

Granulocytes and their secretory products, myeloperoxidase and eosinophil cationic protein, in bronchoalveolar lavage fluids from two lung lobes in normal subjects

B. Schmekel^{*†}, B. Blom-Bülow^{*}, Y. Hörnblad^{*}, L.A. Laitinen^{*†}, M. Linden[†], P. Venge^{**}

Granulocytes and their secretory products, myeloperoxidase and eosinophil cationic protein, in bronchoalveolar lavage fluids from two lung lobes in normal subjects. B. Schmekel, B. Blom-Bülow, Y. Hörnblad, L.A. Laitinen, M. Linden, P. Venge.

ABSTRACT: The interlobar variability of lavage neutrophils and eosinophils was studied in twelve healthy subjects. In addition, the interlobar variation of the neutrophil cell marker myeloperoxidase (MPO) and the eosinophil cell marker eosinophil cationic protein (ECP) was assessed. Bronchial washes (BW), as defined by the first aspirated lavage aliquot, and bronchoalveolar lavage (BAL) fluids were compared. One subsegment of the right middle lobe and one subsegment of the right lower lobe were lavaged in the same session.

Interlobar consistency of neutrophil and eosinophil cell recoveries was observed but, in contrast, the levels of MPO or ECP did not correlate in lavage fluids aspirated from the two lobes. These results suggest that BAL cell content from a single lobe of the lung in healthy subjects does reflect the cell populations throughout the airways, while the levels of soluble proteins may differ between the lobes. Such a variation questions the correlation between cells and their secretory products or the correlation between levels of solutes in lavage fluid and in the underlying tissue.

Further methodological studies appear warranted to elucidate whether cell and solute recoveries accurately reflect the underlying pathology. *Eur Respir J*, 1991, 4, 867-871.

* Dept of Lung Medicine, University Hospital, Lund, Sweden.

† Dept of Expl. Clin Research, AB Draco, Lund, Sweden.

** Dept of Clin. Chemistry, University Hospital, Uppsala, Sweden.

Correspondence: B. Schmekel, Dept of Exploratory Clinical Research, AB Draco, Box 34, S-21100 Lund, Sweden.

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Bronchoalveolar lavage (BAL) is a well-established research tool, used to study cells and constituents of bronchoalveolar lining fluids. Even under carefully controlled conditions, the variability of cell numbers within and between healthy subjects in BAL was reported to be considerable [1, 2]. Due to the large variability, it was suggested that the study of cell numbers alone in BAL fluids would be of limited value in clinical medicine.

The present investigation focused on the smallest cell compartment in lavage, the neutrophils and the eosinophils. The mere presence of increased numbers of these cells is not, in itself, the contributing factor to a potential worsening of the clinical status. Rather, it is the various active factors that these cells may liberate that determine their significance in local inflammation. The secretion of toxic granular products, such as eosinophil cationic protein (ECP) or myeloperoxidase (MPO) from activated eosinophils or neutrophils, respectively, may result in local damage to the tissue [3, 4]. Measurement of the secretory products from these cells may be more important in assessing cellular activity than merely counting the cells in BAL.

This study set out to determine the correlation between eosinophil and neutrophil cell numbers and levels of ECP and MPO in lavages from different segments of the lung. Lung lavage was performed in twelve healthy volunteers, six of whom were smokers. One subsegment of the right middle and one of the right lower lobe were lavaged in the same session.

Materials and methods

Subjects

In twelve healthy male volunteers with a mean age of 35.3 yrs (SD 9.6 yrs), bronchoalveolar lavage was performed in both an anterior subsegment of the middle lobe and an anterior subsegment of the lower lobe. Six of the subjects were smokers. They had no clinical history of allergy or lung disorders and chest radiographs were normal. Two lung function parameters, forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) measured with a Bernstein spirometer, were >80% of predicted [5] in all subjects studied.

Informed consent was obtained from the subjects and the study was approved by the local Research Ethics Committee.

