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

M. Spatafora*, A. Mirabella**, G.A. Rossi***, A. Bonanno*, L. Riccobono*, A. Merendino**, V. Bellia**, G. Bonsignore**

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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 ^{67}Gallium (^{67}Ga) lung scan). Therapy significantly lowered T-lymphocyte percentages in BAL and ^{67}Ga 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 ^{67}Ga lung uptake, but also by the decreased Ig levels in the alveolar spaces.

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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 ^{67}Gallium (^{67}Ga) 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 ^{67}Ga 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, ^{67}Ga 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].

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

Patient	Age	Sex	Rx stage	VC	TLC	FEV_1	DLCO	BAL T-lymphocytes	^{67}Ga
				% pred values				% of total cells	Units
1) FS	54	M	II	103	106	110	80	32	100
2) RF	49	F	III	87	93	100	61	34	70
3) SF	33	F	I	90	81	87	72	40	150
4) NL	60	F	III	59	56	54	69	31	60
5) GN	26	M	I	102	100	108	88	32	110
6) LP	27	F	I	98	100	100	71	38	160
7) CL	49	M	II	78	83	75	68	47	180
8) TR	35	F	II	41	46	40	46	52	120
9) RS	32	F	II	90	77	90	78	45	190
10) IF	25	M	II	100	98	100	97	55	200
Mean	40.6			84.8	84	86.4	73	40.6	134
SEM	3.9			6.4	6.3	7.4	4.5	2.8	15.6

VC: vital capacity; TLC: total lung capacity; FEV_1: forced expiratory volume in one second; DLCO: transfer factor for carbon monoxide; BAL: bronchoalveolar lavage.

Functional evaluation was carried out by measuring the following four indices: 1) vital capacity (VC) and 2) forced expiratory volume in the first second (FEV_1) were calculated by electronic integration of measured flow; 3) total lung capacity (TLC) by constant volume body plethysmography (Bodytest System, Jaeger, Wuerzburg, W.G.); 4) transfer factor for carbon monoxide (DLCO) by the single breath-holding method (Morgan Transfer Test, Chatham, U.K.). All results are expressed as percentages of the mean predicted values reported by AMREIN *et al.* [16] for VC, FEV_1 and TLC and by COTES [17] for DLCO respectively.

^{67}Ga lung scans were performed by a rectilinear scanner 48 h after intravenous administration of ^{67}Ga citrate (50 $\mu Ci \cdot kg^{-1}$ body weight). ^{67}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.

BAL was performed by infusing five aliquots of 20 ml of sterile 0.9% NaCl solution through a bronchofibrescope, as described previously [8]. After recovery the aliquots were collected, filtered and centrifuged (500 g, 10 min). Cytological analysis of BAL was performed by assessing: 1) cell viability by using a trypan-blue dye exclusion method; 2) total counts by using a haemocytometer and 3) differential counts by identifying at least 400 elements on Diff-Quick (Merz-Dade AG, Dudingen, Switzerland) stained cytocentrifuge preparations. Cells were then resuspended in Hanks' balanced salt solution and percentages of T-lymphocytes, with respect to total cells recovered, were determined by a rosette-forming method [2, 8], using neuraminidase-treated sheep erythrocytes. Biochemical analysis of BAL was carried out by nephelometric measurements (Immunochemistry Analyzer 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.

Analysis of data. All data are presented as mean $\pm$ standard error of the means (SEM); comparisons were made by using the Student's t-test for paired data.

Results

Chest roentgenograms performed at the beginning of the study allowed staging of the patients as follows: three patients (one male, two females) were considered to be affected by pulmonary sarcoidosis stage I, five patients (three males, two females) stage II and two patients (both females) stage III. After prednisone therapy a slight reduction in mediastinal node dimensions was observed in one of the three stage I patients and in three of the five stage II patients. No major changes in chest X-rays were seen in stage III patients.

