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Comparison of human lung surface protein profiles from the central and peripheral airways sampled using two regional lavage techniques

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ABSTRACT: This study describes two new techniques of lung lavage which selectively remove material from the central airways, or from the lung below the seventh generation. Bronchograms confirmed that discrete regions of the lung were washed by central lavage (CL; maximum airway diameter ~6.5 mm) and peripheral lavage (PL; maximum airway diameter ~1.3 mm), and that both could be clearly distinguished from conventional bronchoalveolar lavage (BAL). These techniques were used to establish whether or not large-airway proteins made a major contribution to the protein profile of BAL. Twenty consecutive patients undergoing routine fibreoptic bronchoscopy were investigated. More bronchial mucus proteinase inhibitor per unit albumin and per unit total measured antiproteinase was present in CL than PL or BAL. In contrast α_1-proteinase inhibitor per unit albumin and as a percentage of total measured antiproteinase was lower in CL than in other lavage types. There were no differences in elastase activity, irrespective of the way in which the data were expressed. As no differences were found between BAL and PL for any of the variables measured, it was concluded that in the subjects studied the contribution of CL proteins to BAL was minimal.

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Bronchoalveolar lavage (BAL) is a widely used, relatively non-invasive method of sampling epithelial lining fluid and cells from the human lung surface, for diagnostic and research use. It is generally assumed that BAL fluid contains material predominantly from the peripheral lung surface, and that the central airways make little or no contribution to the protein profile of the collected fluid. However, biochemical and physical studies have shown that material collected by BAL may potentially be derived from many levels of the airway [1–4], from the second or third generation down. Thus, BAL data may not accurately reflect the composition of the fluid lining the peripheral epithelial surface. A method of regional lung lavage might help to determine whether or not this is so.

The initial aim of this study was, therefore, to develop lavage techniques with which to selectively sample the central and peripheral regions of the lung. One of the methods developed samples of material from above the 5th or 6th generations (central lavage, CL), whilst the other contains epithelial lung fluid from the lung distal to the 7th or 8th generations of the airways (peripheral lavage, PL). Since locally produced proteinase inhibitors have been shown by immunohistochemistry to be abundant in the central and upper airways [5, 6], one such protein, bronchial mucus proteinase inhibitor (BMPI) [7, 8], has been used as an indicator of the presence of central airways components in these lavages. α_1-proteinase inhibitor (α_1-PI) is believed to be the predominant antielastase of the lower respiratory tract [9]. Therefore, this protein has been analysed in peripheral lavage fluid in order to confirm that it is the major proteinase inhibitor below the 7th generation. We have used our analyses of these proteins in BAL, CL and PL collected from the same subjects to establish the contribution made by central airways components to the content of BAL fluid. Part of this study has been presented in preliminary form [10, 11].

Methods

Subjects

The study was carried out in two parts, each part on a group of ten consecutive patients undergoing routine fibreoptic bronchoscopy for diagnostic purposes. Only areas of lung which were radiologically normal and

which had airways of normal appearance on inspection were lavaged. Initially, ten subjects (seven males) underwent CL (5x4 ml) and PL (5x20 ml) in the same lung. Five fractions of 4 ml were chosen for CL because it was tentatively estimated that 4 ml would be the maximum volume of airway lavaged, and the aim was maximum recovery. The PL protocol had therefore to consist of five fractions to be comparable to CL. 20 ml was chosen because it was estimated that the volume of lung filled would be roughly one third of that filled by a 50 ml bolus of NaCl.

In the second part of this study, ten patients (six males) underwent BAL (4x50 ml) in one lung, in addition to PL (4x20 ml) and two CLs each (4x2 ml) in the contralateral lung. The fluid for CL and PL was instilled in four fractions in order to achieve parity with the standard BAL protocol used in this laboratory. CL was instilled in 2 ml fractions because the results of the preliminary study indicated that if 4 ml were instilled, the majority of the fluid spilled into adjacent airways and was lost. The wash was repeated in a second airway in order to increase the amount of material obtained for analysis.

Clinical data for each patient including the final diagnosis is presented in table 1.

Lavage collection and processing

Premedication of patients was as previously described [12] except that in patients undergoing BAL as well as PL and CL, 5% cocaine given by transtracheal injection was used to anaesthetize the vocal cords and upper airways. An Olympus BF_1T fibreoptic bronchoscope was used for all types of lung lavage. BAL was performed as described previously [12], using 4x50 ml portions of warmed 0.15 M NaCl.

