Experimentally Challenged Reactivity of the Hypothalamic Pituitary Adrenal Axis in Patients with Recently Diagnosed Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** There is evidence that the hypothalamic pituitary adrenal (HPA) axis is subresponsive in patients with rheumatoid arthritis (RA). We assessed HPA axis responses to experimental stressors mimicking daily life challenges in patients with RA to determine whether HPA axis activity is associated with Th1 and Th2 activity.

Methods. ACTH and cortisol responses in reaction to the succession of a bicycle ergometer task, a cold pressor task, and a computerized Stroop Color-Word interference test, as well as basal Th1 and Th2 cell activity, were assessed in 29 patients (21 female, 8 male) with recently diagnosed RA (mean disease duration 29 wks, range 5–69), mean age 55.7 years, none receiving glucocorticoid treatment, and 30 (20 female, 10 male) healthy age and sex matched controls (mean age 54.1 yrs).

Results. Mean ACTH and cortisol levels did not differ between the groups ($p > 0.10$). Patients tended to have a less pronounced ACTH response ($F_{2,50} = 2.7$, $p = 0.08$) and had a significantly smaller cortisol response ($P\ F_{2,50} = 6.1$, $p < 0.01$) than healthy controls in reaction to the stressors. This difference in cortisol response was reduced, but remained significant when ACTH responsiveness was accounted for by entering it as a covariate ($P\ F_{2,49} = 3.7$, $p = 0.03$). ACTH and cortisol levels and responses were not associated (all $p > 0.19$) with basal interferon-γ and interleukin 4 as reflections of Th1 and Th2 cell activity, respectively. HPA axis activity was not linked to current disease activity.

Conclusion. Our findings show reduced HPA axis responsiveness in RA patients with recent diagnosis receiving longterm medication that is suggested to be located both at a hypothalamic/pituitary and at an adrenal level. It appears that common HPA axis activity accomplishes low amounts of cortisol release, which makes it difficult to determine an influence of endogenous cortisol changes on the Th1/Th2 balance. (J Rheumatol 2001;28:1496–504)

Key Indexing Terms:

RHEUMATOID ARTHRITIS	ACTH	CORTISOL
HYPOTHALAMUS	PITUITARY	ADRENALS

During inflammatory stress, proinflammatory cytokines such as interleukin 1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) activate the release of corticotrophin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus. CRH stimulates the release of adrenocorticotrophic hormone (ACTH) from the pituitary, followed by the secretion of cortisol by the adrenals. Thus, inflammation stimulates the production of cortisol that, in turn, is able to dampen inflammation. It has been suggested that activity of the hypothalamic pituitary adrenal (HPA) axis in rheumatoid arthritis (RA) is inappropriately normal for the degree of inflammation, resulting in an amount of cortisol that is insufficient to dampen the ongoing inflammation[1,2].

ACTH and cortisol responses to assess HPA axis function in RA have been investigated in animal models[3] and in human patients in response to pharmacological stimulants, such as injection of ovine CRH in the CRH test[4,5] or injection of ACTH[6], and in reaction to psychological and physiological stressors such as mental effort tasks or surgery[7,8].

Studies in arthritis susceptible Lewis rats showed deficient HPA responses that were implicated to be due to impaired regulation of CRH secretion and biosynthesis at the hypothalamic level[3]. In reaction to injection of ACTH, patients with RA and healthy controls showed similar cortisol reactions[6]. In response to the CRH test impaired adrenal function has been implied by relatively low cortisol levels in relation to ACTH levels[9], but also normal pituitary and adrenal function has been observed[10]. The suggestion of

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impairment at hypothalamic level in studies using the CRH test is speculative, because hypothalamic function is not assessed directly in this paradigm. In contrast, studies using natural stressors challenge the HPA axis at the hypothalamic level[11] and may help clarify whether the hypothalamus is able to adequately stimulate the pituitary and adrenals.

