

REVIEW

Markers of active airway inflammation and remodelling in chronic obstructive pulmonary disease

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Introduction

Chronic obstructive pulmonary disease (COPD) is defined as a disorder characterized by reduced maximum expiratory flow and slow forced emptying of the lungs, features which do not change markedly over several months [1]. Most of the airflow limitation is slowly progressive and irreversible. COPD is a broad term and includes three clinical conditions: chronic bronchitis, small airways disease and emphysema. The airflow limitation is due to varying combinations of destruction and remodelling of the small airways and the lung parenchyma, which cause an increase in airway resistance mainly in the small airways [2]. Cigarette smoking has been shown to be the most important risk factor and accounts for 80–90% of the risk of developing COPD [3]. Airway hyperresponsiveness, α_1 -antitrypsin deficiency and certain occupational exposures are other established risk factors for the development of COPD [4]. The mechanism via which the presence of these risk factors results in airflow limitation has been investigated intensively in the last decades, but has not been clarified yet.

It is probable that the development of airflow limitation in COPD is mainly caused by inflammatory changes in the airways and lung parenchyma. The introduction of fiberoptic bronchoscopy in the early seventies opened the way to perform airway wall biopsies, thereby facilitating invasive research of the airways and providing tissue samples to study inflammation. It has demonstrated directly, combined with results of analysis of induced sputum, and indirectly via investigation of exhaled air and hyperresponsiveness, the presence of airway inflammation. With regard to the development of airflow limitation, the emphasis until recently has been on tissue destruction caused by mediators of inflammatory cells. The parenchymal destruction as seen in emphysema was suggested to be caused by an inflammatory process due to a protease-antiprotease-imbalance and oxidant-antioxidant-imbalance [5]. The basis for this

theory of parenchymal destruction was the association of emphysema with congenital α_1 -antitrypsin deficiency and the occurrence of emphysema-like pathological changes in hamsters after intratracheal instillation of elastase. The protease-antiprotease-imbalance suggests that proteases with elastin degrading capacity are insufficiently inhibited by antiproteases and thereby induce emphysema. The oxidant-antioxidant-imbalance suggests that oxidants, which can injure lung tissue, interfere with repair of the extracellular matrix and inactivate antiproteases, are insufficiently inhibited by antioxidants and thereby induce emphysema. The theory about oxidant-antioxidant-imbalance was excellently reviewed by Rahman and McNee recently [6]. However, as the activity of proteases and oxidants can be expected to be quite similar in all smokers, the protease-antiprotease-imbalance and the oxidant-antioxidant-imbalance theories do not explain why only 10–20% of all smokers develop emphysema. Moreover, these theories explain the parenchymal destruction of emphysema but do not explain changes in the small airways. Therefore, the concept of airway remodelling caused by abnormal repair of the small airways and parenchyma has become more and more accepted [7].

Airway remodelling

The concept of airway remodelling is that tissue destruction induces a repair process with proliferation and activation of resident airway cells, which produce proteins for the extracellular matrix (ECM), matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs). In a part of the smoking population this remodelling process is insufficient, resulting in airflow limitation. This process is characterized by structural changes, which can be found in the large and small airways and in the parenchyma [8]. In the large airways, structural changes in COPD have been observed in mucous glands and epithelium. Mucous gland hypertrophy and goblet cell hyperplasia occur in chronic bronchitis and contribute to excess mucus production. Epithelial changes include atrophy, squamous metaplasia and loss of ciliated epithelial cells and decrease of cilia

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length and function. There have also been reports of thickening of the epithelial basement membrane [9,10]. In the small airways, important features are peribronchiolar fibrosis, smooth muscle hypertrophy and destruction of alveolar walls which attach to and surround the airways. Other changes are, mural oedema, goblet cell metaplasia, lumen occlusion by mucous plugging and a relative excess of airways < 400 µm in internal diameter. In the parenchyma, there may be an abnormal permanent enlargement of air spaces distal to the terminal bronchioles. Elastin sheets are disrupted and perforated with multiple fenestrations and collagen fibrils are thickened and deposited in a disorganized way [11].

Airway remodelling is a complex process of ECM degradation and synthesis and deposition of new matrix. All inflammatory cells have the potential to degrade tissue and to influence each other via cytokines and to modulate repair as well. Important mediators involved in destruction and repair are elastase released by neutrophils and macrophages, MMPs released by macrophages, eosinophils, lymphocytes, neutrophils, and fibroblasts and cathepsins released by macrophages. These mediators have a synergistic effect on tissue destruction. These proteinases are inhibited by antiproteinases, of which α_1 -antitrypsin, α_2 -macroglobulin, elafin and TIMPs are the most important. The balance between proteinases and their inhibitors is critical in tissue repair and remodelling. The fibroblast is the cell which synthesizes and modulates most of the components of the ECM. The ECM consists of fibrous, elastic and adhesive proteins (elastin, collagen, fibronectin and laminin), which are embedded in a hydrated polysaccharide gel containing several glycosaminoglycans, e.g. hyaluronic acid. Fibroblast proliferation and protein synthesis are stimulated by growth factors, in particular TGF- β , endothelin, EGF, GM-CSF and IGF. Disruption of the ECM leads to tissue instability, but also activates resident cells and causes recruitment of inflammatory cells.

