Subclinical alveolar inflammation in rheumatoid arthritis: superoxide anion, neutrophil chemotactic activity and fibronectin generation by alveolar macrophages


ABSTRACT: Interstitial lung disease (ILD) can be detected by pulmonary function testing (PFT) in 30-40% of rheumatoid arthritis (RA) patients. We assessed by bronchoalveolar lavage (BAL) the patterns of alveolitis in 21 RA patients: group 1 comprised 12 patients without evidence of ILD, and group 29 patients with clinical ILD defined by abnormal pulmonary function tests and/or chest X-ray. Cellular characteristics of BAL were studied in both groups. In addition, alveolar macrophages (AM) from patients in group 1 were isolated, and three parameters of cellular activation were studied: superoxide anion, fibronectin and neutrophil chemotactic activity generation. Total cell counts were not increased in group 1 but significantly increased in group 2 compared to controls. In group 1, 9/12 patients had elevated lymphocyte percentage (>18%) suggesting subclinical lymphocyte alveolitis. In contrast, neutrophil alveolitis (>4%) was found in 7/9 patients in group 2, mean percentage 12.9±4.2, compared with 1.2±6.4% in controls and 1.9±0.5% in group 1. These changes were not correlated with disease duration nor rheumatoid factor titres. Marked elevation of lymphocyte percentage was observed in patients with abnormal serum beta-2-microglobulin. Alveolar macrophages from group 1 patients released increased amounts of superoxide anion (7260±2780 vs controls 850±120 URL/5·10⁶ cells), neutrophil chemotactic activity (21±4.8 vs controls 8.1±4.7 cells/10² cells/hour), and fibronectin (61±1.6 vs controls 1.3±0.2 ng·10⁶ cells/hour). Whether or not lymphocyte alveolitis and/or AM dysfunction are pathogenic mechanisms of subsequent interstitial lung disease in patients who are still free of symptoms remains to be determined.


Pulmonary involvement is a major extra-articular manifestation of rheumatoid arthritis (RA). Interstitial lung disease (ILD) was reported in less than 5% of RA patients in studies using radiographic evaluation [1]. However, there is evidence that chronic infiltrative lung disease may be present in patients with normal chest roentgenograms [2]. More recently pulmonary function tests studies detected abnormalities in 30 to 40% of RA patients [3, 4]. Moreover, combined use of diffusing capacity, X-ray and lung biopsy showed disturbances in up to 80% of patients [5]. Thus subclinical ILD as a frequent feature of RA requires precise tools for diagnosis, pathophysiologic approach and prognostic evaluation.

Alveolitis is usually recognized as the first stage of chronic interstitial lung disorders, preceding irreversible fibrosis [6, 7]. Bronchoalveolar lavage (BAL) has emerged as a useful investigative method for evaluation of alveolitis [8, 9]. In addition, a good correlation has been shown between BAL disturbances and histological findings for parenchymal inflammation assessment [10]. In this context, a subclinical alveolitis was demonstrated by BAL in 48% of patients with various collagen vascular diseases [11] and recent studies described BAL differential cell count abnormalities in RA patients without evidence of ILD [12, 13].

With this in mind, we initiated this study to evaluate by BAL the cellular characteristics of alveolitis in RA patients without evidence of ILD and compared our results with those obtained from healthy controls and from RA patients with ILD.

The occurrence of pulmonary fibrosis requires a
combination of parenchymal injury, recruitment of inflammatory cells, and fibroblast proliferation. In this regard, AM are considered to play a key role in different steps of alveolitis by producing a variety of inflammatory and cell recruiting mediators. Consequently, free radical generation, neutrophil chemotactic activity (NCA) and fibronectin production by AM from symptom free patients (group 1) were evaluated excluding corticosteroid and/or immunosuppressive treated patients with ILD (group 2).

Subjects and Methods

Patients

Twenty-one patients with classical or definite RA [14] were prospectively studied. They comprised 3 males and 18 females with a mean age of 55 yrs (range 29–71). Pulmonary symptoms, clinical signs, and medication history were determined in all patients. All patients were non smokers and none had a significant environmental exposure to toxic gases or dusts, or previous pulmonary disease unrelated to RA. The control group (18 subjects) included hospital staff and healthy volunteers. All were non smokers.

