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Alveolar lavage fluid (ALF) of normal volunteer subjects: cytologic, immunocytochemical, and biochemical reference values

S. SUTINEN*, H. RISKA, R. BACKMAN, S. H. SUTINEN AND B. FRÖSETH

Mjölbolsta Hospital, SF-10350 Meltolan sairaala, Finland

Objective: Pooled bronchoalveolar lavage fluid (BALF), the return of lavage, contains both bronchial and alveolar material which differ from each other. Artifacts may be created by filtering, centrifuging and washing cells before cytopreparation. This study presents reference values of healthy volunteers for the alveolar sample, ALF, cytopreparation being performed without filtration or centrifugation.

Methods: Eighteen healthy, non-smoking volunteers underwent a standard bronchoalveolar lavage using 10 aliquots of 20 ml of saline. Excluding the return of the first and second aliquots, the rest were pooled and examined cytologically, immunocytochemically and biochemically. The mean, standard deviation, and 95% confidence limits were calculated for the following variables: amount of return, estimated content of epithelial lining fluid (ELF), total and differential cell counts on filter and cytocentrifuge (CCF) preparations, computed cell counts per unit volume of ALF, distribution of lymphocyte subgroups CD3+CD2, CD4, CD8, CD19, CD25 and CD57, and the ratio of CD4 to CD8, the amounts of lymphocytes in the same subgroups per volume of ALF, and the concentrations of total protein, albumin, immunoglobulins A, G and M, hyaluronic acid, eosinophilic cationic protein (ECP), procollagen III aminoterminal propeptide (PCP) and β_2-microglobulin in ALF and in ELF, as well as the ratios of the concentrations of the solutes in ALF to the same in serum.

Results: The 95% confidence limits of means for the most important variables were as follows: estimated ELF content 0·42–0·74%; total cells in ALF 76·6–143·0 × 10^6 l^{-1}; distribution of inflammatory cells on filter and CCF slides: macrophages 74·9–83·6 and 81·4–90·1%, lymphocytes 13·1–22·5 and 8·1–16·4%, and neutrophils 1·0–4·1 and 0·7–2·7%, respectively; distribution of lymphocyte subsets: CD3+CD2 85·6–90·6%, CD4 44·3–53·1%, CD8 26·9–35·8%; concentration of solutes in ALF: total protein 44·8–61·3 mg l^{-1}, albumin 15·4–22·2 mg l^{-1}, IgA 1·8–3·4 mg l^{-1}, IgG 3·1–6·1 mg l^{-1}, IgM 0·05–0·26 mg l^{-1}, hyaluronic acid 8·8–11·1 μg l^{-1}, ECP 0·19–0·77 μg l^{-1}, PCP 0·005–0·058 μg l^{-1}, β_2-microglobulin 62·2–81·5 μg l^{-1}.

Conclusions: Our results show that excluding the bronchial sample from ALF of volunteer subjects and omitting filtering and washing before cytopreparation produces cytologic, immunocytochemical and biochemical reference values with reasonable 95% confidence limits to be used in clinical settings.

Introduction

Lung lavage is commonly used to harvest cellular and chemical material from the respiratory parenchyma of the lung for research and diagnosis of interstitial pulmonary diseases (1). Bronchoalveolar lavage fluid (BALF), the return of lavage, represents the contents of both peripheral conducting airways and alveoli. The return of the first part, the bronchial sample, comes mainly from conducting airways while the rest, the alveolar sample, represents the contents of alveoli (2). The cellular profiles of bronchial and alveolar samples differ significantly from each other (3). Thus, reference values for pooled BALF are apparently not valid for the alveolar sample, alveolar lavage fluid (ALF). Unfortunately, very few studies exist in which this distinction has been made.

In pooled BALF there is often bronchial mucus which gives a disturbing background in cytocentrifuge (CCF) preparations. Many laboratories, therefore, filter BALF through gauze, separate cells from supernatant by centrifugation, and wash the cells before making CCF slides (4); sometimes even total cell count has been made after these procedures (5). Saltini *et al.* (6) and Mordelet-Dambrine *et al.* (7)

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*To whom correspondence should be addressed.