These findings demonstrate the close relationship between the presence of inflammatory cells and their mediators and airway remodelling. Airway inflammation and remodelling can be studied via different approaches. In this review we summarize the findings in induced sputum, bronchoalveolar lavage (BAL) fluid, bronchial biopsies, resected tissue, exhaled air and bronchial hyperresponsiveness.

Induced sputum

The presence of increased amounts of sputum implies some abnormal process of the bronchopulmonary system, the cellular and soluble components reflecting the pathological process. Until recently, mainly spontaneously expectorated sputum has been used in research in COPD. In recent years, induced sputum has been applied more frequently, since it

was shown to be useful in studying the airway inflammation in asthma [12]. Sputum induction was introduced in the late 1950s in a screening programme for the early detection of lung cancer in apparently healthy individuals [13]. Inhalation of a hypertonic saline aerosol allowed these individuals to raise sputum for cytological screening. The advantage of sputum induction in research is that it is a non-invasive and well-tolerated method for the collection of airway-derived fluid with cells and mediators. Moreover, sputum can also be induced in healthy control subjects as well as patients with COPD who cannot produce it spontaneously. It has been shown in subjects with COPD that the cellular composition of induced sputum does not differ from spontaneously produced sputum [14].

Several studies have shown that subjects with COPD have manifestations of airway inflammation in induced sputum. They have higher numbers of inflammatory cells and especially higher percentages of neutrophils and corresponding lower percentages of macrophages in induced sputum compared with healthy controls (Table 1). The increased percentages of neutrophils are not associated with the level of FEV₁ nor with the presence of chronic bronchitis. Several studies reported higher percentages of eosinophils. Recently we studied total cell counts and differentiation of inflammatory cells in induced sputum and lavage fluid in COPD and found that the percentages of inflammatory cells did not correlate between the two compartments (Fig. 1) [15]. Subjects with COPD may also have higher concentrations of the cytokines tumour necrosis factor- α (TNF- α), interleukin-6 (IL-6) and IL-8 [16,17], and the cell activation markers eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), myeloperoxidase (MPO), human neutrophil lipocalin (HNL) [18] and histamine [19] in sputum supernatant compared with healthy controls (Table 2).

Induced sputum is thought to be mainly derived from the large airways and it represents a different compartment than BAL fluid and bronchial biopsies [15]. The predominance of neutrophils in induced sputum is not found in BAL fluid or biopsies. Neutrophils are part of the non-specific immunological defence, which is the first line of defence against invading microorganisms and inhaled noxious agents, such as tobacco smoke. Thus it can be easily explained that smoking, the major pathogenic risk factor of COPD, increases neutrophil numbers. This may be partly due to increased production of IL-8 by airway cells, as shown for epithelial cells after stimulation with tobacco smoke *in vitro* [20]. These luminal neutrophils may secrete elastase, which can lead to mucus secretion [21]. Apart from changes in kinetics, activation of neutrophils in sputum is indicated by upregulation of CD11b and CD18 [22]. It remains to be determined, however, whether sputum neutrophilia indicates a pathogenetic role for these cells in

Table 1. Cell differential counts in induced sputum in subjects with COPD and smoking (HS) and non-smoking healthy controls (HNS)

Ref.	Pat. no.	FEV ₁ (% pred.)	CB (+/?)	Smokers (C/E/N)	Neutro, %			Macro, %			Eosinophil, %		
					COPD	HS	HNS	COPD	HS	HNS	COPD	HS	HNS
[26]	18	28	?	?/?/0	78						3.2		
[16]	14	35	?	?/?/0	70*	36	25	28*	61	74	1.9		0.4
[25]	13	35	?	6/7/0	68			29			2.7		
[14]	27	38	+	8/17/2	89			10			0.3		
[18]	16	42	?	?/?/0	62*		28	35*		72	2.0		0.5
[103]	10	47	?	?/?/0	78*	33	36	19*	61	57	0.7*	0.2	0.2
[104]	18	48	+	6/?/?	48*		9	50*		89	0.8		0.6
[105]	21	54	?	0/21/0	75*		23	21*		74	2.4		
[106]	10	57	?	0/10/0	58		46	39		49	1.5		0.7
[15]	18	59	?	0/14/4	77*		36	19*		57	1.2*		0.2
[24]	34	60	?	34/0/0	72			21			2.7		
[107]	7	62	+	0/6/1	79*		35	15*		56	1.8		0
[84]	16	63	?	8/8/0	78/72*	59	50	17/20*	37	45	1.2/1.3	0.7	0.4

*Significantly different between COPD and controls. CB: chronic bronchitis. C/E/N: current smoker/ex-smoker/never smoker.

the progressive airflow limitation of COPD, or whether this neutrophilia is solely a non-specific indication of airway disease. The importance of sputum eosinophilia is unknown yet. These eosinophils are probably not activated, but may have been recruited non-specifically into the airways as part of ongoing inflammation of the airways. This may occur via adhesion molecules that play a role in leucocyte recruitment, e.g. VCAM-1 or ICAM-1, which have been shown to be upregulated in COPD [23].

