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Bronchoalveolar lavage cell analysis and lung function impairment in patients with systemic lupus erythematosus (SLE)

H. GROEN*†, M. ASLANDER*, H. BOOTSMA†, Th. W. VAN DER MARK*, C. G. M. KALLENBERG† & D. S. POSTMA* *Department of Internal Medicine, Divisions of *Pulmonology and †Clinical Immunology, State University Hospital, Groningen, The Netherlands*

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SUMMARY

We examined the relationship between peripheral blood and bronchoalveolar lavage (BAL) lymphocyte phenotypes and lung function in 19 patients with SLE, and evaluated their association with disease activity. Lung function assessment showed a mildly restrictive pattern with frequent impairment of transfer factor for carbon monoxide ($T_{I,CO}$) and diffusing capacity of the alveolocapillary membrane (Dm), of late-expiratory airflow rates and with a high prevalence of increased airway resistance. $T_{I,CO}$, K_{CO} and Dm correlated inversely with the numbers of $CD8^+$ cells and $CD56^+/CD16^+/CD3^-$ (NK) cells in BAL. Oxygen radical production, both by stimulated and unstimulated BAL cells and blood polymorphonuclear leucocytes (PMN) was significantly increased in SLE. In comparison with healthy controls, patients with SLE had a lower percentage of $CD19^+$ B cells in the BAL *versus* an increased percentage of these cells in peripheral blood. HLA-DR expression on $CD4^+$ and $CD8^+$ lung lymphocytes was markedly increased in SLE. Current SLE disease activity was not associated with changes in BAL or peripheral blood lymphocyte phenotypes. Our data suggest that an ongoing cell-mediated immune response is present in the lungs in SLE, particularly involving activated $CD8^+$ T cells and $CD56^+/CD16^+/CD3^-$ NK cells. It is associated with up-regulated local production of oxygen radicals and with impaired pulmonary diffusing capacity. This inflammatory process seems to be independent of general SLE disease activity.

Keywords systemic lupus erythematosus bronchoalveolar lavage lymphocyte phenotypes lung function

INTRODUCTION

Among the connective tissue diseases, SLE is characterized by widespread inflammatory changes in several organs, in particular the kidneys, joints, skin, central nervous system, heart and lungs. The pathogenesis of kidney involvement is generally accepted to be immune complex-mediated, particularly immune complexes in which anti-dsDNA are involved [1]. However, immunopathological studies of lung tissue in patients with pulmonary involvement have not yielded consistent results [2–4]. In acute pneumonitis immune complexes probably play a major role [5], whereas in chronic interstitial pneumonitis their role may be less well delineated and T lymphocyte infiltration is more prevalent. Moreover, the slowly progressive clinical course of interstitial lung involvement in SLE, as opposed to the acute systemic exacerbations of the disease, may suggest different underlying pathophysiologic mechanisms.

Correspondence: D. S. Postma, Dept. of Internal Medicine, Division of Pulmonology, State University Hospital, PO Box 30.001, 9700 RB Groningen, The Netherlands.

Interstitial lung involvement in SLE and scleroderma have some similarities, e.g. with respect to their insidious clinical course and their association with extra-pulmonary signs of microvascular damage [6–8]. In addition, lymphocyte alveolitis is a frequent finding in patients both with SLE and with scleroderma who have developed interstitial lung disease [9–11]. This suggests a common pathophysiology probably related to cell-mediated immune processes that have been implicated in the pathophysiology of scleroderma [12].

We hypothesized that interstitial lung involvement in SLE is a T lymphocyte-mediated immunological process, in which a DTH-like reaction or a cytotoxic reaction directed at as yet unknown antigens is involved. As an approach to this hypothesis we performed bronchoalveolar lavage (BAL) in 19 SLE patients, representing a continuum ranging from severe to mild lung function impairment, and examined the relationship between lung function and the cellular characteristics of BAL fluid, in particular lung lymphocyte phenotypes and oxygen radical production as a functional measure of BAL cell activity. In addition, the relationship between lung lymphocyte phenotypes and general SLE disease activity was investigated.