Results of studies using natural challenges to stimulate the HPA axis in patients with RA have also been conflicting. In one study patients failed to show an increase in cortisol level in reaction to major surgery despite increased IL-1 and IL-6 levels[7]. Since in reaction to the CRH test these same patients showed similar ACTH and cortisol responses as noninflammatory arthritis patients, a defect was suggested to reside at the hypothalamic level, and not at the pituitary or the adrenals. In contrast to these results, Eijsbouts, *et al*[12] observed similar ACTH and cortisol responses in reaction to major surgery in patients with RA and with osteoarthritis, and consequently questioned a defective hypothalamic response in patients with RA. An objection to the natural stressor in these studies is that surgery has a strong physical component also involving HPA activity[13]. It is therefore unlikely that these studies will give insight into common, natural HPA axis responsiveness. Standardized mental and physical stress tasks may be more suitable for determining day-to-day HPA responsiveness. In one study[8] cortisol responses were determined in response to such healthy stressors and were found to be similar for patients and controls. However, the sample size was small and ACTH responsiveness had not been assessed. In the current study, we have used standardized mental and physical stressors to examine both ACTH and cortisol responsiveness. The first aim of our study was to examine whether in patients with RA the hypothalamus is able, in response to natural everyday stressors, to react with an adequate response to activate the pituitary and adrenals. Considering the aforementioned results, we hypothesized reduced HPA axis responsiveness in patients, which would be largely ascribed to impaired hypothalamic function.

It has been suggested that the HPA axis influences inflammatory processes through the differential effect of cortisol on T helper 1 (Th1) and T helper 2 (Th2) immune cells[11]. T helper cells can be distinguished according to their cytokine secretion profiles[14-16]. Th1 cells predominantly secrete interferon-γ (IFN-γ), IL-2, and TNF-α, and induce macrophages to produce the proinflammatory cytokines TNF-α, IL-1, and IL-6. Th2 cells primarily secrete the anti-inflammatory cytokines IL-4, IL-10, and IL-13, of which IL-4 and IL-10 are known to inhibit the activation of macrophages and the production of proinflammatory cytokines.

RA is believed to be characterized by a predominance of Th1 cell activity and to improve during periods in which antiinflammatory Th2 cytokines increase[14,17]. *In vitro*/*ex vivo* animal studies[18-20] revealed a stimulatory effect of dexamethasone on Th2 cell activity, while Th1 cell activity was inhibited[18,19], and a co-occurrence of increased HPA axis activity with a shift from Th1 to Th2 cell activity[20]. Dexamethasone pulse therapy in RA patients resulted in an upregulation of IL-10 and a transient decrease in IFN-γ[21]. Taken together, there is evidence that the Th1/Th2 balance is of importance for disease activity in RA, and that exogenous administered cortisol differentially affects Th1 and Th2 immune cell activity. It is not clear whether naturally occurring HPA axis responsiveness is meaningfully associated with Th1/Th2 balance. The second aim of our study was to verify whether day-to-day HPA axis responsiveness is associated with Th1 and Th2 cell activity.

MATERIALS AND METHODS

Subjects. Twenty-nine patients (21 female, 8 male) with recently diagnosed RA (mean age 55.7 yrs, range 25–77) and 30 healthy subjects (20 female, 10 male) (mean age 54.1 yrs, range 33–76) were included. All patients fulfilled the classification criteria of the American College of Rheumatology[22]. Age and sex matched healthy individuals were recruited as controls via the RA patients and by the researchers. Exclusion criteria for healthy subjects were the presence of a chronic disease, chronic pain, heart problems, or hypertension. The ethical committee of the University Medical Center Utrecht had approved the study.

Patient characteristics are shown in Table 1. Patients had a mean erythrocyte sedimentation rate (ESR) of 25 mm/h (range 2–130), a mean Thompson joint score of 102 (range 0–459), and a mean disease duration of 29 weeks (range 5–69). Patients were taking part in a larger population based study on the efficacy of medication treatment strategies among outpatients with RA of recent onset[23]. All patients were asked to participate in the current study. Thirty-eight patients were willing to participate. At the time of the study 6 patients were taking prednisone (5 to 15 mg daily). Three patients, of whom one was taking prednisone, received a corticosteroid joint injection within 3 months prior to the start of the study. Patients taking prednisone or having received a corticosteroid injection within 3 months prior to the start of the study were omitted from analyses. One patient was omitted from analyses because of extreme ACTH and cortisol values due to fainting during insertion of the catheter. Omission of these patients reduced the original 38 patients to a sample size of 29 eligible patients. Two patients and 3 healthy controls had incidental ACTH and cortisol values missing. Immune variables were missing for 3 healthy controls.