Airway inflammation is a dynamic process and numbers of inflammatory cells and concentrations of their mediators may change with treatment and exacerbations. One study

reported a reduction of the percentage of sputum neutrophils after 2 months treatment with inhaled beclomethasone 1500 µg/day [24]. Another study, however, failed to show any decrease in inflammatory cell markers after a 2-week treatment with inhaled budesonide 1600 µg/day or oral prednisolone 30 mg/day in a small group of patients [25]. These difference may be attributable to the differences in duration of treatment and the study population. The latter study group was smaller ($n = 8$) and contained patients with more severe COPD who had lower percentages of sputum eosinophils than the individuals in the first study. Finally, Pizzichini *et al.* found that the presence of sputum

Table 2. Mediator concentrations in induced sputum in subjects with COPD and smoking (HS) and non-smoking healthy controls (HNS)

Ref.	Pat. no.	FEV ₁ (% pred.)	CB (+/?)	Smokers (C/E/N)	ECP, µg/mL			MPO, ng/mL		IL-8, ng/mL		
					COPD	HS	HNS	COPD	HNS	COPD	HS	HNS
[26]	18	28	?	?/?/0	3800			2.6				
[16]	14	35	?	?/?/0						9.2*	4.0	1.6
[108]	9		?	7/2/0						16.3		
[25]	13	35	?	6/7/0	836					26.4		
[18]	16	42	?	?/?/0	936*		36	8.1	0.2			
[109]	33	45	?	11/22/0	610*		70	9.1*	42.6	21.0*		3.3
[103]	10	47	?	0/?/0	711*		82	83.9				
[30]	13	50	?	9/4/0						10.9		
[106]	10	57	?	0/10/0	1687					23.4		17.6
[15]	18	59	?	0/14/4	750*		138			13.4*		4.6
[84]	16	63	?	8/8/0	97/273*	29	47			11/19.8*	3.6	5.7

*Significantly different between COPD and controls. CB: chronic bronchitis. C/E/N: current smoker/ex-smoker/never smoker.