Central lavage was achieved following passage of a 5F double lumened balloon-tipped Swan-Ganz catheter (Kimal Scientific Products Ltd, Uxbridge, UK) through the biopsy channel of the unwedged bronchoscope. The tip of the catheter was extended up to 2.5 cm beyond the tip of the bronchoscope (above the 5th or 6th generation). The catheter was then wedged by inflating the balloon. A 2 or 4 ml sample of warmed 0.15 M NaCl was then introduced into the airway immediately proximal to the balloon via the opening of the second lumen in the outer wall of the catheter. The lavage fluid was then aspirated back through the same opening. This was repeated a further three or four times (table 1) and the aspirates pooled [13].

Table 1. – Clinical and lung function data of patients studied in this investigation

Subjects	Sex	Age	Smoking status	Final diagnosis	FEV_1	FVC	FRC	VC	TLC	Kco
n		yr			% predicted value					
1	M	37	NS	Normal lung	127	145	121	126	125	106
2	F	59	NS	Normal lung	106	96	51	93	77	105
3	F	54	S	Normal lung	88	81	72	78	77	102
4	M	61	S	Resolving pneumonia	–	–	–		–	–
5	M	63	S	Right middle lobe collapse	38	75	178	77	122	83
6	F	54	S	COAD	–	–	–	–	–	–
7	M	64	S	COAD	77	64	68	80	75	122
8	M	44	S	Normal lung	103	107	-	-	-	-
9	M	72	ES	Bronchial Ca	30	72	83	76	90	94
10	M	53	S	Bronchial Ca	–	–	–	–	–	–
11	M	52	S	Bronchial Ca	90	79	70	74	73	–
12	F	94	S	Bronchial Ca	–	–	–	–	–	–
13	M	72	ES	COAD and resolving pneumonia	43	76	–	–	–	–
14	M	35	S	COAD and resolving pneumonia	77	71	57	81	70	71
15	M	71	S	Fibrosing alveolitis	88	93	73	90	76	106
16	F	77	NS	Fibrosing alveolitis	115	89	74	89	68	54
17	F	58	ES	Pleural effusion	95	101	92	85	85	69
18	M	69	ES	Pleural effusion	54	60	43	65	61	142
19	M	59	S	Mesothelioma	–	–	–	–	–	–
20	F	71	ES	Scleroderma	73	66	–	–	–	–
Mean (SD)		61 (14)			80 (29)	85 (22)	82 (36)	85 (15)	83 (20)	96 (25)

NS: non-smoker; ES: ex-smoker; S: smoker; Ca: carcinoma; COAD: chronic obstructive airway disease; normal lung: bronchoscoped for reported haemoptysis or cancerphobia; – : data not available; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; FRC: functional residual capacity; VC: vital capacity; TLC: total lung capacity; Kco: carbon monoxide transfer coefficient.

Peripheral lavage was achieved in a similar way to CL. Following removal of the CL catheter, the bronchoscope was wedged at the 4th generation and a 6F Swan-Ganz catheter passed through the biopsy channel. This was extended up to 5 cm beyond the tip of the bronchoscope (about the 7th or 8th generation) and the balloon inflated. Twenty millilitre portions of warmed 0.15 M NaCl were instilled into the peripheral lung through a hole in the catheter distal to the balloon. During aspiration of PL, the patient inhaled several times to prevent airway collapse and facilitate fluid recovery [13].

The aspirated lavage fluid of each type was pooled, filtered through gauze to remove mucus and concentrated ten-fold by ultrafiltration under nitrogen in a stirred cell (Amicon Ltd) containing a membrane with a nominal cut-off of 500 Da (Amicon Ltd) [12]. Unpublished data from this laboratory show a 5–10% protein loss during this procedure (SMITH and TETLEY, unpublished observation).

Validation of lavage techniques

In order to establish which regions of the lung were being washed by the three techniques described above, the lavages were carried out using a single instillation of a radio-opaque contrast medium (propyliodone 50% w/v in aqueous suspension, benzylalcohol 1.15% w/v, sodium citrate 0.75% w/v) diluted 1:1 in 0.15 M saline. The volumes of fluid instilled were identical to those used during saline lavage: *i.e.*, BAL, 50 ml; CL, 2 ml; PL, 20 ml. Bronchograms were taken within five minutes of instillation of the medium. The BAL, CL and PL bronchograms were each taken in a different subject. Two observers each measured the diameters of the two largest airways (one for CL) visualized by the contrast medium. Approximately eight measurements were made along the length of each airway, half by each observer. The magnification factor of the X-rays was calculated by measuring the diameter of the bronchoscope tip image on the X-ray and relating it to the diameter of the actual instrument. The real airway diameter was then related to a published anatomical description of the human respiratory tract [14].

Protein levels

Total protein was measured by the method of LOWRY *et al.* [15] using bovine serum albumin as a standard. Albumin, α_1-PI and α_1-antichymotrypsin (α_1-ACh) were measured by rocket immunoelectrophoresis [16] using monospecific antisera obtained from DAKO Ltd, High Wycombe, UK and standardized against protein standard plasma and standard human serum supplied by Behring, Hounslow, UK. Bronchial mucus proteinase inhibitor (BMPI) was estimated by radial immunodiffusion using antisera and standards prepared in this laboratory as described previously [7, 17].

