RELATIONSHIP BETWEEN ENZYMATIC MARKERS OF PULMONARY CELL DAMAGE AND CELLULAR PROFILE:

A Study in Bronchoalveolar Lavage Fluid

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□ *It has been suggested that alterations in bronchoalveolar lavage fluid (BALF) reflect pathologic changes in the lung. Cytoplasmatic enzymes such as lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and LDH isoenzymes are recognized indicators of cell damage or death. The aim of this study was to determine whether there is a relation between the enzyme activity and the cell types present in BALF. Therefore, BALF samples obtained from patients with various pulmonary disorders were studied. Out of these samples a group with mainly polymorphonuclear neutrophils (PMNs; n = 15; Group I) and another with mainly alveolar macrophages (AMs; n = 10; Group II) were selected. Additionally, the value of analysis of lysed cells in BALF for assessment of LDH-isoenzyme patterns was examined. The cell-free fraction of BALF of Group II showed lower LDH and ALP activity compared to Group I. The LDH-isoenzyme pattern also differed, with the LDH3/LDH5 ratios being lower in all BALF samples with predominantly PMNs than in BALF samples with predominantly AMs. Lysis of the cells present in the BALF samples by sonication prior to LDH-isoenzyme analysis provided no additional information beyond that found by analysis of the cell-free BALF. In conclusion, determination of enzyme activity appears to be useful in monitoring pulmonary inflammation.*

Parameters measured in bronchoalveolar lavage fluid (BALF) to detect pulmonary damage or inflammation are most often quantitative measures of the degree of the inflammatory response [1–5]. Cellular changes in BALF during inflammation include an activation of alveolar macrophages (AMs) and an influx of polymorphonuclear neutrophils (PMNs) [1–5]. AMs constitute one of the first lines of cellular defense against inhaled particles and pathogens. AMs release factors that attract neutrophils and other macrophages into the lung [3–5]. It has been suggested that the neutrophil influx plays a major role in increasing the permeability of the alveolar/capillary barrier and producing cellular toxicity during the inflammatory response. Furthermore, AMs clear inhaled particles by phagocytosis [4]. During phagocytosis, a number of active oxygen species are generated which can also be injurious to the host organism [6, 7]. A rapid screening test for the early detection of pulmonary inflammation is needed to assess this pulmonary damage.

Recently, it has been suggested that biochemical changes in BALF may be useful for this purpose [8–10]. An increase in the activity of lactate dehydrogenase (LDH) in the recovered BALF or of other enzymes which are normally intracellular reflects cell damage or cell death in the airways. Several pulmonary disorders have been associated with elevated serum as well as BALF LDH activity [9, 11]. An increase in airway LDH activity might arise from diverse sources, including rupture (necrosis) of airway and/or alveolar epithelial cells, AMs, or other pulmonary cell types [12]. Lung parenchymal cells, or local inflammatory cells including AMs and PMNs, may be a potential source of elevation of LDH associated with pulmonary diseases [1, 11–14]. Transudation of serum proteins due to increased permeability of the alveolar/capillary barrier is another potential source of LDH activity [9, 15]. However, less is known about the characteristics of LDH isoenzyme patterns related to pulmonary inflammation and their clinical relevance is generally poor. Alkaline phosphatase (ALP) is a membrane-bound enzyme secreted by pulmonary type II cells along with surfactant and is also present in neutrophils [16–18]. In BALF ALP activity has been associated with type II cell damage or stimulation [16–18].

The purpose of the present study was to determine (1) whether it is possible to identify different ALP, LDH, and LDH isoenzyme patterns of AMs and PMNs, respectively, and (2) whether sonication of cells present in BALF has additional value in identifying the relation between the LDH isoenzyme enzyme patterns in cell-free BALF and the nature of the cells. We hypothesized that AMs and PMNs release different enzymes, particularly LDH isoenzymes. With this knowledge, the LDH isoenzyme pattern, therefore, could

be used as an index of the cellular response involved in lung damage and/or inflammation. To test this hypothesis, we used BALF samples with predominantly PMNs and mainly AMs, respectively, and compared both groups with lung tissue specimens.

