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# NeuroImmunoModulation

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**KARGER**

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# Impairment of Adrenocortical Function Associated with Increased Plasma Tumor Necrosis Factor-Alpha and Interleukin-6 Concentrations in African Trypanosomiasis

## Abstract

African sleeping sickness (SS) is a severe, potentially lethal parasitic disease. The treatments of choice are the antiparasitic agents suramin, which is adrenotoxic, and/or melarsoprol. We evaluated the functional integrity of the hypothalamic-pituitary-adrenal (HPA) axis of patients with SS before, during, and after therapy with suramin and/or melarsoprol, in two sequential stages. First, we employed the standard adrenocorticotrophic hormone (ACTH) 1-24 stimulation test (250 µg i.v.) to assess the maximal adrenocortical responsiveness of 69 patients with SS and 38 normal controls. We demonstrated paradoxically subnormal cortisol responses before suramin therapy [net cortisol response 60 min after stimulation:  $10.5 \pm 2.9$  (mean  $\pm$  SE) vs.  $17.5 \pm 1.0$  µg/dl for controls,  $p = 0.004$ ], with 27% of the patients falling within the adrenal insufficiency range (stimulated cortisol concentration  $<20$  µg/dl). These responses subsequently and unexpectedly improved with suramin and/or melarsoprol therapy. Second, we performed a human corticotropin-releasing hormone (hCRH) test (100 µg i.v.) in 68 additional patients with SS and 14 control subjects to examine whether the glucocorticoid deficiency observed was primary and/or secondary. Compared to controls, the ACTH and cortisol responses to hCRH were blunted (ACTH after 60 min:  $29 \pm 7$  vs.  $58 \pm 8$  pg/ml in controls,  $p = 0.014$ ; cortisol:  $15.2 \pm 1.5$  vs.  $19.6 \pm 0.7$  µg/dl,  $p = 0.018$ ), suggesting the presence of secondary adrenal insufficiency. There was improvement of both ACTH and cortisol responsiveness to hCRH with therapy, with cortisol recovery occurring before ACTH, suggesting an additional primary component of adrenal dysfunction in these patients. Plasma concentrations of tumor necrosis factor (TNF)- $\alpha$  ( $16.0 \pm 4.1$  vs.  $2.9 \pm 1.4$  pg/ml in controls,  $p = 0.003$ ) and interleukin (IL)-6 ( $19.2 \pm 7.3$  vs.  $1.3 \pm 0.2$  pg/ml,  $p = 0.0001$ ), but not IL-1 $\beta$  ( $2.0 \pm 0.2$  vs.  $0.9 \pm 0.2$ ,  $p = \text{NS}$ ), were elevated when adrenocortical function impairment and disease activity were at their maximum, but gradually decreased into the normal range with therapy. We found a negative correlation between baseline cytokine concentrations and maximal cortisol concentrations during hCRH testing (TNF- $\alpha$ :  $r = -0.31$ ,  $p = 0.003$ ; IL-6:  $r = -0.34$ ,  $p = 0.002$ ). We conclude that unmedicated SS is associated with significant impairment of adrenocortical function which is reversed with suramin and/or melarsoprol therapy in the majority of patients. This impairment may be due to the elevated plasma cytokine concentrations, and may represent a natural adaptation of the HPA axis in inflammatory states. A controlled therapeutic trial is necessary to demonstrate whether supplemental glucocorticoids could be beneficial in SS.

## Key Words

Tumor necrosis factor- $\alpha$   
Interleukin-1 $\beta$   
Interleukin-6  
*Trypanosoma brucei*  
Hypothalamic-pituitary-adrenal axis  
Cortisol  
Adrenal cortex  
Adrenocorticotrophic hormone

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## Introduction

Sleeping sickness (SS; African trypanosomiasis) is an anthroponosis transmitted cyclically by the bite of the tsetse fly of the genus *Glossina*. It is caused by the protozoans *Trypanosoma brucei gambiense* (West-African SS with a more chronic course) or *T.b. rhodesiense* (East-African SS with rapid progression). Fifty million Africans are living in areas where SS is endemic, of which only 10 million have access to health services capable of diagnosing trypanosomiasis [1]. Approximately 20,000 new cases are reported each year to the World Health Organization, although there is considerable fluctuation in number due to epidemics [2]. Clinically, the early acute disease is characterized by hemolympathic involvement with irregular fever, rash, weight loss, localized edema, lymphadenopathy and chronic fatigue, which can last from months to years in West-African SS, but has a more acute onset and rapid progression in East-African SS. After invasion of the central nervous system by the parasite, a meningoencephalitis with a broad spectrum of neurologic and psychiatric symptoms evolves. Untreated, the disease is fatal and death is due to secondary bacterial infection, coma, or cachexia [3, 4].