Methods

Informed consent was obtained from all patients. Mean disease duration was 10 yrs (range 1-23). Screening of secondary Sjögren syndrome was systematically performed based on clinical criteria (xerophthalmia and/or xerostomia, positive Schirmer test) and minor salivary gland biopsies. Rheumatoid factor was assessed by nephelometry (Hyland disc 120, Hyland Lab). Beta-2-microglobulin was radioimmunoassayed using a commercial test (Phadebas Pharmacia, Uppsala, Sweden). Results were considered abnormal if over 2.5 mg/l.

Definition of patient groups

Chest roentgenograms. Posteroanterior and lateral roentgenograms were evaluated by a radiologist unaware of clinical and BAL data.

Pulmonary function tests (PFT). Forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and total lung capacity (TLC) were determined using a Jaeger spirometer. Carbon monoxide diffusing capacity (DLco) was obtained by a single breath method and height were obtained from standard tables [15]. Data were expressed as a percentage of the predicted values. Pulmonary function tests were considered abnormal if at least two of the following parameters were reduced: FEV1, TLC, FVC<80% predicted, and/or DLco<75% predicted.

According to clinical data, radiological and PFT findings, patients with RA were divided in 2 groups: group 1 included 12 patients without evidence of interstitial lung disease; group 2 included 9 patients with clinical interstitial lung disease defined as abnormal X-ray consistent with an interstitial disorder and/or a restrictive pattern of PFT. Clinical and biological data in both groups are shown in table 1.

Table 1. – Clinical biological and pulmonary function data in group 1 and group 2 patients. Results are expressed as mean±SEM.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Age yrs</th>
<th>Disease duration yrs</th>
<th>Sjogren's syndrome</th>
<th>Dyspnoea</th>
<th>ESR mm·h</th>
<th>Beta-2-microglobulin mg·l⁻¹</th>
<th>FVC % predicted</th>
<th>TLC % predicted</th>
<th>TLco % predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>53.9±4.7</td>
<td>11.7±2.1</td>
<td>4/12</td>
<td>2/12</td>
<td>40±6</td>
<td>3.3±0.3 (n=10)</td>
<td>97±2.6</td>
<td>11±±1.2</td>
<td>107±6.8</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>60.9±3</td>
<td>10.5±2.6</td>
<td>2/9</td>
<td>6/9</td>
<td>39±7</td>
<td>2.5±0.2 (n=6)</td>
<td>59±2.4</td>
<td>69±±5.1</td>
<td>66±4±5.5</td>
</tr>
</tbody>
</table>

ESR: erythrocyte sedimentation rate; FVC: forced vital capacity; TLC: total lung capacity; TLco: diffusing capacity for carbon monoxide; *; mean-B2-microglobulin is not significantly different between the two groups and between each group and the controls. †; 1 versus 2: p<0.05. FVC, TLC and TLco are expressed as % of predicted value.

Bronchoalveolar lavage

Bronchoalveolar lavage was performed after premedication with atropine under local anaesthesia with lignocaine using a wedged fibroptic bronchoscope (Olympus model BF-B3; Olympus Corps of America, New Hyde Park, N.Y.). 250 ml of sterile saline solution were instilled in five 50 ml aliquots with immediate gentle vacuum aspiration after each aliquot, as previously described [11]. Total number and differential cell counts were determined and the cellular variability of alveolar macrophages was assessed by trypan blue exclusion.

Alveolar macrophage isolation

The fluid was filtered through several layers of sterile surgical gauze and centrifuged at 400 g for 10 min at 4°C. After three washings the pellet was resuspended at a cell concentration of 1.5x10⁶ ml⁻¹ in Hank’s balanced salt solution (HBSS). 2 ml of the cell suspension were cultured at 37°C in humidified air with 5% CO₂ in 35 mm diameter Petri dishes. The cell population consisted of at least 95% alveolar macrophages after a 2 h adherence phase; basophil cells and mastocytes were absent from the culture as previously demonstrated [16].
Evaluation of alveolar macrophage function

To determine whether alveolar macrophages from patients in group 1 without clinical interstitial lung disease were activated, three secretory products were evaluated: superoxide anion generation before and after stimulation by phorbol-myristate-acetate (PMA), release of neutrophil chemotactic activity for neutrophils (NCA) and of fibronectin.