Table 1. Demographic and disease characteristics of 19 patients with SLE undergoing bronchoalveolar lavage

Age (mean ± s.d.)	36·9 ± 12·7
Sex (female/male)	17/2
Disease duration (mean ± s.d.)	6·1 ± 5·7
Smoker (yes/no)	4/15
Liang score (median (range))	6 (0–19)

ARA criterion (cumulative data)	Positive (%)
1. Malar rash	8 (42)
2. Discoid rash	5 (26)
3. Photosensitivity	11 (58)
4. Oral ulceration	4 (21)
5. Arthritis	14 (74)
6. Serositis	5 (26)
7. Renal disorder	8 (42)
8. Neurological disorder	5 (26)
9. Haematological disorder	12 (63)
10. Immunological disorder	15 (79)
11. ANA	19 (100)

SUBJECTS AND METHODS

Subjects

A large population of patients with SLE, diagnosed according to the 1982 revised ARA criteria [13], is followed up at the Department of Clinical Immunology in our hospital [14]. As part of this follow up, serological studies and lung function tests are performed at regular intervals. For this study, 30 consecutive patients were asked by their own physician (H.B./C.G.M.K.) to undergo BAL in addition to the routine follow up. Twenty-two patients consented to participate. In three patients BAL could not be performed due to abnormally high pharyngeal reflexes, and they were excluded from further analysis. The demographic characteristics of the remaining 19 patients are given in Table 1. Thirteen patients used immunomodulating drugs, either low-dose prednisolone ($n=5$) or chloroquine ($n=3$) or both ($n=4$) or a combination of prednisolone and azathioprine ($n=1$). BAL data of 23 healthy controls, 16 male and seven female, mean age 40·6 years, s.d. 12·4, consisting of six smokers and 17 non-smokers, available from our laboratory database were used for comparative purposes.

Lung function

Lung volumes were measured by helium dilution according to standardized techniques. Other spirometric parameters such as the slow inspiratory vital capacity (IVC), forced expiratory volume in one second (FEV_1), inspiratory volume in one second (FIV_1) were measured with a standard water-sealed spirometer. Values of IVC, FEV_1, FIV_1 and total lung capacity (TLC) were expressed in litres BTPS (body temperature, pressure, saturated). Predicted values according to Quanjer *et al.* [15] were applied. Transfer factor for carbon monoxide ($T_{l,CO}$) was determined using the single breath technique of Krogh, as modified by Ogilvie [16] and Cotes [17]. The components of $T_{l,CO}$, i.e. the diffusing capacity of the alveolocapillary membrane (Dm) and the pulmonary capillary blood volume (Vc), were determined from triplicate measurements of $T_{l,CO}$ at high (88%) and low (19·2%) inspiratory oxygen concentrations under otherwise identical conditions and at comparable inspiratory volumes and breath holding times. The calculations were done following the equation originally devised by Roughton & Forster [18]. The $T_{l,CO}$ values, breathing air, were corrected for haemoglobin concentrations according to Cotes [17] to obtain $T_{l,CO}$ values under standard conditions. $T_{l,CO}$ and Dm were expressed in mmol/kPa per min, Vc was expressed in ml. Predicted values were taken from Cotes [17]. K_{CO} was calculated by dividing $T_{l,CO}$ by the alveolar volume. Flow-volume curves with determination of expiratory flow rates at 25%, 50% and 75% of the expired volume (FEF_{25}, FEF_{50} and FEF_{75}) and airway resistance (R_{aw}) were assessed with a body plethysmograph. All pulmonary function tests were performed at room temperature, with the patient in a steady state condition. Pulmonary function parameters were considered abnormal when below 80% of the predicted value.

Bronchoalveolar lavage

On the day of the BAL patients did not take their morning medication. BAL was performed with a flexible bronchoscope (Olympus B1-IT20; Olympus Optical Co., Tokyo, Japan) after local anaesthesia of the airways with lidocaine spray. The tip of the bronchoscope was wedged in the lateral segment of the right middle lobe. Subsequently, 10 aliquots of 20 ml of sterile PBS at 37°C were instilled and recovered by gentle suction (−50 cm H_2O) after each aliquot. Suction was continued as long as possible to increase cell yield without causing unnecessary discomfort for the patient.