METHODS

General Experimental Design

The initial BALF specimens of 68 patients with various pulmonary disorders were studied. The study population included 4 patients who suffered from sarcoidosis (all male; 1 smoker and 3 nonsmokers), 4 patients with drug-induced pneumonitis (all female; all nonsmokers) 5 patients with pulmonary fibrosis (3 male and 2 female; 3 smokers and 2 nonsmokers), 16 patients with other interstitial lung disorders (9 male and 7 female; 8 smokers and 8 nonsmokers), 34 patients who suffered from pneumonia (24 male and 10 female; 20 smokers and 14 nonsmokers) and 5 patients with lung cancer (all male; 3 smokers and 2 nonsmokers). Out of this population, BALF samples with mainly ($> 86\%$) polymorphonuclear neutrophils (PMNs; n $= 15$: Group I; 12 patients with pneumonia, 3 with idiopathic pulmonary fibrosis; 6 nonsmokers, 9 smokers) and predominantly ($> 86\%$) alveolar macrophages (AMs; n $= 10$; Group II; 4 patients with pneumonia, 6 patients with noninfectious diffuse interstitial disease of unknown origin; 4 nonsmokers, 6 smokers) were selected. A group of 8 healthy subjects (8 nonsmokers), without a relevant medical history, was used as a control group (Group III). Biochemical analysis of these samples were only done in the original BALF.

The lung tissue samples were obtained from additional resected normal lung tissue from patients with a $T_1N_0M_0$ squamous cell bronchial carcinoma without further relevant pulmonary history who underwent a lobectomy (n $= 9$: Group IV).

Bronchoalveolar Lavage

BAL was performed as reported previously during fiberoptic bronchoscopy [19]. The procedure is briefly described here. After premedication (atropine and sometimes diazepam) and local anaesthesia of the larynx and bronchial tree (lidocaine 0.5%), BAL was performed by standardized washing of the middle lobe with 4 aliquots of 50 mL sterile saline (0.9% NaCl) at $37°$C. After careful mixing, the BALF recovered was split into 2 portions and kept on ice in a siliconized specimen trap. The first portion was separated from cellular compounds by centrifugation (for 5 minutes with a force of 350g). After an additional centrifugation step (for 10 minutes with a

force of 1000*g*), supernatants were directly stored at -70°C. The cells were washed twice, counted, and suspended in minimal essential medium (MEM; Gibco, Grand Island, New York, USA) supplemented with 1% bovine serum albumin (BSA; Organon, Teknika, Boxtel, the Netherlands). Preparations of the cell suspensions were made in a cytocentrifuge (Shandon; Scientific Ltd.; Ashmoor, England). Cytospin slides of BAL cells were stained with May-Grünwald-Giemsa (MGG; Merck, Darmstadt, Germany) for cell differentiation. At least 500 cells were counted.

Before chemical analyses the second BALF portion was carefully mixed and divided into two portions. One portion was centrifuged at 2000*g* for 15 minutes. LDH, LDH-isoenzymes, and ALP were determined in the obtained cell-free supernatant (Portion I). The other portion of BALF (Portion II) was sonicated in five bursts of 60 seconds each (Sonorex Baudelin, type RK 102 H, 120–240 W, 35 kHz, Berlin, Germany). To prevent heat inactivation of the enzymes, the BALF-containing tubes were kept on ice during sonication. Sonication caused lysis of all cellular constituents whereafter the cytoplasmic enzymes were released into the BALF. After lysis of the cells, this portion of the BALF was also centrifuged at 2000*g* for 15 minutes. LDH, LDH-isoenzymes, and ALP were determined in the sonicated BALF, now containing the cellular enzymes plus the enzymes already present before sonication. Cellular enzyme content or enzyme distribution was obtained by subtracting the total enzyme activity of the sonicated BALF (Portion II) from the enzyme activity present in the original cell-free supernatant (Portion I).