Procedure. Subjects arrived at the laboratory at 9:45 AM. In order to assess hormonal and immunological variables, an indwelling catheter was inserted into a prominent upper side forearm vein, about 1 hour before the first actual blood draw. This first blood sample was drawn after a 20 min relaxation period in which subjects listened to easy listening music and watched a wildlife documentary.

After this relaxation period, subjects performed physical and mental stress tasks in the following fixed order: a bicycle ergometer task, a cold pressor task, and the computerized Stroop Color-Word Interference test, each of which was preceded by a 5 to 10 min rest period. During this period subjects could recover from the previous task, receive instructions, and practice the upcoming task. The bicycle ergometer task involved cycling at a rate of 50 revolutions per minute against a resistance of 12.5 watt for 3 min. During the cold pressor task, subjects immersed their right hand in a bowl filled with water at 10°C for 3 min. During a computerized Stroop test, 150 words were successively presented on the screen during 2.5 min. These words are color names (blue, yellow, red, or green) that were printed in another color. Subjects had to name the color they saw while reading of the word caused interference.

At the end of this experimental period, which lasted about 50 min, a

Table 1. Patient characteristics.

Patient	Age (yrs)	Sex	Disease Duration (wks)	ESR (mm/h)	DMARD	NSAID
1121	58	F	23	15	MTX	–
1124	54	F	17	16	MTX	–
3089	65	F	11	20	HCQ	+
3092	67	F	8	9	HCQ	+
1134	43	M	7	130	HCQ	+
3093	77	F	14	10	MTX	+
5511	29	F	23	5	MTX	–
4121	73	F	56	10	—	+
3097	53	M	10	16	—	+
6028	41	F	55	2	DPA	+
6043	66	M	24	19	HCQ	–
6030	41	F	58	24	DPA, MTX	+
1137	76	M	16	80	MTX	+
4125	68	F	39	5	MTX	+
6035	69	F	69	21	IM gold	–
4123	53	F	51	14	MTX	+
6040	44	F	53	10	HCQ	–
5038	65	F	19	4	IM gold	+
5037	51	F	26	2	MTX	+
4131	66	F	39	17	MTX	+
3107	52	M	5	13	HCQ	+
3103	45	F	18	50	MTX	+
6050	54	M	48	6	—	+
6053	51	M	28	—	HCQ	–
4142	50	F	40	21	MTX	+
4146	54	F	30	55	DPA	+
1023	60	F	13	92	MTX	+
6002	25	F	25	29	MTX	+
3005	64	M	5	10	MTX	+
Mean ± SD	55.7 ± 13.0		28.6 ± 18.4	25.2 ± 30.2		

DPA: D-penicillamine; DMARD: disease modifying antirheumatic drugs; ESR: erythrocyte sedimentation rate; HCQ: hydroxychloroquine; IM gold: intramuscular gold; MTX: methotrexate; NSAID: nonsteroidal antiinflammatory drugs.

post-stressor blood sample was drawn in order to determine hormonal and immunological variables. After a 30 min post-stress relaxation period, in which patients listened to easy listening music and watched a wildlife documentary, a third blood sample was drawn. Throughout the duration of the study, subjects were comfortably seated in a reclined position in a chair with movable back and footrest. An overview of the protocol is shown in Table 2.

Assessment of cortisol and ACTH. For the assessment of ACTH and cortisol, peripheral blood was collected in a 5 ml ethylenediamine tetraacetic acid (EDTA) tube. ACTH was measured using an immunometric technique on an Advantage Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The lower limit of detection was 1.0 ng/l and the interassay variation was 11.4, 10.7, and 6.8% at 11, 68, and 310 ng/l, respectively (n = 32). Cortisol was measured by a competitive chemiluminescence immunoassay performed on an Advia Centaur automated immunoassay platform (Bayer Diagnostics; Leverkusen, Germany). Interassay variation was 8.3, 7.4, and 9.2% at 0.17, 0.42, and 1.08 µMol/l, respectively (n = 94).

Assessment of IFN-γ and IL-4. IFN-γ and IL-4 production was measured to assess Th1 and Th2 cell activity, respectively. For the assessment of IFN-γ and IL-4, peripheral blood was collected in a 10 ml heparin tube. Next, peripheral blood was diluted 1:1 with Dulbecco's modified Eagle's medium

Table 2. Overview of study protocol.