Severe illnesses or stress of any kind are usually accompanied by activation of the hypothalamic-pituitary-adrenal (HPA) axis [5–8] and the sympathetic nervous system [9, 10]. The activation of the HPA axis in the course of inflammatory disease has been considered to be a result of elevated inflammatory cytokine levels, such as those of tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , and IL-6, which have corticotropin-releasing hormone (CRH)-, adrenocorticotropin hormone (ACTH)- and/or cortisol-releasing properties [for a review see ref. 11]. In turn, and primarily via glucocorticoids, the HPA axis exerts major anti-inflammatory and immunosuppressive effects, thus, participating in the natural control of inflammation [12]. In addition, via CRH and opioid peptidergic-mediated, as well as through glucocorticoid-mediated mechanisms, the HPA axis suppresses appetite, induces catabolism and inhibits thyroid and gonadal function [11]. The presence of cachexia associated with chronic parasitic disease has been explained both as a result of activation of the HPA axis and sympathetic nervous system and as a consequence of circulating cytokines, such as TNF- $\alpha$  (or cachexin), with cachexia-promoting properties [13].

Endocrine abnormalities in African SS have been observed since the early 1950s and include hypothyroidism, and hypogonadism [14–18]. Sporadic cases of adrenal

insufficiency have also been reported in SS [19], but the HPA axis has not been systematically evaluated in this condition. Suramin, used since the early 1920s as the treatment of choice for SS in the hemolympathic stage [20], cures SS, and corrects the known endocrine and metabolic manifestations. This drug, given in large doses to patients with AIDS or to macaque monkeys, has significant adrenotoxicity and results in adrenocortical insufficiency [21–23]. To investigate the HPA axis in African SS and the potential effects of suramin treatment on this axis, we studied cross-sectionally 137 patients with SS and 52 normal Ugandan controls by means of ACTH 1-24 and human (h)CRH stimulation tests. Unmedicated SS was associated with impairment of HPA axis function, despite high plasma cytokine concentrations. Paradoxically, HPA axis function was corrected by specific treatment with suramin and/or melarsoprol in the majority of the patients.

## Patients and Methods

### Patients and Controls

Patients and controls were recruited through the National Sleeping Sickness Control Program in south-east Uganda. The diagnosis of SS was established by microscopical demonstration of parasites in the peripheral blood (hemolympathic stage), and/or in cerebrospinal fluid obtained by lumbar puncture (cerebral stage). All patients with SS initially received a small i.v. dosage of suramin (day 1: 250  $\mu$ g, day 3: 500  $\mu$ g). Thereafter, patients without cerebral involvement (hemolympathic stage) received weekly 1-gram injections of suramin up to a total dose of 5.75 g, whereas patients with cerebral SS were treated with a 5-week course of melarsoprol (total dose: 20 mg/kg body weight).

A total of 137 patients (67 female, 70 male; mean age 36 years) with *T.b. rhodesiense* infection and 52 healthy Ugandan control subjects (24 female, 28 male; mean age 30 years) were enrolled in two different sequential protocols (1 and 2, see below), after giving informed consent. None of the healthy subjects had a previous history of SS, adrenal disease or had received suramin, melarsoprol, or glucocorticoid treatment. Every patient was studied during the course of treatment (1) while acutely ill, within the first 5 days after admission to the health care center; (2) in the middle of the course of treatment (days 8–21); (3) at the end of the treatment period (days 23–38), and (4) after permanent cure (at least 3 months after the end of treatment).

The study was approved by the ethics committee of the University of Cologne and by the Ministry of Health, Uganda, and post hoc by the ICRS of the NICHD.

