EXTENDED REPORT

Bioenergetics of immune cells to assess rheumatic disease activity and efficacy of glucocorticoid treatment

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Objective: To investigate whether activity and glucocorticoid treatment of rheumatic diseases are reflected by selected parameters of cellular energy metabolism of peripheral blood mononuclear cells (PBMC).

Methods: PBMC were obtained from 30 healthy volunteers, 28 patients (16 inactive; 12 active) with rheumatoid arthritis, systemic lupus erythematosus, vasculitis, or other autoimmune diseases, and five patients with infectious diseases. Patients with active rheumatic diseases were examined before and 4–5 days after starting, restarting, or increasing the dose of glucocorticoids. Cellular oxygen consumption (as a measure of ATP production), bioenergetic ability to be stimulated, and major ATP consuming processes were measured amperometrically with a Clark electrode.

Results: A normal value for oxygen consumption of 3.84 (SEM 0.1) (all data in nmol O₂/min/10⁷ cells) independent of sex was found. In patients with inactive disease the respiration rate was slightly higher, but was significantly increased in active patients to 4.82 (SEM 0.33) (p<0.001). PBMC from active patients showed a significantly lower bioenergetic response to a mitogenic stimulus than controls (p<0.05). In stimulated cells from active patients there was a significant reduction in cation transport and protein synthesis. All parameters above were almost normalised within 4–5 days upon optimised treatment with glucocorticoids. For comparison, PBMC from patients with active infectious diseases also showed an increased respiration rate; their response to mitogenic stimulation was even higher.

Conclusions: This study shows for the first time that parameters describing the cellular function of PBMC in bioenergetic terms are suitable for (a) describing semiquantitatively the activity of a rheumatic disease and (b) assessing the therapeutic effect on the disease.

An important aspect of bioenergetics is the investigation of the energy turnover of biological processes at a cellular level. From a rheumatological point of view, bioenergetics is of special importance, because immune reactions are important consumers of energy, especially in the form of ATP. Both the housekeeping functions (maintenance of ionic integrity, volume regulation, cell growth) and the specialised activities of immune cells (for example, cytokinesis, phagocytosis, signalling, antigen processing, effector functions) depend on their energy supply.^{1,2} Without adequate energy, proper immune function would fail; this explains why processes of energy metabolism are important targets for immunotherapy¹ and why reduced immunocompetence is observed with increasing age, in severe shock, or in the case of malnutrition. This may also explain, in part, why fasting followed by vegetarian diets might be useful in the treatment of rheumatoid arthritis (RA).

Against this backdrop, we examined the question of whether and how rheumatic diseases can be described bioenergetically. The present paper for the first time presents data on patients with both inactive and active rheumatic diseases before and after glucocorticoid treatment. This first systematic clinical study is an extension of our comprehensive preliminary experiments of various cellular models in the past. In stimulated rat thymocytes and in stimulated human peripheral blood mononuclear cells (PBMC), there is significantly higher ATP production to balance the higher ATP demand of specific processes resulting from activation.^{3–5} Activated lymphocytes accordingly differ from inactive cells in their (a) increased consumption of oxygen; (b) greater ATP consumption for protein synthesis and Na⁺K⁺-ATPase; and (c) the detection of a significant energy requirement for Ca²⁺-ATPase and RNA/DNA synthesis.^{3–5} In other studies we have shown that this bioenergetic machinery can be targeted by glucocor-

ticoids when they are used therapeutically at high doses. High dose glucocorticoids have been shown recently to interfere with processes that are essential for the activation and maintenance of lymphocytes, such as sodium and potassium transport.^{6–9} Here, we show that the activity of rheumatic diseases and their treatment are indeed also reflected bioenergetically at the cellular level.

PATIENTS AND METHODS

Patients with rheumatic diseases

Sixteen consecutive patients with inactive rheumatic diseases (age 20–79 years) ("inactive patients") and 12 patients with active rheumatic diseases (age 24–72 years) ("active patients") admitted to our rheumatology unit were studied. Each patient was assigned to one of the following diagnostic categories: systemic lupus erythematosus (SLE), RA, polymyalgia rheumatica or giant cell arteritis, or other autoimmune diseases (table 1).