Time	Activity
9:45 AM	Arrival at the laboratory
10:00 AM	Insertion of catheter
10:05 AM	Explanation of protocol
	Attachment of equipment
10:40 AM	Relaxation period
	Easy-listening music
	Wildlife documentary
11:00 AM	Blood draw
11:05 AM	Stress period
	Bicycle ergometer test
	Cold pressor task
	Stroop test
11:55 AM	Blood draw
12:00 PM	Post-stress period
	Easy-listening music
	Wildlife documentary
12:30 PM	Blood draw

(DMEM, Gibco 074-01600), supplemented with penicillin, streptomycin and glutamine (PSG), and then mononuclear cells were isolated by density centrifugation using Ficoll-Paque (Pharmacia, Biotech, Roosendaal, The Netherlands). Subsequently, mononuclear cells (0.5×10^6/ml) were cultured for 48 h in DMEM supplemented with 10% human pooled adult male AB+ serum (Red Cross Blood Transfusion Centre, Utrecht, The Netherlands). Because of undetectable low spontaneous production of IFN-γ and IL-4, T cells were costimulated specifically with CD3-CD28 monoclonal antibodies[24] (CLB, Amsterdam, The Netherlands). After 48 h culture, the culture media were harvested and rendered cell-free by centrifugation. Next they were frozen in liquid nitrogen and stored at –70°C. IFN-γ and IL-4 concentrations were determined by ELISA (Cytosets, Biosource, Fleurus, Belgium), according to the manufacturer's guidelines. Detection limits were 15 pg/ml for IFN-γ and 10 pg/ml for IL-4.

ESR. ESR (Westergren) was assessed through standardized laboratory measurement as a measure of inflammatory activity. The Thompson joint score[25] was assessed after completion of the study around 12:30 PM by a researcher who was trained by a certified rheumatologist. Joints that were both painful and swollen were scored. The theoretical range varies from 0 to 534.

Statistical analysis. To examine hypothalamic/pituitary and adrenal function, repeated measures analysis of variance (ANOVA) was used, with condition as within-subject factor and group as between-subject factor to determine whether ACTH and cortisol levels and courses differed between patients and healthy controls across the 3 experimental conditions: baseline, stressor, and 30 min post-stressor.

To examine whether adrenal responsiveness could be explained by pituitary responsiveness, repeated measures ANOVA of the cortisol course across conditions was repeated, adding a second group factor consisting of subjects showing an ACTH increase (N = 29, range 1 to 16 ng/l) and no change or a decrease (N = 24, range –24 to 0 ng/l) in reaction to the experimental stressors.

To verify whether cortisol responsiveness was completely explained by ACTH responsiveness, the cortisol courses across conditions of patients and controls were compared in repeated measures ANOVA while controlling for ACTH responsiveness (stressor minus baseline level) by entering it as a covariate.

To ascertain whether differences in HPA axis responsiveness were related to current disease activity, repeated measures ANOVA for ACTH and cortisol were done in which ESR was entered as a covariate to control for its effect.

To examine differences in Th1 and Th2 cell activity between patients and controls, independent samples T tests were performed. Pearson's product moment correlation coefficients were calculated to analyze the associations of basal ACTH and cortisol levels with Th1 and Th2 cell activity and the Th1/Th2 ratio.

P values reported are based on 2 tailed tests, and p values below 0.05 were considered to be significant.

RESULTS

ACTH responsiveness. Figure 1 shows mean (standard error) ACTH levels at baseline, immediately after the stressors, and at 30 min after the stressors for patients and controls. Repeated measures ANOVA showed that the course of ACTH approached significant difference between patients and controls ($F_{2,50} = 2.68$, $p = 0.08$). This marginally significant group × condition interaction reflected that RA patients tended to mount a smaller ACTH response to the series of stressors than did controls. No significant main group effect for ACTH was found ($F_{1,51} = 2.27$, $p = 0.13$), reflecting that the average ACTH level did not differ between patients and controls. Across the groups, ACTH