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showed that washing destroys an unpredictable amount and variety of cells, and Lam *et al.* (8) observed that even filtering through gauze significantly decreases the total recovery of cells in BALF. A little later, Willcox *et al.* (9) demonstrated that mere centrifugation with resuspension causes a generalized loss of cells. Nevertheless, the routine of filtering and centrifuging pooled BALF is still in common use, especially in immunocytochemical studies (4).

BALF also contains a large amount of active soluble substances (4), but the significance of them is problematic because a reliable method for the determination of the so-called dilution factor does not exist (10). We, however, have demonstrated significant differences in the concentration of several proteins in lavage fluid between healthy controls and patients with interstitial lung diseases (11), as well as differences of the same associated with the activity of sarcoidosis (12). Rennard *et al.* (2) observed considerable differences in the concentrations of several proteins between bronchial and alveolar samples of both normal volunteers and patients. However, most biochemical reference values published so far have been determined on pooled BALF (4). Although the proportion of bronchial material in BALF is relatively small, it may still significantly increase the existing confusion.

In our laboratory the bronchial sample is separated from ALF which is not filtered nor centrifuged before cytopreparation. In this article we describe the method and give some cytologic, immunocytochemical and biochemical reference values for ALF obtained by lavaging a group of healthy non-smoking volunteers.

Materials and Methods

STUDY SUBJECTS

The material consisted of 18 healthy volunteers, 10 men and eight women, with a mean age of 33·4 years (SD 8·3 years), who used no drugs, had a normal result in spirometry and no skin reactivity, nor showed any bronchial obstruction or hyperreactivity to histamine. All were lifetime non-smokers. For some immunocytochemical studies CCF preparations from the specimens of only 14 or 13 subjects were available (Tables 4 and 5) because the supply had been inadvertently exhausted, i.e. in some cases too many CCF preparations had been inadvertently used for technical testing purposes. All subjects had given their informed consent before participation, and the study has been approved by the Ethics Committee at Mjölbolsta Hospital.

STUDY DESIGN

A standard bronchoalveolar lavage was performed on each subject and the fluid recovered was examined cytologically, immunocytochemically and biochemically, and the mean, standard deviation, and 95% confidence limits of the mean were calculated for each variable. The variables determined were as follows: amount of return, estimated content of epithelial lining fluid (ELF) by urea method (13), total and differential cell counts on filter and CCF preparations, computed cell counts per unit volume of ALF, distribution of lymphocytes with surface markers for CD3+CD2, CD4, CD8, CD19, CD25, CD57 and the ratio of CD4 to CD8, as well as the amounts of the same per volume of ALF, and the concentrations of total protein, albumin, immunoglobulins A, G and M, hyaluronic acid, eosinophilic cationic protein (ECP), procollagen III aminoterminal propeptide (PCP) and β_2-microglobulin in ALF and ELF, as well as the ratios of the concentrations of the solutes in ALF to the same in serum.

METHODS

Lavage protocol

BAL was performed as recommended by the European Task Group on BAL (4). The night before lavage the subjects received diazepam 10 mg p.o., and 1–1·5 h prior to the investigation diazepam 10 mg, atropine 0·5 mg, pethidine 50 mg, glycopyrrobromide 0·2 mg, and chlobutinol hydrochloride 20 mg were given i.m. for premedication. Immediately before lavage 10 ml of 2% lidocaine were given via intermittent positive pressure breathing. After local anaesthesia of the pharynx, larynx and trachea with 2% lidocaine, examination of the airways was carried out with a flexible, fibre optic bronchoscope. When necessary, lidocaine was also given also via the bronchoscope and removed immediately with suction.

After general inspection of the airways, a subsegment of the right middle lobe was lavaged. With the end of the bronchoscope wedged, an aliquot of 20 ml of sterile 0·9% NaCl at 37·5°C was instilled 10 times. The effluent was aspirated manually by gentle suction using a syringe.

The fluid recovered from the first and second aliquots (bronchial sample) was excluded from the study. The other aliquots (ALF) were pooled and examined. All fluid recovered was sent to the laboratory in an ice-bath without preservative and processed immediately, always within half an hour.