Isolation of BAL cells

The lavage fluid recovered from aliquots two to 10 was filtered through a venous infusion filter (Curapharm; Medica B.V. Hospital Supplies, The Netherlands) to remove mucus and centrifuged at 400 *g* at 4°C for 5 min. The cell pellet was resuspended in ammonium chloride (pH 7·6) for 5 min at 4°C followed by centrifugation at 400 *g* at 4°C for 5 min to dispose of erythrocytes. Subsequently the cell pellet was washed three times in PBS with 0·1% glucose with centrifugation at 4°C for 5 min at 400 *g*. The cells were counted and viability was assessed by cellular exclusion of trypane blue. Cytospin preparations were made from 100-μl fractions of the lavage cell suspension ($0{\cdot}3 \times 10^6$ cells/ml in PBS with 0·5% bovine serum albumin (BSA)). Two preparations from each patient were stained with a May–Grünwald–Giemsa stain. Cell differential counts were performed on two slides and the average of 300 cell counts on each slide was taken as the definite cell differential count. The remaining cell suspension was used for lymphocyte phenotyping and measurement of oxygen radical production. In case of poor cell recovery, determination of lymphocyte phenotypes had the highest priority. When sufficient cells remained, unstimulated and, if possible, phorbol myristate acetate (PMA)-stimulated oxygen radical production were measured.

Isolation of blood mononuclear cells

Twenty millilitres of EDTA blood were obtained by venapuncture and the mononuclear cells were separated by Ficoll–Paque density gradient centrifugation. The mononuclear cell fraction obtained was resuspended in ammonium chloride for 5 min at 4°C and centrifuged at 970 *g* during 10 min at 4°C. The cells were washed twice in PBS with 0·1% glucose with centrifugation steps at 970 *g* for 5 min and at 170 *g* for 10 min, both at 4°C. The

Table 2. Mononclonal antibodies used to determine lymphocyte phenotypes in bronchoalveolar lavage fluid and peripheral blood of patients with SLE

MoAb	Target cell type
CD3/Leu-4	T cells
CD4/Leu-3a	T helper/inducer cells
CD8/Leu-2a	T cytotoxic/suppressor cells, NK cells
CD14/Leu-M3	Monocytes, macrophages, granulocytes
CD16/Leu-11c	Granulocytes, some macrophages, NK cells
CD19/Leu-12	B cells
CD25	Activated T cells, $CD4^+$ T cells, some B cells
CD45	All human leucocytes
CD56/Leu-19	NK cells, cytotoxic T cells
HLA-DR	B cells, monocytes, macrophages, activated T cells
FITC/PE	Negative control for immunofluorescence

cells were counted and viability was assessed by cellular exclusion of trypane blue. The cell suspension was used for FACS lymphocyte phenotyping and cytospin preparations.

Lymphocyte phenotype determination

Isolated blood mononuclear cells and lavage cells were prepared for two-colour immunofluorescence analysis with MoAbs conjugated to PE or FITC. The final cell concentration in each sample was adjusted to 10^6 cells/ml in PBS with 0·5% BSA.

The cells were centrifuged at 530 ***g*** at 10°C for 5 min and subsequently incubated for 30 min at 4°C with the appropriate dilutions of MoAbs against CD4, CD8, CD3, CD19, CD56, CD16, CD25, HLA-DR, CD45 and CD14 (Becton Dickinson; see Table 2). Aspecific binding was checked using FITC- and PE-labelled control antibodies.

After incubation the cells were washed twice with PBS containing 0·5% BSA with centrifugation at 530 ***g*** at 10°C for 5 min. After adding 200 μl PBS with 0·5% BSA the labelled cells were stored in the dark at 4°C until analysis.

Fluorescence-activated cell sorting was done using a FACStar (Becton Dickinson), equipped with an argon laser. Ten thousand events were counted for each MoAb or combination of MoAbs. The data were collected with the Consort 30 program. Lymphocytes were identified on the forward and side scatter image. Subsequently, the lympocyte gate was set on the image of the combined CD14 and CD45 scatter in order to exclude contamination from erythrocytes and debris and to exclude monocytes, granulocytes and alveolar macrophages.

Isolation of polymorphonuclear cells from blood

The cell pellet obtained after Ficoll–Paque separation, containing polymorphonuclear leucocytes (PMN) and some erythrocytes, was collected, resuspended in ammonium chloride for 10 min at 4°C and centrifuged at 970 ***g*** for 5 min at the same temperature. Subsequently, the cells were again suspended in ammonium chloride for 5 min at 4°C and centrifuged at 970 ***g*** for 5 min at 4°C.