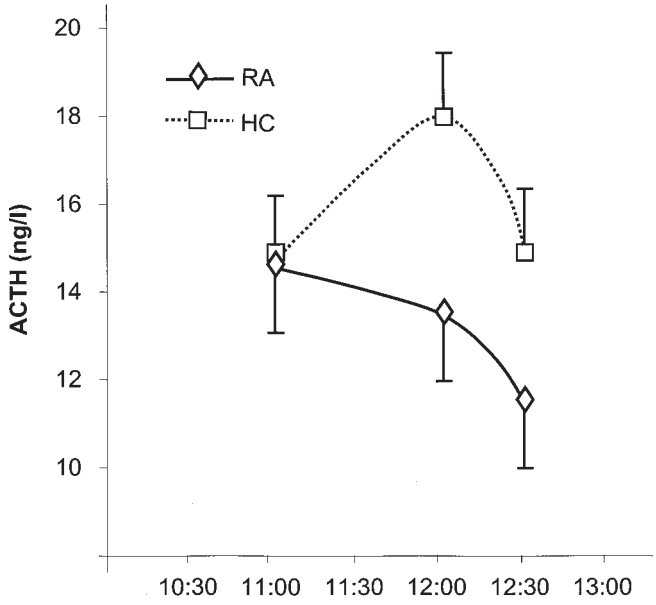

Figure 1. ACTH levels (and SE) of patients (RA) and healthy controls (HC).

varied significantly as a function of condition ($F_{2,50} = 5.99$, $p = 0.005$).

Cortisol responsiveness. A decrease in cortisol was significantly more marked in patients than in controls ($F_{2,50} = 6.06$, $p = 0.004$) (Figure 2). The decrease in cortisol was stronger in patients than in controls, progressing from a higher basal level for patients to about the same post-stressor levels for the 2 groups. When mean cortisol level was corrected for by entering it as a covariate, the different cortisol response between patients and controls remained significant ($F_{2,49} = 5.66$, $p = 0.006$), reflecting that the more marked cortisol decrease in patients was independent of mean cortisol level. Average cortisol level did not differ between patients and

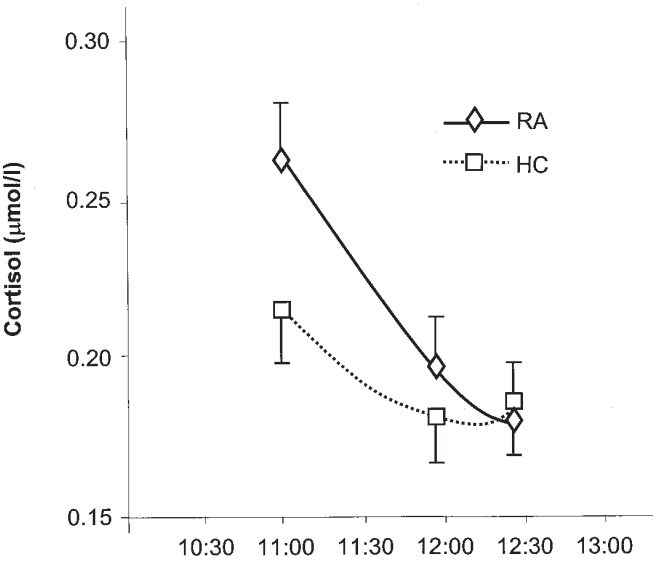

Figure 2. Cortisol levels (and SE) of patients (RA) and healthy controls (HC).

controls ($F_{1,51}$ = 0.92, p = 0.34). Cortisol level varied significantly as a function of condition ($F_{2,50}$ = 32.44, p = 0.000).

To verify whether the difference in cortisol course between the 2 groups was not a consequence of spurious fluctuations, cortisol responses for each individual participant sorted on cortisol responsiveness were inspected (Table 3). Inspection of the individual cortisol responses showed that although there was considerable overlap between the groups, relatively more patients (59%) than controls (39%) were in the lower part of the distribution. Most pronounced was that no patient showed an increase in cortisol in reaction to the stressors, while 29% of the healthy subjects had a more pronounced cortisol response than the patient that responded most to the experimental stressors. Thus, inspection of individual scores shows that the significant effect was not a consequence of spurious fluctuations or single outliers. When combining the data of Tables 1 and 3, we observed that patients in the upper and lower part of the score distribution did not differ considerably with respect to type of medication, age, or sex, implying that differences in cortisol responsiveness between patients did not depend on type of medication, age, or sex.