### Protocol 1

Sixty-nine patients and 38 controls underwent an ACTH stimulation test between 10.00 and 14.00. The clinical profile of these patients is given in table 1. 250  $\mu$ g ACTH 1-24 (Synacthen<sup>®</sup>, Ciba-Geigy, Wehr, FRG) was given as an i.v. bolus. Venous blood was

**Table 1.** Clinical profile of patients and controls studied with ACTH 1-24

	n	Sex (f/m)	Hemo-lymphatic stage	Cerebral stage	Mean age <sup>a</sup>	Mean time of test after onset of therapy <sup>a</sup>
Normal controls	38	17/21	–	–	28 (18–42)	–
Patients shortly after admission	11	5/6	7	4	34 (19–60)	2.4 (1–4) days
Patients after 2 weeks of treatment	22	11/11	12	10	34 (19–60)	13.3 (9–18) days
Patients after 4 weeks of treatment	22	9/13	14	8	35 (18–65)	29.5 (23–33) days
Patients after cure	14	9/5	10	4	44 (18–65)	7 (0.3–14) years

<sup>a</sup> The range is given in parentheses.

**Table 2.** Clinical profile of patients and controls studied with hCRH

	n	Sex (f/m)	Hemo-lymphatic stage	Cerebral stage	Mean age <sup>a</sup> years	Mean time of test after onset of therapy <sup>a</sup>
Normal controls	14	7/7	–	–	34 (21–60)	–
Patients shortly after admission	15	5/10	6	9	34 (20–60)	3.6 (2–5) days
Patients after 2 weeks of therapy	19	10/9	9	10	36 (21–56)	14.4 (8–21) days
Patients after 4 weeks of therapy	20	11/9	11	9	38 (18–65)	29.9 (23–38) days
Patients after cure	14	7/7	6	8	39 (20–66)	25 (9–43) months

<sup>a</sup> The range is given in parentheses.

drawn at 0 and 60 min for determination of serum cortisol. Serum suramin concentrations were measured in the baseline samples.

The samples were stored on ice for up to 6 h, then centrifuged and stored at  $-20^{\circ}\text{C}$ . After transportation to the FRG or USA on dry ice, all samples from a single patient were run in the same assay.

#### Protocol 2

Sixty-eight patients with SS and 14 normal subjects had a hCRH stimulation test between 10.00 and 14.00. The clinical profile of these patients is given in table 2. After placing an indwelling catheter in the antecubital vein, blood was drawn for determination of plasma ACTH and serum cortisol at baseline and 15, 30 and 60 min after a bolus injection of 100  $\mu\text{g}$  hCRH i.v. (Corticobiss, Bissendorf GmbH, Hannover, FRG). The concentrations of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 were determined in the baseline plasma samples. The samples were immediately chilled at  $4^{\circ}\text{C}$ , centrifuged and stored at  $-20^{\circ}\text{C}$ .

#### Assays

Plasma ACTH was determined in duplicate by a commercial two-site immunoradiometric assay (Nichols, Bad Nauheim, FRG). The interassay and intraassay variability were 7.3 and 2.6%, respectively. Serum cortisol (Serono, Freiburg, FRG) was determined by specific RIA with commercial reagents. The inter- and intra-assay variability were 5.1 and 2.2%, respectively. Serum suramin concentrations were determined by HPLC in a modification of the method of Klecker and

Collins [24]. TNF- $\alpha$ , IL-1 $\beta$  and IL-6 were determined by specific ELISA using commercial assays (R & D Systems, Minneapolis, Minn., USA). The lower limits of detection of these assays were, 2.8, 1.0 and 1.0 pg/ml, respectively, and the inter- and intra-assay variability 6.4 and 7.8, 3.0 and 5.0, and 7.8 and 7.5%, respectively.

#### Statistics

The data of patients with hemolymphatic and cerebral SS were analyzed separately, to exclude effects of disease stage and treatment on the HPA axis. Since no significant differences were found, the combined data of both groups are shown. All data are expressed as mean  $\pm$  SEM, if not otherwise stated. The normal range of hormones and peptides was defined as the mean of the controls  $\pm$  2 SD. The total integrated ACTH and cortisol responses to hCRH were calculated by the trapezoid method and expressed as area under the hormone concentration time curve (AUC) from 0 to 60 min. The net integrated ACTH and cortisol responses to hCRH were expressed as the AUC from 0 to 60 min minus the area generated by the baseline value multiplied by 60 min. Differences between group means were assessed using a nonparametric one-way ANOVA (Kruskal-Wallis test) and the Mann-Whitney U test for unpaired data, as appropriate. Correlations were examined with linear-regression analysis after logarithmic transformation of cytokine concentrations and expressed as Pearson's correlation coefficient. A  $p \leq 0.05$  was considered statistically significant.