Tissue Preparation

Lung tissue samples were washed in phosphate-buffered saline (pH 7.4) and after gentle blotting to remove adhering moisture, the samples were weighed and immediately frozen at -70°C until use. Frozen samples of between 0.3 to 0.5 g tissue were homogenized (5% weight/volume) in ice-cold phosphate-buffered saline (pH 7.4), in bursts of 7 seconds, with 7-second intervals (Sonorex Bandelinn, type RK 102 H, 120–140 W, 35 kHz Berlin, Germany). Sample homogenization and sonication were performed in tubes kept on ice. One part of the homogenate was used for a total protein determination, to allow determination of the LDH content per gram protein; 2 mL was used to determine the dry weight per mL homogenate, to allow determination of LDH per gram dry weight of tissue.

The suspension was then centrifuged at 3000*g* for 10 minutes in a cooled centrifuge. The supernatant was diluted 1:1 with a pasteurized plasma protein solution (40 g/L from the Dutch Red Cross Blood Transfusion Centre, Amsterdam), LDH and LDH isoenzymes were stable upon freezing in this plasma protein solution. Total protein was performed on a Beckman Synchron CX-7 analyzer, using a timed endpoint biuret method with Beckman reagents (testkit 442740).

Laboratory Tests

The LDH activity was measured at 37 °C by an enzymatic rate method, using pyruvate as a substrate. The test was performed on a Beckman Synchron CX-7 system with Beckman reagents (testkit 442660) and was optimized according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie (DGKC-recommendations) [20]. The reduction of pyruvate to $_L$-lactate with the concurrent oxidation of β-nicotinamide adenine dinucleotide (NADH; reduced form) was monitored at 340 nm. The change in absorbance at 340 nm, caused by the disappearance of NADH, was measured over a fixed time interval and was directly proportional to the LDH activity. LDH activity was expressed in micromoles of substrate (pyruvate) converted per minute (U), per liter serum at 37 °C. The measuring range was 10–3800 U/L; for higher concentrations, the samples were manually diluted with saline and reanalysed. The reference range in serum for LDH is 200–450 U/L.

The surface charge difference was the basis on which the 5 LDH isoenzymes were separated by electrophoresis using the Beckman appraise system (the LDH isoenzyme electrophoresis kit P/N 655940) [20]. After separation of the LDH isoenzymes by electrophoresis, the agarose gel was incubated with a reaction mixture containing the LDH substrate lactate, the coenzyme NAD^+, and a tetrazolium salt. During this incubation NADH was formed at the zones on the gel, where the LDH isoenzymes were present. The NADH generated was detected by its reduction of the tetrazolium salt to the coloured bands, which could be quantitated by scanning the gel at 600 nm.

The ALP activity was measured at 37 °C by an enzymatic rate method using *p*-nitrophenylphosphate as a substrate. The test was performed on a Beckman Synchron CX-7 system with Beckman reagents (testkit 442670). At an alkaline pH of 10.3, using a 2-amino-2-methyl-1-propanol (AMP) buffer, ALP catalyses the hydrolysis of the colourless organic phosphate ester substrate, *p*-nitrophenylphosphate, to the yellow coloured product *p*-nitrophenol and phosphate. The rate of change in absorbance at 410 nm was monitored over a fixed-time interval. The rate of change in absorbance, directly proportional to the ALP activity, was expressed in micromoles substrate (*p*-nitrophenylphosphate) converted per minute (U) per litre serum at 37 °C. The measuring range was 10–1800 U/L; for higher concentrations, the samples were manually diluted with saline and reanalysed. The same procedure was used for ALP measurements in both serum and BALF.

Statistical Evaluation

Pearson coefficient of correlation (r) was estimated in order to test against a linear relation in the different groups between LDH, its isoenzyme

pattern, ALP, and the detected cells in BALF. A probability value of less than .05 was considered to be significant. In the whole group a partial correlation was estimated, with correction made for group effect. The Mann-Whitney test was subsequently used to evaluate the differences between selected BALF samples and lung tissue samples. A Wilcoxon matched-pairs signed ranks test was done to compare the percentages of LDH isoenzymes before and after sonification of BALF.