Systemic lupus erythematosus

SLE was diagnosed in six (three inactive, three active) patients who met four or more of the American College of Rheumatology (formerly American Rheumatism Association) criteria.¹⁰ Clinical activity of SLE was assessed by the European Consensus Lupus Activity Measurement (ECLAM) scale.^{11,12} Typical manifestations of clinical activity (arthritis, vasculitis, myositis, serositis, fever, and other) accompanied by a significant

Abbreviations: Con A, concanavalin A; ECLAM, European Consensus Lupus Activity Measurement; MCTD, mixed connective tissue disease; PBMC, peripheral blood mononuclear cells; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus

Table 1 Distribution of patients with rheumatic diseases according to diagnostic categories

Diagnostic categories	Number of patients		
	Inactive	Active (before treatment)	Active (after treatment)
Systemic lupus erythematosus	3	3	3
Rheumatoid arthritis	5	3	3
Polymyalgia rheumatica/giant cell arteritis	4	2	2
Other autoimmune diseases	4	4	4
Progressive systemic sclerosis	1		
Discoid lupus erythematosus	1		
Mixed connective tissue disease		1	1
Sjögren's syndrome		2	2
RS ₃ PE syndrome	1		
Behçet's disease		1	1
Cogan's syndrome	1		

increase in DNA binding and a significant decrease in C3 or C4 level with or without other laboratory abnormalities (proteinuria, leucopenia, and other) were criteria for starting, restarting, or increasing the dose of glucocorticoids. Patients classified as having active SLE had ECLAM scores of at least 6.

Rheumatoid arthritis

Eight patients (five inactive, three active) met the 1987 American Rheumatism Association criteria for RA.¹³ Criteria for starting, restarting, or increasing the dose of glucocorticoids were active disease as defined by more than three swollen joints, morning stiffness of greater than 45 minutes' duration, and raised C reactive protein or erythrocyte sedimentation rate.

Polymyalgia rheumatica or giant cell arteritis

Six patients (four inactive, two active) had clinically diagnosed and/or histologically confirmed polymyalgia rheumatica or giant cell arteritis. The indication for starting, restarting, or increasing the dose of glucocorticoids was defined on the basis of typical clinical and laboratory criteria for disease activity.

Other autoimmune diseases

This group comprised eight patients with the following inactive or active diseases (table 1) all diagnosed by standard methods: progressive systemic sclerosis, discoid lupus erythematosus, mixed connective tissue disease (MCTD), Sjögren's syndrome (two patients), remitting seronegative symmetrical synovitis with pitting oedema (RS₃PE syndrome), Behçet's disease, or Cogan's syndrome. For these diseases the indication for starting, restarting, or increasing the dose of glucocorticoids was defined on the basis of typical clinical and laboratory criteria for disease activity.

Treatment of active patients with glucocorticoids

In the 12 cases of active diseases the indication for starting, restarting, or increasing the dose of glucocorticoids was defined as described above. Four patients (two with RA, one with MCTD, one with Sjögren's syndrome) received initially 30–≤50 mg, five patients (two with giant cell arteritis, one with Behçet's disease, one with Sjögren's syndrome, one with SLE) received initially 50–≤100 mg, and three patients (two with SLE, one with RA) received initially ≥250 mg of prednisolone equivalent a day. Concomitant drugs remained unchanged in these patients. The second measurement was taken after 4.6±1.1 (median 4.5, minimum 3, maximum 7) days. The clinical course of all patients included in our study was accurately monitored, but no case of glucocorticoid resistance was seen. The laboratory and clinical outcome parameters of all 12 patients treated with glucocorticoids responded

well. Also, there were no unexpected glucocorticoid related side effects.

Patients with infectious diseases

Five patients (age 18–50 years) with active forms of one of the following infectious diseases were also studied: hepatitis A, hepatitis B (two patients), infectious mononucleosis or erysipelas. The high activity of these diseases was defined on the basis of typical clinical and laboratory criteria. There were no signs of any other underlying chronic or acute disease at the time of measurements.