Hypothalamic pituitary adrenal responsiveness. To assess whether changes in cortisol could be explained by pituitary responsiveness, cortisol courses across the 3 conditions were studied in subgroups of subjects showing an increase (12 patients, 17 controls) or no change or a decrease in ACTH (14 patients, 10 controls) in response to the experimental stressors. The interaction for condition × subgroup of ACTH was significant ($F_{2,48}$ = 4.98, p = 0.01), reflecting that cortisol course across the 3 conditions was different for the 2 ACTH subgroups. Subjects showing an ACTH increase in response to the stressors showed a smaller decrease of cortisol than subjects who did not show an increase in ACTH in reaction to the stressors (data not shown). This shows that our experimental protocol was effective in testing pituitary effects on adrenal function. The group (patient or control) × ACTH subgroup × condition interaction was not significant ($F_{2,48}$ = 2.10, p = 0.13), reflecting that the effect of a slower decline of cortisol in the ACTH response groups was not significantly different for controls and patients. Because the power of this analysis was small, we nevertheless repeated this analysis in the patient and control groups separately. The analysis showed no difference in cortisol course in the ACTH subgroups of patients with RA ($F_{2,23}$ = 1.38, p = 0.27), and a significant difference in cortisol course in the ACTH subgroups of controls ($F_{2,24}$ = 4.93, p = 0.02).

Hypothalamic/pituitary responsiveness. To ascertain whether the different cortisol course in patients and controls was explained by reduced ACTH production in patients, ACTH responsiveness (stressor minus baseline level) was entered as a covariate in the repeated measures ANOVA of cortisol to remove its effect. Despite removal of the ACTH response, the difference in cortisol course between patients and controls remained significant ($F_{2,49}$ = 3.69, p = 0.03), reflecting that reduced adrenal responsiveness of patients was not explained merely by reduced hypothalamic/pituitary responsiveness.

Disease activity. To assess whether differences in HPA axis responsiveness were due to disease activity, ACTH and cortisol were examined in repeated measures ANOVA in which ESR was entered as a covariate. When the influence of ESR was controlled in this way, the almost significant time × group interaction for ACTH became (more) significant ($F_{2,48}$ = 4.97, p = 0.01), and the difference in cortisol response between patients and controls remained significant ($F_{2,48}$ = 5.30, p = 0.008). Thus, the difference in ACTH and cortisol courses of patients and controls did not depend on current disease activity.

Basal Th1 and Th2 cell activity. The production of basal IFN-γ and IL-4 in patients and controls is shown in Figure 3. Although Figure 3 suggests higher IL-4 production in patients than controls, independent samples T tests revealed no significant differences in mean basal levels of IFN-γ, IL-4, or the IFN-γ/IL-4 ratio between patients and controls. No correlation of basal levels of IFN-γ, IL-4, and IFN-γ/IL-4 ratio with basal cortisol or ACTH levels and with ACTH or cortisol reactivity was significant (data not shown). When disease activity was corrected for by computing partial correlations with ESR as a covariate, the correlations hardly changed.

DISCUSSION

Our aim was to examine HPA responsiveness as it most likely occurs in response to everyday challenges. Instead of using CRH infusions or strong real-life stressors, such as surgery, our subjects performed common physical and mental tasks as they occur in everyday life.

Before measuring HPA axis responsiveness, we first had

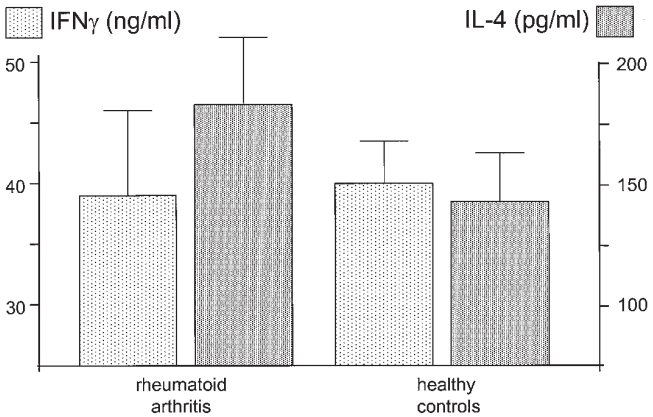

Figure 3. IFN-γ and IL-4 production (and SE) of patients with RA and healthy controls.