RESULTS

The cellular BALF sample analysis results are summarized in Table 1. BALF samples of Group I contained mainly PMNs ($91 \pm 3.7\%$), whereas BALF samples of Group II contained predominantly AMs ($91.8 \pm 3.2\%$). Group I (mainly PMNs) contained significantly more cells than Group II (mainly AMs). In Table 2, the LDH, ALP, and LDH isoenzymes of the different groups, in the original, nonsonicated BALF samples are presented. Group I contained significantly higher LDH and ALP and showed a different isoenzyme pattern. Furthermore, the LDH and LDH isoenzyme levels in BALF in the different cell types present in BALF were examined in both groups. The respective levels in BALF were evaluated before and after lysis of the cells by sonification. The calculated levels (substracting the enzyme activity of the sonicated BALF from the enzyme activity present in the original cell-free supernatant) indicated the LDH and LDH isoenzyme activities of the different cells present in BALF. Group I showed higher LDH activity ($P < .02$) as well as ALP activity ($P < .02$) compared to Group II. Moreover, the 2 selected groups showed a significant different LDH isoenzyme pattern (Table 3).

The LDH isoenzyme pattern of Group II, with mainly AMs, resembled the isoenzyme pattern of the lung tissue (Group IV) the most, although

TABLE 1 Cellular Bronchoalveolar Lavage Fluid (BALF) Characteristics of Group I: Mainly Polymorphonuclear Neutrophils (PMNs); Group II: Mainly Alveolar Macrophages (AMs); Group III: BALF Obtained From a Healthy Control Group

	Group I (n = 15)	Group II (n = 10)	Group III (n = 8)
Total cell count $\times$ 10^4/mL	496.8 (174.5) $\pm$ 834.6[aef]	24.2 (17.5) $\pm$ 18.4[e]	14.3 (14.3) $\pm$ 3.1
AMs $\times$ 10^4/mL	39.1 (5.3) $\pm$ 71.1[d]	22.3 (16.3) $\pm$ 17.2[e]	12.7 (12.1) $\pm$ 3.1
AMs %	5.7 (5.7) $\pm$ 3.9[cf]	91.8 (93.4) $\pm$ 3.2	89.1 (89.0) $\pm$ 4.4
PMNs $\times$ 10^4/mL	460.5 (133.5) $\pm$ 772.6[bf]	0.55 (0.44) $\pm$ 0.48[e]	0.24 (0.20) $\pm$ 0.16
PMNs %	91.4 (91.8) $\pm$ 3.7[cf]	2.3 (2.4) $\pm$ 1.3	1.7 (1.6) $\pm$ 1.2
AMs/PMNs	0.07 (0.07) $\pm$ 0.05[bf]	46.7 (39.7) $\pm$ 25.5[e]	86.1 (65.1) $\pm$ 72.1
Lymphocytes $\times$ 10^4/mL	10.1 (1.7) $\pm$ 20.5[aed]	0.84 (0.55) $\pm$ 0.94	0.99 (0.82) $\pm$ 0.80
Lymphocytes %	2.0 (1.2) $\pm$ 1.87[d]	3.7 (3.1) $\pm$ 2.3	6.8 (5.4) $\pm$ 5.1
Smoker/nonsmoker	9/6	6/4	0/8

Values are expressed as mean $\pm$ SD, with median in parentheses.
[a] $P < .01$, [b] $P < .001$, and [c] $P = .0001$: Group I versus Group II.
[d] $P = .01$, [e] $P < .003$, and [f] $P = .001$: versus controls (Group III).