Control subjects

The control group of healthy subjects comprised 15 women and 15 men (age 20–49 years).

Preparation and incubation of cells

After a thorough physical examination had been performed and informed consent obtained, blood samples of a maximum of 46 ml were taken in heparinised tubes. PBMC were isolated by density centrifugation using the Ficoll-Hypaque method. Each sample of venous blood was diluted with saline, and density gradient centrifugation was performed at 400 *g* for 20 minutes. The PBMC enriched interphase was isolated, washed with saline, and resuspended at 2×10⁷ cells/ml. The incubation medium was a 1.5:1 mixture of Eagle's basal medium with a salt mixture according to Hanks (+0.15M Tris/HCl, –glutamine, –NaHCO₃, pH 7.4) supplemented by the addition of 19 L-amino acids each at 0.2 mmol/l final concentration and filtered through a 0.2 µm pore size filter to remove undissolved particles as previously described.⁵ In this medium the contribution of glycolysis to ATP production is negligible.³ Cells were stored and incubated for up to three hours in wide plastic flasks to ensure aeration at 4°C. The PBMC consisted of 85–95% lymphocytes and 5–15% monocytes, as determined by FACS analysis. Trypan blue staining showed that the viability of freshly isolated cells was >95%.

Measurement of oxygen consumption

Oxygen consumption was measured amperometrically in a 0.7 ml aliquot of cell suspension with a Clark electrode for up to 15 minutes as described previously.^{3,5,7,9} The cell suspensions in the Perspex incubation chamber of the electrode were magnetically stirred and thermostatically maintained at 37°C. Concanavalin A (Con A) (Sigma, Deisenhofen, FRG) was dissolved in water and in all experiments was added to cell suspensions at 50 µg/ml. In previous experiments⁵ this concentration of Con A was shown to produce a reproducible cell stimulation that was reflected by a significant increase of steady state respiration that persisted over the time of measurements and for at least 10 minutes (the time at which

Figure 1 Respiration rate of PBMC in healthy controls. PBMC were separated by Ficoll-Hypaque density centrifugation. Oxygen consumption was measured as described in "Patients and methods". There were no statistically significant differences between male, female, and all control groups for respiration rate. NS, not significant.

all oxygen is removed from the chamber due to respiration of the cells). At first glance this concentration appears to be very high, but we have found that the Con A effect on respiration is also dependent on the cell count.⁵ The cell concentration we need to use in our experiments is about 5–40 times higher than that used by other investigators, who add Con A only at 1, 5, or 20 $\mu\text{g}/\text{ml}$. Therefore, related to the cell number our Con A concentration corresponds to 2.5 $\mu\text{g}/10^6$ cells, which is within the same range reported by other authors.^{5–14} To quantify main ATP consuming pathways in PBMC, inhibitors of protein synthesis (1 mM cycloheximide), Na^+/K^+ -ATPase (1 mM ouabain), RNA/DNA synthesis (60 μM actinomycin D),

and Ca^{2+} -ATPase (2 mM lanthanum(III) chloride 7-hydrate) (all from Sigma, Deisenhofen, Germany) were applied as described previously.^{3,5,7,9} Respiration rates were measured 1–3 minutes after each addition of Con A or inhibitor. Specific inhibitors have been used successfully in several different model systems and the technique been tested for validity in different ways.^{1,3,5,7,9} Thus, we have (a) previously quantified protein synthesis directly and shown it gives the same numerical result as the inhibition method; (b) presented data showing that inhibition of one ATP consuming process does not significantly affect the rates of the others; (c) shown that secondary effects of the inhibitors are negligible in our experimental setting.⁷

Respiration rates were measured after 1–3 minutes to evaluate the basal respiration rate (untreated cells) and another 1–3 minutes after each addition of Con A or inhibitor. After each addition of substances the onset of maximal effect was achieved in seconds, which was constant during the time of experiments. In active patients, it was not always possible to prepare sufficient cells because of the often disease related cytopenia, so that the quantification of the ATP consuming processes in inactive cells could only be of an orientational nature.