The chemiluminescence of AM was investigated using a lucigenin-dependent chemiluminescence method adapted from Williams and Cole [17, 18]. Lucigenin (10⁻⁴ M) (Sigma) was dissolved in HBSS buffered with 18 mM HEPES (N-2-hydroxethylpiperazine-N-2-ethane-sulphonic-acid). PMA (Sigma) was dissolved in dimethylsulphoxide at a concentration of 0.5 mg·mL⁻¹. PMA (10µl) was dissolved just before use in 5 ml of HBSS-HEPES. Superoxide dismutase (SOD) (Sigma) was dissolved in HBSS-HEPES (120 µg·ml⁻¹). AM with HBSS were centrifuged at 800 g (10 min, 4°C) and resuspended in HBSS-HEPES to a concentration of 1x10⁶ viable AM per ml. The suspension was kept on ice in a siliconized glass container until use. Chemiluminescence (CL) was measured at 37°C using a nucleometer 107. AM suspension aliquots (500µl) were added to vials containing lucigenin aliquots (900µl) with or without PMA (100µl) and/or SOD (100µl). The total volume in each vial was brought to 1650 µl by adding a 50µl aliquot from 3% gelatin solution and the appropriate amount of HBSS-HEPES. Intensity of luminescence was integrated for 60 sec after a 12 min incubation at 37°C. The results are expressed in relative luminescent units (RLU) per 0.5x10⁶ viable AM.

Alveolar macrophage derived chemotactic factor for neutrophils was measured as previously described [19]. In brief, alveolar macrophages were cultured at 1.5x10⁶ cells per ml in serum-free RPMI-1640 at 37°C for three hours. The supernates were collected and assayed for neutrophil chemotactic activity (NCA) by counting the number of neutrophils that passed through a 3µm micropore filter (Nucleopore Corp., Pleasanton, California) from the upper compartment of a 48-well microchemotaxis chamber (Neutro Probe, Cabin John, Md) in response to the macrophage supernate placed in the lower chamber. In order to distinguish chemokinesis from chemotaxis, experiments were performed in which supernatant samples were placed on both sides of the filter membrane. This procedure demonstrated no significant difference between the negative controls and the experimental wells and proved the existence of a chemotaxis mechanism. The number of neutrophils that had migrated through the filter was determined microscopically by the use of an oil immersion lens. Four fields were read per well. Experiments were conducted in groups of four. The results were expressed as the difference between the mean number of cells per field in the experimental well and the mean number of cells per field on the control well (migration towards medium). Positive control experiments consisted of migration towards FMLP 10⁻⁷M (Peninsula Laboratories Inc., San Carlos, California).

Alveolar macrophage fibronectin production was measured by competitive radioimmunoassay as previously described [20]. AM were cultured in the same conditions and the fibronectin secreted in the medium was measured. The results were expressed as nanogram of fibronectin per 10⁶ cells per hour.

Statistical analysis

Results are expressed as mean±SEM. Comparison between groups used Mann-Whitney U-test since most data are non parametric. Correlations were analysed with the Spearman rank test using a 5% significance level.

Results

Bronchoalveolar lavage results

Total cell count: Total cell numbers were not significantly elevated in group 1 (12±7±2.0x10⁴·ml⁻¹) compared with controls (10.2±1.1±1x10⁴·ml⁻¹). Group 2 had the highest cellular concentration (20±4.7±1x10⁴·cell·ml⁻¹) significantly different from controls (p<0.05) but there was no statistical difference between group 1 and group 2.

Cell Differential: Results are expressed as a percentage of (1) lymphocytes, (2) neutrophils and (3) eosinophils.

(1) Lymphocytes. Interestingly lymphocyte percentage was markedly elevated (>18%) in 5 out of 12 patients without ILD (group 1) (range 22-43%). However, no significant difference could be seen between mean lymphocyte percentage in group 1 (17.3±3.8%) and the control group (9.1±2.2%) (fig. 1). In contrast only 1 of the 9 group 2 patients exhibited an abnormal lymphocyte percentage (24%).