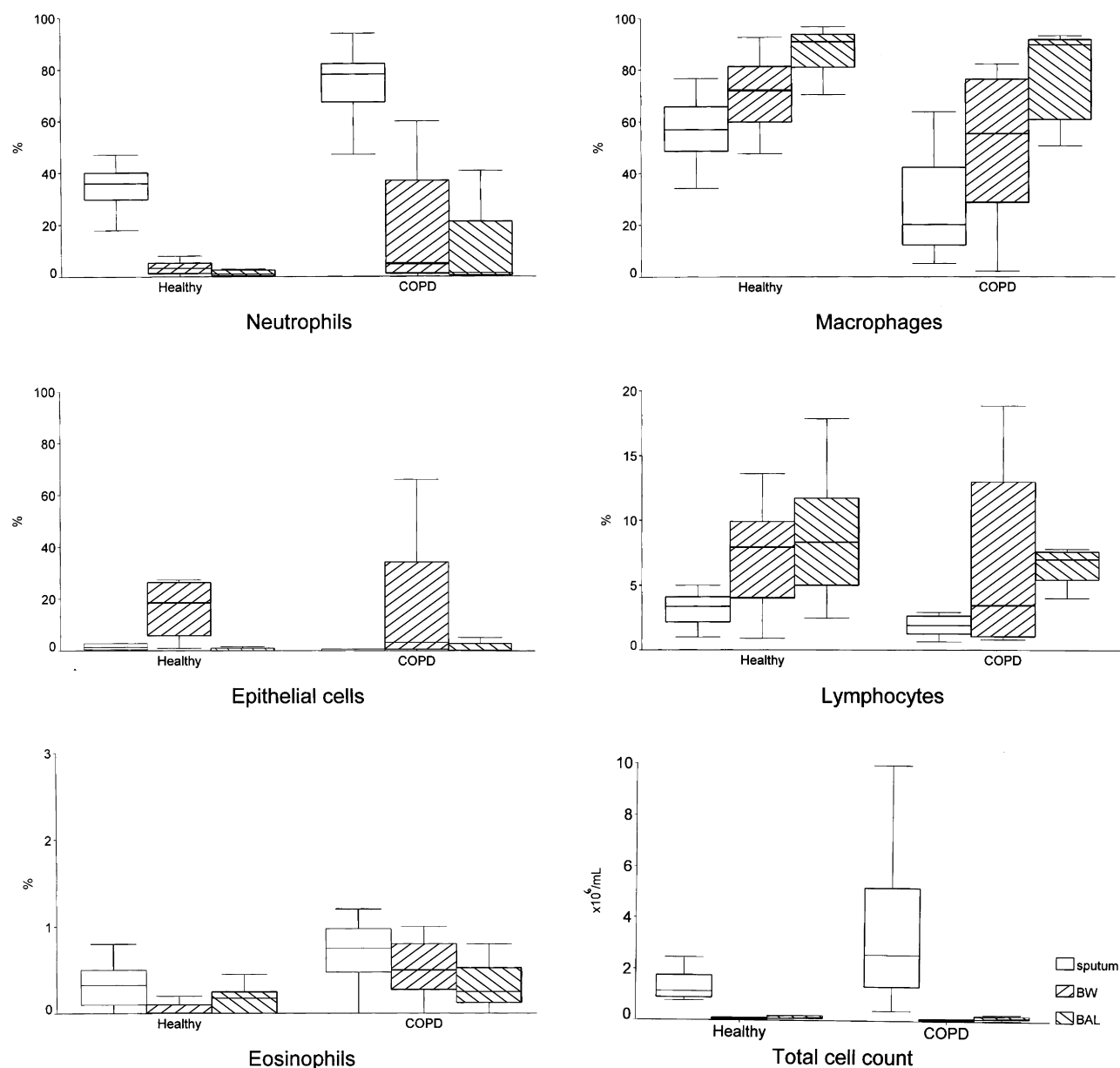


Fig. 1. Box plot with median values, interquartile range and 95% confidence intervals of total cell count and differential cell count in sputum, bronchial wash (BW) and bronchoalveolar lavage (BAL) fluid in subjects with COPD and healthy subjects.

eosinophilia was a predictor of response to corticosteroids [26]. Thus, sputum eosinophilia could be a marker of airway inflammation that is sensitive to corticosteroids.

During an exacerbation, the percentage of sputum eosinophils has been shown to be higher than in subjects without symptoms of an exacerbation [27]. Sputum IL-6 increases during exacerbations and is correlated to the total cell count and eosinophil and lymphocyte numbers [17]. IL-6 is mainly produced by bronchial epithelial cells and airway macrophages and its increased concentrations

during exacerbations probably reflect the high activation state of epithelial cells during exacerbations.

The first studies investigating markers of remodelling in sputum in COPD have been performed with spontaneous sputum. The predominance of neutrophils in sputum made investigators focus on neutrophil mediators initially. Neutrophil elastase was found to be present in a complex and inactivated form, bound to α_1 -antitrypsin [28]. The concentration of neutrophil elastase was higher in purulent sputum during exacerbations, when free enzyme activity was

measurable. Concentrations of MMPs and their inhibitors TIMPs were higher in subjects with COPD than in healthy subjects [29]. The molar ratio of MMP-9 and TIMP-1, however, was lower in subjects with chronic bronchitis than in healthy subjects and correlated positively with FEV₁.

This relative increase of TIMP-1 concentrations may lead to an increased deposition of ECM components, thereby leading to bronchiolar fibrosis and airflow limitation. A 4-week treatment with inhaled fluticasone 1000 µg/day did not alter the concentrations of MMPs, TIMPs and the secretory leukoprotease inhibitor nor the elastase activity [30], whereas in another study 8 weeks of treatment with fluticasone 1500 µg/day resulted in a doubling of the elastase inhibitory potential in spontaneous sputum of bronchitics with more severe airflow limitation [31]. The findings of these studies can be explained by the occurrence of an antiproteolytic effect of inhaled steroids only after prolonged treatment, in high doses and in severe COPD, or by the differences in laboratory techniques used. Concentrations of endothelin (ET)-1, a vasoactive peptide with bronchoconstrictive and mitogenic properties, are higher in subjects with COPD than in healthy controls [32]. ET-1 is a comitogen with a number of other growth factors, and may contribute to increased collagen deposition and smooth muscle hypertrophy.

In conclusion, the non-invasiveness, easy performance and applicability in both subjects with COPD and healthy controls makes sputum induction a valuable tool for investigation of airway inflammation and remodelling. It may be useful for studying the priming and activity of airway neutrophils and eosinophils and quantification of soluble mediators and degradation products involved in airway remodelling in the large airways.

BAL

BAL yields fluid from yet another compartment than induced sputum [15]. The difference in technique of fluid sampling contributes to the differences between lavage fluid and induced sputum. Sputum can be regarded as a depository for cells and mediators with concentrations that do not change markedly over time [33]. Lavage fluid can be regarded as a quick shower that gets whatever might be passing by, which may be dependent on the site lavaged and the amount of fluid used. The first fraction of BAL, the bronchial wash, samples fluid from the bronchiolar airways, whereas the subsequent fractions sample fluid from the distal airways and the alveolar compartment. Analysis of lavage fluid can give information about the number, differentiation and activity of inflammatory cells [34–39], the concentration of soluble mediators of inflammation [34–37,39,40], the concentration of cytokines and soluble adhesion molecules [39–41] and markers of airway remodelling [42–46].

