

## ***In vitro* release of neutrophil elastase, myeloperoxidase and $\beta$ -glucuronidase in patients with emphysema and healthy subjects**

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*In vitro* release of neutrophil elastase, myeloperoxidase and  $\beta$ -glucuronidase in patients with emphysema and healthy subjects. T.E.J. Renkema, D.S. Postma, J.A. Noordhoek, H.J. Sluiter, H.F. Kauffman.

**ABSTRACT:** Evidence is accumulating that cigarette smoking plays an important role in the protease-antiprotease imbalance in  $\alpha_1$ -antitrypsin-sufficient emphysema. Since most smokers, however, do not develop emphysema, it has to be presumed that other factors in addition to smoking contribute to the origin of the imbalance.

The major source of proteases is the polymorphonuclear leucocyte (PMN). We tested the hypothesis that an abnormality in the releasability of PMN might predispose for the development of emphysema. Therefore, the release of elastase, myeloperoxidase, and  $\beta$ -glucuronidase from PMN was investigated in patients with emphysema and healthy controls, matched for sex, age, and smoking habits.

PMN were isolated from peripheral blood and stimulated with calcium-ionophore A23187, formyl-methionyl-leucyl-phenylalanine (FMLP), and serum-treated zymosan (STZ). Total enzyme content of PMN was measured after cell lysis with Triton X-100.

Total elastase, myeloperoxidase, and  $\beta$ -glucuronidase content of PMN were not significantly different in healthy subjects and patients with emphysema. *In vitro* release of elastase and myeloperoxidase from both stimulated and unstimulated PMN was not significantly different in healthy subjects and emphysematous patients. Moreover, no differences were found between smoking and ex-smoking individuals. Beta-glucuronidase release tended to be lower in patients with emphysema than in healthy controls.

We conclude that an abnormality in the releasability of peripheral PMN is unlikely to be a pathogenetic factor in emphysema.

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Pulmonary emphysema is characterized by enlargement of alveolar airspaces accompanied by destruction of their walls [1]. The disease is associated with changes in all components of connective tissue, including elastin [2].

It is generally accepted that emphysema results from an imbalance between protease and antiprotease activity in the lung; the so-called "protease-antiprotease imbalance" hypothesis [3]. In addition, evidence is accumulating that emphysema is related to smoking [4], although only one smoker in six develops disabling airflow obstruction [5].

Only proteases with the capacity to degrade elastin *in vitro* (elastases) can produce pulmonary emphysema *in vivo* [5]. For this reason attention has been focused on the neutrophil or polymorphonuclear leucocyte (PMN), as this cell type represents the major endogenous source of elastase in human lungs. Human

neutrophil elastase (HNE) is intracellularly stored in lysosomes, and it can be released extracellularly after activation of these cells or after cell death [6]. HNE is capable of cleaving elastin [7]; moreover, it can destroy all the proteins that provide the structural backbone of the alveolar walls [8, 9]. Other PMN lysosomal enzymes may also contribute to the development of emphysema. Beta-glucuronidase is capable of breaking down proteoglycans of the connective tissue ground substance [10]. Myeloperoxidase is known to diminish the activity of antiprotease in lungs [11], due to formation of toxic oxygen metabolites [12].

The observation that smokers have more PMN in peripheral blood [13] and in bronchoalveolar lavage fluid [14] than nonsmokers suggests an increased proteolytic burden in smokers' lungs. Moreover, tobacco smoke has been shown to oxidize and thereby inactivate  $\alpha_1$ -antitrypsin ( $\alpha_1$ -AT) [15] and antileucoprotease

(ALP) [16], the major antiproteases of the lower respiratory tract. In addition, tobacco smoke inhalation may indirectly lead to oxidative inactivation of antiprotease through stimulation of the release of reactive oxidants from pulmonary PMN and alveolar macrophages [16, 17]. Most smokers, however, do not develop emphysema. Therefore, these findings do not explain the proposed imbalance between proteolytic and antiproteolytic activity in patients with emphysema and it has to be presumed that other factors in addition to smoking contribute to the origin of this imbalance.