Cytologic methods

ALF was mixed thoroughly but gently. The total number of cells per μl and % of viable cells were

counted in a Bürker haemocytometer after staining with 0·02% trypan blue (exclusion test) directly from ALF without former centrifugation or resuspension. Then 1 ml of ALF was fixed in an equal amount of 95% ethanol and a Millipore filter preparation was made and stained according to Papanicolaou.

The amount of ALF used for CCF specimens was directed by the total amount of cells so that a monolayer of a total of about 100 000 cells was spread on each CCF slide. The slides were prepared in a Cyto-Tek cytocentrifuge (Ames Division, Miles Laboratories, Elkhart, Indiana 46515, U.S.A.) at 900 rpm for 7 min, air-dried overnight in room temperature, protected from dust, and stained with May-Grünwald-Giemsa.

For differential counts 200 cells were counted on filter preparations and 400 cells on CCF slides. Epithelial cells were counted on filter preparations and their number was subtracted from the total count before calculating the differentials of the inflammatory cells. Epithelial cells were omitted when counting cells on CCF slides.

Immunocytochemical methods

After air-drying, CCF slides for immunocytochemistry were fixed in acetone at −20°C for 10 min and stored at −70°C until used. Primary antisera (Becton-Dickinson Immunocytometry Systems, San Jose, California 95131-1807, U.S.A.) included Anti-Leu-4+Anti-Leu-5b (CD3+CD2), Anti-Leu-3a+3b (CD4), Anti-Leu2a (CD8), Anti-Leu2a (CD8), Anti-Leu-12 (CD19), Anti-Leu-7 (CD57, and Anti-Interleukin-2 Receptor (CD25). Immunostaining of CCF slides was performed using the Vectastain ABC kit (Vector Laboratories, Burlingame, California 94010, U.S.A.). Briefly, the slides were incubated in a moist chamber at room temperature in (1) normal horse serum for 20 min; (2) in primary antiserum for 60 min; (3) in biotinylated secondary antiserum for 30 min; and (4) in avidin-biotin complex for 45 min. After steps 2–4 the slides were washed in phosphate buffered saline. Visualization was performed with diaminobenzidine using the Vector PK 4002 Kit (Vector Laboratories), and finally, the slides were counterstained with Mayer's haematoxylin and covered using a normal mounting medium (Permount, Fisher Scientific, Fair Lawn, New Jersey 07410, U.S.A.).

Biochemical methods

After separating the cytologic samples the rest of ALF was filtered through gauze and centrifuged at 4°C 1000 rpm for 10 min. All biochemical determinations were performed from the ALF supernatant and the serum of each subject.

Urea was determined by an enzymatic method with a spectrophotometer using the BUN reagent provided by Ciba-Corning, Gilford Systems (Oberlin, Ohio 44074, U.S.A.). The apparent epithelial lining fluid content (ELF) in ALF was evaluated by means of the ratio of the urea content of ALF to that of serum (12). For total protein 5 ml of ALF supernatant and 2 ml of 20% trichloracetic acid were centrifuged and a biuret reaction was performed on the sediment. Albumin was determined in a Behring Nephelometer 100 (Behringwerke Aktiengesellschaft, Marburg, Germany) using a procedure recommended by the manufacturer for microalbumin in urine, and immunoglobulins A, G and M in Behring Nephelometer 100 using a procedure recommended for cerebrospinal fluid, with a 10-fold concentration, if necessary, in a Minicon Macrosolute Concentrator (Amicon, Danvers, Minnesota 61923, U.S.A.). Hyaluronic acid was determined using the Pharmacia HA Test 50 RIA kit (Pharmacia Diagnostics AB, S-75182 Uppsala, Sweden) modified for 200 μl of ALF, eosinophil cationic protein using the Pharmacia ECP RIA kit, procollagen III amino-terminal propeptide using the Orion Diagnostica Farmos kit (Orion-Yhtymä Oy, Orion Diagnostica, SF-02101 Espoo, Finland) modified for 400 μl of ALF, and β_2-microglobulin was determined using the Pharmacia $\beta_2$micro RIA kit, modified for low concentrations.