Pulmonary function tests at the beginning of the study showed a moderate mean reduction in static and dynamic lung volumes (VC = 84.8 $\pm$ 6.4% of predicted; TLC = 84 $\pm$ 6.3% of predicted; FEV_1 = 86.4 $\pm$ 7.4% of predicted), as well as a more marked impairment in diffusing capacity (DLCO = 73 $\pm$ 4.5% of predicted). The same tests performed at the end of the study showed

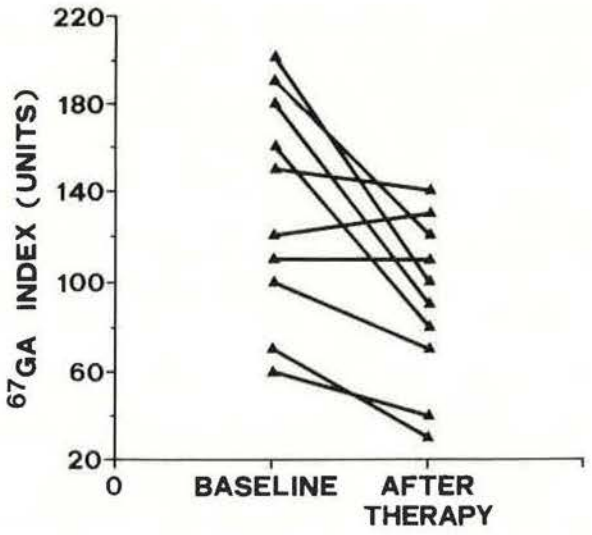

Fig. 1. Effects of oral prednisone therapy upon ^{67}Ga lung uptake of patients with active pulmonary sarcoidosis. The data are expressed as ^{67}Ga index Units (see reference [18] for details).

a significant mean increase of all the considered indices with respect to baseline mean values (VC = $+10.2\pm4.4\%$, $p<0.05$; TLC = $+9\pm3.5\%$, $p<0.05$; FEV_1 = $+11.1\pm4.5\%$, $p<0.05$; DLCO = $+13\pm3.8\%$, $p<0.01$).

Evaluation of ^{67}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 $57.3\pm4.5\%$, after therapy $55.2\pm5.8\%$; $p>0.2$). Comparative evaluation of cytological parameters showed that total cell counts (baseline $40.5\pm4.7\times10^6$ cells, after therapy $20.5\pm3.2\times10^6$ cells; $p<0.02$) and percentage of T-lymphocytes (baseline $40.6\pm2.8\%$; 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; $p>0.2$).

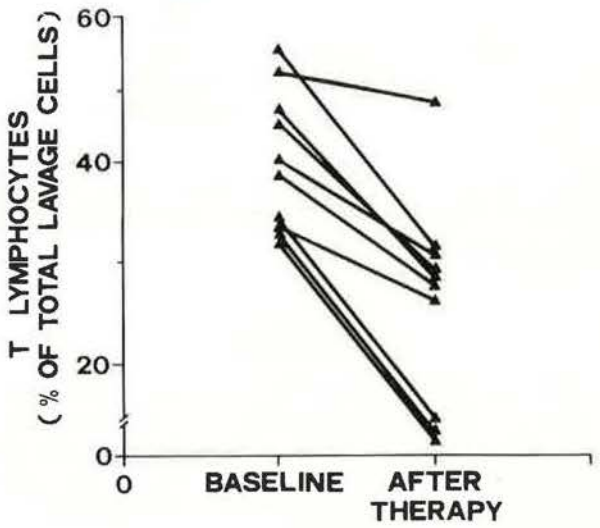

Fig. 2. Effects of oral prednisone therapy upon T-lymphocyte component of the alveolitis in patients with active pulmonary sarcoidosis. The data are expressed as percentages of BAL recovered cells (see Methods for details).

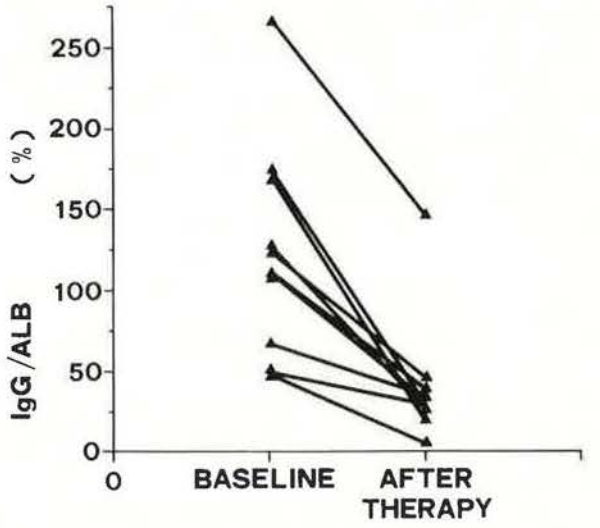

Fig. 3. Effects of oral prednisone therapy upon local IgG production, as assessed by comparisons of IgG/albumin ratios in BAL before and after six months treatment in patients with active pulmonary sarcoidosis.