Elastolytic activity

Elastase activities were assessed in those lavages in which sufficient material was available. Elastolytic activity was measured against a tritiated insoluble elastin substrate by the method of BANDA and WERB [18] modified as previously described [12]. Human neutrophil elastase (NE) obtained from Dr N.A. Roberts of Roche Products Ltd, Welwyn Garden City, UK was used as a standard.

Statistics

The diameters of the largest airways visualized on the bronchograms were compared by means of a Student's t-test for unpaired data and also using a Mann-Whitney U test. Similar statistical significances were obtained with parametric and non-parametric statistics. Differences between the lavage types were evaluated by the Wilcoxon signed rank test for paired data, *i.e.* lavages were compared only within, not between individual subjects. In all analyses, $p<0.05$ in a 2-tailed test was taken as the level of statistical significance. All the data are expressed as individual values and summarized as means and standard deviations, and as medians with the interquartile ranges. For PL and CL the complete data and the subgroup of values paired with BAL data are summarized separately.

Results

Validation of lavage techniques

The bronchograms shown in figure 1 illustrate the regions of lung lavaged during BAL, PL and CL. BAL fills a greater area of lung and washes larger airways than PL, whilst CL lavages a small, localized region of the central airway (fig. 1). The mean diameters of the largest airways washed by each technique are all significantly different from each other (table 2). Using these measurements, it is possible to estimate the largest generation of airway lavaged (table 2) by reference to anatomical descriptions of the lung [14].

Table 2. – Comparison of largest airways observed on bronchograms following bronchoalveolar, central and peripheral lavage

	BAL	CL	PL
Number of measurements	16	9	15
Diameter of largest radio-opaque airways mm**	3.0±0.3	6.5±0.2	1.3±0.2
Estimated generation of largest radio-opaque airway	4–5	2–3	8

**: CL>BAL>PL, $p<0.05$; Mean±SD calculated from at least n=9 measurements made by two observers measuring the X-rays independently as described in the text. BAL: bronchoalveolar lavage; CL: central lavage; PL: peripheral lavage.

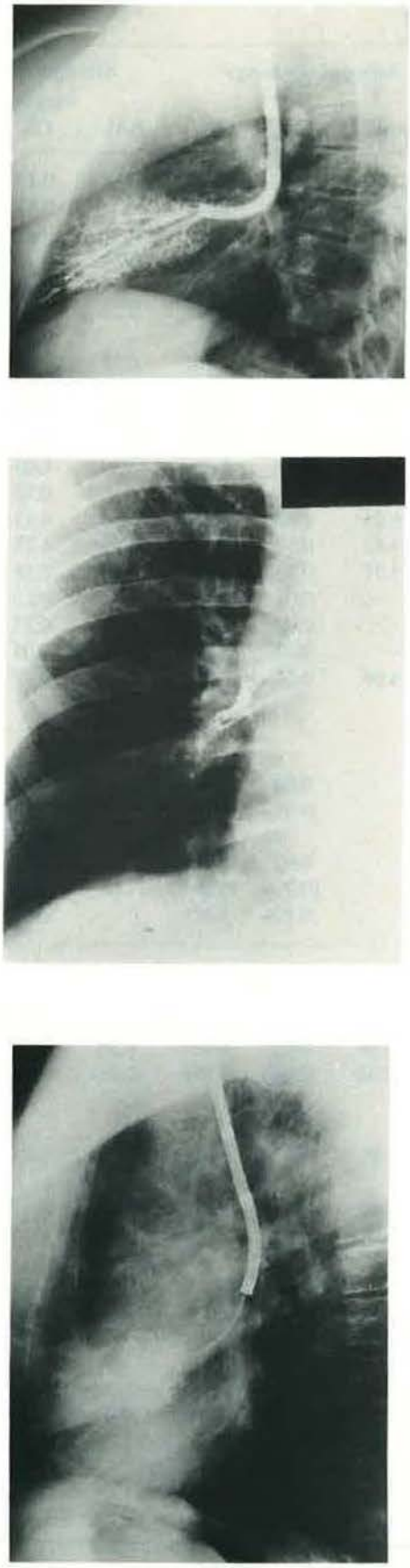

Fig. 1. – Bronchograms demonstrating the distribution of fluid during: a) bronchoalveolar; b) central and; c) peripheral lavage techniques.

Comparison of differential lavage techniques

The data from the preliminary and second studies are presented as a single group.

