Effects of oral steroids on immunoglobulins in bronchoalveolar lavage fluid in active sarcoidosis


ABSTRACT: Immunoglobulin (Ig) levels increase in the lower respiratory tract of patients with pulmonary sarcoidosis. We evaluated the effects of prednisone therapy upon Ig concentrations in bronchoalveolar lavage (BAL) fluid of ten patients with active disease (>30% T-lymphocytes in BAL and positive 67Ga lung scan). Therapy significantly lowered T-lymphocyte percentages in BAL and 67Ga lung scan indices and was followed by a slight improvement of the studied functional parameters. Biochemical analysis of BAL showed a significant decrease of both IgG/albumin (baseline 1.24±0.21; after therapy 0.40±0.12) and IgA/albumin (baseline 0.55±0.07; after therapy 0.14±0.03) ratios in all patients. Conversely, comparisons of IgM/albumin ratios did not show any change over the study period (baseline 0.05±0.01; after therapy 0.06±0.03). Thus oral steroid treatment suppresses the alveolitis of pulmonary sarcoidosis, as shown not only by the reduction of lung T-cells and 67Ga lung uptake, but also by the decreased Ig levels in the alveolar spaces.

Eur Respir J 1988, 1, 505-509.

It has been suggested that in pulmonary sarcoidosis the activation of lung T-cells and the elevation of local helper/suppressor T-cell ratio [1-3] are at least partly responsible for the stimulation of immunoglobulin (Ig) production by lung B-cells [4-7].

Recent reports have also demonstrated a marked increase of Ig concentrations in bronchoalveolar lavage (BAL) fluid of patients with active disease as compared to patients with inactive disease and to normal controls [8-10]. Therefore, assessment of local Ig levels may be considered as an additional parameter suitable for evaluation of the activity of the inflammatory process in the lower respiratory tract.

The classical treatment for pulmonary sarcoidosis is based upon oral corticosteroids [11]. Although considerable debate remains about the effects of steroid treatment upon the ultimate prognosis [12], recent investigations have shown that steroid treatment is able to suppress the alveolitis of pulmonary sarcoidosis [3, 13].

This study was designed to evaluate whether oral prednisone therapy reduces Ig levels in the lower respiratory tract. Therefore, biochemical analysis of BAL, together with evaluation of cellular, functional and scintigraphic parameters was performed in ten patients with active pulmonary sarcoidosis (T-lymphocytes in BAL >30% and positive 67Ga lung scans) before and after six months of oral prednisone therapy.

Patients and methods

A diagnosis of pulmonary sarcoidosis was established in ten non-smoking patients (four males, six females; aged 40.6±3.9 yr) according to previously described criteria [2]. Seven patients (three males, four females) had never been treated, whilst three (one male, two females), who had previously been treated, had not received steroids in the previous twelve months. Patients were considered to be affected by persistent active disease [14], as demonstrated by: 1) T-lymphocytes in BAL >30% and 2) positive 67Ga lung scan in two consecutive assessments of alveolitis activity separated by therapy-free periods lasting 4-6 months. After demonstrating that spontaneous remission of the alveolitis had not occurred in any of the selected patients, the decision to start steroid therapy was taken.

Patients' evaluations were performed by chest X-rays, pulmonary function tests, 67Ga lung scans and BAL immediately before treatment (data hereafter referred to as 'baseline') (table 1) and after six months of oral prednisone therapy (0.75 mg·kg⁻¹ body weight for six weeks, then dosage was reduced by 2.5 mg each week to a daily maintenance dose of 0.25 mg·kg⁻¹). Patients were still receiving therapy at the time of final evaluation.

Chest roentgenograms were typed according to conventional three stage classification [15].
chemical analysis of BAL was carried out by centrifugation. Cell suspensions were then resuspended in RPMI 1640, with respect to total cells recovered, and percentages of T-lymphocytes were determined by a rosette-forming method [2, 8], using neuraminidase-treated sheep erythrocytes. Biochemical analysis of BAL was carried out by nephelometric measurements (Immunoclinic Analyser II, Beckman Instruments, Brea, CA, USA) of IgG, IgA, IgM, and albumin concentrations on supernatants, as described previously [8]. The supernatants were concentrated ten times by using an Amicon 8MC ultrafiltration system and stored at −20°C. Loss of the investigated proteins, due to concentration procedures, was excluded by random comparisons of determinations performed on both concentrated and unconcentrated lavage fluids and by demonstrating lack of proteins in the filtrate fluids.

*n°Ga lung scans* were performed by a rectilinear scanner 48 h after intravenous administration of *n°Ga citrate (50 μCi·kg⁻¹ body weight). *n°Ga indices were calculated according to the semiquantitative scoring method proposed by Line et al. [18], the score of 50 Units being considered as the normal threshold value.