The total cell count and differentiation of inflammatory cells in COPD are shown in Fig. 1 [15]. The predominant cell type is the macrophage (Table 3). Several studies have reported higher numbers of neutrophils in smokers with COPD than in healthy subjects [34–37,39], whereas others did not report elevated numbers in subjects with COPD who do not currently smoke [15,38]. An interesting finding is the increased numbers of eosinophils in subjects with COPD [15,35,37]. The importance of BAL fluid eosinophilia is yet unknown. The eosinophilia, as in sputum, is probably the effect of non-specific recruitment, as has been discussed above. Allergy does not play a role in this eosinophilia in COPD, since atopic subjects were excluded from the studies.

The activation state of inflammatory cells in the peripheral airways can be assessed via measurement of soluble mediators. Increased concentrations of ECP [36,40] and MPO [36,37,39,40] have been reported in smokers with COPD (Table 4), which suggests increased activity of eosinophils and neutrophils in the lumen of the peripheral airways. Although higher numbers of BAL fluid neutrophils have been associated with lower FEV₁ values [34–36], it remains to be established whether these luminal cells have a direct role in tissue damage. Concentrations of glutathione have also been shown to be increased in smokers with COPD [36], suggesting an increased defence against oxidative stress in these patients.

Factors that mediate chemotaxis of inflammatory cells have been shown to be increased in BAL fluid of smokers with COPD. Concentrations of the chemoattractant cytokine IL-8 (Table 4) and LTB₄ were significantly higher than in non-smoking healthy subjects and were significantly higher with increasing neutrophil counts [39]. Concentrations of the circulating ICAM-1 were higher than in non-smoking healthy subjects [41]. An increase of chemotactic factors may explain the higher number of BAL fluid neutrophils and eosinophils that were found in patients with COPD in these and other studies [34–37,39].

Several findings in BAL fluid suggest increased tissue injury and repair. Increased concentrations of MMP-1, MMP-8, MMP-9, HNL and neutrophil elastase α_1 -antitrypsin complexes have been found in subjects with emphysema [43,45]. Approximately 50% of the elastolytic activity originates from the MMPs. It has not been established yet whether the neutrophils or the macrophages account for most of the elastolytic activity [44,45]. Increased concentrations of hyaluronan and fibronectin [40,42], as well as elastin-derived peptides, have been found [46,47]. The latter correlated significantly with elastin breakdown [43,46].

Thus, BAL fluid may be useful for studying the priming and activity of neutrophils, eosinophils, soluble mediators and degradation products of inflammation and airway

Table 3. Cell differential counts in BAL fluid in subjects with COPD and smoking (HS) and non-smoking healthy controls (HNS)

Ref.	Pat. no.	FEV ₁ (% pred.)	CB (+sol;?)	Smokers (C/E/N)	Neutro, %			Macro, %			Eosinophil, %			Lymphocytes, %		
					COPD	HS	HNS	COPD	HS	HNS	COPD	HS	HNS	COPD	HS	HNS
[34]	21	43	±	11/10/0	22*			77						9		
	23	75	?	13/10/0	9			84						3		
[10]	13	45	+	?/?/0	5.5*		0.5				1		0			
	12	56	+	?/?/0	3*		0.5				3*		0			
[35]	28	51	+	28/0/0	15*	6*	3	77	89	80	1.2*	0.7	0.4	2.6*	2.3*	13.1
[37]	17	51	+	?/?/0	34*	5*	0.5	55*	88	91	1.0	1.1	0.1	3.5	4.8	8.3
[36]	11	53	+	11/0/0	1.4*	1.6*	0.9	96*	87	86	0.2	0.6*	0.2	1.3	1.8	7.2
[15]	18	59	?	0/14/4	1.7		1.1	86		92	0.4*		0.2	6.9		2.1
[38]	22/25	63	—	22/25/0	3/1	2	36/2	84/94	95	83	0/0	0	0	14/2	3	3
[39]	36	65	+	36/0/0	3.5		2				1.3			6		
[55]	25	73	+	4/21/0	3.3		0.5	83		92	0.6		0	12.8		6.9
[110]	24	77	+	6/12/6	3*		0.1	83*		94	0.6*		0.1	13*		6
[111]	12	80	+	?/?/0	16			77			1.3			6		

*Significantly different from non-smoking healthy controls. CB: chronic bronchitis. C/E/N: current smoker/ex-smoker/never smoker.

remodelling in COPD. The relationship between these findings and those in airway wall and parenchyma, however, has not yet been established.

Biopsies

Airway wall inflammation has been studied in the large airway by means of bronchial biopsies and in small airways and lung parenchyma by means of resected tissue from subjects who had an operation for a peripheral tumour or from post-mortem tissue. For many years study of the airway wall in COPD focused on the small airways, which had been shown to be the main site of airflow limitation [2]. The large airways, which are the main site of excess mucus production [48], have received renewed attention in recent years since the introduction of fiberoptic bronchoscopy.

Several biopsy studies in volunteers have been performed and have shown both inflammatory and structural changes in large and small airways and in the lung parenchyma [23,37,49–52].