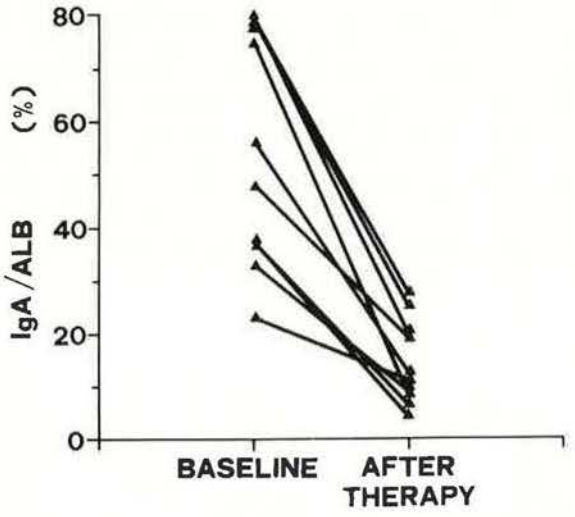

Fig. 4. Effects of oral prednisone therapy upon local IgA production, as assessed by comparisons of IgA/albumin ratios in BAL before and after six months treatment in patients with active pulmonary sarcoidosis.

Discussion

We have demonstrated that IgG and IgA concentrations in BAL fluid of patients with active pulmonary sarcoidosis are reduced after steroid therapy. In agreement with other reports [3, 13], this provides evidence that steroids can reduce T-cell percentages in BAL and ^{67}Ga lung uptake.

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

1. Hunninghake GW, Crystal RG. – Pulmonary sarcoidosis. A disorder mediated by excess helper T lymphocyte activity at sites of disease activity. *New Engl J Med*, 1981, 305, 429–434.
2. Rossi GA, Sacco O, Cosulich E, Risso A, Balbi B, Ravazzoni C. – Helper T lymphocytes in pulmonary sarcoidosis. Functional analysis of a lung T cell population in patients with active disease. *Am Rev Respir Dis*, 1986, 133, 1086–1090.
3. Ceuppens JL, Lacquet LM, Marieu G, Demedts M, van den Eeckout, Stevens E. – Alveolar T cell subsets in pulmonary sarcoidosis. Correlation with disease activity and effect of steroid treatment. *Am Rev Respir Dis*, 1984, 129, 563–568.
4. Daniele RP, Dauber JH, Rossman MD. – Immunological abnormalities in sarcoidosis. *Ann Intern Med*, 1980, 92, 406–416.
5. Chretien J, Venet A, Danel C, Israel-Biet D, Sandron D, Arnoux D. – Bronchoalveolar lavage in sarcoidosis. *Respiration*, 1985, 48, 222–230.
6. Hunninghake GW, Crystal RG. – Mechanisms of hypergammaglobulinemia in pulmonary sarcoidosis. *J Clin Invest*, 1981, 67, 86–92.
7. Lawrence EC, Martin RR, Blaese RM, Teague RB, Awe RJ,