Considering the importance of PMN in the pathogenesis of emphysema, we put forward the hypothesis that an abnormality of PMN may be a predisposing factor for the development of emphysema. Therefore, we investigated whether differences in the *in vitro* release of elastase, myeloperoxidase, and  $\beta$ -glucuronidase by PMN existed between patients with emphysema and healthy controls.

### Patients and methods

#### Patients and healthy controls

Fifty seven male patients with chronic obstructive pulmonary disease and clinical signs of emphysema (25 smokers and 32 ex-smokers) participated in the study. All patients met the following selection criteria: 1) clinical diagnosis of pulmonary emphysema according to history (persistent dyspnoea, mainly on exertion, without sudden attacks of dyspnoea), physical examination (hyperresonant percussion of the chest with decreased intensity of breath sounds) and chest radiography (flat and/or low diaphragm, increased retrosternal space, vessel narrowing or loss) [18]; 2) forced expiratory volume in one second ( $FEV_1$ ) <80% of the predicted value; 3) residual volume (RV) >100% of the predicted value; 4) specific compliance expressed as a percentage of the predicted value ( $C_{sp} \% \text{ pred}$ ) >100% after bronchodilation (when, however, air trapping (calculated as thoracic gas volume measured by body plethysmography minus functional residual capacity measured by an indicator gas) [19] was greater than 1.5 l, specific compliance was allowed to be <100% of the predicted value); 5) no signs of allergy (negative skin tests, total immunoglobulin E (IgE) <200 I.U., eosinophils in peripheral blood <250  $\text{mm}^{-3}$ ).

All patients had serum  $\alpha_1$ -AT levels within the normal range. All were in a stable phase of their disease. All patients used anticholinergics, betamimetics, theophylline, or a combination of these drugs as maintenance therapy. They had not used oral or inhaled corticosteroids for at least three months before entering the study. Smoking history was expressed as pack years (estimated average number of packs of 25 cigarettes smoked per day  $\times$  number of years of smoking). All ex-smokers had stopped smoking at least one year before entering the study except for one patient who stopped four months before entry.

A group of 21 healthy volunteers (9 smokers and 12 ex-smokers), matched for age, sex and smoking habits, was taken as a control group. No subject had signs or symptoms of dyspnoea, cough or sputum production. None had airflow obstruction as measured by spirometry. All had a normal chest radiograph. Informed consent was obtained from all participants. The study protocol was approved by the hospital Ethical Committee.

#### Materials and methods

**Cells.** PMN were isolated from peripheral venous blood essentially according to the method of Bøyum [20]. Immediately after collection, 50 ml of heparinized venous blood was diluted 1:1 with saline solution and gently layered on a Ficoll-Paque solution (Pharmacia Fine Chemicals) with a density of 1.077  $\text{g}\cdot\text{ml}^{-1}$ . After centrifugation for 20 min at 2,600 rpm (18°C), plasma, lymphocytes, monocytes and Ficoll-Paque were removed. The red-cell pellet was resuspended in the recollected plasma to a final volume of 50 ml. Dextran (6.68 ml 4.5% Dextran T-500, Pharmacia Fine Chemicals) in 4.5% glucose and 0.9% NaCl solution was added and the suspension was kept at 37°C for 45 min for sedimentation of the red cells. The dextran plasma layer, containing PMN and some erythrocytes, was collected and centrifuged for 10 min at 2,000 rpm. After decantation, the cell pellet was washed twice with a phosphate-buffered saline solution (PBS +0.1% glucose, 4°C) and centrifuged at 2,000 rpm for 10 min. Finally, the cell pellet was resuspended in PBS and gently layered on a Percoll (Pharmacia Fine Chemicals) solution with a density of 1.095  $\text{g}\cdot\text{ml}^{-1}$  (prepared according to methodology and applications of Percoll, Pharmacia Fine Chemicals). After centrifugation for 20 min at 2,600 rpm (18°C), the PMN layer was removed, diluted with PBS, and centrifuged for 10 min at 2,000 rpm (4°C). After decantation, the cell pellet was washed two times with PBS and finally resuspended in Hank's balanced salt solution (HBSS +0.1% glucose). Cyto-centrifuge smears were made from this suspension and differential counts were done after staining with May-Grünwald-Giemsa. The number of cells was counted with a Coulter Counter. Contamination with lymphocytes and monocytes by this method was always less than 5%. Viability as tested by Trypan blue exclusion was always higher than 95%. All reagents used for PMN isolation were free of endotoxin. The isolation procedure was always performed over roughly the same time period.