The PMN were washed three times in PBS with two 5-min centrifugation steps at 970 ***g*** and a final centrifugation step at 170 ***g*** for 10 min, all at a temperature of 4°C. The cells were counted and viability was assessed by cellular exclusion of trypane blue. The cell suspension was used for the preparation of cytospin preparations and for the measurement of the oxygen radical production. All materials used were lipopolysaccharide (LPS) free.

Oxygen radical production

Oxygen radical production was measured as the superoxide dismutase (SOD) inhibitable reduction of cytochrome C as described previously [19]. In short, a volume of the cell suspension, containing 4×10^5 cells, was incubated with a mixture containing cytochrome C with or without SOD and PMA (Sigma) as a soluble stimulus at concentrations of 2·5, 5 and 10 ng/ml. The incubation mixture, at a final volume of 0·5 ml, was collected in polystyrene tubes. Tubes without PMA were included to measure spontaneous reduction, and the total reduction was checked by including tubes with 0·085 ml of a saturated sodium hydrosulphite solution (Sigma). The reaction mixtures were prepared at room temperature and placed in a water bath at 37°C for 10 min. After termination of the reaction by centrifugation, the optical density at 550 nm was determined in a spectrophotometer.

Disease activity

Minor and major exacerbations were defined as previously reported [14]. In addition, disease activity was scored according to a validated activity scoring system as described by Liang *et al.* [20], which scores 32 aspects of disease activity.

Statistical analysis

Data were analysed with the SPSS/PC+ statistical package, version 3.0. For between-group comparisons Student's *t*-test or Mann–Whitney's *U*-test was used. In order to investigate the relationship between BAL parameters and lung function scores, Pearson's correlation coefficient (r) was calculated, on ranked values (ρ) where applicable, and averages of BAL parameters were compared for groups distinguished by lung function values above and below 80% of predicted.

The null hypothesis was rejected when it had a probability (P value) of 5% or less. Individual P values are indicated in parentheses in text and by symbols in figures.

RESULTS

Lung function

Lung function tests were performed in 18/19 patients. One patient was unable to complete the lung function tests. Average results are shown in Table 3. The main features were a mildly restrictive pattern, frequent impairment of $T_{I,CO}$ and Dm, of late-expiratory airflow (FEF_{75}), and increased airway resistance (R_{aw}) in a high number of patients.

BAL cell content and differentiation

The cell content and differentiation of the BAL fluid is shown in Table 4. Due to a low recovery (26%) and a low cell yield, cell differentials could not be assessed in one patient. One non-smoking patient had a high percentage of neutrophils (25%) for no clinically apparent reason and without bacteria in the BAL. One other patient, also a non-smoker, had an increased percentage of lymphocytes (23%). Except for a tendency toward an increase of neutrophils, no abnormalities were present in this group of patients with SLE. In comparison with the healthy

Table 3. Average lung function data of 18/19 patients with SLE undergoing bronchoalveolar lavage

Parameter	Mean ± s.d. (% pred)	Range (% pred)	≤80% pred (*n* (%))
TLC	92·2 ± 11·6	73–113	4 (22)
IVC	88·9 ± 12·8	70–111	5 (28)
FEV_1/IVC	109·1 ± 8·2	95–126	0 (0)
FIV_1/IVC	115·7 ± 7·3	100–125	0 (0)
$T_{l,CO}$	84·6 ± 16·0	52–110	8 (44)
K_{CO}	98·1 ± 15·8	65–129	3 (17)
Dm	71·9 ± 17·8	40–109	13 (72)
Vc	97·7 ± 17·5	67–124	5 (28)
FEF_{25}	99·1 ± 17·8	73–146	2 (11)
FEF_{50}	84·3 ± 19·9	51–112	7 (39)
FEF_{75}	65·1 ± 20·7	29–114	14 (78)
R_{aw}*	0·32 ± 0·14	0·15–0·60	13† (72)

* Expressed as kPa per litre/s.

† Number (percentage) of patients with increased airway resistance.