Bronchoalveolar lavage procedure

All bronchoscopies were performed in a standardized way, by one of two persons (BS or YH). The subjects received an intramuscular injection of 10 or 15 mg morphine-scopolamine one hour before the bronchoscopy. A local anaesthetic (Xylocaine® 40 mg·ml⁻¹, Astra Sweden) was nebulized to the oropharyngeal and nasal mucosa, three times during the hour preceding the bronchoscopy. In addition, Xylocaine was infused *via* the bronchoscope to the bronchial mucosa. The working channel of the fiberoptic bronchoscope (Olympus BFI T10), was connected with a central venous catheter equipped with a three way valve, which in turn was connected to both the wall suction and the hand held infusion syringes. The bronchoscope was passed *via* the nasal pathway to the airways, wedged and kept steady in an anterior subsegmental bronchus of the right middle or lower lobe. Warm saline (37°C) was gently infused in boluses of 50 ml. The infused aliquots were aspirated immediately after each infusion. The first aspirated aliquot, defined as bronchial wash (BW), was kept separate from the following two pooled aliquots, which were defined as bronchoalveolar lavage (BAL). The aspirated fluids were collected in siliconized glass bottles and kept on ice, until centrifuged at +4°C at 200 × g for 10 min. The cell pellet was resuspended in RPMI 1640 (Gibco, Scotland), supplemented with 5% foetal calf serum and gentamicin (50 µg·ml⁻¹). The lavage fluid supernatants were kept frozen at -70°C until analysed.

Cell analysis. Bürker chambers were used for total cell counts. Viability of the cells was determined using trypan blue exclusion. Differential cell counts were performed on cytocentrifuge preparations (Shandon, Cytospin 2, England, 500 rpm for 10 min) stained with May-Grünwald Giemsa. Four hundred cells were differentiated on each of two separate cytospin preparations, mean values of two readings were determined. The variability within the observations was 1.5% and 0.7% for neutrophils aspirated in the first and second aliquots, respectively. Variability for the corresponding eosinophil counts was 0.3% and 0.2%, respectively.

Lavage fluid analyses. ECP and MPO concentrations were measured using radio-immunoassay methods described previously [6, 7]. Total contents of the soluble substances in BAL supernatants were calculated by multiplying the concentration with the volume of the corresponding aspirated fluids.

Statistical analysis

Mean±SD have been used to describe normally distributed values while median values and ranges has been used when non-normally distributed values were

described. Non-parametric statistical tests were used: Wilcoxon's rank sum test has been used for comparison of differences between groups. Spearman's rank correlation test was used for estimation of the significance of correlations. A probability value (p-value) $p \leq 0.05$ was set to indicate the level of statistically significant difference. An analysis of variance was used for estimation of the intraobservation variability.

Results

The lavage procedure was well-tolerated by all subjects, with no apparent clinical complication. The lavage volumes recovered from the lower lobe tended to be smaller than from the middle lobe. However, the variability was large and there was no significant interlobar difference in BW volumes (18.2±4.8 ml and 14.6±4.0 ml, from middle and lower lobes, respectively, (mean±SD; $p > 0.05$) or the BAL volumes (57.8±21.7 ml and 45.9±19.2 ml, from middle and lower lobes, respectively, $p > 0.05$). Total and differential counts in BWs and BALs aspirated from the two lobes are shown in table 1.

Determination of interlobar difference

The numbers of neutrophils or eosinophils in BWs or BALs varied greatly, whether expressed as total number or as number of cells·ml⁻¹. There was no significant interlobar difference. The neutrophil, but not the eosinophil, concentration was higher in BWs than in BALs ($p = 0.005$ for values obtained in the middle lobe and $p = 0.08$ for those obtained in the lower lobe), as shown in table 2.

The intersubject variability in concentration or total amount of the secretory products from neutrophils and eosinophils, was also large. There was no significant interlobar difference. The concentrations of MPO or ECP were higher in BWs than in BALs (MPO values: $p < 0.05$ for values obtained in the middle lobe and $p = 0.05$ for values obtained in the lower lobe; and ECP values: $p < 0.001$ for values obtained in the middle lobe and $p > 0.05$ for values obtained from the lower lobe). In contrast to the findings with MPO, the total amounts of ECP tended to be higher in BALs than in BWs (table 3), but the difference was statistically significant only in the lower lobe ($p < 0.01$). There was no significant interlobar difference between the total amounts of MPO or ECP, either in BWs or in BALs, as is shown in table 3.

Determination of interlobar correlations

Although there was considerable spread in the observed values, significant correlations were noted for neutrophils, whether expressed as total number or cells·ml⁻¹ (BW; $r = 0.82$, $p < 0.01$ or BALs, $r = 0.67$, $p < 0.05$) and for eosinophils (BW; $r = 0.74$, $p < 0.05$ or BALs, $r = 0.65$, $p < 0.05$). The granular products secreted

Table 1. - Total number of cells and differential counts of fluid aspirated from twelve healthy subjects in bronchial wash (BW) or bronchoalveolar lavage (BAL) of the right middle or lower lobes

		BW	BW	BAL	BAL
	B5	B8	B5	B8	B8
Total cells	10 ⁶	2.4(0.4-7.5)	2.3(0.6-4.3)	10.3(5.8-35.4)	14.8(2.8-40.7)
Differentials:					
Macrophages	%	82.5(41-92.9)	81.5(18.5-97.5)	91.9(85.1-98.6)	95.1(81.3-98.1)
Lymphocytes	%	3(0.5-8.4)	2.8(0.5-10.5)	4.5(0.4-8.9)	2.2(0.3-9.5)
Neutrophils	%	5.8(0.9-54.3)	11.9(0.8-26.6)	1.3(0.1-11.1)	1.1(0.1-2.3)
Eosinophils	%	0.3(0-1.3)	0.3(0-2.4)	0.2(0-0.8)	0.3(0-1.3)

B5 and B8 signifies the middle and lower lobe, respectively. Median values and ranges in parentheses are given.