TABLE 2 Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP) Levels, and Percentage of LDH Isoenzymes in Bronchoalveolar Lavage Fluid (BALF) in Group I, Mainly Polymorphonuclear Neutrophils (PMNs); in Group II, Mainly Alveolar Macrophages (AMs); and in Group III, Healthy Controls, Data of Original Nonsonicated BALF Samples

	Group I (n = 15)	Group II (n = 10)	Group III (n = 8)
LDH U/L	465 (329) ± 434[b,e]	87.6 (83) ± 20.3[e]	63.5 (65.0) ± 9.7
ALP U/L	51.1 (30.0) ± 57.0[a,e]	15.2 (8.5) ± 17.7[d]	7.3 (8.5) ± 4.7
LDH 1 (%)	4.3 (3.3) ± 4.9[b,e]	10.4 (8.8) ± 5.2[e]	14.5 (12.7) ± 5.12
LDH 2 (%)	8.3 (6.8) ± 1.1[c,f]	20.5 (20.8) ± 3.5[d]	23.1 (21.9) ± 3.6
LDH 3 (%)	15.6 (15.1) ± 4.5[c,f]	28.9 (29.6) ± 3.1	29.1 (29.3) ± 1.9
LDH 4 (%)	22.8 (23.0) ± 1.9[a]	26.0 (25.6) ± 4.0	22.9 (24.2) ± 4.1
LDH 5 (%)	48.9 (51.5) ± 13.7[b,f]	14.2 (13.7) ± 6.2[d]	10.5 (10.4) ± 2.1
LDH3/LDH4	0.68 (0.66) ± 0.15[c,f]	1.12 (1.10) ± 0.12[e]	1.31 (1.27) ± 0.24
LDH3/LDH5	0.42 (0.30) ± 0.43[c,f]	2.32 (2.26) ± 0.81[e]	2.86 (2.63) ± 0.59
LDH4/LDH5	0.56 (0.44) ± 0.41[c,f]	2.04 (2.03) ± 0.61	2.21 (2.05) ± 0.36

Values are expressed as mean ± SD with median in parentheses.
[a] $P < .05$, [b] $P < .003$, and [c] $P \leq .0001$: Group I versus Group II.
[d] $P < .05$, [e] $P < .01$, and [f] $P < .001$: versus controls (Group III).

LDH5 was significantly different between Groups II and IV (Table 3). The LDH-isoenzyme pattern of the cells present in BALF in Group I (mainly PMNs) showed a low LDH3/LDH4, LDH3/LDH5, and LDH4/LDH5 ratio compared to Group II (mainly AMs; $P < .005$; see Tables 2 and 3). The LDH/albumin ratio and the ALP/albumin ratio were $8.2(7.1) ± 7.4$ and

TABLE 3 Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP) Levels, and Percentage of LDH Isoenzymes in Bronchoalveolar Lavage Fluid (BALF) in Group I, Mainly Polymorphonuclear Neutrophils (PMNs); in Group II, Mainly Alveolar Macrophages (AMs), (Calculated Levels by Subtracting the Enzyme Activity of the Sonicated BALF from the Enzyme Activity Present in the Original Cell-Free Supernatant); and in Group IV, Lung Tissue

	Group I (n = 15)	Group II (n = 10)	Group IV (n = 9)
LDH U/L	497 (209) ± 691	87.6 (83) 71.2[a]	
U/g wet weight			51.9 (50.3) ± 15.4
ALP U/L	34.9 (11.0) ± 63	1.1 (0.5) ± 3.2[a]	
U/g wet weight			2.09 (2.18) ± 0.86
LDH 1 (%)	5.5 (1.9) ± 8.7[d]	12.4 (4.9) ± 19.7	7.2 (7.3) ± 1.3
LDH 2 (%)	9.4 (7.5) ± 7.5[b,d]	25.0 (22.7) ± 8.3	19.5 (19.6) ± 1.9
LDH 3 (%)	14.5 (13.8) ± 5.7[b,e]	31.9 (33.3) ± 6.7[e]	29.3 (29.6) ± 1.5
LDH 4 (%)	21.2 (21.5) ± 6.5	24.0 (24.9) ± 8.9	23.9 (24.8) ± 2.6
LDH 5 (%)	50.0 (53.0) ± 15.7[b,f]	8.9 (9.8) ± 5.4[f]	20.0 (20.3) ± 2.2
LDH3/LDH4	0.83 (0.67) ± 0.65[b,e]	1.90 (1.33) ± 1.62	1.23 (1.27) ± 0.13
LDH3/LDH5	0.35 (0.25) ± 0.34[c,f]	5.26 (2.73) ± 5.05[f]	1.48 (1.45) ± 0.21
LDH4/LDH5	0.44 (0.38) ± 0.32[c,e]	3.56 (2.27) ± 2.83[f]	1.21 (1.18) 0.23

