

## Treatment of chronic sarcoidosis with an azathioprine/ prednisolone regimen

J. Müller-Quernheim\*, K. Kienast<sup>#</sup>, M. Held<sup>+</sup>, S. Pfeifer<sup>#</sup>, U. Costabel<sup>+</sup>

*Treatment of chronic sarcoidosis with an azathioprine/prednisolone regimen. J. Müller-Quernheim, K. Kienast, M. Held, S. Pfeifer, U. Costabel. ©ERS Journals Ltd 1999.*

**ABSTRACT:** In a few patients with chronic sarcoidosis, prolonged, unacceptably high doses of corticosteroids are required to achieve symptomatic relief. In these cases, a corticosteroid-sparing drug might be administered to allow long-term treatment without the adverse effects of corticosteroids. This study examines azathioprine as a prednisolone-sparing treatment.

In an open study, the course of 11 patients with chronic sarcoidosis was analysed. In an induction phase, 2 mg azathioprine·kg body weight (BW)<sup>-1</sup>·day<sup>-1</sup> in combination with 0.6–0.8 mg prednisolone·kg BW<sup>-1</sup>·day<sup>-1</sup> were administered with prednisolone being reduced to 0.1 mg·kg BW<sup>-1</sup>·day<sup>-1</sup> within 2–3 months. This was followed by a 21–22-month maintenance phase with 2 mg azathioprine·kg BW<sup>-1</sup>·day<sup>-1</sup> and 0.1 mg prednisolone·kg BW<sup>-1</sup>·day<sup>-1</sup>. Clinical parameters and immunological findings of bronchoalveolar lavage (BAL) were analysed.

All patients had significant symptomatic relief and improvements or resolutions of physiological, serological and radiographic findings without suffering from serious adverse effects. Nine of 11 patients completed therapy after 19–26 months, and 2/11 patients terminated therapy after 8 and 12 months, respectively. Eight patients had remissions lasting 4–73 months. Three relapses occurred after 8, 18, and 22 months. During the induction phase, BAL cell composition changed and their activity in terms of cytokine release was suppressed.

This preliminary study suggests that azathioprine may be effective as a corticosteroid-sparing agent in long-term therapy of sarcoidosis, but a much larger study is necessary to give the definitive answer.

*Eur Respir J 1999; 14: 1117–1122.*

Corticosteroids still provide the principal therapy for symptomatic sarcoidosis, although their long-term benefit is disputed. However, a recently published study in which only patients with objective evidence of deterioration were treated with standard doses of corticosteroids, demonstrated the therapeutic benefit of this approach. After discontinuation of therapy the resultant improvement in most patients was maintained over the observation period, while 14% of the patients suffered from relapses [1]. Studies analysing the course of the disease after corticosteroids have been tapered off report relapses requiring therapy in 14% [1], >25% [2] and up to 74% [3] of patients. In treating relapses after prednisolone therapy cytotoxic and non-cytotoxic agents such as chlorambucil [4], methotrexate [5], cyclosporine A [6, 7], azathioprine [7–9], cyclophosphamide [10], melatonin [11], thalidomide [12] and pentoxifylline [13] have been employed. The effect of these drugs on the prognosis of sarcoidosis has not been adequately evaluated and at present such therapies should be considered experimental.

Patients with relapses frequently require long-term corticosteroid therapy [14] and to enable the dose to be lowered when unacceptably high doses have to be used, corticosteroid-sparing combinations have been applied.

\*Research Centre Borstel, Medical Hospital, Borstel, Germany. <sup>#</sup>Third Dept of Medicine, Pulmonary Division, Johannes Gutenberg-University, Mainz, Germany. <sup>+</sup>Ruhrlandklinik, Dept of Pneumology and Allergy, Essen, Germany.

Correspondence: J. Müller-Quernheim  
Medical Hospital  
Research Centre Borstel  
Parkallee 35  
23845 Borstel  
Germany  
Fax: 49 4537188313

Keywords: Azathioprine  
bronchoalveolar lavage  
prednisolone  
sarcoidosis  
tumour necrosis factor- $\alpha$

Received: February 24 1999  
Accepted after revision June 30 1999

This study was supported in part by a grant from the "Deutsche Forschungsgemeinschaft", No. MU 692/3-3.

Azathioprine is one agent which can be used for this purpose [15]. The combination of prednisolone and azathioprine has been evaluated for the treatment of idiopathic pulmonary fibrosis (IPF), proved to be safe, and shown to lower the mortality rate of this disorder in a double-blind, randomized, placebo-controlled trial [16]. Anecdotal reports of the successful treatment of sarcoidosis with azathioprine monotherapy encouraged pulmonary physicians to use azathioprine as a corticosteroid-sparing partner in the long-term therapy of sarcoidosis; however, larger series or controlled studies have not yet been reported.