Large airways

Inflammatory changes have been observed in the epithelium and in the mucosa of smokers with COPD. The epithelium of large airways has been shown to be infiltrated with increased numbers of neutrophils and CD8+ T cells [53,54]. Increased numbers of epithelial mast cells have been reported in a group of obstructed and non-obstructed smokers with chronic bronchitis [55]. The mucosa has been shown to be infiltrated with increased numbers of macrophages and activated lymphocytes [50,51]. A higher

Table 4. Mediator concentrations in BAL fluid in subjects with COPD and smoking (HS) and non-smoking healthy controls (HNS)

Ref.	Pat. no.	FEV ₁ (% pred.)	CB (+/?)	Smokers (C/E/N)	ECP, ng/mL			MPO, ng/mL			IL-8, ng/mL		
					COPD	HS	HNS	COPD	HS	HNS	COPD	HS	HNS
[10]	13	45	+	?/?/0	0		0				1		0
	12	56	+	?/?/0	5.2*		0				3*		0
[37]	17	51	+	?/?/0	0–11.3	0–2.2	0	0–174*	0–28*	0–10			
[36]	11	53	+	11/0/0	5*	5*	0.5	18*	16*	4			
[15]	18	59	?	0/14/4	0–62		0				40		8
[40]	19	63	+	19/0/0	146*	60	12	1263*	1028	125	10.7	5.6	1.2
[39]	36	65	+	36/0/0	1		1	19*		1	7*		1

*Significantly different from non-smoking healthy controls. CB: chronic bronchitis. C/E/N: current smoker/ex-smoker/never smoker.

number of lymphocytes was associated with a lower FEV₁ value in smokers [50,51] and specific immunostaining revealed that these lymphocytes consisted of CD8+ T cells [51]. An increase in CD8+ cells was not observed in subjects with COPD who did not currently smoke [56], which might indicate that smoking itself influences the differentiation of inflammatory cells in the large airways. Other cell types have inconsistently found to be increased and their role in COPD is less clear. Increased numbers of eosinophils have been found deep in the submucosa, but these cells were not degranulated, indicating that probably they were not activated [37]. Their numbers may increase dramatically during exacerbations [27]. Increased numbers of neutrophils have been reported in the mucosa of smokers with severe airflow limitation [57] and in bronchial glands of smokers with moderate airflow limitation [54]. Mast cell numbers were increased in a group of obstructed and non-obstructed smokers with chronic bronchitis [55]. Inflammatory changes in COPD have also been shown to include increased numbers of epithelial and vascular adhesion molecules [23,52] and increased epithelial expression of MIP-1 α [57].

The increase in TGF- β expression and synthesis in epithelial cells and in the submucosa of subjects with chronic bronchitis suggests active remodelling [9]. The expression of TGF- β correlated significantly with the number of fibroblasts and the basement membrane thickness. No such correlation was found for EGF or GM-CSF. TGF- β may act as both a chemoattractant inducing recruitment of macrophages, lymphocytes, mast cells and eosinophils, as well as a growth factor stimulating fibroblasts to synthesize ECM molecules.

Small airways

The major inflammatory changes in smokers with COPD are increased numbers of CD8+ T lymphocytes in the airway wall [58] and increased numbers of B-lymphocytes in the adventitia [59]. The CD8+ T lymphocytes also correlated with the degree of airflow limitation [58]. Other studies have reported increased numbers of epithelial mast cells [60,61] and macrophages [61].

Analogous to the large airways, increased TGF- β mRNA expression and protein synthesis was also found in epithelial and alveolar cells [62]. The mRNA expression and protein synthesis correlated significantly with the number of macrophages and mRNA expression with the number of mast cells in the bronchiolar epithelium. Moreover, they also correlated significantly with the FEV₁ in the combined group of subjects with COPD and healthy controls.

Parenchyma

The group of Saetta also found increased numbers of

CD8+ T cells in the lung parenchyma of smokers with COPD [63]. It is tempting to think that this cell type, with its potential for tissue injury via granzymes and perforin, contributes to airway and parenchymal destruction. Other studies have reported increased numbers of macrophages as well [64].

The increased staining of MMP-2 and MT1-MMP and of mRNA of MMP-2 and MMP-9 in tissue of subjects with emphysema suggests an increased tissue destruction in emphysema [65]. The staining for MMP-2 and MMP-9 correlated with elastolytic activity.

Hyperresponsiveness

Bronchial hyperresponsiveness is defined as an abnormal sensitivity and exaggerated response of the airways to pharmacological, chemical, physical or physiological stimuli [66]. Airway luminal diameter, smooth muscle mass, elastic recoil, airway inflammation, epithelial injury and neural activity may all play a role in hyperresponsiveness in COPD [67]. Increase in smooth muscle mass contributes more to hyperresponsiveness than adventitial or submucosal thickening [68].

There are only a few studies on the relationship between hyperresponsiveness and airway inflammation in COPD. One study reported a negative correlation between PC₂₀histamine or methacholine and membranous bronchiolar inflammation in cigarette smokers, i.e. more severe hyperresponsiveness was associated with more extensive inflammation [69]. In another study smokers with centrilobular emphysema (CLE) were compared with smokers with panlobular emphysema and non-smoking controls. The wall area internal to the layer of smooth muscle of the small airways was found to be increased [70]. This inner thickness correlated negatively with PC₂₀methacholine in the patients with CLE. The number of T lymphocytes also correlated negatively with PC₂₀methacholine in these patients.