Fluid and cells. Less fluid was recovered from CL than PL, and from PL than BAL (table 3). In contrast, a significantly lower percentage of the instilled saline was recovered from PL than from other lavage types, although there were no differences in the percentage fluid recovery between BAL and CL (BAL, 50±9%; CL, 36±9%; PL, 23±12%; PL<BAL and CL; $p<0.05$). Since the proportion of CL fluid recovered in the preliminary study was only 30% and the bronchoscopist could see fluid spilling into the proximal airways, the lavage protocol was modified for the subjects studied in the second part of the investigation. Saline was instilled in 2 ml rather than 4 ml fractions. Two airways were lavaged rather than one, in order to increase the amount of material recovered for analysis.

Significantly fewer cells were recovered in total from CL than PL, and from PL than BAL (table 3). However, when the cell number was expressed per millilitre recovered fluid, there was no difference between BAL and PL, but both were significantly greater than CL (BAL, $0.69\pm0.52\times10^{6}\cdot ml^{-1}$; CL, $0.05\pm0.07\times10^{6}\cdot ml^{-1}$; PL, $0.46\pm0.50\times10^{6}\cdot ml^{-1}$; CL<BAL and PL; $p<0.05$). Cell

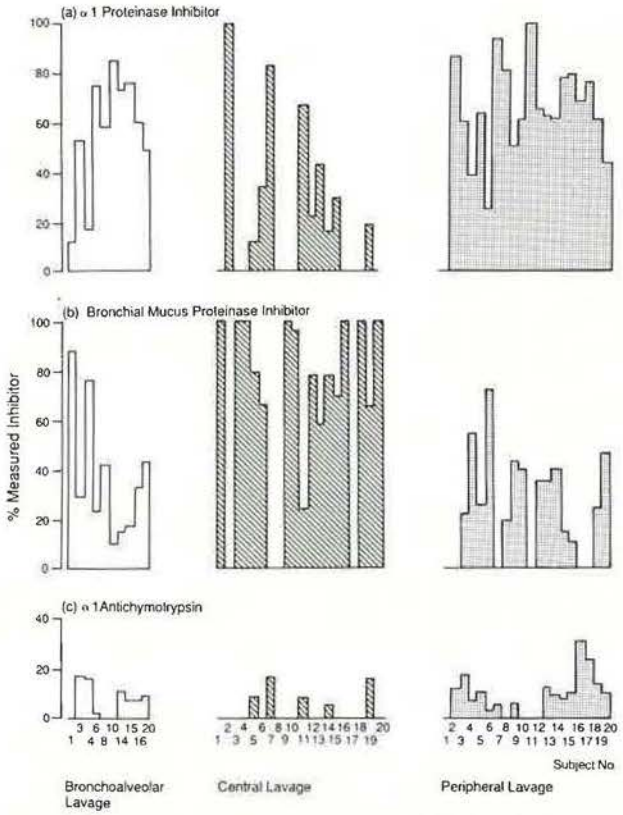

Fig. 2. – Proportions of measured inhibitors in bronchoalveolar, central and peripheral lavages: a) α_1-PI, BAL and PL>CL, $p<0.05$; b) BMPI, BAL and PL<CL, $p<0.05$; c) α_1-ACh, BAL and PL>CL, $p<0.05$. α_1-PI: α_1-proteinase inhibitor; BAL: bronchoalveolar lavage; PL: peripheral lavage; CL: central lavage; BMPI: bronchial mucus proteinase inhibitor; α_1-ACh: α_1-antichymotrypsin.