Values are expressed as mean ± SD with median in parentheses.
[a] $P < .05$, [b] $P < .005$, [c] $P \leq .0001$; Group I versus Group II.
[d] $P < .03$, [e] $P \leq .007$, [f] $P \leq .0005$; versus Group IV.
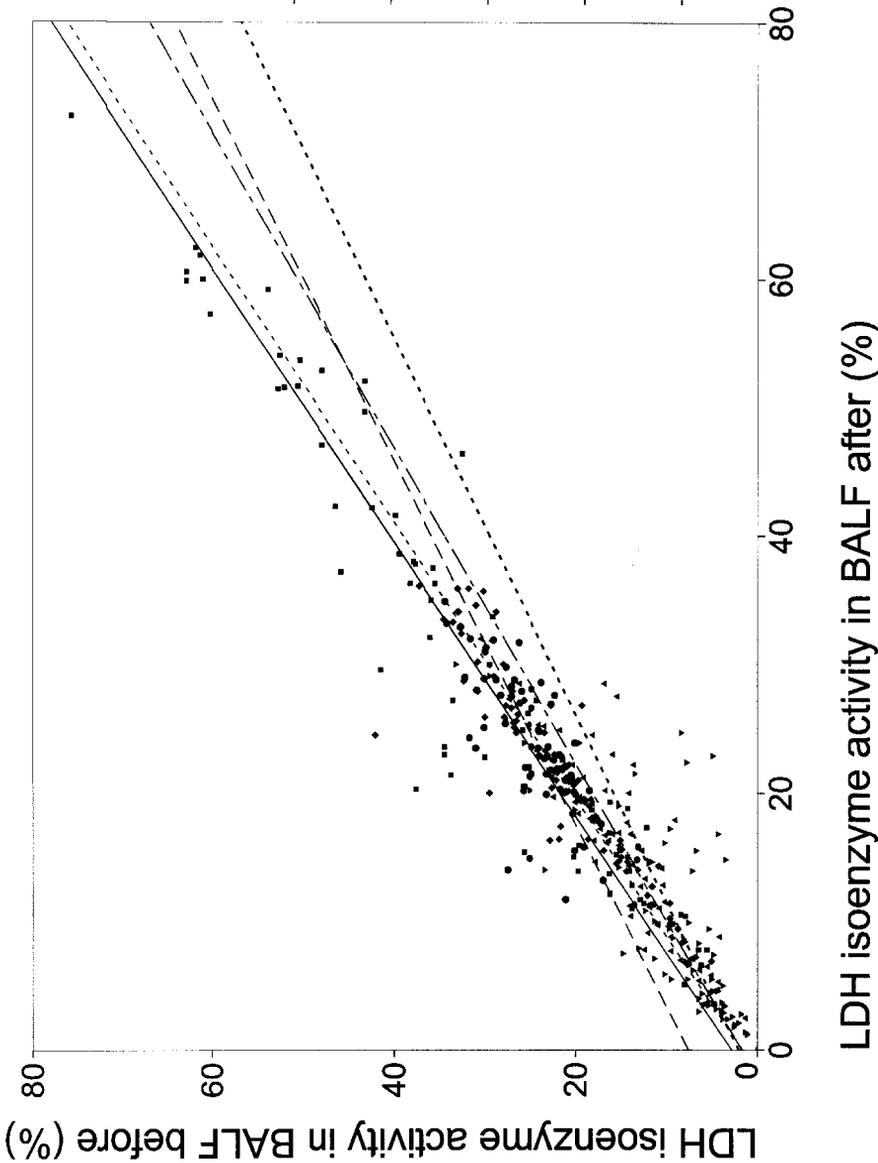
FIGURE 1 Bronchoalveolar lavage fluid (BALF) lactate dehydrogenase (LDH) isoenzyme percentages before (original BALF) and after lysis of cells present in BALF by sonication.