Therefore, an open prospective trial to evaluate the effectiveness of a combination therapy of prednisolone with azathioprine in the treatment of sarcoidosis was performed. In some of the patients, the opportunity was available to repeatedly perform bronchoalveolar lavages (BAL) and use the immune cells obtained to evaluate the immunosuppressive effects of the therapy on sarcoid immunopathogenic phenomena.

### Materials and methods

#### *Patients and treatment protocol*

Eleven patients (six female, five male) with sarcoidosis diagnosed according to defined criteria including

Table 1. – Characteristics of the study population

	Mean	Median	Range
Age at diagnosis yrs	39.1	37	23–64
Number of episodes requiring corticosteroid therapy	2.6	3	1–4
Months under prior corticosteroid therapy	26.4	27	13–45
Age at start of azathioprine/prednisolone therapy yrs	42.5	40	26–67

transbronchial biopsy [14, 17], entered the study after giving informed consent. Nine suffered from chronic relapsing disease requiring the repeated reinitiation of corticosteroid-therapy after its cessation. Two had stigmata of chronic disease (*i.e.*, skin disease, musculoskeletal problems) and suffered from relapses during the tapering off of the first course of prednisolone, over 13 and 34 months, respectively, which prevented the termination of therapy (table 1). Previous treatments with corticosteroids employed a standard protocol using a starting dose of 0.6–0.8 mg prednisolone·kg body weight<sup>-1</sup>·day<sup>-1</sup>. After achieving a control of the symptoms, the dose was tapered-off resulting in a total treatment time of 6–12 months [1]. Patients underwent a standard evaluation that included a thorough history and physical examination, pulmonary function tests, blood chemistry profiles and chest radiography. All patients included in the study demonstrated progressive clinical disease with progressive dyspnoea, cough, skin lesion or other symptoms, and a  $\geq 10\%$  decrease in vital capacity (VC) or increasing hypoxaemia with exercise. In addition, typical radiographic parenchymal and hilar abnormalities were noted (table 2). Restrictive lung disease was diagnosed when VC and total lung capacity were  $< 80\%$  of the predicted value. The follow-up examinations were performed as previously described [18]. Changes  $> 10\%$  of the baseline value were

considered significant and the patients were categorized into the following groups: resolved, improved, stable and deteriorated (deteriorations were not observed). To test for a diffusion defect, symptom-limited incremental exercise tests using cycle ergometry were performed. For the purpose of this study, a significant hypoxaemia with exercise was diagnosed if the arterial oxygen tension ( $P_{a,O_2}$ ) fell below 9.31 kPa (70 mmHg) [19]. Improvement was noted, when at the same work rate  $P_{a,O_2}$  did not fall below 9.31 kPa (70 mmHg) and the difference between the two exercise tests was at least 1.33 kPa (10 mmHg).

Treatment consisted of a combination of prednisolone and azathioprine for 2 yrs. In an induction phase prednisolone was given in a dose of 0.6–0.8 mg·kg BW<sup>-1</sup> daily for two weeks followed by a gradual reduction of the dose to 0.1 mg·kg BW<sup>-1</sup>·day<sup>-1</sup> over a period of 8–12 weeks. During this induction phase azathioprine was given in a dose of 2 mg·kg BW<sup>-1</sup>·day<sup>-1</sup>. A maintenance phase of 21–22 months followed with a constant dose of prednisolone (0.1 mg·kg BW<sup>-1</sup>·day<sup>-1</sup>) and azathioprine at the specified dose resulting in a treatment with  $\sim 5.0$ – $10.0$  mg prednisolone·day<sup>-1</sup> and 100–150 mg azathioprine·day<sup>-1</sup>. Two patients terminated therapy after 8 and 12 months, respectively. In the remaining group, the entire treatment period was 19–26 months. At the end of the maintenance phase azathioprine was discontinued and prednisolone was gradually reduced over another 4–6 weeks. Patients were monitored at 2–4 week intervals at the out-patient clinics of the institutions involved and/or by their practitioners. Discontinuation or dose reduction of azathioprine was permitted to allow for haematological recovery if bone marrow suppression was observed on full blood counts. However, such haematological side-effects were not observed. The patients were monitored by clinical examination and routine laboratory tests including differential blood cell and platelet count and lipase measurements.