Statistical methods

All data are expressed as mean (SEM) or mean (SD). Results between the groups were compared using Student's unpaired *t* test. Probability values of $p < 0.05$ were considered to be significant.

RESULTS

Respiration rate of quiescent PBMC in healthy controls

The oxygen consumption of PBMC in healthy controls was 3.84 (SEM 0.1) units (nmol $\text{O}_2/\text{min}/10^7$ cells) ($n=30$). These

Figure 2 Respiration rate and stimulated respiration rate of PBMC in healthy controls and patients with rheumatic and infectious diseases. PBMC preparation, measurement of oxygen consumption, and stimulation with Con A were as described in "Patients and methods". (A) The respiration of PBMC from patients with active rheumatic diseases before treatment was significantly higher than in healthy controls ($***p < 0.001$), whereas the value after treatment was not significantly different from that found in healthy controls. Also, the respiration rate of PBMC from patients with infectious diseases was significantly higher than in controls ($***p < 0.001$). (B) The increase in respiration rate as induced by Con A. The most striking effects are (a) the significantly reduced stimulation in patients with active rheumatic diseases ($*p < 0.05$ versus healthy controls) and (b) the significantly greater PBMC stimulation in patients with active infectious diseases ($**p < 0.05$ versus all groups).

data are in agreement with previous results measured for human PBMC.^{5,14} No statistically significant differences between men (n=15) and women (n=15) nor age related differences were found (figs 1 and 2A).

Respiration rate of quiescent PBMC in patients

The mean respiration rate of quiescent PBMC in patients with inactive rheumatic diseases was 4.18 (0.28) units (n=16). There were no statistically significant differences between men and women nor age related differences. The respiration in this group of patients was slightly higher, but not significantly different from the value found for healthy controls (fig 2A). However, in the 12 patients with active rheumatic diseases, the respiration of PBMC was significantly increased at 4.82 (0.33) nmol O₂/min/10⁷ cells (n=12) as compared with healthy controls (p<0.001) (fig 2A, column 3). All these active patients received effective glucocorticoid treatment, and second values for PBMC oxygen consumption were determined four to five days after initiation of treatment. The results of these measurements are shown in fig 2A (column 4). It shows a significant reduction of oxygen consumption by PBMC to 3.84 (0.27) nmol O₂/min/10⁷ cells (n=12) as compared with the value before treatment (p<0.05). Thus, glucocorticoid treatment reduced the respiration rate to a value not statistically different from that found in healthy controls. The respiration of PBMC in patients with active infectious disease was found to be even higher than in patients with active rheumatic diseases, reaching 5.1 (0.29) nmol O₂/min/10⁷ cells (n=5) (fig 2A, column 5). This value was significantly higher than that found for healthy controls (p<0.001). In summary, PBMC basal respiration of healthy controls and patients with inactive rheumatic diseases was similar, whereas it was significantly higher in patients with active rheumatic diseases and was highest in patients with active infectious diseases (fig 2A).

Stimulated respiration rate of PBMC in healthy controls and patients

To quantify the ability to react to a defined stimulus, we exposed the PBMC to 2.5 µg Con A/10⁶ cells. Con A stimulated respiration to a significant, but variable, extent in all groups. In healthy controls it significantly increased oxygen consumption by 2.03 (0.1) nmol O₂/min/10⁷ cells (52.8% with respect to basal respiration) to 5.87 (0.17) nmol O₂/min/10⁷ cells (n=30, p<0.001 as compared with basal respiration; fig 2B, column 1). Similarly, in patients with inactive rheumatic diseases Con A also produced a significant increase of respiration by 2.15 (0.28) nmol O₂/min/10⁷ cells (or 52% with respect to basal respiration) to 6.33 (0.48) nmol O₂/min/10⁷ cells (n=16, p<0.001; fig 2B, column 2). In patients with active rheumatic diseases in the pretreatment measurements, Con A also significantly increased oxygen consumption by PBMC, but only by 1.51 (0.33) nmol O₂/min/10⁷ cells (33 % with respect to basal respiration) to 6.33 (0.43) nmol O₂/min/10⁷ cells (n=12, p<0.002; fig 2B, column 3). Interestingly, the measurements 4–5 days after initiation of glucocorticoid treatment showed a partial