(2) Neutrophils. Neutrophil count abnormalities were more prominent (fig. 1). In group 1, 3 out of 12 patients exhibited a slightly elevated percentage (range 5-6%). Mean proportion of neutrophils (2.2±0.6%) was not significantly different from controls (1.2±0.4%). An increased proportion of neutrophils was detected in group 2 (12.9±4.2%), significantly different from controls and from group 1 (p<0.01); 5 out of 9 patients had a marked elevation of neutrophil percentage (>10%).

(3) Eosinophils. No difference appeared between patient groups and controls. Only one patient in group 2 had a slightly elevated percentage (3.5%).

Relationship between differential counts, clinical and biological data

Neutrophil and lymphocyte percentages did not differ significantly between patients with recent or long term disease duration. Surprisingly, secondary sicca syndrome was not associated with higher lymphocyte counts. Similarly, serum rheumatoid factor titres were unrelated to cell differential abnormalities. But
There was no correlation between clinical data and neutrophil percentage.

Interestingly, patients with abnormal serum beta-2-microglobulin had significantly more lymphocytes in BAL (mean 21±3.7%) than did patients with normal serum beta-2-microglobulin (7.8±1.4%, p<0.05). There was no correlation between clinical data and neutrophil percentage.

<table>
<thead>
<tr>
<th>Secretory products</th>
<th>units</th>
<th>Control</th>
<th>Group 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide anion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Spontaneous</td>
<td>RLU</td>
<td>850±120</td>
<td>7260±2700**</td>
</tr>
<tr>
<td>- PMA induced</td>
<td>RLU</td>
<td>16320±1440</td>
<td>28700±6220*</td>
</tr>
<tr>
<td>Neutrophil chemotactic activity</td>
<td>cells/HPF</td>
<td>8.1±0.7</td>
<td>2±0.8*</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>ng·10^6cells h^-1</td>
<td>1.3±0.2</td>
<td>6.1±1.6*</td>
</tr>
</tbody>
</table>

*Group 1 versus controls p<0.05; **Group 1 versus controls p<0.01. PMA: phorbol-myrisate-acetate, RLU: relative luminescent units.

Alveolar macrophage secretory products

Superoxide anion release: When studied at baseline, CL response of AM was significantly greater (7260±2700 RLU) compared with controls (850±120 RLU; p<0.01) (table 2). Mean SOD inhibition was 79% (range 57–96%).

In contrast, after stimulation with PMA there was only a mild increase in group 1 (28700±6220 RLU) compared with controls (16320±1440 RLU, p<0.05). SOD inhibited 83 to 97% of PMA induced CL.

Neutrophil chemotactic activity: An increased NCA was detected in AM supernatants from six group 1 patients (range 14–41 cells/HPF, mean 21±4.8) and a significant difference appeared with controls (8.1±0.7 cells/HPF, p<0.05) (table 2). No correlation was found between NCA and neutrophil proportion in BAL fluid, but low chemotaxis values (<10 cells/HPF) were associated with low polymorpho nuclear neutrophil (PMN) percentage (0–1%) in BAL fluid.

Fibronectin release: Compared to levels usually recovered from control alveolar macrophages 6 out of 9 patients released abnormal amounts of fibronectin (>3 ng·10^6 cells/hour, range 6–13) (table 2) and mean production was significantly greater than in controls (6.1±1.6 vs 1.3±0.2 ng·10^6 cells/h^-1).

Correlation between alveolar macrophage function. There were no correlations between spontaneous CL, NCA and fibronectin. A significant correlation was found between PMA induced CL and NCA (r=0.76 p<0.05).

Correlation between cell differentials and alveolar macrophage functions. There were no correlations between lymphocyte proportion and CL, NCA or fibronectin production. In contrast, a significant correlation was found between neutrophil percentage in BAL fluid and spontaneous CL (r=0.64 p<0.05). Group 1 was divided into a subgroup of patients who had normal BAL differential counts and a subgroup of those who had elevated neutrophil and/or lymphocyte percentages (fig. 2).

Interestingly, AM from some patients with normal BAL differential counts released increased amounts of superoxide anion and/or NCA, and/or fibronectin.
Conversely, normal AM secretory functions were only found in those patients with normal BAL differential counts.

**Discussion**

Our results demonstrate a high incidence of BAL abnormalities in RA patients with and without clinical evidence of ILD and allow definition of two preferential patterns of alveolitis according to the group studied.