Table 2. – Clinical, radiographic, pulmonary function test, and serological findings at the start and end of azathioprine/prednisolone therapy in the 11 patients studied

Findings	Start	End			
		Resolved	Improved	Stable	Deteriorated
<b>Clinical findings</b>					
Dyspnoea	11	9	1	1	0
Cough	11	10	1	0	0
Skin disease	5	4	1	0	0
Fatigue	4	3	1	0	0
Arthralgia	4	2	2	0	0
Musculoskeletal problems	3	3	0	0	0
Weight loss	1	1	0	0	0
<b>Pulmonary function test</b>					
Restrictive lung disease	9	5	2	2	0
Hypoxaemia under exercise	8	4	4	0	0
<b>Chest radiograph</b>					
Reticulonodular infiltrates	11	1	8	2	0
Hilar adenopathy	8	3	2	3	0
<b>Serological findings*</b>					
ACE	9	6	2	1	0
Soluble IL-2R	6/7**	5	1	0	0
Neoprotein	7/7**	7	0	0	0

\*: Number of patients with elevated values are given in the "start" column, and the number of patients with the indicated changes are given in the "end" column; \*\*: the test was only performed in the number of patients given in the denominator and was positive in the number of patients shown in the numerator. ACE: angiotensin converting enzyme; IL-2R: interleukin-2 receptor.

Patients were thoroughly evaluated before starting therapy, at the end of the induction phase, after 1 yr of treatment, at the end of the maintenance phase, and at 3–6 month intervals after discontinuation of therapy. Evaluation included: posterioranterior and lateral chest radiographs, pulmonary function tests, arterial blood gas analyses at rest and exercise, blood chemistry profiles, serum angiotensin converting enzyme (ACE) tests, and in cases treated at the clinic of the University of Mainz, serum tests for soluble interleukin-2 receptor (sIL-2R) and neopterin. In addition, BAL was performed in cooperative patients before starting therapy and after terminating the induction phase. BAL cells were used to perform several immunological tests (see below).

The induction phase was considered effective when symptoms vanished or improved and/or VC or exercise tests improved. After a stable remission was reached maintenance therapy lasting 21–22 months was intended, although two patients terminated this phase prematurely, as stated previously.

#### *Bronchoalveolar lavage and immunobiological tests*

**Reagents.** Adhesion slides were obtained from Biorad (Munich, Germany) and the antibodies for immunoperoxidase-staining were purchased from Ortho Diagnostic Systems (Neckargemünd, Germany). Bromodeoxyuridine, deoxycytidine, phytohaemagglutinine (PHA), phorbolmyristate acetate (PMA) and lipopolysaccharide (LPS) were purchased from Sigma (St. Louis, MO, USA). Roswell Park Memorial Institute medium (RPMI) 1640 from Seromed (Berlin, Germany); L-glutamine, penicillin and streptomycin from Gibco (Grand Island, NY, USA); foetal calf serum (FCS) from Vitromex (Vilshofen, Germany) and human blood group AB serum from the Blood Bank Luetjensee, Germany. The cytotoxic T-lymphocyte line (CTLL) cell line as bioindicator of interleukin (IL)-2 was a kind gift of R. Bartlett (Hoechst, Wiesbaden, Germany). Antibodies (clone 195) and standards (recombinant human tumour necrosis factor (TNF)- $\alpha$ ) for enzyme-linked immunosorbent assays (ELISA) of TNF- $\alpha$  were kindly provided by Knoll AG (Ludwigshafen, Germany). The tissue culture plates and cryotubes were obtained from Nunc (Wiesbaden, Germany).

**Bronchoalveolar lavage, cell differentials, and immunophenotyping.** BAL with 200–300 mL sterile saline (0.9% NaCl), cell preparation, differential cell counts and immunophenotyping were performed as previously reported [20, 21].

**Cell cultures.** Washed BAL cells were cultured in RPMI 1640 supplemented with 2% heat inactivated endotoxin-free human AB serum, 2 mM L-glutamine, 100 U·mL<sup>-1</sup> penicillin and 100 mg streptomycin at a density of  $1 \times 10^6$  cells·mL<sup>-1</sup> over a period of 24 h without any stimulation to measure the spontaneous release of cytokines. Control cultures received *Salmonella abortus equi* LPS 1  $\mu$ g·mL<sup>-1</sup> or PHA (5 mg·mL<sup>-1</sup>) and PMA (2 ng·mL<sup>-1</sup>). After 24 h the supernatants were harvested and stored at -70°C until assayed for TNF- $\alpha$  and IL-2 activity. The viability of the cells after culture always exceeded 95% as determined by trypan blue exclusion.