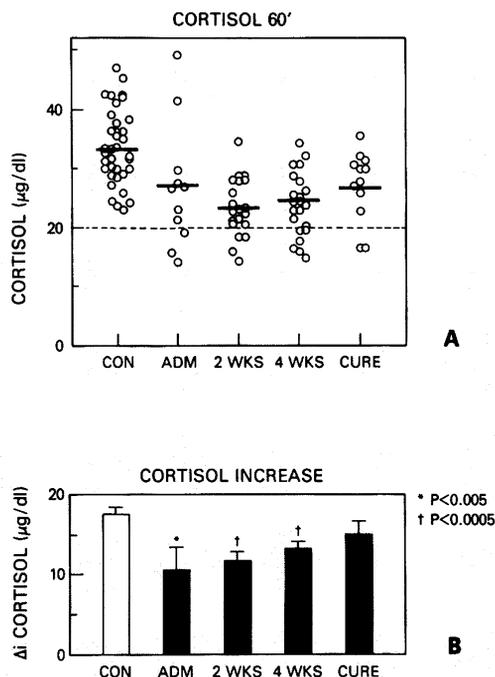
## Results

### ACTH Stimulation Test

Sixty-nine patients with SS and 39 age- and sex-matched normal Ugandan controls underwent an ACTH stimulation test to assess the maximum cortisol secretory capacity of the adrenal cortex (fig. 1). Compared to normal controls, total and net ACTH 1-24-stimulated cortisol concentrations were significantly lower in patients with SS than in controls (Kruskal-Wallis test:  $p = 0.0001$  and  $0.0009$ , respectively). Shortly after onset of therapy, SS patients had low total ACTH 1-24-stimulated cortisol concentrations ( $26.8 \pm 3.2$  vs.  $33.0 \pm 1.0$   $\mu\text{g/dl}$ ,  $p = 0.01$ ) and a subnormal net cortisol response ( $10.5 \pm 2.9$  vs.  $17.4 \pm 1.0$   $\mu\text{g/dl}$ ,  $p = 0.004$ ). After 2 and 4 weeks of treatment, which resulted in marked clinical improvement, stimulated cortisol concentrations in the patients were subnormal, whereas the net cortisol responses were still significantly lower than in controls ( $11.7 \pm 1.1$   $\mu\text{g/dl}$  after 2 weeks of treatment,  $p = 0.0005$  vs. controls;  $13.2 \pm 0.7$   $\mu\text{g/dl}$  after 4 weeks of treatment,  $p = 0.0004$ ). After cure, adrenocortical function improved somewhat, but not completely. Maximal circulating cortisol concentrations after ACTH 1-24 stimulation were significantly lower than in controls ( $25.6 \pm 1.4$   $\mu\text{g/dl}$ ,  $p = 0.006$ ); however, the mean cortisol increase in these patients was normal ( $15.0 \pm 1.7$   $\mu\text{g/dl}$  vs.  $17.4 \pm 1.0$   $\mu\text{g/dl}$ ,  $p = 0.5$ ).

Three of 11 (27%) patients on admission, 4 of 22 (18%) after 2 weeks of treatment, 6 of 22 (27%) after 4 weeks of treatment and 2 of 14 (14%) patients after cure had stimulated cortisol concentrations below the conventionally accepted level of 20  $\mu\text{g/dl}$  [25].