TLC, Total lung capacity; IVC, inspiratory vital capacity; FEV_1, forced expiratory volume in one second; FIV_1, inspiratory volume in one second; $T_{l,CO}$, transfer factor for carbon monoxide; Dm, diffusing capacity of the alveolocapillary membrane; Vc, pulmonary capillary blood volume; R_{aw}, airway resistance; K_{CO}, $T_{l\,CO}$ divided by the alveolar volume; FEF_{25}, FEF_{50}, FEF_{75}, forced expiratory flow rates at 25%, 50% and 75% of the expired volume.

controls, patients with SLE had a significantly lower percentage and number of lymphocytes.

Lymphocyte phenotypes

The results of lymphocyte phenotype determinations are given in Table 5. The distribution of phenotypes in the SLE patients was compared with available values in healthy controls, four male and four female, all non-smoking. A significantly lowered percentage of $CD19^+$ (B) lymphocytes was observed in the BAL of SLE patients. The number, but not the percentage, of $CD4^+$ cells was significantly decreased in patients with SLE. HLA-DR expression on $CD4^+$ cells was markedly increased in SLE compared with data of six healthy controls. No control data were available for HLA-DR expression on $CD8^+$ cells, for CD25 expression, and for the percentage of CD56/CD16 positive cells. In comparison with blood lymphocytes, HLA-DR expression on $CD4^+$ and $CD8^+$ cells from the lungs was markedly increased, whereas CD25 expression was similar. In sharp contrast with their occurrence in the lungs, peripheral blood $CD19^+$ lymphocytes were significantly increased in patients with SLE compared with the healthy controls. Otherwise, no significant differences among these two populations were observed.

Oxygen radical production

Compared with the healthy controls, both unstimulated and PMA-stimulated O_2^- production by BAL cells were significantly increased in the patients with SLE. The differences remained unchanged when the patient with extremely high oxygen radical production was excluded. For PMN the same phenomenon was observed, although unstimulated O_2^- production was not significantly different from normal (Fig. 1). Oxygen radical production did not differ significantly among patients with normal and impaired TLC, Vc, $T_{l,CO}$ or Dm, nor did it correlate with SLE disease activity.

Relationships between lung function and BAL lymphocyte phenotypes

The presence of restrictive lung function impairment, i.e. either decreased TLC or Vc, was not associated with significant alterations of BAL lymphocytes. Parameters of pulmonary diffusing capacity were correlated with both proportional and absolute numbers of BAL lymphocyte phenotypes. $T_{l,CO}$ showed a significant inverse correlation with the percentage of $CD56^+/CD16^+/CD3^-$ cells ($\rho = -0{\cdot}72$, $P = 0{\cdot}019$) and tended to correlate inversely with the percentage of $CD8^+$ lymphocytes ($r = -0{\cdot}47$, $P = 0{\cdot}07$). K_{CO} also correlated with the percentage of $CD56^+/CD16^+/CD3^-$ cells ($\rho = -0{\cdot}70$, $P = 0{\cdot}025$). These correlations were reflected in a tendency toward a correlation between Dm, the diffusing capacity of the alveolocapillary membrane, and the percentage of $CD56^+/CD16^+/CD3^-$ cells ($r = -0{\cdot}58$, $P = 0{\cdot}08$) and the number of $CD8^+$ lymphocytes ($r = -0{\cdot}47$, $P = 0{\cdot}06$). In accordance with the above correlations, patients with $T_{l,CO}$ below 80% predicted tended to have higher percentages of $CD8^+$ and $CD56^+/CD16^+/CD3^-$ cells than those with normal $T_{l,CO}$ values ($P = 0{\cdot}06$ and $P = 0{\cdot}09$, respectively). Also, the percentage of $CD56^+/CD16^+/CD3^-$

Table 4. Bronchoalveolar lavage cell counts and differentiation in 19 patients with SLE and healthy controls

		SLE			Controls			
Parameter	Unit	Median	Range	*n*	Median	Range	*n*	*P*
Fluid recovery	%	74·4	26–85	19	77·0	38–93	23	NS
Cell concentration	$\times 10^6$/ml	0·05	0·01–0·58	19	0·11	0·03–0·66	23	NS
Alveolar macrophages	%	94·5	66–98	18	91·0	79–100	23	NS
	$\times 10^3$/ml	55·2	9·0–55·7	18	97·2	25·8–640·2	23	0·10
Lymphocytes	%	3·5	1–23	18	9·0	0–17	23	0·015
	$\times 10^3$/ml	2·8	0·3–12·2	18	8·8	0·0–34·3	23	0·002
Neutrophils	%	2·0	0–25	18	1·0	0–6	23	0·07
	$\times 10^3$/ml	1·0	0–26·6	18	1·1	0·0–15·0	23	NS
Eosinophils	%	0·0	0–5	18	0·0	0–2	23	NS
	$\times 10^3$/ml	0·0	0–5·3	18	0·0	0·0–11·2	23	NS

NS, Not significant ($P > 0{\cdot}10$).

