways (16, 43–45). In this study, we found a remarkable increase in sputum levels of sTNF-R55, sTNF-R75, and IL-8 in ex-smoking patients with COPD compared with currently smoking patients with COPD, but not in ex-smoking versus currently smoking healthy controls. In addition, sputum levels of sTNF-R55 and IL-8 in ex-smoking patients with COPD were also significantly raised in comparison with levels in ex-smoking healthy subjects. In contrast, the systemic proinflammatory state was not associated with the smoking status in both study groups. Our results are strongly supported by the recently reported data of Willemse and colleagues (46), who collected induced sputum before and 1 year after smoking cessation in smokers with COPD and without COPD. They showed that IL-8 levels in sputum decreased without changes in sputum neutrophils after 1 year of smoking cessation in smokers without COPD. In contrast, in smokers with COPD, levels of IL-8 and neutrophils in sputum tended to increase ($p < 0.1$). Taken together, these data suggest an ongoing inflammatory response in the respiratory compartment of patients with COPD after smoking cessation.

In summary, patients with COPD had significantly higher percentages of neutrophils and increased levels of sTNF-R55 and IL-8 in their induced sputum samples as compared with healthy smokers. A comparison of sTNF-receptors in sputum and plasma, however, did not demonstrate direct correlations between local and systemic inflammation, despite the significantly higher circulating levels of sTNF-R75 in patients with COPD. Moreover, sputum sTNF receptors were related with the degree of airflow limitation in patients with COPD, whereas circulating sTNF receptors were not, suggesting different regulation of the inflammatory response in the local and systemic compartment. Sputum levels of sTNF-R55, sTNF-R75, and IL-8 were increased in ex-smoking versus currently smoking patients with COPD as well as ex-smoking control subjects, whereas the current smoking status was not associated with the systemic proinflammatory state in the study groups. These results confirm the presence of inflammatory processes in the airways and circulation of patients with COPD despite smoking cessation.

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