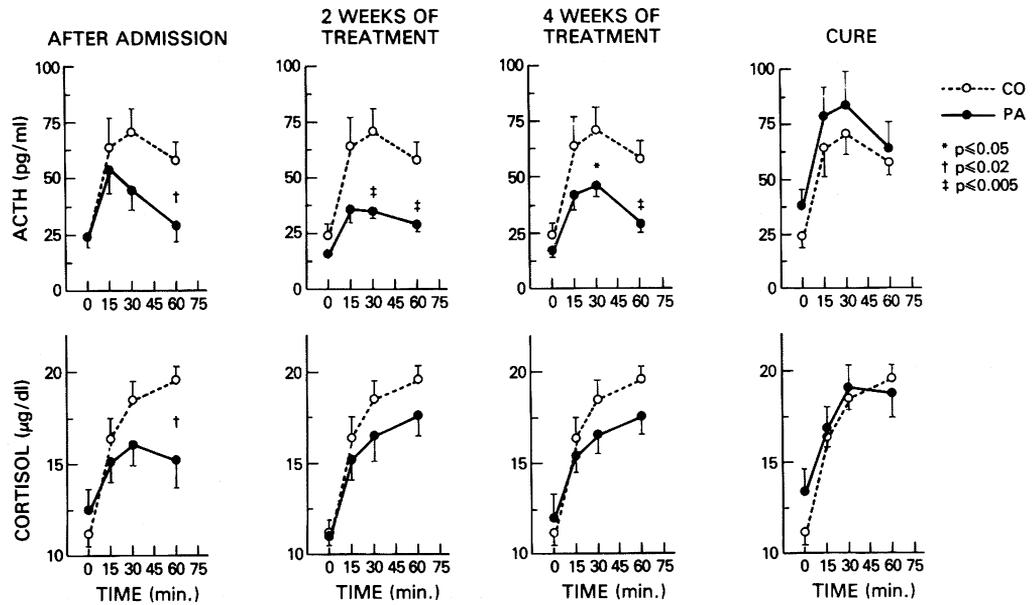
Serum suramin concentrations in patients with SS ranged from 9.0 to 135.4  $\mu\text{g/ml}$  and correlated well with the individual dose given to the patient. Mean suramin levels increased gradually during the course of suramin treatment in patients with hemolymphatic SS, but remained low in patients with cerebral SS on melarsoprol therapy, who received only the initial suramin dose of 0.75 g. All patients receiving suramin had therapeutic levels above 50  $\mu\text{g/ml}$  at the end of the treatment period. After cure, 5 patients had still measurable suramin levels (the half-life of suramin in plasma is approximately 50 days), whereas in the remaining patients the levels were under the limit of detection ( $<1.5$   $\mu\text{g/ml}$ ). No correlation was found between suramin concentrations and the maximal cortisol concentrations ( $r = 0.13$ ) or the net cortisol response ( $r = 0.005$ ) after ACTH stimulation.



**Fig. 1. A** Serum cortisol concentrations 60 min after i.v. administration of 250  $\mu\text{g}$  of ACTH 1-24 in 69 African patients with SS and 38 age- and sex-matched controls studied in parallel. Controls (CON), newly admitted, unmedicated patients (ADM), after 2 (2 WKS) and 4 (4 WKS) weeks of suramin and/or melarsoprol treatment, and after cure. The means of each group are depicted as horizontal lines. Patients were significantly different from controls: ADM,  $p = 0.007$ ; 2 WKS,  $p = 0.0001$ ; 4 WKS,  $p = 0.0001$ ; CURE,  $p = 0.006$ . The interrupted line represents the conventionally accepted minimal normal cortisol concentration after ACTH stimulation [25]. **B** Mean ( $\pm$  SE) serum cortisol increases in controls and patients with SS: Patients were significantly different from controls, as indicated.

### hCRH-Stimulation Test

Sixty-eight patients with SS and 14 Ugandan age- and sex-matched control subjects underwent a hCRH stimulation test (fig. 2). Baseline ACTH and cortisol concentrations did not differ significantly between patients with SS and control subjects (Kruskal-Wallis test:  $p > 0.05$  for ACTH,  $p = 0.4$  for cortisol). Although severely ill, patients studied shortly after admission showed no stress-induced activation of the HPA axis (baseline ACTH:  $24 \pm 5$  vs.  $24 \pm 5$   $\text{pg/ml}$  in controls; cortisol:  $12.5 \pm 1.1$  vs.  $11.2 \pm 0.7$   $\mu\text{g/dl}$ ).



**Fig. 2.** Mean ( $\pm$  SE) baseline and hCRH-stimulated (100  $\mu$ g i.v.) plasma ACTH (upper panel) and serum cortisol (lower panel) concentrations in African patients with SS and age- and sex-matched controls studied in parallel. The symbols denote significant changes between patients (PA) and controls (CO).