Table 5. Phenotypes of lymphocytes in blood and bronchoalveolar lavage (BAL) of 19 patients with SLE and healthy controls

		SLE			Controls			
	Unit	Median	Range	*n*	Median	Range	*n*	*P*
Lung lymphocytes								
$CD3^+$ lymphocytes	%	83·0	54–93	15	72·5	62–80	8	0·012
	$\times 10^3$/ml	2·6	0·4–11·4	15	5·3	0·9–10·1	8	NS
$CD4^+$ lymphocytes	%	48·0	1–86	17	47·0	35–53	8	NS
	$\times 10^3$/ml	1·1	0·01–8·5	17	3·5	0·7–6·5	8	0·04
$CD4^+HLA\text{-}DR^+$	% of $CD4^+$	72·0	37·5–87·0	7	27·5	17–37	6	0·001
	$\times 10^3$/ml	0·8	0·59–6·3	7	NA	—	—	—
$CD8^+$ lymphocytes	%	34·0	2–69	17	33·0	25–38	8	NS
	$\times 10^3$/ml	1·1	0·01–7·6	17	2·5	0·5–4·8	8	0·10
$CD8^+HLA\text{-}DR^+$	% of $CD8^+$	77·8	37·9–96·9	7	NA	—	—	—
	$\times 10^3$/ml	0·6	0·30–7·6	7	NA	—	—	—
$CD25^+$ lymphocytes	%	3·0	0–7	9	NA	—	—	—
	$\times 10^3$/ml	0·1	0–0·3	9	NA	—	—	—
$CD19^+$ lymphocytes	%	0·0	0–1	6	10·5	9–15	8	0·002
	$\times 10^3$/ml	0·0	0–0·04	6	0·9	0·1–1·5	8	0·002
NK lymphocytes	%	4·0	1–19	11	NA	—	—	—
	$\times 10^3$/ml	0·1	0·01–1·0	11	NA	—	—	—
$CD4^+/CD8^+$ ratio		1·32	0·3–9·6	17	1·40	1·32–1·50	8	NS
Blood lymphocytes								
$CD3^+$ lymphocytes	%	72·0	44–91	19	71·5	63–78	8	NS
$CD4^+$ lymphocytes	%	40·0	19–63	19	43·5	36–52	8	NS
$CD4^+HLA\text{-}DR^+$	% of $CD4^+$	8·3	2·3–55·1	18	3·0	2–6	25*	0·001
$CD8^+$ lymphocytes	%	29·0	13–50	19	31·0	25–39	8	NS
$CD8^+HLA\text{-}DR^+$	% of $CD8^+$	13·0	2·4–34·3	18	2·0	1–8	25*	0·001
$CD25^+$ lymphocytes	%	3·0	0–15	19	NA	—	—	—
$CD19^+$ lymphocytes	%	13·0	2–26	18	6·0	5–9	8	0·02
NK lymphocytes	%	6·0	2–27	19	14·0	6–38	25*	0·001
CD4/CD8 ratio		1·41	0·57–3·77	19	1·41	1·28–1·55	8	NS

NA, Not available; NS, not significant ($P > 0·10$).
* Blood lymphocyte data only, no BAL data available.