Table 3. – Basic bronchoalveolar, central and peripheral lavage data

n	Fluid Recovery ml·lavage^{-1}			Cell Recovery x10^6·lavage^{-1}			Protein mg·lavage^{-1}			Albumin mg·lavage^{-1}			Albumin/Protein mg/mg		
	BAL	CL	PL	BAL	CL	PL	BAL	CL	PL	BAL	CL	PL	BAL	CL	PL
1	114	4	13	9.6	0	2.2	4.49	0.04	0.09	2.90	0.01	0.04	0.65	0.17	0.44
2*	–	6	53	–	0	9.6	–	0.73	14.52	–	0.27	1.65	–	0.37	0.11
3	123	4	26	55.4	0.2	12.0	18.30	0.05	2.51	8.79	0.02	1.16	0.48	0.44	0.46
4	97	4	10	54.3	0.1	2.5	6.67	0.04	12.54	1.41	0	1.50	0.21	0	0.12
5*	–	6	36	–	0	13.7	–	0.08	3.81	–	0.02	1.29	–	0.25	0.34
6	100	5	18	71.0	0.8	5.9	8.21	0.08	0.86	1.68	0.02	0.23	0.21	0.23	0.27
7*	–	6	26	–	0	8.6	–	0.09	1.70	–	0.07	0.45	–	0.78	0.27
8	110	4	15	58.3	0.2	5.9	5.46	0.02	0.37	1.91	0	0.10	0.35	0	0.27
9*	–	6	16	–	0	1.4	–	0.13	1.68	–	0.01	0.55	–	0.08	0.33
10	80	5	10	138.4	0.2	5.0	5.40	0.06	0.93	1.34	0.01	0.37	0.25	0.18	0.40
11*	–	8	8	–	0	NA	–	0.69	0.40	–	0.10	0.11	–	0.15	0.28
12*	–	5	31	–	0	7.7	–	1.08	3.15	–	0.10	1.60	–	0.09	0.51
13*	–	5	12	–	0	26.3	–	0.42	2.50	–	0.11	1.67	–	0.26	0.67
14	35	5	37	52.2	0.5	43.5	10.92	0.22	3.20	6.08	0.07	1.51	0.56	0.33	0.47
15	95	5	14	35.2	1.3	11.9	12.40	0.24	2.40	3.65	0.30	0.41	0.29	1.25	0.17
16	120	5	15	56.8	0.8	1.8	17.10	0.10	1.63	5.21	0.03	0.49	0.31	0.28	0.30
17*	–	8	40	–	0	2.6	–	0.04	4.88	–	0.01	0.92	–	0.25	0.19
18*	–	5	36	–	0.4	7.7	–	0.33	2.93	–	0.09	0.74	–	0.27	0.25
19*	–	5	52	–	0	27.3	–	0.42	1.88	–	0.02	0.68	–	0.05	0.36
20	110	4	15	52.8	0.2	4.2	28.40	0.07	1.21	6.66	0.01	0.49	0.24	0.17	0.41
All CL and PL data															
Mean		5	24		0.2	10.5		0.25	3.16		0.06	0.80		0.28	0.33
(SD)		(1)	(14)		(0.4)	(10.9)		(0.29)	(3.76)		(0.08)	(0.57)		(0.29)	(0.14)
Median		5	17		0.1	7.7		0.10	2.14		0.02	0.59		0.24	0.32
(IQ range)		(5–6)	(14–36)		(0–0.3)	(3.0–12.0)		(0.06–0.38)	(1.07–3.18)		(0.01–0.10)	(0.39–1.40)		(0.12–0.31)	(0.26–0.43)
Data paired to BAL															
Mean															
	98	5	17	58.4	0.4	9.5	11.72	0.09	2.6	3.96	0.05	0.63	0.35	0.31	0.33
(SD)	(26)	(1)	(8)	(32.6)	(0.4)	(12.5)	(7.60)	(0.08)	(3.6)	(2.60)	(0.09)	(0.55)	(0.16)	(0.36)	(0.12)
Median															
	105	5	15	54.9	0.2	5.5	9.57	0.07	1.42	3.28	0.02	0.45	0.30	0.21	0.35
(IQ range)															
	(95–114)	(4–5)	(13–18)	(52.2–58.3)	(0.2–0.8)	(2.5–11.9)	(5.46–17.10)	(0.04–0.10)	(0.86–2.51)	(1.68–6.08)	(0.01–0.03)	(0.23–1.16)	(0.24–0.48)	(0.17–0.33)	(0.27–0.44)

*: CL (5x4 ml) plus PL (5x20 ml); lavage protocol of other subjects described in text; NA: not assessed; -: no BAL; IQ: interquartile range; BAL>PL>CL, $p<0.05$ for fluid, cell, protein and albumin data. No significant differences for albumin/protein. BAL: bronchoalveolar lavage; CL: central lavage; PL: peripheral lavage; n: subject number.

profiles were established only for a minority of CL (n=3) and PL (n=2) due to the small numbers of cells recovered. In BAL the majority of recovered cells were alveolar macrophages (AM, 77.6±12.2%) with fewer polymorphonuclear neutrophils (PMN, 10.9±11%) and lymphocytes (10.6±7.6%). Where analysed, PL was found to be similar to BAL in cell profile, but CL was different (AM 24±14%, PMN 70±10%, lymphocytes 5±3%)

Protein levels. Significantly more total protein was recovered in BAL than in either type of selective lavage (table 3). In addition, more total protein was recovered in PL than CL (table 3). Similarly albumin recovery was higher in BAL than PL and in PL than CL (table 3). There were no statistically significant differences between lavages in the recovery of albumin per unit of total protein, although albumin/protein tended to be lower in CL than in the other lavage types