recovered ability of PBMC to become activated. In these experiments Con A was found to significantly stimulate respiration rate by 1.82 (0.34) nmol O₂/min/10⁷ cells (or 45% with respect to basal respiration) to 5.66 (0.58) nmol O₂/min/10⁷ cells (n=12, p<0.001; fig 2B, column 4). Surprisingly, Con A caused the greatest stimulation in patients with infectious diseases, with an increase of respiration by 2.9 (0.49) nmol O₂/min/10⁷ cells (or 56.6%) to 8.0 (0.67) nmol O₂/min/10⁷ cells (n=5, p<0.004; fig 2B, column 5) notwithstanding the high basal respiration. Thus, the most striking effect is the significantly reduced (p<0.05 versus healthy controls) ability of PBMC obtained from patients with active rheumatic diseases to react on Con A activation, which is partially recovered by successful glucocorticoid treatment. In contrast, patients with active infectious diseases show a significantly higher Con A effect than found in all other groups investigated (p<0.05) (fig 2B).

Identification of processes accounting for oxygen consumption

Oxygen consumption results mostly from ATP consuming processes, whereas only about 15%⁵ is due to uncoupled respiration (to drive mitochondrial proton leak),¹ and extramitochondrial oxygen consumption is negligible.^{1,5,15} Therefore, we quantified the coupled oxygen consumption to drive ATP consuming pathways by measuring the rates of processes known to be the main ATP consuming pathways^{3,5,7,9}: protein synthesis, plasma membrane sodium cycling, plasma membrane calcium cycling, and RNA/DNA synthesis.

Quiescent cells

In healthy controls and in patients in the inactive stage of the disease, only 15–17% of the oxygen consumption could be attributed to the processes investigated. Around half of this consumption is caused by Na⁺K⁺-ATPase and the other half by protein synthesis, which consume 0.25 and 0.38 nmol O₂/min/10⁷ cells, respectively. In contrast, neither Ca²⁺-ATPase nor RNA/DNA synthesis are of measurable bioenergetic importance. These results are consistent with our previous investigations on PBMC isolated from buffy coats.³ For the investigation of these parameters in patients with active rheumatic and infectious diseases, we did not always have enough cells available. In these investigations, which could therefore only be of an orientational nature, no reliable differences in the main energy consuming processes were measurable compared with the healthy controls and inactive patients.

Stimulated cells

In Con A stimulated PBMC of healthy controls the Na⁺K⁺-ATPase consumes 0.94 (0.10) nmol O₂/min/10⁷ cells. Under these conditions the oxygen consumption for the Ca²⁺-ATPase was 1.23 (0.14) nmol O₂/min/10⁷, whereas protein synthesis and RNA/DNA synthesis consumed 1.29 (0.16) and 0.56 (0.09) nmol O₂/min/10⁷, respectively (table 2). These data are similar to those previously published.³ We found no significant

Table 2 Processes accounting for oxygen consumption in stimulated cells*

	Control subjects	Patients with rheumatic diseases			Patients with infectious diseases
		Inactive	Active before treatment	Active after treatment	
Protein synthesis	1.29	1.36	0.98	0.97	1.58
Ca ²⁺ -ATPase	1.23	1.25	0.75†	1.03	1.32
Na ⁺ , K ⁺ -ATPase	0.94	0.70	0.54†	0.45†	0.98
RNA/DNA-synthesis	0.56	0.37	0.24†	0.39	0.78
Other processes (calculated)	1.85	2.65	3.82†	2.82	3.34

*Values are the mean given in nmol O₂/min/10⁷ cells; for clarity, SEM values for the ATP consuming processes are not shown; they were between 0.05 and 0.21.

†p<0.05 versus healthy controls.