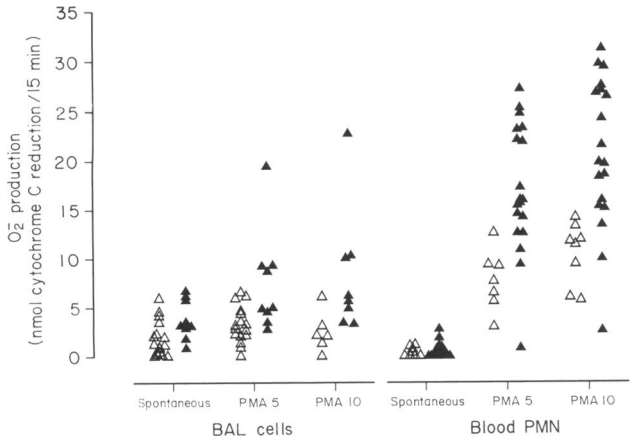

Fig. 1. Oxygen radical production by bronchoalveolar lavage (BAL) cells and blood polymorphonuclear cells in 19 patients with SLE and healthy controls. △, Healthy controls; ▲, SLE patients; PMA 5, after stimulation with phorbol myristate acetate (PMA) 5 ng/ml; PMA 10, after stimulation with PMA 10 ng/ml.

positive cells was significantly higher in patients with impaired Dm *versus* those with a normal Dm ($P = 0·04$). No correlations with HLA-DR and CD25 expression were found.

As opposed to the parameters of pulmonary diffusing capacity, there were no significant correlations between parameters of small airways disease, i.e. late-expiratory flow rates and airway resistance, and BAL lymphocyte percentages.

Disease activity

Of the 19 patients, one patient had a major exacerbation and one had a minor exacerbation. The median Liang score was 6, and ranged from 0 to 19. The Liang score did not correlate with either blood or lung lymphocyte phenotypes or with lung function.

Treated patients did not differ significantly from untreated patients regarding Liang score, lung and blood lymphocyte phenotypes or lung function pattern, and were therefore not analysed separately.

DISCUSSION

In this study we examined the relationship between peripheral blood and BAL lymphocyte phenotypes and lung function in 19 patients with SLE, and evaluated their association with disease

activity. Lung function assessment showed a mildly restrictive pattern with frequent impairment of $T_{l,CO}$, Dm and late-expiratory airflow rates. $T_{l,CO}$, K_{CO} and Dm correlated inversely with the numbers of $CD8^+$ cells and $CD56^+/CD16^+/CD3^-$ (NK) cells in BAL. Oxygen radical production by BAL cells and blood PMN was significantly increased in SLE. In comparison with healthy controls, patients with SLE had a lower percentage of $CD19^+$ cells in the BAL *versus* an increased percentage of these cells in peripheral blood. HLA-DR expression on $CD4^+$ $CD8^+$ lung lymphocytes was markedly increased in SLE. Current SLE disease activity was not associated with changes in BAL or peripheral blood lymphocyte phenotypes.

Nowadays, BAL is widely used to obtain pulmonary cells and alveolar epithelial lining fluid in patients with pulmonary diseases. BAL has previously been performed in SLE, usually showing an increased percentage of lymphocytes [9,10]. However, little is known about the distribution and state of activation of lymphocyte phenotypes and their relationship with SLE disease activity. Such knowledge is essential to provide insight into the pathogenic mechanisms of lung involvement in SLE. Currently, these mechanisms remain the subject of controversy, with immune complexes implicated by some investigators and T lymphocyte dysregulation by others [21]. Pathological studies have not shown consistent results. Pulmonary immune complex depositions have been found but could not be confirmed in other studies [2–4,22]. Given the similarities between interstitial lung involvement in SLE and scleroderma, the fact that a scleroderma-like connective tissue disease pattern, including interstitial lung disease, can occur in chronic graft-*versus*-host disease [23–25] may suggest that pulmonary involvement is an immunological process with a pivotal role for the T lymphocyte in both scleroderma and SLE.

Results of lung function assessments in our population were compatible with a mildly restrictive pattern, with impairment of the pulmonary diffusing capacity in most patients. This is in accordance with previous studies, which have shown impaired $T_{l,CO}$ to be a frequent finding [22,26–29]. Late-expiratory flow rates have also been shown to be decreased [27] and are considered to reflect small airways disease. Thus, pulmonary abnormalities in our SLE population may be considered as representative for those occurring in SLE in general.