Statistical methods

The means, standard deviations and 95% confidence limits of means for each variable, as well as Wilcoxon signed rank-tests for significance of differences between results of filter and CCF preparations were computed using the statistical package SOLO (BMDP Statistical Software, Inc, Los Angeles, California, U.S.A.) in an Osborne MiStation 3S computer (Osborne Computer, Espoo, Finland). A nonparametric test was used because BAL data are not normally distributed (14).

Results

There were no complications. The numeric results are presented in Tables 1–8. The proportion of monocytes was so small on both filter and CCF preparations (means: 0·08% and 0·69%, respectively) that they were included in macrophages. The proportion and number of monocytes per volume of macrophages were smaller on filter than on CCF preparations (Wilcoxon test, P=0·0010 and P= 0·0007, respectively), and the same of lymphocytes

Table 1 Means, standard deviations and 95% confidence limits of means for lavage return, ELF content, total cells in ALF and ELF, and proportions of living cells and epithelial cells in ALF from 18 healthy non-smoking volunteers

Variable	Unit	Mean	SD	95% confidence limits
Lavage	ml	200		
Total return	ml	171·1	15·1	163·6–178·6
	%	86·0	7·3	82·0–89·1
Estimated ELF content	ml	1·0	0·64	0·71–1·3
	%	0·59	0·34	0·43–0·76
Total cell count				
in ALF	$\times 10^6\,l^{-1}$	107·7	70·3	72·7–142·6
in ELF	$\times 10^8\,l^{-1}$	219·1	135·3	151·9–286·4
Viable cells	%	87·2	7·0	83·7–90·7
Epithelial cells	%	3·2	2·2	2·1–4·3

ALF, alveolar lavage fluid, alveolar sample of bronchoalveolar lavage return;
ELF, epithelial lining fluid, estimated by urea method (10).

Table 2 Means, standard deviations and 95% confidence limits of means for distribution of inflammatory cells in ALF from 18 healthy non-smoking volunteers

Variable	Unit	Mean	SD	95% confidence limits
Filter preparation, Papanicolaou stain				
Macrophages[a]	%	78·5	8·7	74·2–82·8
Lymphocytes	%	18 4	9·6	13·7–23·2
Neutrophils	%	2·7	3·2	1·1–4·3
Eosinophils	%	0·41	0·53	0·14–0·67
CCF-preparation, MGG-stain				
Macrophages[a]	%	85·3	9·0	80·8–89·8
Lymphocytes	%	12·6	8·6	8·3–16·9
Neutrophils	%	1·7	2·1	0·66–2·8
Eosinophils	%	0·35	0·62	0·04–0·66
Mast cells	%	0·08	0·16	0·00–0·16

ALF, alveolar lavage fluid, alveolar sample of bronchoalveolar lavage return;
CCF, cytocentrifuge; MGG, May–Grünwald–Giemsa; [a], including monocytes.

were larger on filter than on CCF preparations (P=0·0015 and P=0·0017, respectively).

Discussion

In this report we give reference values for some common cytologic, immunocytochemical and biochemical variables of the alveolar sample of BALF, which we call alveolar lavage fluid (ALF), determined on 18 healthy non-smoking volunteers using a standard technique of lavaging (4) and handling of the specimen with the least possible damage to the cells. Despite a trend to standardize the procedures, reference values from different laboratories are not directly comparable because significant technical variations still exist. Although the deleterious effects of filtration (8) and centrifugation before cytopreparation (9) have been clearly demonstrated, the procedures are still commonly used (4). Similarly, although significant differences have been demonstrated in cell differentials between bronchial and alveolar samples in patients with interstitial diseases (15) and in healthy controls (2,3), examination of pooled BALF continues in many centres. In fact, we have not found a directly comparable report of reference values for ALF in the literature, in which all these technical pitfalls would have been avoided. Even a very comprehensive recent study on BAL cellularity in normal volunteers has been performed on pooled BALF using conventional methods (14). According to our experience, filtration and centrifugation before cytopreparation of ALF are not needed in order to get rid of mucus if the return of both first and second aliquots are separated from