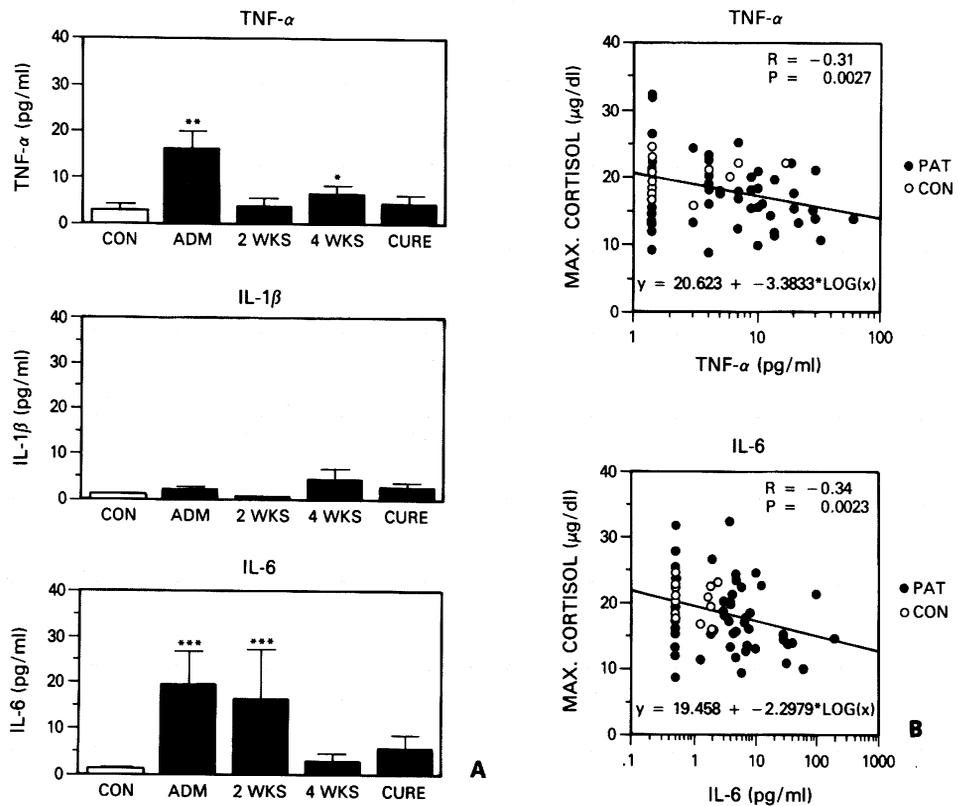
After stimulation with hCRH, the ACTH response was blunted in patients with SS (Kruskal-Wallis test for ACTH at 30 and 60 min:  $p = 0.01$  and  $0.005$ , respectively). ACTH concentrations in untreated patients were low compared to controls after 60 min ( $29 \pm 7$  vs.  $58 \pm 8$  pg/ml,  $p = 0.014$ ). In addition, the net integrated ACTH increase was reduced in the patients ( $1,131 \pm 289$  vs.  $2,149 \pm 357$  pg/ml  $\times$  min;  $p = 0.045$ ). The subnormal ACTH response to hCRH was even more pronounced after 2 and 4 weeks of treatment, but was not different from controls after permanent cure (fig. 2).

The cortisol response to hCRH was also subnormal (Kruskal-Wallis test for maximum cortisol increase:  $p = 0.006$ ). Patients studied shortly after admission had low cortisol concentrations after 60 min ( $15.2 \pm 1.5$  vs.  $19.6 \pm 0.7$   $\mu$ g/dl in controls,  $p = 0.018$ ), a low net integrated cortisol increase ( $182 \pm 46$  vs.  $377 \pm 36$   $\mu$ g/dl  $\times$  min,  $p = 0.035$ ) and a significantly reduced maximum cortisol increase ( $4.4 \pm 1.1$  vs.  $9.36 \pm 0.7$   $\mu$ g/dl,  $p = 0.0012$ ). After 2 and 4 weeks of treatment, a normalization of the cortisol response to hCRH was observed, despite subnormal levels of the corresponding ACTH concentrations. After permanent cure, the cortisol response to hCRH was normal.

Defining the normal response intervals to hCRH as the peak cortisol concentration of the controls  $\pm 2$  SD (15.3–25.7  $\mu$ g/dl), 50% of the patients at admission, 37 and 15% after 2 and 4 weeks of treatment, respectively, and 14% after cure of SS had a subnormal cortisol response.