Wilson RK, Deaton WJ, Bloom K, Greenberg SD, Stevens DM. – Increased bronchoalveolar Ig-secreting cells in interstitial lung diseases. *N Engl J Med*, 1980, 302, 1186–1188.
8. Spatafora M, Mirabella A, Rossi GA, Bonanno A, Riccobono L, Carini C, Sacco O, Ravazzoni C. – Lung inflammation in sarcoidosis: analysis of immunoglobulin levels in bronchoalveolar lavage fluid in active and inactive disease. *Respiration*, 1985, 48, 127–135.
9. Rankin JA, Naegel GP, Schrader CE, Matthay RA, Reynolds HY. – Air-space immunoglobulin production and levels in bronchoalveolar lavage fluid of normal subjects and patients with sarcoidosis. *Am Rev Respir Dis*, 1983, 127, 442–448.
10. Bauer W, Gorny MK, Baumann HR, Morell A. – T-lymphocyte subsets and immunoglobulin concentrations in bronchoalveolar lavage of patients with sarcoidosis and high and low intensity alveolitis. *Am Rev Respir Dis*, 1985, 132, 1060–1065.
11. Hunninghake GW, Keogh BA, Line BR, Gadek JE, Kawanami O, Ferrans VJ, Crystal RG. – Pulmonary sarcoidosis: pathogenesis and therapy. *In:* Basic and clinical aspects of granulomatous diseases. D.L. Boros, T. Yoshida eds, Elsevier-North Holland, New York, 1980, 275–287.
12. De Remee RA. – The present status of pulmonary sarcoidosis: a house divided. *Chest*, 1977, 71, 388–393.
13. Rossi GA, Bernabo'Di Negro G, Balzano E, Cerri E, Sacco O, Balbi B, Venturini A, Ramoino R, Ravazzoni C. – Suppression of the alveolitis of pulmonary sarcoidosis by oral corticosteroids. *Lung*, 1985, 163, 83–93.
14. Keogh BA, Hunninghake GW, Line BR, Crystal RG. – The alveolitis of pulmonary sarcoidosis. Evaluation of natural history and alveolitis dependent changes in lung function. *Am Rev Respir Dis*, 1983, 128, 256–265.
15. Crystal RG, Roberts WC, Hunninghake GW, Gadek JE, Fulmer JD, Line BR. – Pulmonary sarcoidosis: a disease characterized and perpetuated by activated lung T cells. *Ann Intern Med*, 1981, 94, 73–94.
16. Amrein R, Keller R, Joos H, Herzog H. – Valeurs theoriques nouvelles de l'exploration de la fonction ventilatoire du poumon. *Bull Eur Physiopathol Respir*, 1970, 6, 317–349.
17. Cotes JA. – *In:* Lung function: assessment and application in medicine. 4th edition. Blackwell, Oxford. 1979.
18. Line BR, Hunninghake GW, Keogh BA, Jones AE, Johnson GS, Crystal RG. – Gallium-67 scanning to stage the alveolitis of sarcoidosis: correlation with clinical studies, pulmonary function studies and bronchoalveolar lavage. *Am Rev Respir Dis*, 1981, 123, 440–446.
19. Buchalter S, Rennard SI, Fulmer JD, Basset G, Crystal RG. – Evidence for alveolar edema and capillary leak in the lower respiratory tract of patients with sarcoidosis and idiopathic pulmonary fibrosis. *Am Rev Respir Dis*, 1984, 129, A 64 (abstract).
20. Rossi GA. – Bronchoalveolar lavage in the investigation of disorders of the lower respiratory tract. *Eur J Respir Dis*, 1986, 69, 293–315.
21. Hance AJ , Douches S, Winchester RJ, Ferrans VJ, Crystal RG. – Characterization of mononuclear phagocyte subpopulation in the human lung by using monoclonal antibodies: change in alveolar macrophage phenotype associated with pulmonary sarcoidosis. *J Immunol*, 1985, 134, 284–292.
22. Snyder DS, Unanue ER. – Corticosteroids inhibit murine macrophage Ia expression and interleukin 1 production. *J Immunol*, 1982, 129, 1803–1805.
23. Pinkston P, Bitterman PB, Crystal RG. – Spontaneous release of interleukin 2 by lung T-lymphocytes in active pulmonary sarcoidosis. *N Engl J Med*, 1983, 308, 793–800.
24. Robinson BWS, McLemore TL, Crystal RG. – Gamma interferon is spontaneously released by alveolar macrohages and lung T lymphocytes in patients with active pulmonary sarcoidosis. *J Clin Invest*, 1985, 75, 1488–1495.
25. Ayra S, Wong-Staal F, Gallo RC. – Dexamethasone-mediated inhibition of human T cell growth factor and gamma interferon messenger RNA. *J Immunol*, 1984, 133, 273–276.
26. Pinkston P, Saltini C, Muller-Quernein J, Crystal RG. – Corticosteroid suppresses spontaneous interleukin 2 release and spontaneous proliferation of the lung T lymphocytes of patients with active pulmonary sarcoidosis. *J Immunol*, 1987, 139, 755–760.
27. Spector WG, Heeson N. – The production of granulomata by antigen-antibody complexes. *Am J Pathol*, 1969, 98, 31–39.
28. Ghose T, Landrigan P, Asif A. – Localization of immunoglobulin and complement in pulmonary sarcoid granulomas. *Chest*, 1974, 66, 264–268.

RÉSUMÉ: Les niveaux d'immunoglobulines augmentent dans l'arbre respiratoire inférieur des patients atteints de sarcoïdose 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 (0.55 ± 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 (valeur de base 0.05 ± 0.01; valeur après traitement 0.06 ± 0.03; $p > 0.2$). Une corticothérapie orale diminue l'alvéolite de la sarcoïdose pulmonaire, ainsi que le démontrent, aussi bien la réduction du pourcentage de cellules T pulmonaires et la captation pulmonaire de Gallium, que par ailleurs la diminution des taux d'immunoglobulines dans les espaces alvéolaires.