Table 4. – Proteinase inhibitor and elastase activity data from bronchoalveolar, central and peripheral lavage

n	α_1-PI/Albumin mg/mg			α_1-ACh/Albumin mg/mg			BMPI/Albumin mg/mg			EA/Albumin pmol NEE/mg			EA/Inhibitor pmol NEE/mg		
	BAL	CL	PL	BAL	CL	PL	BAL	CL	PL	BAL	CL	PL	BAL	CL	PL
1	0.01	<0.01	<0.01	<0.01	0	0	0.03	0.33	<0.01	344	556	677	6.16	0.95	NA
2	–	0.02	0.24	–	0	0.04	–	<0.01	<0.01	–	NA	0	–	NA	0
3	0.02	<0.01	0.06	0.01	0	0.03	0.01	0.10	0.01	5	45	9	0.16	0.25	0.11
4	0.01	*	0.06	0.03	0	0.01	0.04	*	0.03	3545	*	1	41.78	0.08	0.01
5	–	0.20	0.14	–	0.20	0.03	–	0.50	0.02	–	NA	0	–	NA	0
6	0.17	0.69	0.06	0.01	0	0.01	0.02	0.46	0.06	3	124	11	0.02	0.10	0.07
7	–	0.14	0.21	–	0.04	0.02	–	0	0	–	NA	70	–	NA	0.51
8	0.05	0	0.12	<0.01	*	<0.01	0.01	0	0.01	6	*	NA	0.12	NA	NA
9	–	<0.01	0.13	–	0	0.02	–	0.20	0.04	–	NA	0	–	NA	0
10	0.19	0	0.10	<0.01	<0.01	0	0.01	0.02	0.02	8	100	3	0.06	0.38	0.03
11	–	0.27	0.18	–	0.05	<0.01	–	0.04	0	–	NA	262	–	NA	2.38
12	–	0.13	0.12	–	<0.01	<0.01	–	0.17	0.02	–	NA	0	–	NA	0
13	–	0.24	0.21	–	<0.01	0.06	–	0.12	0.03	–	NA	10	–	NA	0.05
14	0.08	0.04	0.09	0.02	0.02	0.02	0.01	0.07	0.02	2	38	2	0.03	0.23	0.02
15	0.15	0.02	0.16	0.02	<0.01	0.02	0.01	0.01	0.01	2	NA	NA	0.02	NA	NA
16	0.06	<0.01	0.10	0.01	0	0.02	0.01	0.13	<0.01	181	900	36	3.10	0.39	0.48
17	–	<0.01	0.06	–	0	0.04	–	0	0	–	NA	0	–	NA	0
18	–	<0.01	0.09	–	<0.01	0.04	–	0.07	0	–	18	2	–	0.15	0.03
19	–	0.35	0.41	–	0.45	0.12	–	0.45	0.06	–	NA	0	–	NA	0
20	0.08	<0.01	0.05	0.02	0	0.01	0.03	0.17	0.02	<1	30	1	<0.01	0.10	0.01
All data															
Mean		0.10+	0.13		0.04	0.02		0.15**	0.02		125	60		0.29	0.22
(SD)		(0.18)	(0.09)		(0.11)	(0.03)		(0.17)	(0.02)		(178)	(166)		(0.27)	(0.58)
Median		0.02	0.11		<0.01	0.02		0.10	0.02		73	2		0.23	0.02
(IQ)		(<0.01–	(0.07–		(0–	(0.01–		(0.01–	(<0.01–		(34–	(0–		(0.10–	(0–
		0.19)	0.17)		0.01)	0.06)		0.19)	0.03)		340)	11)		0.38)	0.08)
Data paired to BAL															
Mean															
	0.08	0.08	0.08	0.01	<0.01"	0.01	0.02	0.14**	0.02	410	256	93	5.14	0.31	0.11
(SD)	(0.07)	(0.22)	(0.04)	(0.01)	(0.01)	(0.01)	(0.01)	(0.16)	(0.02)	(1108)	(339)	(236)	(13.03)	(0.29)	(0.17)
Median															
	0.07	<0.01	0.09	0.01	0	0.01	0.01	0.10	0.02	6	100	6	0.09	0.24	0.03
(IQ)	(0.02–	(<0.01–	(0.06–	(<0.01–	(0–	(<0.01–	(0.01–	(0.02–	(0.01–	(2–	(40–	(2–	(0.02–	(0.10–	(0.02–
	0.15)	0.03)	0.10)	0.02)	<0.01)	0.02)	0.03)	0.21)	0.02)	181)	424)	24)	3.10)	0.38)	0.10)

NA: not analysed; *: albumin not detected; -: lavage not available; NEE: neutrophil elastase equivalents, molecular wt, 30,000; α_1-PI: α_1-proteinase inhibitor; α_1-Ach: α_1-antichymotrypsin; BMPI: bronchial mucus proteinase inhibitor; EA: elastase activity; IQ: interquartile range; +:<PL, p<0.05; ": <BAL, p<0.05; **: >PL and BAL, p<0.05; (statistics relate to paired data only). n: subject number.