**Cytokine assays.** TNF- $\alpha$  concentrations in the BAL supernatants were measured by an ELISA technique as published previously [20]. The background release of TNF- $\alpha$  in the system employed was  $219 \pm 149$  pg·mL<sup>-1</sup> as measured in 20 control subjects for BAL and in 18 for peripheral blood. The upper limit of normal TNF- $\alpha$  release was set at 650 pg·mL<sup>-1</sup>. It was demonstrated for a wide range of TNF- $\alpha$  concentrations released by alveolar macrophages either spontaneously or after *in vitro* stimulation that the TNF- $\alpha$  content of the supernatants measured by ELISA correlated with the bioactivity measured by the L-cell (lytic fibroblasts) assay ( $r_s=0.85$ ,  $p<0.001$ ,  $n=23$ , range 20–5,000 pg·mL<sup>-1</sup>, data not shown).

IL-2 biological activity was determined by CTLL bioassay as previously described [22]. In the test system employed in the present study, a background release of IL-2 by BAL cells of  $<1.0$  IU·mL<sup>-1</sup>  $10^6$  cells<sup>-1</sup>·24 h<sup>-1</sup> was established using the BAL cells of eight normal volunteers and 20 patients being retrospectively free of any inflammatory lung disease, and peripheral blood cells of nine healthy donors.

#### *Serum parameters*

The concentrations of sIL-2R and neopterin were measured by commercially available ELISA (sIL-2R: Immulite IL-2R; DPC Biermann, Bad Nauheim, Germany; Neopterin: Neopterin ELISA; Merck, Darmstadt, Germany). ACE was measured by a kinetic test obtained from Bühlmann Laboratories (Allschwil, Switzerland).

#### *Statistical methods*

Student's t-tests were used to test for significant differences. All data are presented as mean $\pm$ SD.

## **Results**

#### *Clinical findings*

The study population exhibited the clinical characteristics of chronic sarcoidosis. All suffered from active disease after several corticosteroid courses lasting on average 2 yrs. Chronic recalcitrant disease was followed on average for 3 yrs before the need for another therapeutical approach was seen (table 1) and the described regimen commenced. Constitutional symptoms and signs of extrathoracic manifestations were observed (table 2). Pulmonary function tests identified restrictive lung disease in 9/11 patients, no obstructive patterns were observed. Exercise tests showed hypoxaemia in 8/11 patients. Chest radiographic examinations revealed reticulonodular infiltrates without evidence of fibrosis in 11/11 patients. ACE serum levels indicating granuloma burden were elevated in 9/11 patients. sIL-2R serum concentration demonstrating T-cell activation was increased in 6/7, and serum neopterin indicating monocyte activation was elevated in 7/7 patients (table 2).

#### *Therapy and follow-up*

All patients showed improvement in their symptoms and pathological findings during the induction phase. Remaining symptoms, functional defects and chest radiographic

findings gradually improved over the maintenance phase. At the time of final evaluation, 8/11 patients were free of symptoms. New symptoms or deterioration of pre-existing findings or defects due to sarcoidosis were not observed. For the entire study group VC improved from  $74.8 \pm 12.4\%$  pred at the start of therapy to  $89.2 \pm 15.2$  when the maintenance phase ended ( $p < 0.024$ , paired t-test). In two patients restrictive lung disease with dyspnoea persisted, although the overall physical condition of these patients improved. Hilar adenopathy resolved in three and reticulonodular infiltrates in one patient, however, an improvement in radiological findings was observed in most patients (table 2).

An ineffective induction phase occurred in one patient, therefore a second phase which was effective was administered. One patient together with his physician decided to stop therapy while having a remission after 12 months, and one patient stopped therapy after 8 months due to mental illness. In the remaining 9/11 patients, the entire therapy (*i.e.*, induction plus maintenance phase) lasted 19–26 ( $23.1 \pm 2.6$ ) months.

The only side-effect observed was a transient elevation of lipase associated with upper abdominal pain, which returned to normal without interruption of treatment. No sonographic evidence of pancreatitis was found. No haematological side-effects were noted.

Serum ACE concentration was elevated in 9/11 patients at the start of therapy which resolved during therapy in six, decreased in two, and remained stable in one patient. Neopterin and sIL-2R serum levels were analysed in seven patients. All exhibited elevated neopterin levels that normalized under therapy. Six had increased sIL-2R levels which resolved in 6 and improved in one patient.