Table 3. Basal ACTH and cortisol levels and responses to the stressors of patients (RA, none receiving glucocorticoid treatment) and healthy controls (HC), sorted on post-stressor minus baseline cortisol responses.

Subject	Baseline ACTH (ng/l)	ACTH Response (ng/l)	Baseline Cortisol (µmol/l)	Cortisol Response (µmol/l)
RA	10	—	0.18	—
RA	7	—	0.51	—
HC	17	—	0.28	—
HC	—	—	—	—
RA	12	–2	0.31	–0.16
RA	13	–4	0.58	–0.14
RA	9	—	0.27	–0.12
RA	15	1	0.22	–0.11
RA	16	–7	0.31	–0.11
HC	11	–2	0.31	–0.11
HC	21	–2	0.35	–0.11
HC	14	0	0.26	–0.10
RA	6	2	0.24	–0.10
RA	14	1	0.38	–0.10
RA	4	0	0.30	–0.09
RA	33	–24	0.25	–0.09
RA	25	–14	0.37	–0.09
RA	26	–2	0.27	–0.08
HC	12	–1	0.23	–0.08
RA	6	11	0.24	-0.08
HC	23	–6	0.35	–0.08
RA	27	6	0.22	–0.07
HC	14	4	0.19	–0.07
HC	6	2	0.17	–0.07
HC	11	4	0.19	–0.07
HC	18	12	0.17	–0.06
RA	13	–6	0.24	–0.06
RA	10	1	0.20	–0.06
RA	21	–10	0.48	–0.06
HC	15	–7	0.22	–0.06
HC	27	–2	0.35	–0.06
HC	8	5	0.22	–0.06
HC	8	1	0.17	–0.05
HC	15	–1	0.24	–0.05
HC	13	–5	0.29	–0.05
HC	20	5	0.28	–0.04
RA	16	0	0.17	–0.04
RA	3	3	0.17	–0.04
RA	6	–1	0.26	–0.04
RA	5	2	0.17	–0.04
RA	12	4	0.14	–0.04
HC	12	–3	0.25	–0.04
RA	10	0	0.18	–0.04
RA	8	–2	0.14	–0.03
RA	16	5	0.18	–0.02
RA	20	–7	0.22	–0.02
HC	11	–2	0.15	–0.02
HC	12	12	0.15	–0.02
HC	25	5	0.22	–0.02
RA	8	13	0.37	–0.01
RA	35	6	0.25	–0.01
HC	12	16	0.18	0
HC	13	5	0.13	0
HC	21	2	0.18	0.01
HC	11	6	0.15	0.01
HC	29	7	0.20	0.03
HC	13	14	0.21	0.05
HC	16	2	0.23	0.05
HC	18	11	0.22	0.13

to be sure that our experimental protocol had been effective in testing pituitary effects at the adrenal level. Our patients and healthy controls showed a decrease in cortisol in the baseline stressor interval. This is likely due to the circadian rhythm of cortisol, which is strongly declining in the morning[8,26]. Experimental stressors attenuate, but do not reverse, the decline of cortisol in the morning hours. Our observation that subjects who showed an increase in ACTH in reaction to the stressors showed a smaller decrease in cortisol than subjects showing no change or a decrease in ACTH reflected that our experimental protocol was a valid way to examine HPA reactivity under standardized baseline and stressor manipulations.

Patients tended to have a smaller ACTH increase than controls in reaction to the stressors. This may suggest hyporesponsiveness of the HPA axis on the part of patients at a pituitary or hypothalamic level. It is unlikely that the pituitary is not sufficiently responsive to hypothalamic stimulation (for example, because the sensitivity for CRH is downregulated at the pituitary level) because studies of ACTH responsiveness to CRH tests suggest normal pituitary function[7,10]. This suggests that hyporesponsiveness resides at a hypothalamic level rather than a pituitary level. Possibly, the chronic inflammation present in RA alters the synthesizing capacity of CRH by the hypothalamus to some extent, resulting in the hypothalamus being unable to mount an adequate CRH response in reaction to natural stressors. Higher centers such as the cerebral cortex and limbic system structures that play a role in the processing of emotions[27] may also be involved in the blunted ACTH response. It is possible, for instance, that the experimental stressors were perceived as less stressful by patients than by controls, and consequently, the hypothalamus was stimulated less. In conclusion, the reduced ACTH response may reside at a hypothalamic or pituitary level, or a higher level, such as the cerebral cortex and limbic structures.