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**Table 1.** Baseline roentgenological, functional, scintigraphic and bronchoalveolar lavage data from patients with active pulmonary sarcoidosis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Rx stage</th>
<th>VC% pred values</th>
<th>TLC% pred values</th>
<th>FEV₁% pred values</th>
<th>Dlco Units</th>
<th>BAL T-lymphocytes % of total cells</th>
<th>⁶⁷Ga Units</th>
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<td>M</td>
<td>II</td>
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<td>F</td>
<td>III</td>
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**Results**

Chest roentgenograms performed at the beginning of the study showed a moderate mean reduction in static and dynamic lung volumes (VC = 84.8 ± 6.4% of predicted; TLC = 84 ± 6.3% of predicted; FEV₁ = 86.4 ± 7.4% of predicted), as well as a more marked impairment in diffusing capacity (Dlco = 73 ± 4.5% of predicted). The same tests performed at the end of the study showed
Fig. 1. Effects of oral prednisone therapy upon \( ^{67} \text{Ga} \) lung uptake of patients with active pulmonary sarcoidosis. The data are expressed as \( ^{67} \text{Ga} \) index Units (see reference [18] for details).

![Graph showing effects of oral prednisone therapy on \( ^{67} \text{Ga} \) lung uptake](image)

A significant mean increase of all the considered indices with respect to baseline mean values (VC = +10.2±4.4%, \( p < 0.05 \); TLC = +9±3.5%, \( p < 0.05 \); FEV\textsubscript{1} = +11.1±4.5%, \( p < 0.05 \); DLCO = +13±3.8%, \( p < 0.01 \)).

Evaluation of \( ^{67} \text{Ga} \) lung scans showed a significant reduction of lung uptake after prednisone therapy with respect to baseline values (baseline 134±15.6 Units, after therapy 91±11.5 Units; \( p < 0.01 \)) (fig. 1); however, analysis of individual patients demonstrated no score modifications in two subjects and a slight elevation in one patient, whilst in two subjects scores dropped below the threshold value.

BAL was performed without difficulty in all patients. No bronchoscopic abnormalities were detected in the tracheobronchial tree and no differences were shown in terms of percentage of recovered fluid (baseline 57.3±4.5%, after therapy 55.2±5.8%; \( p > 0.2 \)). Comparative evaluation of cytological parameters showed that total cell counts (baseline 40.5±4.7 x 10\(^6\) cells, after therapy 20.5±3.2 x 10\(^6\) cells; \( p < 0.02 \)) and percentage of T-lymphocytes (baseline 40.6±2.8%, after therapy 20.6±4.5%; \( p < 0.01 \)) (fig. 2) were reduced after prednisone therapy. Biochemical analysis of BAL demonstrated a significant reduction of both IgG and IgA concentrations with respect to baseline values (IgG: baseline 6.99±1.27 mg%, after therapy 1.78±0.37 mg%; \( p < 0.005 \); IgA: baseline 2.9±0.31 mg%, after therapy 0.64±0.13 mg%; \( p < 0.001 \)); in contrast, IgM was detected at very low levels in all cases with no differences between the study groups (IgM: baseline 0.27±0.11 mg%, after therapy 0.21±0.09 mg%; \( p > 0.2 \)). In addition, although mean albumin concentrations were shown to be somewhat lower after steroid treatment, the difference was not significant (albumin: baseline 6.04±0.94 mg%, after therapy 4.75±0.59 mg%; \( p > 0.2 \)). In agreement with the previous data, comparisons of IgG/albumin and IgA/albumin ratios demonstrated a marked reduction of both ratios after prednisone therapy (IgG/albumin: baseline 1.24±0.21, after therapy 0.40±0.12; \( p < 0.001 \); IgA/albumin: baseline 0.55±0.07, after therapy 0.14±0.03; \( p < 0.001 \)) (figs 3 and 4), whilst comparisons of IgM/albumin ratios did not show any change over the study period (IgM/albumin: baseline 0.05±0.01, after therapy 0.06±0.03; \( p > 0.2 \)).

Fig. 2. Effects of oral prednisone therapy upon T-lymphocyte component of the alveolitis in patients with active pulmonary sarcoidosis. The data are expressed as percentages of BAL recovered cells (see Methods for details).

![Graph showing effects of oral prednisone therapy on T-lymphocyte component of BAL](image)

Fig. 3. Effects of oral prednisone therapy upon local IgG production, as assessed by comparisons of IgG/albumin ratios in BAL before and after six months treatment in patients with active pulmonary sarcoidosis.

![Graph showing effects of oral prednisone therapy on IgG production](image)
Bronchoalveolar lavage in sarcoidosis.