After an average of  $20.2 \pm 6.3$  months (range 8–26), treatment was discontinued. At that time all patients had resolving or improving symptoms, normalization of physiological findings and an improvement of radiographic findings (table 2). After 8, 18 and 22 months, relapses were observed in three patients. The remaining eight patients have stable remissions persisting for 4, 6, 11, 30, 40, 49, 52, and 73 months at the time of writing.

In one patient, cough and constitutional symptoms (fatigue and night sweat) did not resolve during the induction phase and the chest radiograph showed only a marginal improvement, although a second BAL documented a normalization of the percentage of lymphocytes ( $52.2$  versus  $16.2\%$ ) and a decrease in the CD4/CD8 ratio ( $4.4$  versus  $3.7$ ) at that time (fig. 1). As a consequence, a second induction phase was initiated and all symptoms and findings normalized. Unfortunately this patient terminated the maintenance therapy prematurely after 8 months due to mental illness and suffered from a relapse 22 months later.

#### Immunological findings of bronchoalveolar lavage

At start of therapy the percentage of BAL lymphocytes in seven patients was  $19.9 \pm 17.2\%$  ranging 1.6–52.5% (fig. 1). After the induction phase, BAL was repeated in six patients with a decrease of BAL lymphocytes in four and a considerable increase in two patients. Thus, on average the percentage of BAL lymphocytes remained unchanged at  $20.5 \pm 13.5\%$  (range 7.0–44.0%,  $p > 0.2$ ). However, for the CD4/CD8 ratio of BAL lymphocytes which was

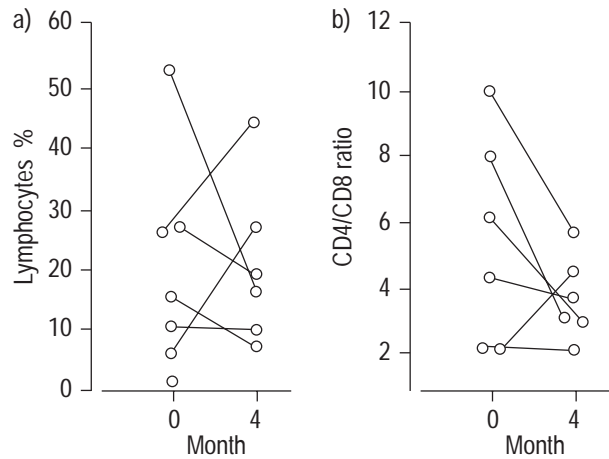


Fig. 1. – Bronchoalveolar lavage (BAL) cytology at the start and after 4 months of therapy when the induction phase was completed and the maintenance phase began. a) Percentage of BAL lymphocytes. This was determined in seven patients at the start of the induction, and then BAL was repeated in six patients after the induction phase. b) The CD4/CD8 ratio of BAL lymphocytes. Data points of patients who underwent two BALs are connected by a line.

$5.5 \pm 3.1$  (range 2.2–10.0,  $p > 0.2$ ) before therapy and  $3.7 \pm 1.2$  (range 2.2–4.5,  $p > 0.2$ ) when the induction phase was completed, a notable decrease was observed. This decrease, however, did not reach a statistically significant level.

Spontaneous release of cytokines was measured in the BAL cell culture supernatants of five patients before and of four patients after completion of the induction phase. TNF- $\alpha$  was elevated in 4/5 and IL-2 in 3/4 patients. In all patients, IL-2 release normalized under therapy showing that the therapeutic approach suppressed the inflammatory activity of BAL T-cells (fig. 2). Three patients with initially elevated spontaneous TNF- $\alpha$  release had follow-up BAL and demonstrated a TNF- $\alpha$  release within the normal range indicating a dampening of alveolar macrophage activity. The observed trends did not reach statistical significance ( $p > 0.1$  in both comparisons).

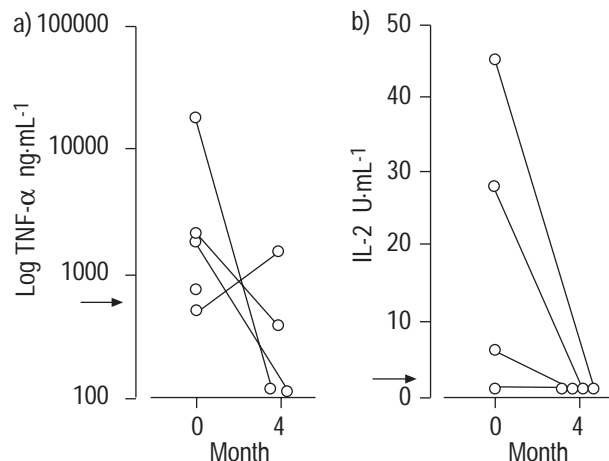


Fig. 2. – Spontaneous cytokine release of bronchoalveolar lavage (BAL) cells at the start and after 4 months of therapy. a) Tumour necrosis factor (TNF)- $\alpha$  release. b) Interleukin (IL)-2 release. Arrows indicate the upper limits of the normal ranges [23]. Data points of patients who underwent two BALs are connected by a line.