Table 2. - Cells recovered in bronchial wash (BW) and bronchoalveolar lavage (BAL) obtained from twelve healthy subjects undergoing lavage in the right middle and lower lobes

		BW	BW	BAL	BAL
	B5	B8	B5	B8	B8
Total amounts:					
Neutrophils	10 ⁶	0.14(0.03-2.17)	0.11(0.04-0.64)	0.11(0.04-0.64)	0.14(0.003-0.87)
Eosinophils	10 ⁶	0.007(0-0.09)	0.005(0-0.6)	0.02(0-0.22)	0.006(0-0.29)
Concentrations:					
Neutrophils	10 ⁴ .ml ⁻¹	70(11-1550)	110(11-720)	20(10-140)	35(1-310)
Eosinophils	10 ⁴ .ml ⁻¹	6.5(0-10)	3.2(0-30)	6.4(0-30)	5.7(0-100)

B5 and B8 signifies the middle and lower lobes, respectively. Median values and ranges in parentheses are given.

Table 3. - Cell secretory products recovered in bronchial wash (BW) and bronchoalveolar lavage (BAL) obtained from twelve healthy subjects undergoing lavage in the right middle and lower lobes

		BW	BW	BAL	BAL
	B5	B8	B5	B8	B8
Total amounts:					
MPO	µg	0.22(0.10-3.86)	0.34(0.05-1.56)	0.42(0-2.70)	0.21(0-5.89)
ECP	µg	0.23(0.14-0.53)	0.21(0.008-0.34)	0.37(0.14-0.60)	0.44(0.10-2.56)
Concentrations:					
MPO	µg.l ⁻¹	14.5(5.1-203)	23(3-86.7)	4.8(0-55)	3(0-131)
ECP	µg.l ⁻¹	10.7(7.6-34.3)	12.6(5.2-21)	6(1.6-9.7)	8.5(1.6-36)

B5 and B8 signifies the middle and lower lobes, respectively. Median values and ranges in parentheses are given. MPO: myeloperoxidase; ECP: eosinophil cationic protein.

from neutrophils or eosinophils showed no interlobar consistency, as suggested by the absence of significant correlations of total amounts of MPO (BW: $r=0.37$, $p>0.05$ and BAL: $r=-0.12$, $p>0.05$) or ECP (BW: $r=0.53$, $p>0.05$ and BAL: $r=0.04$, $p>0.05$).

Discussion

The aspirated BW and BAL volumes were within the previously reported ranges, and tended to be smaller when aspirated from the lower lobe than from the middle lobe, as previously reported [8]. A difference in airway wall flaccidity of these two lobes may explain the lower recovery volumes from the lower lobe, presumably due to closure of the airways in conjunction with aspiration of the fluid. The infused lavage fluid might then cover a smaller area of the mucosal surface, and dilution of the lavage fluid by rapidly diffusing body fluid into the airspace would be less in the central as compared with the peripheral airways, as suggested by KELLY *et al.* [9]. Rapid diffusion of body fluids into peripheral parts of the airways in contrast to central ones, argues against the expression of recovered lavage cells or solutes $\cdot\text{ml}^{-1}$ recovered fluid, *i.e.* concentration, and would rather favour the expression of total cell counts or total amounts of solute, respectively.

A large interindividual, as well as interlobar variability in cells and solutes was noted. In keeping with our previously published data, eosinophil counts and ECP data [10], were similar in the BWs and the BALs, respectively, suggesting that eosinophils are distributed to peripheral as well as central parts of the airways. There was a tendency, albeit not statistically significant, for higher contents of neutrophils, when corrected for volume of recovery, in BWs as compared with BALs. When neutrophil recoveries were expressed as total cell counts, on the other hand, the recovery of neutrophils was not significantly higher in BWs as compared with BALs. These findings contrast with previous observations [11], and may suggest that there are subject dependent parameters or that lavage recoveries may be influenced by uncontrollable factors that contribute to the variability.