An increased number and proportion of neutrophils in BAL suggesting a neutrophil alveolitis was prominent in 7 out of 9 patients with clinical ILD. Indeed neutrophil alveolitis with increased total cell concentration is a classical feature of alveolitis in idiopathic pulmonary fibrosis (IPF) [6] and ILD associated with systemic sclerosis [21].

In patients without clinical ILD, abnormalities in BAL content were less prominent, but 5 out of 12 patients had a pattern of lymphocyte alveolitis. Among them, only one had an associated secondary sicca syndrome. These results parallel histopathologic findings of lung biopsies. Beside rheumatoid nodules, bronchiolitis obliterator and usual interstitial pneumonitis, frequent primary patterns include lymphoid hyperplasia and mononuclear interstitial infiltrates [22].

Our data are in agreement with a recent study of Garcia et al [12]. Elevated percentages of lymphocytes were detected in 5 of the 15 symptom free patients with normal X-ray and PFT. In contrast, Tischler [23] demonstrated a lymphocyte alveolitis only in patients with mild X-ray interstitial changes.

Mechanisms responsible for lymphocyte alveolitis in RA remain unclear. Although lymphocyte alveolitis was reported to be an usual feature of primary Sjögren syndrome [11] abnormal lymphocyte counts were not significantly associated with secondary sicca syndrome. Interestingly, lymphocyte alveolitis was related in our study to high serum levels of beta-2-microglobulin. An elevation of plasma, urinary and synovial beta-2-microglobulin is well known in RA and seems to be correlated with inflammatory parameters, circulating immune complex levels and severity of joint disease [24–26]. Although non specific beta-2-microglobulin appears as a good index of lymphocyte total mass and of cell membrane turnover. Thus lymphocyte proliferation in alveolar spaces might be related to the generalised hyperreactivity of lymphoid tissue associated with RA [27] paralleling synovial disorders.

In the second part of our study, three parameters of alveolar macrophage activation were examined in patients without clinical ILD.

Among a wide range of secretory activities [28, 29] hydrogen peroxide and superoxide anion production by AM are triggered by cell membrane stimulation. Lucigenin dependent chemiluminescence correlates closely with oxygen radical release [17] and inhibition by SOD suggests a close relationship with superoxide anion generation. Our results demonstrate a prominent increase of spontaneous CL indicating oxidant species production by BAL cells. A significant correlation appeared with PMN percentage suggesting a possible participation of PMN. Nevertheless, experimental contamination of AM suspension with similar amounts of PMN produced only a slight increase of lucigenin-dependent CL response, in contrast with luminol dependent method [17]. In addition lymphocytes do not produce detectable amounts of oxygen radical. Thus free radical generation in BAL fluid from our patients probably reflects mainly AM production. PMA induced CL was not significantly different from controls. A previous activation occurring in vivo might explain such a low response to PMA.

Abnormal free radical production by AM is well established in IPF and both pulmonary and extrathoracic sarcoidosis [18, 30]. Lymphokines enhance in vitro generation of oxygen reactive intermediates by monocytes and monocyte-derived macrophages [31] but no correlation was found in our study between lymphocyte percentages in BAL fluid and AM CL. Oxygen radical production is a potent mechanism of respiratory membrane damage [32, 33] but the relative contribution of AM and recruited PMN to lung lesions is not yet determined in ILD. Experimental studies on lung explants showed a significant injury by IPF-AM which was reduced by antioxidants [34]. In addition a marked synergistic oxidant mediated cytotoxicity by IPF cells and alveolar epithelial lining fluid has been recently demonstrated by Cantin et al. [35]. Whatever its relevance in lung damage, oxidant generation reflects an AM activation which likely implicates other mechanisms and mediators.