0.6(0.5) ± 0.6 in the group with mainly PMNs, and 4.6(3.2) ± 5.5 and 0.7(0.3) ± 1.5 in the group with mainly AMs, respectively.

Evaluation of the LDH isoenzyme pattern in BALF obtained from smokers and nonsmokers with mainly AMs and mainly PMNs separately revealed no significant differences. However, only in the group with mainly AMs was the absolute cell count and the absolute LDH activity slightly higher in the smokers group than in the nonsmokers group $(25.5(22.8) \pm 11.2 \times 10^4/mL$ vs. $10.1(10.1) \pm 8.3 \times 10^4/mL$ and $89(91) \pm (8$ U/L vs. 72(74) ± 5 U/L, respectively).

Although the absolute values of the isoenzymes were higher in the sonicated BALF, the relative isoenzyme values were comparable in the sonicated and original, nonsonicated BALF (Figure 1).

Furthermore, we examined possible relations between the variables in Group I and Group II separately. In group I (mainly PMNs), a relation was found between the LDH serum/BALF ratio and PMNs $(P < .0001; r = .99)$, AMs $(P = .001; r = .97)$, as well as lymphocytes $(P = .003; r = .96)$. Also in BALF, a significant correlation between LDH and ALP was found $(P < .0001; r = .88)$. In Group II (mainly AMs), no such correlations were found. When considering the total combined group of BALF, a correction was made for the group effect by estimating partial correlations. In the complete group, a correlation was found between the LDH serum/BALF ratio and AMs $(P < .001; r = .84)$, PMNs $(P < .0001; r = .98)$, and lymphocytes $(P = .0001; r = .90)$. No partial correlation was found between ALP and the different cell types present in BALF.

DISCUSSION

This study showed that the LDH and ALP activities were higher in the cell-free fraction of BALF that contained mainly PMNs compared to the cell-free fraction of BALF that contained predominantly AMs. This finding is consistent with the higher inflammatory response indicated by the PMNs [1, 8, 14, 21]. The LDH isoenzyme pattern also differed, with the LDH3/LDH5 ratio being lower in all BALF samples with predominantly PMNs than in any BALF sample with predominantly AMs; the ratio was lower in the PMN-BALF samples due to mainly high LDH5 values.

Sonication of cells present in BALF appeared not to influence these results. The LDH pattern in BALF with mainly AMs compared most closely with that of lung tissue. The exact mechanism of enzymes with respect to pulmonary cell damage and/or inflammation has to be clarified. In agreement with others, the LDH isoenzyme pattern of the lung was characterized by proportionally higher LDH3 and LDH4 compared to the normal serum isoenzyme pattern [13, 22]. The high levels of LDH5 in BALF obtained from

Group I with mainly PMNs compared to LDH5 in lung tissue indicate that the source of this isoenzyme is more likely the PMNs than lung parenchymal cells. We realize that one of the limitations of the present study is that we did not directly test our hypothesis in isolated, exceptionally pure populations of human neutrophils and AMs. Analysis of these purified cell populations might have significantly strengthened the clinical data; however, differences between Groups I and II already were highly indicative for a different enzyme release pattern between PMNs and AMs.

Many studies in animals report the relationship between LDH and pulmonary disorders [13, 17, 23–31]. Only a few studies on humans have been carried out to investigate the relation between LDH an pulmonary disorders. Increased serum LDH activity was reported after pulmonary embolism [32], *Pneumocystis carinii* pneumonia, tuberculosis, bacterial pneumonia [33], diffuse interstitial pneumonitis [34], extrinsic allergic alveolitis [11], drug-induced respiratory distress [35], lipoid pneumonia [36] and idiopathic pulmonary fibrosis [11, 37]. Previously, we found that coal-dust deposition in the lung—even many years after the actual exposure—was reflected by an increase in the total serum LDH activity, mainly characterized by a high LDH3 activity. Since all other liver function tests were within normal limits, the liver was excluded as another possible source of LDH. Moreover, silica exposure induced pulmonary cell damage, resulting in LDH release; these results indicated that the increased LDH originated from lung parenchymal or inflammatory cells, predominantly AMs [38, 39].