**Enzyme release.** Tests for the release of lysosomal enzymes were performed with the following stimuli:

- 1) Serum-treated zymosan (STZ, Sigma), prepared as described by Busse [21] and as modified by Postma *et al.* [22]. Until use the STZ was stored at -80°C in 1 ml portions.
- 2) Calcium ionophore A23187 (Sigma) dissolved in dimethyl sulfoxide (DMSO, Merck) to a concentration

of  $2 \times 10^{-2}$  M as stock solution and stored at  $-20^{\circ}\text{C}$  until use.

3) N-Formyl-L-Methionyl-L-Leucyl-L-Phenylalanine (FMLP, Sigma) dissolved in DMSO to a concentration of  $1.14 \times 10^{-2}$  M as stock solution and stored at  $-20^{\circ}\text{C}$  until use.

Before addition of the stimuli, PMN were diluted to a concentration of  $1 \times 10^6$  cells·ml<sup>-1</sup> HBSS in polystyrene vials and pre-incubated with cytochalasin B (Serva), 5 µg·ml<sup>-1</sup> cell suspension, for 5 min at  $37^{\circ}\text{C}$ . Then 1 ml samples of PMN suspension were incubated with 0.2 ml HBSS and 0.1 ml stimulus for 30 min at  $37^{\circ}\text{C}$  in a shaking water bath. The final concentrations were 1 µM and 5 µM for A23187, and 0.2 µM and 2 µM for FMLP. For spontaneous enzyme release 0.1 ml HBSS was added instead of a stimulus. The reaction was stopped by pelleting the cells by centrifugation at  $4^{\circ}\text{C}$  for 10 min at 2,300 rpm. Elastase, myeloperoxidase, and β-glucuronidase activity were measured in the supernatants by spectrophotometry (model 25, Beckman Instruments Inc.). Total enzyme content of the cells was obtained after cell lysis with 2.6% Triton X-100 (Sigma) for β-glucuronidase and 2.6% Triton X-100 with 4 M NaCl for elastase and myeloperoxidase. Release of cytoplasmic lactate dehydrogenase (LDH) was measured in the supernatants from all stimulated and non-stimulated PMN, as an indicator of cell damage. It was always less than 15% of the total LDH content of PMN, as measured after cell lysis with Triton X-100.

**Elastase.** Elastase was measured with the substrate S-2484 (Kabi Vitrum) according to the methods of KRAMPS *et al.* [23] (end-point method). Briefly, 0.2 ml Tris-buffer, pH 8.3  $37^{\circ}\text{C}$ , was added to 0.2 ml supernatant. The mixture was thermostated at  $37^{\circ}\text{C}$  for 2–3 min. Then, 0.2 ml S-2484,  $37^{\circ}\text{C}$ , was added, and the mixture was incubated for exactly 3 min. The reaction was terminated with 0.2 ml acetic acid 50%,  $37^{\circ}\text{C}$ . Optical density (OD) was measured at 405 nm.

**Myeloperoxidase.** Myeloperoxidase in the supernatants was determined according to HENSON *et al.* [24]. Briefly, 0.2 ml cell supernatant was mixed with 0.6 ml HBSS, 0.5 ml phosphate buffer, pH 6.2, 0.1 ml 0.05% H<sub>2</sub>O<sub>2</sub>, and 0.1 ml 1.25 mg·ml<sup>-1</sup> dimethoxybenzidine (O-dianisidine HCl, Sigma). After 15 min at room temperature the reaction was stopped with 0.1 ml 1% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. Optical density was measured at 450 nm.