Table 3 Means, standard deviations and 95% confidence limits of means for amounts of inflammatory cells per volume of ALF from 18 healthy non-smoking volunteers

Variable	Unit	Mean	SD	95% confidence limits
Filter preparation, Papanicolaou stain				
Macrophages[a]	$\times 10^6 l^{-1}$	83·6	56·2	55·7–111·5
Lymphocytes	$\times 10^6 l^{-1}$	21·1	16·5	12·9–29·3
Neutrophils	$\times 10^6 l^{-1}$	2·5	3·0	1·0–4·0
Eosinophils	$\times 10^6 l^{-1}$	0·33	0·59	0·04–0·63
CCF-preparation, MGG-stain				
Macrophages[a]	$\times 10^6 l^{-1}$	92·3	64·0	60·5–124·1
Lymphocytes	$\times 10^6 l^{-1}$	13·4	10·7	8·1–18·7
Neutrophils	$\times 10^6 l^{-1}$	1·6	1·6	0·79–2·4
Eosinophils	$\times 10^6 l^{-1}$	0·28	0·57	−0·01–0·56
Mast cells	$\times 10^6 l^{-1}$	0·06	0·24	−0·06–0·17

ALF, alveolar lavage fluid, alveolar sample of bronchoalveolar lavage return; CCF, cytocentrifuge; MGG, May–Grünwald–Giemsa; [a], including monocytes.

Table 4 Means, standard deviations and 95% confidence limits of means for distribution of lymphocytes with different surface markers in ALF from healthy non-smoking volunteers

Variable	(CD class)	Unit	Mean	SD	95% confidence limits
(n=18)					
T cells	(CD3+CD2)	%	88·0	5·2	85·4–90·6
T helper cells	(CD4)	%	49·0	9·2	44 4–53·6
T suppressor cells	(CD8)	%	30·9	9·3	26·3–35·6
CD4/CD8 ratio			1·7	0·6	1·5–2·0
(n=13)[a]					
B cells	(CD19)	%	2·1	0·9	1·6–2·7
Natural killer (NK) cells	(CD57)	%	10·3	4·3	7·7–13·0
(n=14)[a]					
IL2-receptor positive cells	(CD25)	%	6·4	2·7	4·9–7·9

ALF, alveolar lavage fluid, alveolar sample of bronchoalveolar lavage return; IL-2, interleukin 2; [a], number of patients smaller than total because the supply of CCF preparations was inadvertently exhausted.

the rest. Even lymphocyte surface markers may be demonstrated without filtering or washing the cells. However, after taking all samples for cytology we separate the supernatant by centrifuging, and filter it before biochemical determinations in order to protect the capillary tubing of automatic analysers.

In cytology, we have made both millipore filtration with Papanicolaou staining and CCF preparations with May–Grünwald–Giemsa staining. Saltini *et al.* (6) considered filtration more accurate than CCF but Thompson *et al.* (16) showed that filtration may underestimate the number of neutrophils. Wilcox *et al.* (9) did not observe a significant difference between millipore filter and CCF preparations in cell differentials if serum was not added before making cytospins, and they considered the more expensive filter preparations unnecessary. In our material there were significantly more lymphocytes and less mononuclear phagocytes on filter than on CCF preparations. The same observation was made by Taskinen *et al.* (5), who used the same technique in millipore filtration as us, although their CCF preparations were made after filtration and washing. We also did not make esterase staining on either preparation to distinguish immature macrophages from large lymphocytes which may be confused on Papanicolaou stained filters. Taskinen *et al.* concluded that both millipore filtration and CCF preparations have advantages that complement each other (5). On millipore filter, however, the interpretation of lymphocytosis must be more cautious than in CCF preparations. Recently, Moumouni *et al.* have suggested that lymphocyte

Table 5 Means, standard deviations and 95% confidence limits of means for amounts of lymphocytes with different surface markers per volume of ALF from healthy non-smoking volunteers