Further support for a role of airway inflammation in hyperresponsiveness comes from the finding that smokers with COPD are more hyperresponsive to adenosine 5'-monophosphate (AMP) than ex-smokers with COPD, even though both groups had comparable severity in hyperresponsiveness to methacholine [71]. AMP is a stimulus that acts indirectly on the smooth muscle cell, probably via activation of mast cells or neural pathways. It may be that hyperresponsiveness to an indirectly acting stimulus is more indicative for airway inflammation than directly acting stimuli, e.g. methacholine, as has been suggested in asthma [72].

We performed several studies to unravel the mechanism of bronchoconstriction of AMP in COPD [73–75]. Mast cells but not cholinergic nerves seem to be involved in

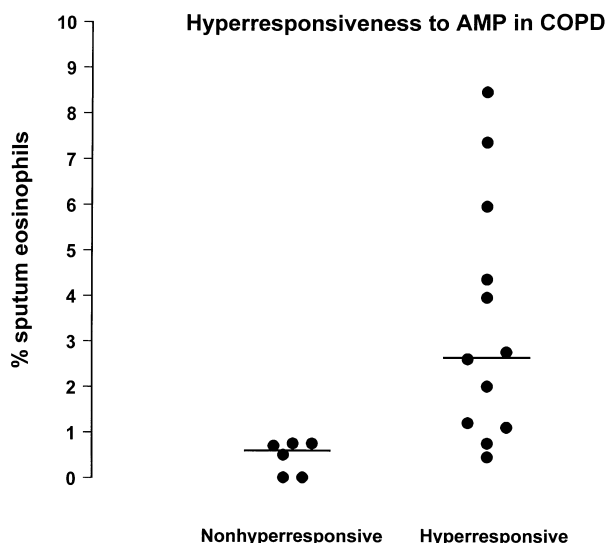


Fig. 2. Percentage of sputum eosinophils in subjects with COPD with or without hyperresponsiveness to adenosine 5'-monophosphate (AMP). Horizontal lines represent median values.

AMP-induced bronchoconstriction in smokers with COPD, since terfenadine protected AMP hyperresponsiveness by 0.9 dose concentration, whereas ipratropium bromide failed to do so [73]. Hyperresponsiveness to AMP was related to increased percentages of eosinophils in induced sputum and increased numbers of mucosal CD8+ cells in bronchial biopsies of subjects with COPD who did not currently smoke (Fig. 2) [74]. The association between hyperresponsiveness and these inflammatory cells is probably not a causative one. It is more likely that the hyperresponsiveness and the increased cell numbers and percentages are a manifestation of an ongoing inflammatory process in the airways. The inhibitory effect of the antihistaminic terfenadine on AMP hyperresponsiveness, the increased numbers of mast cells in the epithelium of central and peripheral airways and the potential of mast cell proteases to injure tissue and mast cell cytokines to influence fibroblast activation, suggests an important role for mast cells in hyperresponsiveness to AMP in COPD.

Hyperresponsiveness to AMP did not improve after a 6-week treatment with budesonide 1600 µg/day in smokers with COPD [75]. This implies that the inflammatory component of hyperresponsiveness in these patients is either small or is insensitive to, or needs prolonged treatment with, inhaled steroids in order to improve.

Exhaled air

Nitric oxide (NO) was measured for the first time in exhaled air in humans in 1991 [76]. Thereafter, exhaled NO

has excited considerable interest, since increased concentrations have been measured in subjects with asthma [77], bronchiectasis and upper respiratory tract infections. The current view is that exhaled NO is a marker of airway inflammation in these diseases and it has been proposed as a new lung function test [77]. This has prompted researchers to investigate whether NO in exhaled air is also a marker of airway inflammation in COPD. So far, it has not been elucidated which part of the inflammatory process is reflected in the level of exhaled NO.

NO is formed via conversion of the amino acid L-arginine into L-citrulline by the enzyme NO synthase (NOS) [78]. There are two constitutive NOS, the neuronal (nNOS) and the endothelial (eNOS), and one inducible NOS (iNOS). The constitutive NOS are involved in physiological regulation of airway functions [79], whereas iNOS is involved in inflammatory diseases of the airways and in host defence against infection [79]. iNOS has been identified in epithelial cells, macrophages, fibroblasts, neutrophils and vascular smooth muscle cells and is induced by inflammatory cytokines and endotoxin. iNOS has the capacity to produce much larger amounts of NO than cNOS.

NO is important in airway physiology, e.g. via mediation of cholinergic bronchoconstriction, regulation of ciliary beat frequency and vasodilation and lysis of microorganisms [79], and may be necessary for endogenous defence mechanisms. However, high concentrations of NO can have toxic effects by causing airway oedema, increased mucus secretion, ventilation-perfusion mismatch and amplification of eosinophilic inflammation [79]. Moreover, NO can combine with superoxide to form the highly reactive peroxynitrite, which damages tissues via oxidation of lipids and proteins [79].