## Discussion

This study demonstrates that the combination of prednisolone and azathioprine administered over a period of 2 yrs or until no further improvements are observed, is a safe and effective second line treatment for recalcitrant or chronic relapsing, prednisolone monotherapy-resistant sarcoidosis capable of inducing long lasting remissions.

In those sarcoidosis patients who require anti-inflammatory therapy, there is a clear-cut indication for corticosteroid treatment, and its effectiveness has been demonstrated [1, 2]. Although only a few patients suffer from corticosteroid-resistant or recalcitrant disease they present a major problem in referral centres [17]. While studying the effectiveness of corticosteroids in sarcoidosis, HUNNINGHAKE *et al.* [1] observed the need for unacceptably high prednisone doses in four of 42 patients which led to the application of cyclophosphamide or methotrexate as corticosteroid-sparing agents.

It was decided to evaluate azathioprine as a corticosteroid-sparing agent in the treatment of recalcitrant sarcoidosis for several reasons. Firstly, azathioprine has successfully been used in diseases sharing some immunopathogenetical mechanisms with sarcoidosis, *i.e.* systemic vasculitis, inflammatory bowel diseases and autoimmune hepatitis [24]. Secondly, azathioprine treatment of sarcoidosis either as monotherapy or in combination with prednisolone is under scrutiny, and encouraging reports have been published [7, 8, 25]. Thirdly, this approach has been demonstrated to be safe and effective in IPF, a pulmonary disease that is frequently corticosteroid-resistant [16]. Fourthly, azathioprine has a strong suppressive effect on T-lymphocyte proliferation and activation [24], and activated T-cells are a critical feature of the immunopathogenesis of sarcoidosis [22]. Their activity determines the prognosis of clinically defined subgroups of sarcoidosis patients [18, 26]. Finally, in chronic inflammatory disorders and allograft rejection, restimulated so-called secondary T-cells maintaining the disease process have to be expected [27] and these cells are difficult to suppress [28]. Among the available immunosuppressive agents, azathioprine has been shown to act on secondary cells as seen by suppression of delayed type hypersensitivity and by preventing allograft rejection [24]. These considerations identify azathioprine as a promising corticosteroid-sparing agent for the treatment of sarcoidosis.

The two step mode of an induction and a 2-year maintenance phase has been developed empirically and demonstrated to be effective in a number of disorders in which prolonged immunosuppression is required to induce long lasting remissions [24, 29, 30]. In addition, over this long period of time symptomatic, physiological, and radiographic improvements take place. High doses of prednisolone given in the induction phase resulted in the expected improvements which stabilized or even further improved in the maintenance phase resulting in a high rate of symptomatic relief and improvement of pulmonary function in an average treatment time of  $20.2 \pm 6.3$  months.

An interesting aspect is the duration of the remission. In eight of 11 patients who completed therapy, a relapse has not been observed at the time of writing. The observed remissions have now lasted 4–73 months. In view of the fact that these patients had had up to four relapses within the previous 3 yrs, these results are noteworthy. The patient

with the remission of 73 months had suffered three relapses within the previous 4 yrs requiring a total of 32 months of prednisolone therapy. Unfortunately, three patients suffered from relapses and no clinical findings could distinguish two of them from the group without relapse. The third, however, required two induction phases and after the second induction phase, which was successful, they terminated the treatment prematurely after 8 months. This raises the possibilities that either the disease processes were resistant to immunosuppression or the maintenance therapy was too short.

The use of azathioprine and prednisolone for the treatment of chronic inflammatory disorders was developed empirically [24] but there is now some evidence for the requirement of a 2-yr maintenance therapy. Immunological findings in sarcoidosis are consistent with the concept of an overexuberant cellular immune response against an unknown antigen or antigens within the lungs and other organs [17, 31]. In acute sarcoidosis with spontaneous resolution of the disease, the immune system is capable of eliminating the antigen and down-regulating the immune response [23], but in chronic disease the causative agent withstands the immune processes inducing a long lasting granulomatous reaction similar to chronic berylliosis in which the causative agent of a granulomatous inflammation persists in the alveolar macrophages [17]. Although corticosteroids suppress the activity of pulmonary immune cells within a few weeks, it takes more than 6 months for lymphocytes to disappear from the lower respiratory tract [32] and the lowering of immunosuppressive therapy too early might allow a reactivation of these cells. Since azathioprine is thought to have a delayed effect [24] a treatment period of 2 yrs seems appropriate.