Variable	(CD class)	Unit	Mean	SD	95% confidence limits
(n=18)					
T cells	(CD3+CD2)	$\times 10^6 l^{-1}$	12·0	9·8	7·1–16·9
T helper cells	(CD4)	$\times 10^6 l^{-1}$	7·0	6·2	3·9–10·1
T suppressor cells	(CD8)	$\times 10^6 l^{-1}$	4·3	4·0	2·3–6·3
(n=13)[a]					
B cells	(CD19)	$\times 10^6 l^{-1}$	0·36	0·31	0·17–0·54
Natural killer (NK) cells	(CD57)	$\times 10^6 l^{-1}$	1·6	1·2	0·90–2·3
(n=14)[a]					
IL2-receptor positive cells	(CD25)	$\times 10^6 l^{-1}$	0·96	1·2	0·29–1·6

ALF, alveolar lavage fluid, alveolar sample of bronchoalveolar lavage return; IL-2, interleukin 2; [a], number of patients smaller than total because the supply of CCF preparations was inadvertently exhausted.

Table 6 Means, standard deviations and 95% confidence limits of means for some biochemical variables in ALF from 18 healthy non-smoking volunteers

Variable	Unit	Mean	SD	95% confidence limits
Urea	$mM\ l^{-1}$	0·036	0·024	0·024–0·048
Total protein	$mg\ l^{-1}$	53·6	17·4	45·0–62·3
Albumin	$mg\ l^{-1}$	18·8	7·2	15·3–22·4
Immunoglobulin A	$mg\ l^{-1}$	2·7	1·6	1·9–3·5
Immunoglobulin G	$mg\ l^{-1}$	4·8	3·1	3·3–6·4
Immunoglobulin M	$mg\ l^{-1}$	0·11	0·32	− 0·05–0·27
Hyaluronic acid	$\mu g\ l^{-1}$	9·8	2·4	8·6–10·9
Eosinophil cationic protein	$\mu g\ l^{-1}$	0·49	0·62	0·19–0·80
Procollagen III propeptide	$\mu g\ l^{-1}$	0·028	0·067	− 0·005–0·061
β_2-microglobulin	$\mu g\ l^{-1}$	72·6	20·4	62·4–82·7

ALF, alveolar lavage fluid, alveolar sample of bronchoalveolar lavage return.

Table 7 Means, standard deviations and 95% confidence limits of means for some biochemical variables in ELF from 18 healthy non-smoking volunteers

Variable	Unit	Mean	SD	95% confidence limits
Total protein	$g\ l^{-1}$	10·7	4·5	8·5–12·9
Albumin	$g\ l^{-1}$	3·8	1·9	2·9–4·7
Immunoglobulin A	$mg\ l^{-1}$	57	42	36–78
Immunoglobulin G	$g\ l^{-1}$	0·89	0·66	0·56–1·22
Immunoglobulin M	$mg\ l^{-1}$	28	83	− 13–69
Hyaluronic acid	$mg\ l^{-1}$	2·1	1·1	1·6–2·6
Eosinophil cationic protein	$\mu g\ l^{-1}$	89	65	57–121
Procollagen III propeptide	$\mu g\ l^{-1}$	5	12	− 1–11
β_2-microglobulin	$mg\ l^{-1}$	15·2	7·7	11·3–19·1

ELF, epithelial lining fluid, estimated by urea method (10).

loss from CCF slides could arise from poor adherence on slides, exacerbated during aqueous staining if no artifice is used to hold them (17).