It has previously been suggested that MPO is a relevant estimate of neutrophil presence in lavage fluid [12]. Similarly, ECP, which is selectively secreted by activated eosinophils, is generally considered to reflect eosinophil activity in lavage. The interlobar conformity of cells and the absence of interlobar correlation of their secretory products, suggests a difference in kinetical recovery of cells and solutes, as previously suggested [13]. The cells that are recovered by lavage in healthy volunteers appear to originate mainly from the surface of the bronchial mucosa and the alveolar epithelial lining layer, and to a lesser extent, if any, from the underlying tissue. Disruption of epithelial integrity by inflammation may facilitate the transfer of cells, with various states of activity, to the airway and alveolar lining, to be transported up the mucociliary escalator or to be collected by lavage. A correlation between

neutrophil or eosinophil counts in lung lavage and tissue has been observed in inflammatory lung diseases [14] and acute lung injury [15]. A significant correlation was obtained in healthy volunteers only between inflammatory cells in tissue and BWs, but not between cells in tissue and BAL [16]. In spite of possible correlations of cell numbers in lavage fluid and tissue, it is questionable whether the functional subpopulations of cells collected by lavage actually reflects subpopulations of cells in the underlying tissue [17-19]. The transfer of tissue cells to the airway and alveolar lumen may be governed not only by the permeability of the barrier, but may also depend on the stickiness of the activated cells hampering their movement through tissue and membranes.

Diffusion of solutes through membranes is governed by a number of factors including physicochemical properties of the solute [20] and the membrane [21]. The gradient of the solutes between tissue and lavage fluid appears to be crucial, and longer dwell times of lavage fluid may allow more of the solutes to be diffused passively through membranes [22]. A relationship of extracellular ECP in the tissue and lavage ECP has recently been suggested [23] and diffusion of ECP through membranes was also suggested in a study of sequential lavage. Whether MPO diffuses through membranes with kinetics similar to ECP remains to be proven, but passive diffusion to lavage fluid of MPO through lung membranes was suggested to be less important than passive diffusion of urea, albumin and ECP [11]. We did not record the dwell times of the lavage fluid in this study, and a variability of the dwell times may have affected the recovery of solutes in the two lobes, and consequently the probability of recovering consistent amounts of solutes may have been very low. Recovery of the solutes might reflect both the presence of solute in the liquid lining of the airspace and, depending on the gradient and the physicochemical properties of the solute, varying proportions of extracellular solute dispersed within the underlying tissue.

In conclusion, we found an interlobar consistency in the neutrophil as well as eosinophil cell recovery. In contrast, no correlation in MPO or ECP recoveries was obtained. The recovery of lavage cells and solutes is determined by many factors and it appears warranted to study further the inherent variability of the yield, the presence and activity state of neutrophils or eosinophils in lavage fluid, and whether lavage findings correspond to findings in the underlying tissue.

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- Les granulocytes et leurs produits de sécrétion (myéloperoxydase et protéine cationique éosinophile) dans les liquides de lavage broncho-alvéolaire de deux lobes pulmonaires chez des sujets normaux.* B. Schmekel, B. Blom-Bülow, Y. Hörnblad, L.A. Laitinen, M. Linden, P. Venge.
- RÉSUMÉ: La variabilité interlobaire des neutrophiles et des éosinophiles de lavage a été étudiée chez douze sujets sains. En outre, la variation interlobaire du marqueur neutrophilique: la myéloperoxydase (MPO), et du marqueur éosinophilique: la protéine cationique éosinophile (ECP), a été étudiée. Les lavages bronchiques, définis comme le premier aliquote de lavage aspiré, ont été comparés aux liquides de lavage broncho-alvéolaire (BAL). Un sous-segment du lobe moyen droit et un sous-segment de lobe inférieur droit ont fait l'objet d'un lavage au cours d'une même session. L'on a observé une bonne corrélation interlobaire dans les recueils de cellules neutrophiliques et éosinophiliques mais, au contraire, les niveaux de MPO et de ECP n'étaient pas en bonne corrélation dans les liquides de lavage aspirés dans ces deux lobes. Ces résultats suggèrent que le contenu cellulaire du BAL d'un seul lobe du poumon chez les sujets normaux, est le reflet des populations cellulaires dans l'ensemble des voies aériennes, alors que les niveaux de protéines solubles peuvent être différents entre les lobes. Cette variation remet en question la corrélation entre les cellules et leurs produits sécrétoires, ou les corrélations entre les niveaux de solutés dans le liquide de lavage et dans le tissu sous-jacent.
- Nous concluons que d'autres études méthodologiques, au moyen de cette technique, sont indispensables pour élucider dans quelle mesure les recueils de cellules et de solutés sont un reflet exact de la pathologie sous-jacente.
- Eur Respir J.*, 1991, 4, 867–871.