Figure 3 Contribution of identified ATP consuming processes to oxygen consumption in stimulated cells. PBMC preparation, measurement of oxygen consumption, stimulation with Con A, and quantification of ATP consuming pathways were as described in "Patients and methods". (A) The relationship between identified ATP consuming processes and "other processes" (see "Discussion") contributing to oxygen consumption among the different groups. (B) The ratio of identified processes:other processes varies between 2.17 (controls), 1.4 (patients with inactive rheumatic diseases and patients with infectious diseases), 1.0 (patients with active rheumatic diseases after treatment) and 0.66 (patients with active rheumatic diseases before treatment).

differences between the patients examined and the healthy controls, with the exception that Na^+K^+ -ATPase, Ca^{2+} -ATPase, and RNA/DNA synthesis all consumed significantly less oxygen in stimulated cells of active patients before treatment than in those of healthy controls ($p < 0.05$). After treatment this was true only for Na^+K^+ -ATPase (table 2). Overall, the major energy consuming processes required only 2.51 units of oxygen in active patients (before treatment) as compared with 4.02 units in healthy controls. After treatment this value rose to 2.84, as compared with 3.68 in inactive patients and 4.66 in patients with infectious diseases (fig 3A). From these data the following percentage ratios of major energy consuming processes to "other processes" (see "Discussion") can be calculated: 68:32 (healthy subjects), 58:42 (inactive patients and patients with infectious diseases), 50:50 (active patients after treatment), and 40:60 (active patients before treatment). Figure 3B gives these ratios in absolute values.

DISCUSSION

Bioenergetic investigations have recently brought decisive gains in knowledge in several fields. These include a description of the hierarchical structure of the energy metabolism in immune cells,¹⁻³ the quantification of processes of signal transduction,²⁻¹⁶ and the awareness that energy deficiency (increasing age, severe shock situations, malnourishment) leads to dysfunctions and to failure of the immune system.¹⁻¹⁷⁻²² On the other hand, the observed reduction in the activity of RA while fasting or dieting²³ can be at least partially explained in this way.

After intensive preliminary work on various cellular systems,¹⁻³⁻⁹ we now for the first time make a direct connection

between clinical rheumatology and cellular bioenergetics. To this end we have chosen PBMC as the cellular model for three reasons. Firstly, they have a central role in the immune system. Secondly, they can be activated by mitogenic stimulation, the resulting bioenergetic processes also being useful as a model of antigenic stimulation. In this study we deliberately refrained from separating PBMC into subpopulations in order to permit the cell-cell interactions required for mitogenic activation of lymphocytes.⁵ Thirdly, PBMC are easily accessible and simple to prepare and, therefore, make standardisation easy.

With the present paper, we show that the clinical activity of a rheumatic disease is indeed reflected at a cellular level in the immune cells. The oxygen consumption of the PBMC of patients with active rheumatic diseases is thus significantly (25%) higher than in healthy controls (figs 1 and 2A). This observation is of particular importance because, under our test conditions, glycolytic ATP production is negligible. In other words the total ATP is produced by cellular respiration. Accordingly, these data reflect the fact that the PBMC of active patients produce significantly more ATP by oxidative phosphorylation. The difference is drastic in light of the fact that probably not all cells are stimulated. What is this extra ATP used for? We suspect an increased demand as a result of energy consuming immune processes, such as activated ion transport and signal transduction processes, macromolecule synthesis processes, cytokinesis, phagocytosis, antigen processing, and effector functions.²⁴⁻³³ The increased oxygen consumption is measurable, because ATP is mainly produced "on demand".³⁴ However, recently published new insights into the regulation of energy metabolism have shown that it is not only a matter

of increased or decreased ATP demand because ATP production is controlled also by many different factors.^{2 16 35-37} In patients with inactive rheumatic diseases, we only observed a tendency towards increased oxygen consumption of the PBMC, without statistical significance (fig 2A). The extra demand for metabolic energy of the PBMC is clearly not very high in an inactive stage of disease.

A second important observation of our study concerns the differences between the groups in the ability of the PBMC to be stimulated. Although the mitogen Con A causes a significant increase in cellular oxygen consumption in all groups, this effect is reduced by more than 25% in active patients. We explain this observation by an increased cellular activity (see above) in the sense of a "pre-stimulation", which is why the effect of a standardised stimulation is less than in healthy controls. In inactive patients, there is again only a tendency towards a reduced ability to be stimulated compared with healthy subjects (fig 2B).