**β-glucuronidase.** Beta-glucuronidase was measured according to FISHMAN *et al.* [25]. In short, 0.2 ml cell supernatant was mixed with 0.1 ml autologous plasma (1:1 diluted with saline), 0.1 ml sodium acetate, pH 4.6, and 0.1 ml phenolphthalein glucuronic acid solution (Sigma), and incubated for 18 h at  $37^{\circ}\text{C}$  in a new Brunswick Scientific Shaker at 150 rpm. Afterwards 1 ml glycyl duponal solution, pH 11.8, and 1.5 ml demineralized water were added and after 10 min the optical density was measured at 550 nm. The activity

was expressed in µg phenolphthalein·ml<sup>-1</sup> per 10<sup>6</sup> PMN per 18 h, as read from a reference phenolphthalein dose-response curve.

### Statistics

Differences between groups of individuals were analysed with analysis of variance. Significance levels were set at 5%. Values are presented as mean±one standard error of the mean (mean±SEM).

### Results

Characteristics of the emphysematous patients are shown in table 1. Within the patient group, no significant differences in age, pack-years of smoking, lung function, and degree of airway hyperresponsiveness were found between smokers and ex-smokers. Sputum production was low in both groups (median production being 8 ml·day<sup>-1</sup> in smokers and 3 ml·day<sup>-1</sup> in ex-smokers). There was no difference in the use of bronchodilators in both subgroups.

Table 1. – Characteristics of the emphysematous patients

	Ex-smokers n=32	Smokers n=25
Age yrs	56 (1.7)	55 (1.6)
Smoking history pack-yrs	25 (3.7)	32 (3.2)
FEV <sub>1</sub> l	2.03 (0.11)	1.92 (0.11)
FEV <sub>1</sub> % pred %	63 (2.7)	62 (3.5)
FIV <sub>1</sub> % pred %	98 (2.4)	99 (3.4)
FEV <sub>1</sub> % VC %	46 (2.0)	44 (1.8)
TLC % pred %	110 (2.8)	115 (3.2)
RV % pred %	149 (6.5)	168 (6.5)
Csp % pred %	110 (7.1)	116 (8.2)
Air trapping l	1.46 (0.15)	1.40 (0.27)
PC <sub>20</sub> histamine mg·ml <sup>-1</sup>	5.75	4.68

All values are expressed as mean (SEM) except PC<sub>20</sub> histamine; geometric mean. No significant differences existed between smoking and ex-smoking patients. FEV<sub>1</sub>: forced expiratory volume in one second; % pred: expressed as a percentage of the predicted value; FIV<sub>1</sub>: forced inspiratory volume in one second; FEV<sub>1</sub> % VC: FEV<sub>1</sub> expressed as a percentage of slow inspiratory vital capacity; TLC: total lung capacity; RV: residual volume; Csp: specific compliance (after bronchodilation); PC<sub>20</sub>: provocative concentration of histamine causing a 20% fall in FEV<sub>1</sub> from baseline after inhalation during 30 s.

Table 2. – Characteristics of the healthy controls

	Ex-smokers n=12	Smokers n=9
Age yrs	56 (1.9)	53 (2.4)
Smoking history pack-yrs	13 (2.9)*	24 (4.3)

All values are expressed as mean (SEM). No significant differences existed between smoking and ex-smoking controls. \*: significantly smaller (p<0.05) as compared with emphysematous smokers.

Mean age of the healthy subjects (table 2), both smokers and ex-smokers, was not significantly different from that of the emphysematous patients. In the group of healthy ex-smokers, mean pack-years of smoking was lower than in the healthy smokers and the patients with emphysema, both smokers and ex-smokers. This difference reached statistical significance between healthy ex-smokers and emphysematous smokers.

#### Enzyme release

**Elastase** (fig. 1). Total elastase content of peripheral PMN was not significantly different in healthy subjects and patients with emphysema. In addition, both spontaneous release of elastase and release after stimulation with A23187 and FMLP were not significantly different between the two groups. Within both groups, no significant differences were observed between smoking and ex-smoking individuals.

**Myeloperoxidase** (fig. 2). Total myeloperoxidase content of PMN and both spontaneous and stimulated release of myeloperoxidase were not significantly different in healthy subjects and patients with emphysema. No significant differences were found between smoking and ex-smoking individuals.