(table 3). α_1-PI/albumin was significantly lower in CL than PL and tended to be lower in CL than in BAL, whilst α_1-ACh/albumin was lower in CL than BAL only (table 4). In contrast, the BMPI/albumin ratio of CL was significantly greater than that of PL or BAL (table 4). When the data were expressed as a percentage of the total measured antiproteinase, BMPI was significantly higher in CL than in BAL or PL, whilst α_1-PI and α_1-ACh were significantly lower (fig. 2).

Elastolytic activity. Due to a shortage of some lavage fluids, assessments of elastase activity were only made in 8 CL and 18 PL although all 10 BAL samples were assayed. Thus, when the data were analysed using the Wilcoxon signed rank test, only paired data were included in the calculations, (BAL *vs* CL, 7 pairs; BAL *vs* PL, 8 pairs; CL *vs* PL, 8 pairs). There were no significant differences between lavages however the data were expressed, although elastase activity per unit inhibitor tended to be higher in CL than PL (table 4).

Discussion

The physical evidence obtained from the use of radio-opaque contrast medium demonstrated that the three techniques used in this study are washing different regions of the lung. The bronchograms show that the regions lavaged vary in size depending on the technique used (BAL>PL>CL, fig. 1, table 2) and this is reflected in the quantity of proteins and the numbers of cells recovered (table 3). Using these techniques we were able to confirm that the protein profiles differed at different levels of the respiratory tract. Concentrations of BMPI were elevated in CL compared to other lavage types, whilst the concentrations of α_1-PI were lower (table 4, fig. 2), confirming the usefulness of these proteins as central and peripheral markers, respectively. In addition, the similarity of the BAL and PL protein profiles suggested that in these subjects at least, the central airways contributed little to the protein content of BAL.

Recoveries of fluid and proteins after BAL were similar to our earlier investigations of patients [12] and to other studies [19, 20], indicating that the BAL data provided a standard comparable to earlier publications against which to assess the differential techniques. Cellular recoveries after BAL were comparable to our earlier study in smoking patients [12] and are higher than for healthy non-smokers, as previously described [19, 20].

After PL only about 30% of the fluid was recovered (table 3). The fluid lost may be passing into adjacent acini or out of the alveolar space and into the interstitium. Data from a sheep model used in this laboratory [21] show that following lung lavage, lymphatic drainage from the lung increases, implying that lavage fluid is crossing into the interstitium and, thus, cannot be aspirated out of the lung. The low fluid recovery observed after PL in the present investigation was not a result of studying patients, since similar volumes are recovered from healthy volunteers after this type of lavage (TETLEY *et al.*, unpublished data).

In contrast, after CL, a constant volume was recovered per aliquot instilled, irrespective of the volume of that aliquot (table 3) and this compared well with recoveries obtained using a similar, recently described technique of airway lavage [22]. For CL, the recovered fluid represents the volume of airway lavaged (above the 5th or 6th generations), whilst it is most likely that the fluid lost reflects spillage into the proximal airways. The very small volume of airway washed by the CL technique explains the low cellular recovery (table 3).

Total protein levels were greater in BAL than PL, and in PL than CL in all but two of the twenty subjects irrespective of their smoking or clinical status. This consistency suggests that the differences actually reflect differences in the lavage type rather than random heterogeneity within the lung. As fluid recoveries are so variable within and between lavages, it is important to consider whether the composition of a lavage sample reflects that of the region washed, particularly when the volume aspirated is low. The BAL data suggest that fluid recovery is not a major determinant of protein recovery, since there is no correlation between the amount of protein and the volume of fluid recovered, although there is a positive correlation between total protein and the number of aliquots of fluid instilled per lavage (table 3). This may be because most of the protein is removed from the epithelial surface by the initial washes or because the volume of saline instilled is so large in relation to the amount of protein to be solubilized, that fluid loss does not prevent recovery of a high proportion of the total protein. In PL, the yields of both fluid and protein are low, but the inhibitor profile is still similar to that of BAL (table 4, fig. 2). Study of the individual data, *e.g.* subjects nos 14 (poor BAL, good PL) and 20 (poor PL, good BAL) reinforce this view. The variability of protein profiles in CL is more likely to arise from the presence or absence of bronchial hypersecretion, than from fluid recovery which is relatively consistent (table 3).

Since the volume of fluid recovered seems to be relatively unimportant in determining protein content, it is clearly unsatisfactory to express data per millilitre of fluid. In this laboratory data are often expressed as amounts recovered per lavage, since this shows up changes resulting from altered epithelial permeability, for example the increase in serum-derived proteins observed in lung lavage fluids from patients with sarcoidosis [23]. The conventional reference standard, albumin, has been used in the current studies to permit comparison with published data. However, the tendency for the albumin/protein ratio to be lower in CL than in the other lavage types (table 3) requires explanation. It is possible that in central airways the amount of albumin per unit area is the same as in the periphery, but that the total protein is boosted by locally-produced proteins derived from epithelial or inflammatory cells, or from the sero-mucous glands (since mucus is filtered off before assay, protein in this fraction will not contribute to the measured protein profile of CL). Alternatively, the presence of a mucous layer and the thick bronchial epithelium could result in a reduction in transport of albumin from the blood to the airway surface by creating a physical barrier which does not exist in the peripheral lung. The current data may be explained by either of these alternatives, or by a combination of both.