We observed a significantly smaller cortisol response in patients than controls, suggesting adrenal hyporesponsiveness. Prior treatment of patients with low doses of prednisolone has been suggested to contribute to adrenal hyporesponsiveness[9,28], but this could not have been the case in our study, since patients receiving corticosteroid treatment within 3 months prior to the study were excluded from analyses. Moreover, although the difference in cortisol response between patients and controls was reduced, when the effect of ACTH responsiveness was eliminated, it remained significant. This suggests that adrenal hyporesponsiveness in our patients is not merely explained by reduced ACTH responsiveness. Hyporesponsiveness also appears to reside at the adrenal level. This suggestion was strengthened by our observation that cortisol responsiveness co-varied with ACTH responsiveness in controls, but not in patients. This latter finding resembles the observation of weakened or abolished positive correlations between ACTH and cortisol in patients with RA[29]. Several physiological mechanisms may explain reduced adrenal responsiveness, for instance, reduced adrenocortical sensitivity to ACTH, altered ACTH receptor density on the adrenal cortex, or diminished synthesizing capacity for cortisol by the adrenal cortex due to other factors.

Overall, studies of HPA responsiveness in RA patients have yielded conflicting results[7,9,10,12]. With our experimental procedure, we expected to verify that HPA responsiveness would be reduced and that this would be largely ascribed to reduced hypothalamic function. We observed reduced HPA responsiveness to experimental stress, which does appear to be located both at a hypothalamic/pituitary and at an adrenal level.

Apart from being a patient with RA, with a history of inflammation and pain related to RA and in some cases other consequences, such as physical deconditioning, our patients also differ from healthy controls with respect to current disease activity and medication use. We were able to ascertain that HPA hyporesponsiveness was not a consequence of current disease activity. We could not reliably check a possible influence of nonsteroidal antiinflammatory (NSAID) and disease modifying antirheumatic drug (DMARD) treatment on HPA axis activity[12], due to the small sample sizes of patients using only NSAID (N = 3) or DMARD (N = 7) in our study. This implies that our results cannot be generalized beyond RA patients that are receiving longterm medication. When studying medication use in combination with cortisol responses for each individual patient, however, cortisol responsiveness did not seem to be considerably influenced by type of medication.

The differences in basal Th1 and Th2 cell activity between patients and healthy controls in this study were in the same direction as results from a previous study[30], but failed to reach significance, probably due to the small sample size and heterogeneity of the group. Basal Th1 and Th2 cell activity were unrelated to ACTH and cortisol levels and responses. We expected elevated Th2 cell activity compared to Th1 cell activity in patients because a relative predominance of Th2 cell activity in peripheral blood has been observed in RA[30]. This was interpreted to follow from the tendency of Th1 cells to migrate into the synovial joint, the site of inflammation[15]. It is possible that disease activity in our patients was too low to induce a considerable migration of Th1 cells into the synovial joint, and thus to yield low Th1 cell activity and relatively high Th2 cell activity in peripheral blood.

From results of studies with exogenous corticosteriod, we expected to observe a more favorable balance between Th1 and Th2 cell activity, hence a relative predominance of Th2 activity in patients having a more pronounced HPA axis response or higher levels, but we observed low correlations between HPA axis activity and Th1 and Th2 cell activity. Thus the natural HPA response to experimental stressors

could not be found to be physiologically relevant for significant changes in Th1/Th2 activity. Changes in endogenous cortisol levels after HPA axis response are considerably smaller than changes induced by injection of corticosteroids. The physical and mental stressors of our study are suitable to examine dynamic responsiveness in a standardized way, but they do not result in very high amounts of cortisol release. Therefore, an influence of endogenous cortisol changes on Th1/Th2 balance with this study design is difficult to confirm.

Our study shows reduced HPA axis responsiveness in patients with recently diagnosed RA receiving longterm medication. The reduced HPA axis responsiveness is suggested to be located both at a hypothalamic/pituitary and at an adrenal level. Moreover, natural HPA axis responsiveness to day-to-day stressors could not be shown to be physiologically meaningful for Th1 and Th2 cell activity.

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