PBMC from patients with active rheumatic diseases thus respire more strongly and are less able to be stimulated than those in healthy controls. They are accordingly on a significantly higher bioenergetic level. We therefore asked ourselves whether stimulated cells display differences in their ATP consuming processes. The main energy consuming processes (Na^+K^+ -ATPase, Ca^{2+} -ATPase, protein synthesis, RNA/DNA synthesis) do indeed consume much less oxygen in active patients than in healthy controls (fig 3A). Apparently, the ATP supply of specific immune functions such as signalling, cytokinesis, and regulatory and effector functions ("other processes") is accorded the highest priority under these conditions (fig 3A). In contrast, there are no significant differences in the uncoupled oxygen consumption (approximately 11%).⁵ In inactive patients, there is a trend towards equivalent results, but the measurements do not achieve statistical significance.

Interestingly, as early as 4–5 days after optimisation of the glucocorticoid treatment, the respiration rate and ability of the PBMC to be stimulated had completely normalised in active patients (fig 2), and the differences in the extent of the ATP consuming processes were no longer so marked after treatment (table 2). The proportion of main ATP consumers was thus again slightly increased compared with the value before the start of treatment, whereas the ratio "main energy consumers": "other processes" approached a ratio of 68:32 determined in healthy subjects (before treatment 40:60, after treatment 50:50) (fig 3B). As the laboratory and clinical outcome parameters of all patients responded well, we conclude that these changes constitute the bioenergetic equivalent of successful immunosuppression. The genomic, specific non-genomic, and non-specific non-genomic effects of the glucocorticoids are responsible for this therapeutic outcome.^{1 5-9 38} In this regard clinically relevant concentrations of glucocorticoids have been found to inhibit calcium and sodium cycling across the plasma membrane and decrease intracellular free calcium concentrations, but to have little effect on protein synthesis.⁶

Are these changes specific to rheumatic diseases, or do they generally only reflect an increased activity of the immune system? We comparatively investigated patients with acute infectious diseases and found three interesting differences in patients with active rheumatic diseases: (a) the PBMC of both patient groups have a significantly higher respiration rate than healthy controls, but the oxygen consumption of the PBMC tends to be even higher in infectious diseases than in patients with active rheumatic diseases (fig 2A); (b) the ability of the cells to be stimulated is significantly lower in active rheumatic diseases than in healthy controls, but is significantly higher in infectious diseases (fig 2B). The reason for this observation is unclear. However, we suspect that the immune system in otherwise healthy subjects reacts as if unmodified and unconsumed if it suddenly has to confront an acute infection. It

behaves differently in patients with rheumatic diseases, where recurrent attacks of disease, constantly changing disease activities, and treatment effects chronically affect cellular activity and reactivity. The observation that the proportion of main energy consumers is 68% in stimulated cells from healthy subjects, 58% in acute infectious diseases, and only 40% in patients with an active rheumatic disease supports this assumption (fig 3B). Clearly more ATP is required for cytokinesis, regulatory and effector processes in an active infection than in healthy subjects, but markedly less than in active rheumatic diseases. This observation possibly reflects a bioenergetic difference between chronic-recurrent and acute activity of the immune system. Preliminary data on follow up observations in some patients (data not shown) seem to confirm this assumption, although further systematic investigations will have to be conducted here.

We conclude that selected bioenergetic parameters, such as oxygen consumption and the ability of PBMC to be stimulated, (a) reflect the activity of a rheumatic disease and (b) are changed towards normalisation if therapeutic effects take place. Although immune functions are reported to be altered with aging, the parameters we measured in this study did not show any age related differences. The data collected on patients in a clinical context for the first time in this study are consistent with the results obtained in basic research^{1-9 35-37} and with the knowledge gained on the bioenergetic effects of glucocorticoids^{1 5-9 38} and other drugs.³⁹⁻⁴¹ Further investigations will (a) examine how this knowledge can be applied in clinical routine and (b) contribute to the specific development of drugs with which a targeted and effective immunosuppression can be achieved by precise influence on bioenergetically relevant processes in immune cells.

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