**$\beta$ -glucuronidase** (fig. 3). Total  $\beta$ -glucuronidase content of PMN was not significantly different in healthy subjects and patients with emphysema. Within both groups, however, PMN of smokers contained significantly less  $\beta$ -glucuronidase than those of ex-smokers. Spontaneous  $\beta$ -glucuronidase release and release after stimulation with STZ were significantly higher in healthy subjects than in patients with emphysema. No significant differences were found between smoking and ex-smoking individuals. After stimulation with A23187 and FMLP,  $\beta$ -glucuronidase release was also higher in healthy subjects than in patients with emphysema,

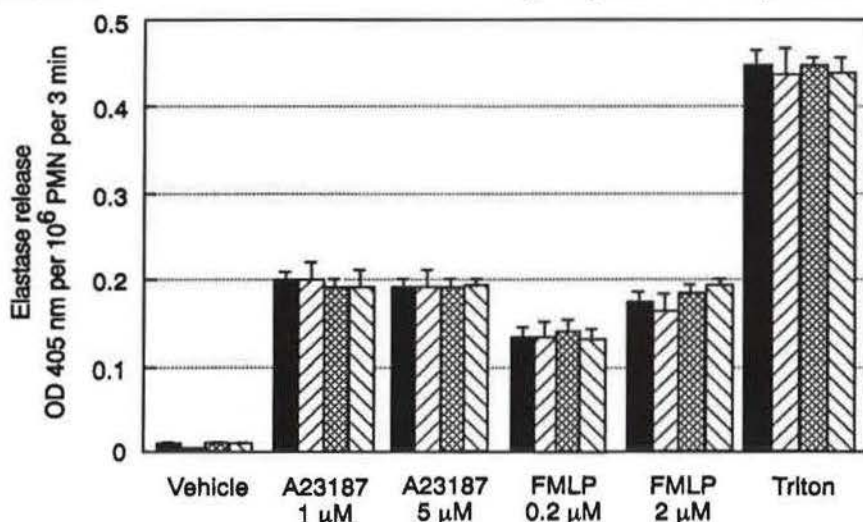


Fig. 1. — Mean (SEM) elastase release (OD 405 nm per  $10^6$  PMN per 3 min) from PMN in healthy subjects and patients with emphysema. Vehicle: no stimulus added (for spontaneous enzyme release); A23187: calcium ionophore A23187; FMLP: N-formyl-L-methionyl-L-leucyl-L-phenylalanine; Triton: Triton X-100 (for total enzyme content); ■: healthy ex-smokers; ●: emphysematous ex-smokers; ▨: healthy smokers; ▩: emphysematous smokers.

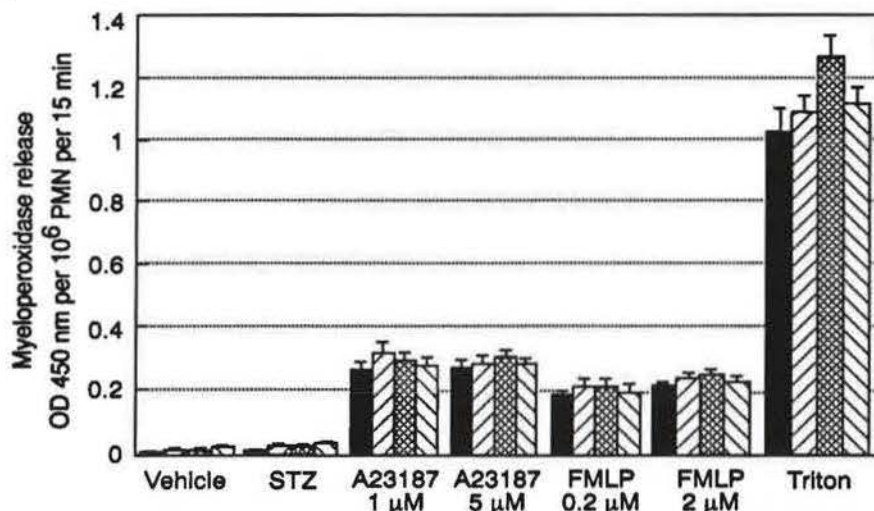


Fig. 2. — Mean (SEM) myeloperoxidase release (OD 450 nm per  $10^6$  PMN per 15 min) from PMN in healthy subjects and patients with emphysema. STZ: serum-treated zymosan. For other abbreviations and key see legend to figure 1.

differences reaching statistical significance for A23187 1  $\mu$ M and FMLP 0.2  $\mu$ M. These differences, however, were entirely attributable to the increased release in healthy ex-smokers; the release in healthy smokers and patients with emphysema was not significantly different.