### Cytokines

In patients undergoing hCRH stimulation, basal circulating TNF- $\alpha$ , IL-1 $\beta$  and IL-6 concentrations were determined (fig. 3A). TNF- $\alpha$  (Kruskal-Wallis test,  $p = 0.005$ ) and IL-6 (Kruskal-Wallis test,  $p = 0.0001$ ), but not IL-1 $\beta$ , were substantially elevated in SS. TNF- $\alpha$  concentrations were high in patients shortly after admission ( $16.0 \pm 4.5$  vs.  $2.9 \pm 1.4$  pg/ml in controls,  $p = 0.003$ ) and returned to normal after 2 and 4 weeks of treatment ( $3.8 \pm 1.6$  and  $6.5 \pm 1.7$  pg/ml, respectively). A weak but significant inverse correlation was observed between basal TNF- $\alpha$  concentrations and peak cortisol concentrations after hCRH stimulation ( $r = -0.31$ ,  $p = 0.003$ ; fig. 3B). IL-1 $\beta$  concentrations were undetectable in most of the patients with SS, and mean immunoreactive concentrations did not differ from control subjects ( $0.9 \pm 0.2$  vs.  $2.0 \pm 0.8$  pg/ml,  $p = \text{NS}$ ), IL-6 concentrations, on the other hand, were dramatically elevated in SS ( $19.2 \pm 7.3$  vs.  $2.9$



**Fig. 3. A** Mean ( $\pm$  SE) plasma cytokine concentrations in controls (CON) and newly admitted, unmedicated SS patients (ADM), after 2 (2 WKS) and 4 weeks (4 WKS) of treatment, and after cure (CURE). \*  $p = 0.02$ , \*\*  $p = 0.003$ , \*\*\*  $p = 0.0001$ , patients vs. controls (Mann-Whitney U test). **B** Correlation between maximal serum cortisol concentrations during the hCRH test and baseline TNF- $\alpha$  (upper graph) and IL-6 concentrations (lower graph) in patients with SS and controls.

$\pm 1.4$  pg/ml in controls,  $p = 0.0001$ ) and slowly returned to normal (after 2 weeks:  $16.3 \pm 10.8$  pg/ml; after 4 weeks:  $2.8 \pm 1.6$  pg/ml). Peak cortisol concentrations after hCRH stimulation were negatively correlated with basal IL-6 concentrations ( $r = -0.34$ ,  $p = 0.002$ ; fig. 3B).

## Discussion

African trypanosomiasis is associated with extensive mononuclear infiltration of organs penetrated by the parasite [26, 27]. Numerous complications of SS have been described, including pancarditis, glomerulonephritis, hepatitis, autoimmune thrombocytopenia, acquired cellular and humoral immunodeficiency, hypothyroidism and hypogonadism [3, 4, 15–18]. Based on the present find-

ings, we add a new frequent complication to this list: impairment of adrenocortical function. In our study, patients with SS as a group had a subnormal cortisol response to ACTH 1-24 and a blunted ACTH and cortisol response to hCRH. These alterations in the HPA axis were almost completely reversed with suramin and/or melarsoprol treatment.

By standard biochemical criteria, the hypocortisolism observed in SS appears to be mild to moderate. Baseline ACTH and cortisol concentrations were in the normal range, and cortisol concentrations after ACTH 1-24 were above 10  $\mu\text{g/dl}$ , with one third of the patients below the conventional criterion of 20  $\mu\text{g/dl}$  [25]. However, one could argue for a clinically relevant glucocorticoid deficit in this condition, even in patients with peak cortisol concentrations exceeding 20  $\mu\text{g/dl}$ . Patients with untreated

SS are exposed to severe disease-related stress and should have an activation of the HPA axis analogous to the severity of their disease [5–8]. ‘Normal’ baseline ACTH and cortisol concentrations in these patients and lack of increased responsiveness to ACTH 1-24 may, thus, reflect a relative glucocorticoid deficit, which may require glucocorticoid replacement therapy. Interestingly, high-dose glucocorticoids have been successfully used as an adjunctive therapy for cerebral SS in uncontrolled studies [4]. The reduced morbidity and mortality seen with this regimen may be attributed not only to the suppressive effects of glucocorticoids on the inflammatory reaction within the CNS, but also to other beneficial effects of appropriate glucocorticoid replacement in these patients, such as prevention of hypotension or a frank adrenal crisis.

What is the locus of the functional defect of the HPA axis in SS? Our results favor a combined (central and peripheral) origin of dysfunction. The low ACTH response to hCRH before and after 2 and 4 weeks of treatment seems to favor a secondary adrenocortical insufficiency. However, the cortisol response to hCRH was improved at 2 and 4 weeks, despite concurrently low ACTH concentrations, arguing for an independent primary adrenocortical dysfunction which resolved earlier than the pituitary ACTH deficiency. The combined nature of adrenal insufficiency in patients with SS is reminiscent of the hypothyroidism and hypogonadism observed in this disease, which also simultaneously involve the hypothalamic-pituitary unit and the gonads [15–18, 28–32].