Steroids upon both macrophage and T-lymphocyte consequence of the suppression provided by cortico-components of the alvcolitis, ultimately leading to seems to be confirmed by comparisons of IgG and IgA concentrations in BAL before and after six months treatment in patients with active pulmonary sarcoidosis.

There are two potential sources for the Ig's that are detectable in the epithelial lining fluid: 1) transudation from plasma and 2) local production by B-lymphocytes (plasma cells) [9]. Since alveolar-capillary membrane permeability has previously been shown to be increased in pulmonary sarcoidosis [8, 19], the data relevant to Ig's were expressed both in terms of absolute concentration (mg%) and as ratios with respect to albumin, the latter protein being considered as a marker of capillary leakage [20]. Comparisons of IgG and IgA absolute concentrations in BAL showed a significant reduction of these classes of Ig after prednisone therapy, whilst analysis of IgM data showed no change over the study period. In addition, although a certain reduction of albumin concentration was found, showing that alveolar-capillary membrane permeability may be reduced along with the intensity of the alveolitis [8, 10], the latter difference was not significant. These data suggest that steroids reduce IgG and IgA concentrations in BAL by suppressing production of these classes of Ig's at sites of disease activity. The latter seems to be confirmed by comparisons of IgG/albumin and IgA/albumin ratios.

The inhibition of IgG and IgA synthesis in the lower respiratory tract could be considered as a consequence of the suppression provided by corticosteroids upon both macrophage and T-lymphocyte components of the alveolitis, ultimately leading to inhibition of B-cell activity. As regards alveolar macrophages, steroids have been shown to be effective in suppressing monocyte recruitment to the alveoli [21] and to inhibit Ig expression, interleukin 1 production and antigen presentation to T-lymphocytes [22]. Considering T-lymphocytes, a major mechanism by which steroids can reduce T-cell proliferation and state of activation is by suppressing production and release of interleukin 2 and gamma interferon, the role of which in the pathogenesis of the disease has been clearly shown [23, 24]. The latter hypothesis was suggested by in vitro investigations showing the suppressive effects provided by steroids on transcription of both lymphokines' genes [25] and was confirmed by recent studies performed on the lung T-cells isolated from sarcoid patients [26].

Modulation of macrophages and T-lymphocyte activities after steroid therapy may be responsible for reduction of IgG and IgA levels in BAL, as demonstrated in the present study. However, it must still be determined whether steroid therapy can directly reduce activation of B-cells and the proportion of Ig-secreting cells in the lungs of sarcoid patients.

The role played by increased local Ig production and immune complex formation in the pathogenesis of pulmonary sarcoidosis is still unclear. However, since immune complex deposition can result in the formation of granulomata in some tissue [27] and Ig's and immune complexes are present in sarcoid granulomata within the lungs [28], it is possible that Ig synthesized at sites of disease activity may be important in modulating at least some of the sarcoid lesions.

Therefore, reduction of local Ig levels after steroid therapy, as suggested by the results of the present study, may not simply mirror suppression of cell-mediated immune processes in the lower respiratory tract, but possibly reflects the inhibition of a process that might be relevant to the derangement of lung parenchyma in this disorder.

References

7. Lawrence EC, Martin RR, Blaee RM, Tangue RB, Ase RJ.
Suppression


RÉSUMÉ: Les niveaux d'immunoglobulines augmentent dans l'arbre respiratoire inférieur des patients atteints de sarcoidose pulmonaire. Nous avons évalué les effets d'une corticothérapie à la prednisone sur les concentrations d'immunoglobulines dans le lavage broncho-alvéolaire de 10 patients atteints de maladie active (T lymphocytes supérieurs à 30% dans le BAL et scan positif au Gallium 67). Les pourcentages de T lymphocytes dans le BAL sont significativement abaissés et les index du scan pulmonaire au Gallium également. De plus, on a noté une légère amélioration des paramètres fonctionnels étudiés. L'analyse biochimique du lavage broncho-alvéolaire a montré une diminution significative, à la fois du rapport IgG/albumine (1.24±0.21 au début; 0.40±0.12 après traitement), On a trouvé également un abaissement du rapport IgA/albumine (0.35±0.07 au début; 0.14±0.03 après traitement) chez tous les patients. De plus, les comparaisons des rapports IgM/albumine n'ont pas montré de modification pendant toute l'étude (valeurs de base 0.05±0.01; valeur après traitement 0.06±0.03; p>0.2). Une corticothérapie orale diminue l'alvéolite de la sarcoidose pulmonaire, ainsi que le démontrent, aussi bien la réduction du pourcentage de cellules T pulmonaires et la capture pulmonaire de Gallium, que par ailleurs la diminution des taux d'immunoglobulines dans les espaces alvéolaires.