HNE is considered to be the protease of crucial importance in the development of emphysema. Therefore, several investigators have studied intracellular elastase levels of peripheral PMN, with somewhat conflicting results. Our results are in agreement with

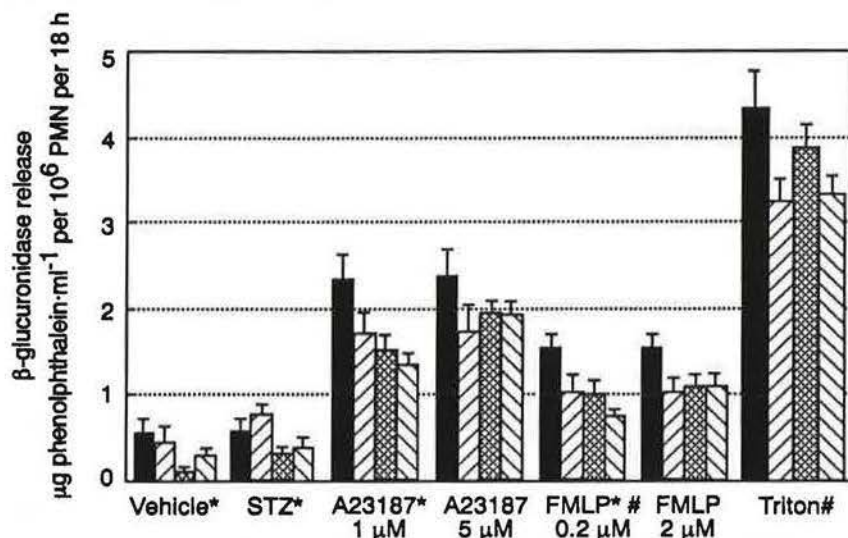


Fig. 3. — Mean (SEM)  $\beta$ -glucuronidase release ( $\mu$ g phenolphthalein  $\cdot$  ml $^{-1}$  per  $10^6$  PMN per 18 h) from PMN in healthy subjects and patients with emphysema. \*: significantly different ( $p < 0.05$ ) in healthy subjects; #: significantly different ( $p < 0.05$ ) in smokers and ex-smokers. For abbreviations and key see legends to figures 1 and 2.

**Relationship between enzyme release and smoking.** Both in patients with emphysema and in healthy subjects, the release of elastase, myeloperoxidase, and  $\beta$ -glucuronidase was not significantly correlated with age, pack-years of smoking, or, in current smokers, with the actual tobacco consumption.

Within the patient group, enzyme release was not significantly correlated to the degree or severity of emphysema, as measured by FEV $_1$  as percentage of predicted, FEV $_1$  as percentage of vital capacity, total lung capacity as percentage of predicted, residual volume as percentage of predicted, specific compliance after bronchodilation as percentage of predicted, or air trapping.

## Discussion

In this study we showed the absence of significant differences in the *in vitro* release of elastase and myeloperoxidase from both stimulated and unstimulated PMN in patients with emphysema as compared with healthy controls. We also found no significant differences in total elastase and myeloperoxidase content of PMN between both groups. Moreover, within both groups no differences were found between smoking and ex-smoking individuals. Beta-glucuronidase release tended to be lower in patients with emphysema, whereas total  $\beta$ -glucuronidase content of PMN was not significantly different in emphysematous patients and healthy subjects. In both groups, release of the studied enzymes was not significantly related to smoking history or current tobacco consumption.

those of ABOUD and co-workers [26] and KRAMPS *et al.* [27], both of whom found no significant differences in intracellular elastase levels of PMN between healthy subjects and patients with emphysema (PiM phenotype). On the other hand, RODRIGUEZ *et al.* [28] and GALDSTON *et al.* [29] demonstrated increased intracellular elastase levels in neutrophils from patients with PiM phenotype and chronic obstructive pulmonary disease. Most of these patients, however, had a history of chronic bronchitis as well, as opposed to our patients.