Suramin has been shown to have adrenotoxic properties *in vivo* [21–23] and *in vitro* [33, 34]. Primary adrenocortical insufficiency was induced in patients with AIDS after 2–6 months treatment with a cumulative dosage of more than 10 g suramin [21, 22]. Suramin concentrations in these patients were invariably higher than 150 µg/ml, and often higher than 250 µg/ml. Because of the toxic effects of suramin on the adrenal cortex, it has been used as an antitumor drug in primary adrenocortical carcinoma [33, 34]. Generally, at this time, patients undergoing suramin treatment are routinely considered at risk for primary adrenal failure and receive concurrent glucocorticoid replacement therapy [33]. Surprisingly, we observed no case of suramin-induced adrenal insufficiency in our patients with SS and, paradoxically, we observed improvement of adrenocortical function with therapy. No correlation was found between the peak cortisol levels after ACTH and the basal serum suramin levels. These results may be explained by the lower cumulative dose of suramin given to our patients (maximum 5.75 g), by the

shorter duration of suramin administration, and/or by the lower serum suramin levels achieved compared to patients with AIDS or cancer patients.

The pathogenesis of the impairment of the pituitary and adrenocortical function that we observed in SS is not clear. It could be explained in two major ways, which are not mutually exclusive. First, it may be due to parasite infiltration and transient inflammatory dysfunction of the pituitary and/or adrenal cortices. Animal and human data support this possibility [8, 27, 30–32]. Hence, experimental infection with *T. brucei* in sheep, resulted in acute coagulative necrosis of the adenohypophysis and leukocytic infiltration of the neurohypophysis, with trypanosomes present in pituitary tissue [35]. In humans, on the other hand, pituitary fibrosis with panhypopituitarism, including adrenal insufficiency, was reported in 2 patients with SS [19]. In both of these paradigms, however, the adrenal insufficiency was severe and permanent, in contrast to the mild to moderate transient dysfunction that we observed. Second, although the inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$  and IL-6 administered acutely stimulate CRH, ACTH and/or cortisol release [36–39], chronic exposure to high endogenous cytokine levels may be associated with suppression of HPA axis activity. The high circulating TNF- $\alpha$  and IL-6 levels in patients with SS correlated negatively with peak cortisol concentrations after hCRH, and TNF- $\alpha$  has been shown to inhibit the ACTH response of cultured dispersed anterior pituitary cells to CRH after 8–24 h, but not 4 h, incubation [40]. In addition, in critically ill patients with extensive burns, high cortisol concentrations correlated significantly with low TNF- $\alpha$  levels [41]. These data are compatible with a direct inhibitory effect of chronically elevated TNF- $\alpha$  and/or IL-6 on the HPA axis in SS. Similar data from patients with rheumatoid arthritis are concordant with this notion [42, 43]. It is, therefore, possible that this phenomenon represents the HPA axis equivalent of the ‘euthyroid sick syndrome’ in inflammatory states.

The correlation between plasma cytokine and cortisol concentrations was weak in this study. The interaction between the immune system and the HPA axis in SS may be, therefore, more indirect. For example, both the elevation of inflammatory cytokines and the suppression of the HPA axis in African trypanosomiasis can be regarded as indices of disease activity. In this paradigm, the observed correlation reflects more the association with the severity of the underlying illness rather than a direct inhibitory effect of TNF- $\alpha$  and/or IL-6 on the HPA axis.

High inflammatory cytokine levels in African trypanosomiasis may contribute positively to the outcome of

infection by increasing the specific immune response against the parasite [44], or through direct toxicity to the parasite itself, as has been shown in mice experimentally infected with *Trypanosoma cruzei* [45]. In patients with burns, the mortality rate correlates positively with endogenous glucocorticoids and negatively with TNF- $\alpha$  concentrations [41]. Thus, the potential clinical significance of the HPA dysfunction observed in SS is not clear at this time. Only a prospective double-blind therapeutic trial of supplemental glucocorticoids will conclusively show whether the HPA axis changes observed in inflamma-

tory states are adaptive, to allow a better antiparasitic defense, or maladaptive, contributing to morbidity and mortality.

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