In two studies on healthy volunteers with comparable, although not identical, technique (2,3), the distributions of main cytologic variables in ALF did

Table 8 Means, standard deviations and 95% confidence limits of means for ratios of some biochemical variables in ALF to the same in serum from 18 healthy non-smoking volunteers

Variable	Units	Coefficient	Mean	SD	95% confidence limits
ALF/serum urea$_a$	$mM \cdot l^{-1}/mM \cdot l^{-1}$	$\times 10^{-2}$	0·64	0·33	0·48–0·81
ALF/serum total protein	$mg \cdot l^{-1}/g \cdot l^{-1}$	$\times 10^{-3}$	0·77	0·25	0·64–0·89
ALF/serum albumin	$mg \cdot l^{-1}/g \cdot l^{-1}$	$\times 10^{-3}$	0·48	0·20	0·38–0·58
ALF/serum immunoglobulin A	$mg \cdot l^{-1}/g \cdot l^{-1}$	$\times 10^{-3}$	1·45	1·08	0·91–1·99
ALF/serum immunoglobulin G	$mg \cdot l^{-1}/g \cdot l^{-1}$	$\times 10^{-3}$	0·40	0·26	0·27–0·52
ALF/serum immunoglobulin M	$mg \cdot l^{-1}/g \cdot l^{-1}$	$\times 10^{-3}$	0·093	0·29	−0·050–0·23
ALF/serum hyaluronic acid	$\mu g \cdot l^{-1}/\mu g \cdot l^{-1}$	$\times 1$	0·97	0·58	0·68–1·26
ALF/serum ECP	$\mu g \cdot l^{-1}/\mu g \cdot l^{-1}$	$\times 1$	0·078	0·091	0·033–0·12
ALF/serum PCP	$\mu g \cdot l^{-1}/\mu g \cdot l^{-1}$	$\times 10^{-2}$	1·0	2·49	2·00–2·27
ALF/serum β_2-microglobulin	$\mu g \cdot l^{-1}/mg \cdot l^{-1}$	$\times 10^{-3}$	53·3	20·3	43·2–63·3

ALF, alveolar lavage fluid, alveolar sample of bronchoalveolar lavage return;
ECP, eosinophil cationic protein; PCP, procollagen III aminoterminal propeptide;
[a], ALF/serum urea × 100 = ELF% (10); ELF, epithelial lining fluid.

Table 9 Means ± standard errors of means of total cells and differentials in cytocentrifuge preparations in three series of 'alveolar' lavages of normal volunteers

Reference	*n*	Total cells $\times 10^6 l^{-1}$	Macrophages %	Macrophages $\times 10^6 l^{-1}$	Lymphocytes %	Lymphocytes $\times 10^6 l^{-1}$	Neutrophils %	Neutrophils $\times 10^6 l^{-1}$
Rennard (2)	18	145 ± 15	81 ± 2		12 ± 2		3 ± 0·4	
van Vyve (3)	31	146 ± 13	77 ± 3	80 ± 10	7 ± 1	8 ± 3	5 ± 1	6 ± 2
Present study	18	108 ± 17	85 ± 2	85 ± 2	13 ± 2	13 ± 3	2 ± 0·5	2 ± 0·4

not essentially differ from those in our series (Table 9), although the study subjects were derived from ethnically very different populations.

The distribution of common lymphocyte surface markers in this study (Table 4) was fairly similar to that obtained by immunoperoxidase slide assay (18). Our method however, is less tedious and time consuming, and the staining may be automated. In economy the present method competes favourably with flow cytometry if the total amount of lavage specimens is not very large. According to our experience, expressing the results as the number of cells per volume of ALF is more meaningful than mere percentage in evaluating the activity of alveolitis in sarcoidosis (19). Thus, these values are also given for normal controls (Table 5).

It has been stated recently that, regarding the quantitation of soluble components, BAL still represents a technical dilemma (10). According to our experience, however, the concentrations of several proteins in ALF from patients with interstitial lung diseases, especially from those with active alveolitis of sarcoidosis, exceed the range of the same from healthy controls (11,12). We therefore think that, regardless of all limitations, reference values with confidence limits should be published for most important solutes in ALF recovered using a standard technique, so that the role of the method in detecting disease states could be evaluated. In fact, Rennard *et al.* (2) have measured the concentrations of selected proteins in bronchial and alveolar samples of BALF in a group consisting of both healthy volunteers and patients, but have not published the data separately. According to the technical recommendation of the European Task Group on BAL (4), the first aliquot recovered should be analysed separately. Thus, if the recommendation is followed, ALF should be studied instead of BALF. Nevertheless, the reference values published so far have been determined on pooled BALF (4,20).

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