The protease-antiprotease imbalance hypothesis proposes that proteolytic damage in emphysema occurs when neutrophils leave the circulation and infiltrate into the alveolar interstitium. In the interstitium, neutrophils are thought to release lysosomal enzymes upon stimulation by several factors which result from lung inflammation [3]. This suggests that it is not the intracellular elastase levels but the quantity of elastase released extracellularly by stimulated cells that might be a crucial factor in the development of emphysema. We, therefore, wanted to investigate the release of elastase from activated PMN. To stimulate PMN we used FMLP, a synthetic chemotactic factor, and calcium ionophore A23187, a stimulus able to raise intracellular calcium levels, which event is held to play a crucial role in neutrophil degranulation [30]. Our results are in accordance with those of ABOUD and co-workers [31] who mentioned that they observed no significantly different enzyme release from PMN in healthy subjects and eight patients with severe irreversible chronic obstructive lung disease, without further definition or characterization of their patient group. HUTCHISON *et al.* [32], who investigated the *in vitro* release of acid

phosphatase and acid ribonuclease from leucocyte suspensions after stimulation by latex phagocytosis, also found no significant differences between normal and emphysematous subjects. It would have been interesting to investigate elastase release after stimulation with STZ. This was not done, however, since it has been shown that the STZ-induced release of elastase from PMN, when measured in the cell supernatant, is underestimated due to binding of elastase to STZ and removal of the STZ-elastase complex by centrifugation of the incubation mixture [22]. BURNETT *et al.* [33], using fibronectin digestion as a marker of elastolytic activity of PMN, found an increased fibronectin proteolysis by both unstimulated and stimulated PMN from patients with emphysema. Their patients, however, also had a diagnosis of chronic bronchitis. Moreover, these investigators studied proteolysis by adherent cells in a functional assay, whereas we measured lysosomal enzyme release from cells in suspension. The differences with our findings, therefore, might stem from a difference in patient selection and/or from different methodology. In addition to the release of elastase, we investigated the release of myeloperoxidase and  $\beta$ -glucuronidase. Increased release of myeloperoxidase would suggest increased oxidative inactivation of antiprotease, thereby contributing to the protease-antiprotease imbalance in lung tissue. As was the case for elastase, however, we could not demonstrate increased extracellular release or intracellular levels of myeloperoxidase in PMN from patients with emphysema as compared with healthy controls. Unlike the role of elastase and myeloperoxidase, the possible role of  $\beta$ -glucuronidase in the pathogenesis of emphysema is unclear. To our surprise, the release of  $\beta$ -glucuronidase tended to be decreased in patients with emphysema. Assuming that  $\beta$ -glucuronidase is stored in the same type of neutrophil granules as elastase and myeloperoxidase, we cannot explain the different pattern of release of this enzyme. Our results, like those of other investigators [28], may at least partly suggest compartmentalization of azurophilic granules, or storage of  $\beta$ -glucuronidase in a different type of granule [34].

Our findings indicate that if excess proteolytic activity is of pathogenetic importance in emphysema, it is unlikely to be due to an abnormality, *i.e.* increased releasability, of peripheral PMN. We cannot with certainty exclude the possibility that we failed to demonstrate differences because of an error of the second type. We investigated a relatively large number of subjects, however, and any differences not observed would be very small. Another factor that may modulate elastase levels is the number of PMN in the lung. Although elastase release was not different in smoking and ex-smoking individuals, the potential proteolytic burden is greater in smokers, as they have more PMN both in peripheral blood and in the lungs than nonsmokers or ex-smokers. The question remains, however, why do not all smokers develop emphysema.