Fortunately, adverse effects requiring treatment or termination of therapy were not observed. In one case, a marginally elevated level of lipase was observed, a rare side-effect of azathioprine known to resolve by lowering the dose or cessation of therapy [15]. Thus, the authors consider azathioprine/prednisolone therapy to be a safe therapeutical option for resistant sarcoidosis.

Analysing BAL cytology before and after the induction phase revealed that the expected decrease in BAL lymphocytes and their CD4/CD8 ratio occurred in 4/6 and in 5/6 patients, respectively. Most interestingly, an increase in the percentage of lymphocytes or CD4/CD8 ratio of these cells accompanied a resolution of the disease in three patients (fig. 1) which supports the notion that immunophenotyping of lymphocytes should not be used to analyse cell migration [33]. Measuring alveolar immune cell cytokine release demonstrated that T-cell activity was suppressed by the tested regimen in three patients with elevated IL-2 release (fig. 2), a result expected from the knowledge that IL-2 gene transcription is blocked by therapeutic doses of prednisolone [32].

Although the conclusions which can be drawn from an open trial are limited, it suggests that azathioprine may be effective as a corticosteroid-sparing agent in the long-term therapy of sarcoidosis. However, further studies are necessary to give a definite answer. In addition, in these future trials, prognostic parameters need to be evaluated to identify those patients who will suffer from relapses and might benefit from other therapeutic approaches including methotrexate [5], pentoxifylline [13] or thalidomide [12].