NO is generally measured in exhaled air by chemiluminescence. Different techniques have been used before recommendations for uniform measurements were made by the ERS and the ATS [80,81]. The differences in measurement technique and study population have contributed to the partial contradictions between several studies and makes interpretation of the results difficult. The concentrations of exhaled NO of subjects with COPD seem to be in the same range as those of healthy subjects [82–84], yet they are lower in subjects with lower FEV₁ [85–87]. Within the group of COPD, smokers have lower concentrations than ex-smokers [85], although similar concentrations have also been reported [84]. The concentrations are elevated during an exacerbation and only return to values in the range of those observed in healthy subjects after several months despite rapid clinical improvement [88].

The source of exhaled NO in COPD is uncertain. The central and peripheral airways seem to contribute equally to the exhaled concentrations during exacerbations [88],

which may point to the epithelial cell as the producing cell. However, only few epithelial cells stained positively for iNOS in bronchial biopsies of patients with COPD and these values did not correlate with exhaled NO concentrations (Rutgers, unpublished data). Macrophages may contribute to exhaled NO concentrations. However, we did not find increased expression of iNOS in sputum macrophages [84] or in CD68+ cells in bronchial biopsies of subjects with COPD (Rutgers, unpublished data).

Although we found a significant correlation between the percentage of sputum eosinophils and NO excretion rate [84], it is unlikely that the eosinophils themselves produce the NO measured in the exhaled air, because we did not see any iNOS staining in eosinophils in sputum [84] nor in bronchial biopsies (Rutgers, unpublished data). It is more probable that the increases in eosinophil numbers in sputum and biopsies [27] and exhaled NO [85,88] during exacerbations are all caused by other inflammatory changes, e.g. epithelial stimulation. Thus, exhaled NO seems to be related to inflammatory changes in the airways in COPD. Its loose relationship with signs and symptoms, however, makes it unsuitable for monitoring of airway inflammation in COPD. The exact role of NO in airway inflammation in COPD needs to be studied further.

Several markers of oxidative stress in the exhaled air are increased in COPD. The condensate of breath contains increased hydrogen peroxide [89,90], probably released by alveolar macrophages, and 8-isoprostane-F, a by product of arachidonic acid [91]. The clinical significance of these parameters remains to be determined.

Blood

Several markers of oxidative stress are present in the plasma of subjects with COPD and have been found to be higher than in healthy subjects [6]. Peripheral blood neutrophils of subjects with COPD have significantly higher superoxide anion production than healthy controls, both after stimulation with phorbol myristate acetate and calcium ionophore A23187 [92] and spontaneously during acute exacerbations [93]. The superoxide anion production decreased after 8 days of treatment with oral prednisolone 40 mg/day [92]. Moreover, plasma levels of antioxidants have been found to be decreased in subjects with COPD. Plasma levels of lipid peroxidation products, measured as thiobarbituric acid-malondialdehyde (TBA-MDA), and of protein degradation, as measured by thiols, were higher in subjects with COPD than in healthy subjects, and were highest during an exacerbation [93].

Elevated levels of elastin-derived peptides (EDP) have been found in plasma in subjects with COPD [47,94–96]. It is not clear, however, whether these peptides reflect elastin turnover in the lung or in other compartments of the body.

It may well be that these elevated EDP levels reflect increased elastin turnover in the lung, as higher EDP levels were significantly correlated with CT scores of severe COPD and with loss of elastic recoil [96].

Urine

There are several findings in urine, which suggest an increased degradation of lung elastin in COPD. Several studies have reported increased EDP in urine of subjects with COPD [47,94]. Additionally, elevated levels of desmosine, a specific marker for degradation of mature crosslinked elastin, have been found in smokers with a rapid decline of lung function [97] and in COPD [98,99], although this was not the case in subjects with α 1-antitrypsin deficiency [100]. Recently it was found that the composition of heparan sulphate, a glycosaminoglycan contributing to the stability of the ECM, was altered in its FM403 epitope [101]. The finding of increased urinary levels of isoprostane F2 α -III, an product of peroxidation of arachidonic acid, may indicate increased oxidative stress in COPD [102].

Conclusion

Markers of inflammation and remodelling can be found in the different compartments of the lung and reveal a dynamic process. Inflammatory cell types may accumulate in a preferential compartment and have an activation state which is different from other compartments. High cell numbers may be due to increased recruitment to that compartment or to prolonged survival, possibly due to delayed apoptosis. There is not one single cell type that causes the tissue destruction and remodelling subsequently leading to airflow limitation. Inflammatory cells have a complex interrelationship and influence each other via a vast range of mediators and cytokines. As the disease process progresses, the role of the inflammatory cells may change. The advent of less invasive techniques like sputum induction and analysis of exhaled air helps to elucidate several aspects of inflammatory processes. However, study of material obtained via bronchoscopy or tissue resection remains necessary for understanding the processes leading to airflow limitation.

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