An increased neutrophil chemotactic activity was detected in AM supernatants from six patients from group 1. Similarly, a significant neutrophil chemotactic activity was found by Garcia et al. [13] in BAL fluid from RA patients without ILD but who demonstrated a subclinical lymphocyte alveolitis. Paradoxically there was no correlation in our study between NCA and PMN proportion in BAL. Such a discrepancy has recently been described in unaffected family members of autosomal idiopathic fibrosis [36]. In contrast, AM from IPF patients were shown to spontaneously release neutrophil chemotactic factor mainly when BAL neutrophils exceeded 10% [37]. NCA was not correlated with spontaneous superoxide anion and fibronectin generation, but an unexpected significant correlation appeared with PMA induced CL. Although mechanisms of secretion, specificity and physical properties of NCA were not determined in our study, several lines of evidence suggest that local immune complexes may be responsible for AM activation generating NCA [37, 38]. The prognostic significance of elevated NCA in symptom free patients with minimal or without neutrophil alveolitis remains unclear. Long term follow-up of group 1 patients is necessary to confirm the hypothesis that NCA secretion by AM leads to a secondary and progressive neutrophil alveolitis and established interstitial disease.

Elevated fibronectin secretion was a third parameter of AM activation detected in 6 out of 9 patients. This
large glycoprotein produced by both fibroblasts and AM mediates cell matrix interactions such as cell adhesion and directed movement of parenchymal cells [39]. It is also a well recognized competence factor for fibroblast growth [40] and an elevated local production by AM is a classical feature of IPF [41]. Whether or not chronic fibronectin elevation could take part in subsequent interstitial structural derangement remains to be determined, since optimal fibroblast proliferation usually requires both a competence factor and a progression factor.

Although unrelated to each other, superoxide anion generation, NCA and fibronectin secretions are indicative of an obvious though subclinical AM activation in our patients. Furthermore activation was detected in some patients despite normal BAL differential counts, suggesting that AM dysfunction might precede recruitment of inflammatory cells such as neutrophils and lymphocytes. Thus study of AM secretory products appears as a useful and sensitive tool to investigate the early stage of subclinical alveolitis. Nevertheless the most relevant problem relates to the long term significance of this subclinical alveolitis in RA patients. In agreement with other studies, we found a high incidence of BAL cell abnormalities in symptom free patients. Moreover, increased AM generation of toxic and cell recruiting mediators was a frequent finding. One could hypothesize that in vivo simultaneous inhibitory mechanisms prevent lung damage in these patients. Such mechanisms include protective antioxidant interventions, namely antioxidant enzymes and oxygen radical scavengers. Similarly, a low molecular weight factor which inhibits PMN chemotaxis and superoxide release was recently shown to be produced by AM [42]. Assessing the pathogenic significance of these abnormalities requires further follow-up studies of clinical and PFT data in these patients.

References

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RÉSUMÉ: Les pneumopathies interstitielles sont fréquentes et volontiers infracliniques au cours de la polyarthrite rhumatoïde puisqu'une altération de transfert du CO est décelée chez 30 à 40% des patients. Le but de cette étude est de préciser grâce au lavage broncho-alvéolaire (LBA) l'incidence et les caractéristiques de l'alvéolite chez 21 patients non fumeurs ayant ou non une atteinte interstitielle décelable. Le groupe 1 (n=12) est constitué de patients asymptomatiques dont l'exploration fonctionnelle respiratoire et la radiographie de thorax sont normales. Le groupe 2 (n=9) rassemble les patients ayant un syndrome interstitiel radiologique et/ou un syndrome restrictif avec trouble du transfert du CO. Une alvéolite lymphocytaire (>18%) est décelée chez 5/12 patients du groupe 1, alors que 7/9 des patients du groupe 2 présentent une alvéolite à polynucléaires neutrophiles (>4%). Le pourcentage de lymphocytes est significativement plus élevé chez les patients ayant un taux sémique de bêta-2-microglobuline anormal. Trois activités sécrétées des macrophages alvéolaires (MA) en culture sont étudiées chez les patients du groupe 1. La production spontanée d'ions superoxyde est significativement élevée par rapport aux MA témoins (7260±2700 versus 850±120 unités luminesces relatives/5.10^6 cellules). La sécrétion de fibronectine est significativement augmentée comparativement aux témoins (6.1±1.6 versus 1.3±0.2 ng/10^6 cellules/heure), ainsi que l'activité chimiotactique du surm auage de culture pour les polynucléaires neutrophiles (PNN) (21±4.8 versus 8.1±0.7 PNN/champ microscopique). La signification pronostique de l'alvéolite lymphocytaire et de l'activation macrophagique décelées chez des patients sans atteinte respiratoire décelable reste à déterminer. Eur Respir J., 1989, 2, 7-13.