The absence of statistically significant differences in elastase activities between lavages may result from either the small number of paired data available for analysis or from the highly varied clinical and smoking status of the subjects studied, since some diseases may preferentially alter elastase activities at only one level of the respiratory tract.

The proteinase inhibitor profile was quite different at different levels of the airway. As predicted by immunohistochemical studies which show locally-produced inhibitors to be particularly abundant in the upper and central airways [5, 6], BMPI was the predominant inhibitor of CL (fig. 2, table 4). In contrast, α_1-PI was the principal inhibitor measured in PL except in a few

individuals, *e.g.* subject no. 1. Despite the absence of α_1-PI in PL and the low levels in BAL from this subject, he was not genetically deficient in this inhibitor since he had normal plasma levels (1.7 $mg \cdot ml^{-1}$) and we cannot explain this unusual observation. α_1-ACh was present only in low proportions in all lavage types (fig. 2, table 4). It may be significant that the only two subjects in whom α_1-ACh contributed more than 20% of the total measured inhibitors in their PL were the two who had pleural effusions (subjects nos 17 and 18, fig. 2). The high levels of α_1-ACh in these subjects could result from changes in epithelial permeability, increases in serum α_1-ACh levels, changes in local production of the inhibitor, or a combination of these factors. The very low levels in all other subjects suggest that, normally, α_1-ACh plays only a minor role in the protection of the lung from proteolytic attack even though it can be produced locally by alveolar macrophages [24, 25].

In this study population the similarity of the BAL and PL inhibitor profiles, which were quite different to those of CL, suggest that, at least in these individuals the airways contributed little to the BAL inhibitor content. However, this may not be true in all subjects. For example, an earlier study from this laboratory suggested that in patients with bronchial hypersecretion, the central airways may make a greater contribution to the protein content of BAL fluid [13]. Furthermore, in this study, we have compared only a small number of variables. Many other parameters such as phospholipids, immunoglobulins, enzymes and inflammatory mediators, are of equal interest and their distribution may or may not be adequately represented by the technique of conventional bronchoalveolar lavage.

In summary, two techniques have been described whereby the central airways or peripheral lung (below the 7th or 8th generation) can be selectively lavaged without any contribution of material from higher levels. In the subjects investigated the central airways components appeared to make only a minor contribution to the proteinase inhibitors detected in BAL fluid. However, this may not be true in all patients or for parameters of interest to other investigators, and we would therefore suggest that a selective lung lavage technique may be more informative for investigation of some subjects, for example, lavage of the central airways in bronchial asthma may be more revealing than BAL containing material from the affected airways and the normal alveoli [22].

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RÉSUMÉ: Cette étude décrit deux nouvelles techniques de lavage pulmonaire, qui prélèvent sélectivement du matériel, soit dans les voies aériennes centrales, soit au niveau pulmonaire au-delà de la 7e génération bronchique. Les bronchogrammes ont confirmé que des régions définies du poumon ont été lavées par le lavage central (CL; diamètre des voies aériennes maximum: environ 6.5 mm) et par le lavage périphérique (PL; diamètre des voies aériennes maximum: environ 1.3 mm), et que les deux pouvaient être distingués clairement du lavage broncho-alvéolaire conventionnel (BAL). Ces techniques ont été utilisées pour établir si oui ou non les protéines des grandes voies aériennes contribuaient de façon importante au profil protéique du lavage broncho-alvéolaire. Nous avons investigué vingt patients consécutifs qui subissaient une fibro-bronchoscopie de routine. Le lavage central ramenait plus d'inhibiteurs des protéinases du mucus bronchique par unité d'albumine et par unité d'anti-protéinase totale mesurée dans le lavage central que dans le lavage périphérique ou le lavage broncho-alvéolaire. Par contre, l'inhibiteur de l'alpha-1-protéinase (par unité d'albumine et en pourcentage des anti-protéinases totales mesurées) s'avère plus faible dans le lavage central que dans les autres types de lavage. Il n'y a pas de différence dans l'activité élastasique, quelle que soit la façon dont les données sont exprimées. Puisqu'il n'y a aucune différence entre lavage broncho-alvéolaire et lavage périphérique pour aucune des variables mesurées, on conclut que chez les sujets étudiés la contribution des protéines du lavage central au lavage broncho-alvéolaire était minimale.