## References

1. Hunninghake GW, Gilbert S, Pueringer R, *et al.* Outcome of the treatment of sarcoidosis. *Am J Respir Crit Care Med* 1994; 149: 893–898.
2. Gibson G, Prescott R, Muers M, *et al.* British Thoracic Society sarcoidosis study: effects of long term corticosteroid treatment. *Thorax* 1996; 51: 238–247.
3. Gottlieb JE, Israel HL, Steiner RM, Triolo J, Patrick H. Outcome in sarcoidosis. The relationship of relapse to corticosteroid therapy. *Chest* 1997; 111: 623–631.
4. Kataria Y. Chlorambucil in sarcoidosis. *Chest* 1980; 78: 36–43.
5. Baughman RP, Lower EE. The effect of corticosteroid or methotrexate therapy on lung lymphocytes and macrophages in sarcoidosis. *Am Rev Respir Dis* 1990; 142: 1268–1271.
6. Martinet Y, Pinkston P, Saltini C, Spurzem J, Müller-Quernheim J, Crystal RG. Evaluation of the *in vitro* and *in vivo* effects of cyclosporine on the T-lymphocyte alveolitis of active pulmonary sarcoidosis. *Am Rev Respir Dis* 1988; 138: 1242–1248.
7. Hammond J, Bateman E. Successful treatment of life-threatening steroid resistant pulmonary sarcoidosis with cyclosporin in a patient with systemic lupus erythematosus. *Respir Med* 1990; 84: 77–80.
8. Pacheco Y, Marechal C, Marechal F, Biot N, Perrin-Favolle M. Azathioprine treatment of chronic pulmonary sarcoidosis. *Sarcoidosis* 1985; 2: 107–113.
9. Sharma O, Hughes D, James D, *et al.* Immunosuppressive therapy with azathioprine in sarcoidosis. In: Levinsky I, Macholda F, eds. Proceedings of the 5th International Conference on Sarcoidosis. Prague, University of Karlova, 1971; pp. 635–637.
10. Demeter S. Myocardial sarcoidosis unresponsive to steroids. *Chest* 1988; 94: 202–203.
11. Cagnoni M, Lombardi A, Matucci-Cerinic M, Dedola G, Pignone A. Melatonin for treatment of chronic refractory sarcoidosis. *Lancet* 1995; 346: 1229–1230.
12. Carlesimo M, Giustini S, Rossi A, Bonaccorsi P, Calvieri S. Treatment of cutaneous and pulmonary sarcoidosis with thalidomide. *J Am Acad Dermatol* 1995; 32: 866–869.
13. Zabel P, Entzian P, Dalhoff K, Schlaak M. Pentoxifylline in treatment of sarcoidosis. *Am J Respir Crit Care Med* 1997; 155: 1665–1669.
14. Lynch III JP, Strieter RM. Sarcoidosis. In: Lynch III JP, DeRemee RA, eds. Immunologically Mediated Pulmonary Diseases. Philadelphia, JB Lippincott, 1991; pp. 189–216.
15. Lynch III JP, McCune WJ. Immunosuppressive and cytotoxic pharmacotherapy for pulmonary disorders. *Am J Respir Crit Care Med* 1997; 155: 395–420.
16. Raghu G, Depaso WJ, Cain K, *et al.* Azathioprine combined with prednisone in the treatment of idiopathic pulmonary fibrosis: a prospective double-blind, randomized, placebo-controlled clinical trial. *Am Rev Respir Dis* 1991; 144: 291–296.
17. Newman LS, Rose CS, Maier LA. Medical progress: Sarcoidosis. *N Engl J Med* 1997; 336: 1224–1234.
18. Ziegenhagen MW, Benner UK, Zissel G, Zabel P, Schlaak M, Müller-Quernheim J. Sarcoidosis: TNF-alpha release from alveolar macrophages and serum level of sIL-2R are prognostic markers. *Am J Respir Crit Care Med* 1997; 156: 1586–1592.
19. Grippi MA, Metzger LF, Krupinski AV, Fishman AP. Pulmonary function testing. In: Fishman AP, ed. Pulmonary Diseases and Disorders. New York, McGraw-Hill, 1988; p. 2499.
20. Müller-Quernheim J, Pfeifer S, Männel D, Strausz J, Ferlinz R. Lung restricted activation of the alveolar macrophage/monocyte system in pulmonary sarcoidosis. *Am Rev Respir Dis* 1992; 145: 187–192.
21. Costabel U, Bross KJ, Rühle KH, Lohr GW, Matthys H. Ia-like antigens on T-cells and their subpopulations in pulmonary sarcoidosis and hypersensitivity pneumonitis. *Am Rev Respir Dis* 1985; 131: 337–342.
22. Müller-Quernheim J, Saltini C, Sondermeyer P, Crystal RG. Compartmentalized activation of the interleukin-2 gene by lung T-lymphocytes in active pulmonary sarcoidosis. *J Immunol* 1986; 137: 3475–3483.
23. Zissel G, Homolka J, Schlaak J, Schlaak M, Müller-Quernheim J. Anti-inflammatory cytokine release by alveolar macrophages in pulmonary sarcoidosis. *Am J Respir Crit Care Med* 1996; 154: 713–719.
24. Spina C. Azathioprine as an immunomodulating drug: clinical applications. *Clin Immunol Allergy* 1984; 4: 415–446.
25. Hof DG, Hof PC, Godfrey WA. Long-term use of azathioprine as a steroid-sparing treatment for chronic sarcoidosis. *Am J Respir Crit Care Med* 1996; 153: A870.
26. Müller-Quernheim J, Pfeifer S, Kienast K, Zissel G. Spontaneous interleukin-2 release of bronchoalveolar lavage cells in sarcoidosis is a co-determinator of prognosis. *Lung* 1996; 174: 243–253.
27. Arnold B, Schönrich G, Hämmerling GJ. Multiple levels of peripheral tolerance. *Immunol Today* 1993; 14: 12–14.
28. Gupta M, Satyaraj E, Durdik JM, Rath S, Bal V. Differential regulation of T cell activation for primary *versus* secondary proliferative responses. *J Immunol* 1997; 158: 4113–4121.
29. Fauci A, Haynes B, Katz P, Wolf S. Wegener's granulomatosis: prospective clinical and therapeutic experience with 85 patients for 21 years. *Ann Intern Med* 1983; 98: 76–85.
30. Pearson D, May G, Fick G, Sutherland L. Azathioprine and 6-mercaptopurine in Crohn's disease: a meta-analysis. *Ann Intern Med* 1995; 122: 132–142.
31. Müller-Quernheim J. Sarcoidosis: immunopathogenetic concepts and their clinical application. *Eur Respir J* 1998; 12: 716–738.
32. Pinkston P, Saltini C, Müller-Quernheim J, Crystal RG. Corticosteroid therapy suppresses spontaneous interleukin-2 release and spontaneous proliferation of lung T-lymphocytes of patients with active pulmonary sarcoidosis. *J Immunol* 1987; 139: 755–760.
33. Westermann J, Pabst R. How organ-specific is the migration of "naive" and "memory" T-cells? *Immunol Today* 1996; 17: 278–282.