Patients and healthy controls were matched for sex, age and, as closely as possible, for smoking habits. A

major difference between our patients and controls was drug therapy. *In vitro* results in the literature suggest that the influence, if any, of anticholinergics, beta-adrenergics or theophylline on neutrophil function is a suppressive one, so that this may have influenced our results [21, 35, 36]. The disturbance of the protease-antiprotease balance in emphysema is supposed to occur in lung tissue. In addition, evidence is accumulating that the proteolytic damage is largely due to localized protease excess in the microenvironment of pulmonary PMN [37, 38]. Therefore, by investigating the releasability of peripheral PMN, we actually studied an *in vitro* model of events that may happen *in vivo*. It is conceivable, however, that the protease-antiprotease imbalance is also detectable in the systemic circulation. For example, it has long been recognized that smokers have increased numbers of circulating PMN [13]. Moreover, WEITZ *et al.* [39] showed that cigarette smoking produces an increase in systemic neutrophil elastase activity by demonstrating increased plasma levels of fibrinopeptide A- $\alpha$ -1-21, a specific cleavage product of unopposed neutrophil elastase, in smokers. This suggests the potential relevance of systemic neutrophils as a source of damaging enzymes. When, however, as our results indicate, the excess proteolytic activity cannot be explained by an abnormality of peripheral PMN, it may be useful to investigate the microenvironment of PMN in the lungs to see whether local differences in number or responsiveness of PMN, or in modulating factors, such as inflammatory mediators, may be a predisposing factor for emphysema.

In summary, the protease-antiprotease imbalance hypothesis suggests that in patients with normal  $\alpha_1$ -AT levels, increased release of lysosomal enzymes from PMN may be a predisposing factor for the development of emphysema. Our results demonstrate that peripheral PMN from carefully selected patients with emphysema do not contain or release more elastase, myeloperoxidase or  $\beta$ -glucuronidase than those from healthy subjects. We conclude that an abnormality in releasability of peripheral PMN is unlikely to be a pathogenetic factor in emphysema.

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*Production in vitro d'élastase neutrophilique, de myéloperoxydase et de bêta-glucuronidase chez les patients emphysémateux et les sujets sains. T.E.J. Renkema, D.S. Postma, J.A. Noordhoek, H.J. Sluiter, H.F. Kauffman.*

RÉSUMÉ: Il est de plus en plus clair que le fait de fumer la cigarette joue un rôle important dans le déséquilibre protéase-antiprotéase chez les emphysémateux sans déficience en alpha<sub>1</sub>-antitrypsine. Toutefois, puisque la plupart des fumeurs ne développent pas d'emphysème, il faut supposer que d'autres facteurs, à côté du tabac, contribuent à créer ce déséquilibre.

La principale source de protéases est le leucocyte polymorphonucléaire (PMN). Nous avons testé l'hypothèse qu'une anomalie de la libération par les polynucléaires pourrait prédisposer au développement de l'emphysème. Dès lors, nous avons investigué la production d'élastase, de myéloperoxydase et de bêta-glucuronidase à partir des polymorphonucléaires chez des patients atteints d'emphysème et chez des contrôles sains, pairés pour le sexe, l'âge et les habitudes tabagiques.

Les polymorphonucléaires ont été isolés du sang périphérique et stimulés au moyen de l'ionophore calcique A23187, de formyl-méthionyl-leucyl-phenylalanine (FMLP), et de zymosan traité au sérum (STZ). Le contenu enzymatique total des polymorphonucléaires a été mesuré après lyse cellulaire au moyen de Triton X-100.

Le contenu total en élastase, myéloperoxydase et bêta-glucuronidase des polymorphonucléaires, n'est pas significativement différent chez les sujets sains et les patients emphysémateux. La libération *in vitro* d'élastase et de myéloperoxydase à partir de polymorphonucléaires à la fois stimulés et non stimulés, ne s'avère pas différente chez les sujets sains et chez les patients emphysémateux. De plus, l'on n'a observé aucune différence entre les individus fumeurs et ex-fumeurs. La libération de glucuronidase tendait à être plus faible chez les patients emphysémateux que chez les contrôles sains.

Nous concluons qu'une anomalie dans la production par les polymorphonucléaires périphériques n'est probablement pas un facteur pathogénique dans l'emphysème.

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