BAL data of our SLE patients showed mild inflammation, without increased cell counts and lymphocyte numbers, but with a tendency toward neutrophil alveolitis, as opposed to the lymphocyte alveolitis reported by others [9,10]. Assessment of BAL lymphocyte phenotypes showed a marked decrease of $CD19^+$ (B) lymphocytes in SLE compared with healthy controls. These data do not support local B cell proliferation, which would be expected in case of local immunoglobulin production and immune complex formation. Although immunohistology directed at lymphocyte subsets has not been performed on a large scale in SLE, such studies in cryptogenic fibrosing alveolitis have shown the occurrence of secondary follicles with germinal centres in the lung, whereas interstitial cells were predominantly of the $CD4^+$ and to a lesser extent of the $CD8^+$ phenotype [30]. Thus, it cannot be excluded that B cells in SLE lungs are present in a germinal centre configuration and not represented in the BAL fluid. In contrast with the lungs, blood B lymphocytes were increased in SLE *versus* healthy controls, which may reflect the excessive B cell proliferation that has been proposed as a factor in the pathogenesis of SLE [1]. HLA-DR expression on lung T lymphocytes was markedly higher than on blood T lymphocytes, which suggests local T cell activation, both of $CD4^+$ and $CD8^+$ cells. The low expression of CD25 on these cells suggests chronic rather than acute antigen-induced activation. Our data suggest a T cell-mediated disease process rather than local B cell activation resulting in antibody and immune complex formation. When interpreting the data two factors which may have influenced the results should be taken into account. First, the sex ratio in patients and healthy controls was clearly different. Lymphocyte subset distributions between males and females were, however, not different in the healthy controls (data not shown). Although this does not exclude an influence of sex ratio on the results, it makes such an influence less probable. Second, the number of specific lymphocytes counted by FACS analysis was rather small for certain subsets. However, FACS analysis allowed the counting of more cells than would any manual procedure.

In the evaluation of the relationship between lung function and lung lymphocyte phenotypes, it appeared that parameters of pulmonary diffusing capacity, such as $T_{l,CO}$, K_{CO} and Dm correlated with the percentage and number of $CD56^+/CD16^+/CD3^-$ (NK) cells and $CD8^+$ cells. Lung volume parameters, on the other hand, did not. The accumulation of NK cells may have resulted from local cytokine production by activated T cells. The primary stimulant causing these T cells to become activated as well as the target for the cytotoxic T cells, however, is not known. It is generally accepted that lung inflammation will, in time, result in damage to the lungs and in worsening of lung function. Decreased $T_{l,CO}$, K_{CO} and Dm are likely to be the earliest signs, while ongoing damage may lead to fibrosis and loss of lung volume as well as deformation of the small airways. However, the time interval between the primary event, i.e. inflammation, and later stages of lung damage may disguise these relationships. We found a correlation between two early parameters of lung damage, i.e. inflammation and impaired diffusing capacity, which is compatible with this theory. Other lung function disturbances, such as decreased late expiratory airflow and increased airway resistance, can be considered as a cumulative effect of earlier damage. The relation of these parameters with the primary event may therefore be obscured and no correlations are likely to be found.

Although disease activity was generally low or absent in our patients, and a number of them used low dose corticosteroids, oxygen radical production by both BAL cells and blood PMN compared with controls was increased. This suggests that BAL cells with the potential to produce oxygen radicals, i.e. alveolar macrophages, neutrophils and eosinophils, as well as blood PMN, are nevertheless activated. In the lungs, this may be related to up-regulated HLA-DR expression on lymphocytes, since activated lymphocytes can produce cytokines that up-regulate O_2^- production of the cells mentioned above [31]. The absence of correlations between disease activity and lung function and lung lymphocyte phenotypes is in accordance with the clinical impression that SLE lung involvement follows a steady progressive course of interstitial lung disease in SLE, more or less independent of exacerbations of the disease.

Taken together, our findings suggest that a chronic cell-mediated immune response plays a role in the development of interstitial lung involvement in SLE. The presence of NK cells, associated with increased HLA-DR expression on T lymphocytes, up-regulation of the local production of oxygen radicals,

primarily by alveolar macrophages, may well fit into the concept of a DTH-like reaction.

Our data support the hypothesis of an important role for the T lymphocyte in the development of interstitial lung disease in SLE. No evidence for local B cell proliferation was found. Our data do suggest that an ongoing cell-mediated immune response with impaired pulmonary diffusing capacity is present in the lungs in SLE, and that this process seems to be independent of general SLE disease activity.

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