A marker of type II cell damage and/or proliferation, such as ALP, was reported to be increased in BALF after exposure to pneumotoxicants [1, 16, 17, 40]. The type II pneumocyte is important in the repair of alveolar epithelium after injury and response to oxidant stress (such as hyperoxia). Capelli and coworkers [18] reported that an increase of the ALP/albumin ratio in BALF obtained from patients with diffuse interstitial disorders was associated with progression of fibrosis. They also found a significant negative correlation of ALP with resting Pa_{O_2}. Hypoxemia at rest is an advanced clinical feature of pulmonary fibrosis, reflecting disease severity rather than disease activity. In contrast to LDH, in the present study an increase in the BALF ALP activity did not correlate with any of the cells identified in BALF, suggesting that the source of the ALP was type II cells, rather than neutrophils. This finding is in agreement with earlier studies in animals [16, 17, 40].

In agreement with Dubar and coworkers [41], we also did not find differences in the BALF LDH levels between smokers and nonsmokers. Dubar et al. [41] studied the immediate effect of cigarette smoke on cell injury, cell viability, and cytokine secretion by AMs from guinea pigs and human healthy subjects. They measured LDH release in a culture medium after smoke exposure together with measurement of interleukin (IL)-6 and tumour necrosis factor (TNF)-α activities. The release of LDH from AMs in the

culture medium was unchanged both immediately after tobacco smoke exposure and at the time of the cytokine evaluation (18–20 hours later). Furthermore, this study [41] demonstrated that the exposure or tobacco smoke produced significant changes in the AM secretory function without alterations of the cell viability. A study which compared BALF of light and heavy smokers showed on differences in release of LDH by AMs between these groups [42]. Despite alterations of cell function, it has been suggested that smoking causes no cell damage or death reflected by LDH release and elevated serum LDH activity [41].

The level of enzyme activity in BALF may provide a quantitative assessment of cell damage and pulmonary defence mechanisms. As mentioned before, not only the amount of the cells involved in an inflammatory response are of importance, but also the activity reflected by the release of, among other inflammatory mediators, enzymes indicating cell damage or death such as LDH and ALP. Moreover, ALP has been associated with type II cell secretion of damage. Type II cells are normally not present in BALF [18]. Therefore, monitoring biochemical changes may be of additional value to a total and differential cell count to establish the inflammatory cell status of a patient. Furthermore, the sensitivity of detecting an increase of the LDH and ALP activity in BALF appears to be minimally dependent on the volume of fluid used for lavage in contrast to cell counting [15, 18, 19]. Moreover, in cases with negative culture results, assessing enzymatic markers of inflammation and cell damage, such as ALP, LDH, and LDH isoenzyme activities, can have the additional value of identifying which inflammatory cells are involved in the pathologic process. More important, these relatively cheap and easy to perform measurements are available in every hospital. Further studies should be conducted to correlate LDH, its isoenzymes, and ALP with different pulmonary disorders.

In conclusion, the LDH isoenzyme pattern of BALF with mainly PMNs differs from BALF with mainly AMs. Sonication of cells in BALF has no additional value in the assessment of the LDH isoenzyme pattern. The LDH3/LDH5 ratio appears to be useful as a rapid screening test for discriminating between lung inflammation in which mainly AMs are involved and inflammation by predominantly PMNs. The isoenzyme pattern of the AMs resembles the isoenzyme pattern of the lung. The LDH isoenzyme pattern of the lung is characterized by proportionally higher LDH3 and LDH4 compared to the normal serum isoenzyme pattern. Moreover, no relation was found between the ALP activity and the cells present in BALF. This suggest that, in contrast to LDH, these latter enzymes originate from cells not present in BALF. Future studies are needed to elucidate the role of the release of various enzymes in the mechanisms of inflammation and pathogenesis of various pulmonary disorders, as well as the clinical relevance of monitoring the enzyme activities in BALF.

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