Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Quantitation of glucocorticoid receptor alpha and NF-κB pathway mRNA and its correlation with disease activity in rheumatoid arthritis patients

L.O. Cavalcante[1], M.R. Melo[2], V.G. Dinis[2], R.B. Castro[2], B.D.B. Souza[1] and C.A. Longui[2]

[1]Disciplina de Reumatologia, Medicina Interna,
Irmandade da Santa Casa de Misericórdia de São Paulo, São Paulo, SP, Brasil
[2]Laboratório de Medicina Molecular, Departamento de Fisiologia,
Faculdade de Ciências Médicas, Santa Casa de São Paulo, São Paulo, SP, Brasil

Corresponding author: M.R. Melo
E-mail: murilomelo@uol.com.br

Genet. Mol. Res. 9 (4): 2300-2310 (2010)
Received August 8, 2010
Accepted September 10, 2010
Published November 23, 2010
DOI 10.4238/vol9-4gmr970

ABSTRACT. We measured NF-κB, IKK, c-Fos, and *GRα* mRNA expression and *in vivo* glucocorticoid sensitivity in patients with rheumatoid arthritis. A very low dose intravenous dexamethasone suppression test and real-time PCR quantitation of mRNA of these genes were performed on blood samples from 21 rheumatoid arthritis patients who were not on glucocorticoids during the previous four months and on blood samples from 20 healthy individuals. Mean rheumatoid arthritis duration was 8.8 years, and mean disease activity, as assessed by Disease Activity Score 28 (DAS28), was 4.45. Basal cortisol and the percentage of cortisol reduction after the very low dose intravenous dexamethasone suppression test, as well as NF-κB, IKK, c-Fos, and *GRα* mRNA expression, were similar among groups. We did not observe significant correlations between glucocorticoid *in vivo* sensitivity and DAS28. There was a positive correlation between

DAS28 and NF-κB, IKK, and *GRα*, but not c-Fos. In the multivariate analysis, only NF-κB mRNA remained as an independent variable for predicting DAS28.

Key words: Adrenal cortex hormones; Corticosteroids; Dexamethasone; Glucocorticoid sensitivity inflammation; Nuclear factor kappa-B; Rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease characterized by symmetrical erosive synovitis. Its prevalence in the Brazilian population ranges from 0.46% (Senna et al., 2004) to 1% (Brenol et al., 2007), while in the world it is about 1% (Brenol et al., 2007). RA causes significant morbidity, increases prevalence of coronary artery disease and increases cardiovascular mortality due to the important role of inflammation in its genesis (Brenol et al., 2007).

In the last years, it has been shown that the transcription factors nuclear factor kappa -B (NF-κB) and activator protein 1 (AP-1) are crucial for the induction of genes involved in inflammation, as well as in a wide range of diseases originating from chronic activation of the immune system, including asthma, atherosclerosis, inflammatory bowel disease, and autoimmune diseases such as multiple sclerosis and rheumatoid arthritis (De Bosscher et al., 2001).

A previous study by our group (Cobra et al., 2009) suggested that the mechanisms involved in resistance to glucocorticoids (GC) observed in RA are not at the glucocorticoid receptor alpha (*GRα*) mRNA expression level but at the post-receptor level, since increased *GRα* expression was not sufficient to reduce the inflammatory activity of the disease estimated by the Disease Activity Score 28 (DAS28). RA patients showed increased *GRα* mRNA expression but decreased dexamethasone suppression capability at the pituitary level.

Elucidating the mechanisms of GC resistance in RA is important not only to better understand the pathophysiology of the disease, but also to identify potential future treatments that may act on the expression of pro-inflammatory factors and improve patients' response to GC and to other medications.

The aim of this study was to determine and compare the expression of NF-κB, IKK, *GRα*, and c-Fos mRNA in RA patients and healthy controls and to correlate their levels with sensitivity to GC as assessed by the dexamethasone suppression test and the use of markers of disease activity.

MATERIAL AND METHODS

Selection of patients and controls

We studied 21 individuals with RA from the Rheumatology Clinic of the Internal Medicine Department of Irmandade da Santa Casa de Misericórdia de São Paulo, who fulfilled RA classification criteria of the American College of Rheumatology (1987) (American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines, 2002).

The control group consisted of 20 apparently healthy individuals selected through

clinical evaluation by history and physical examination. They were selected among the relatives of outpatients not included in this study, in order to match RA and control groups regarding gender and age.

We excluded controls and RA patients who were under the age of 18, had acute or chronic illnesses, such as endocrine disease, asthma and inflammatory bowel disease, and previously used glucocorticoids or drugs that accelerate dexamethasone metabolism (such as phenytoin and barbiturates) in the previous 4 months, as well as those who had basal cortisol <10 μg/dL in order to assure that the hypothalamic-pituitary-adrenal (HPA) axis was not suppressed.

The study protocol was approved by the Institutional Ethics Committee, and all individuals signed a written consent form prior to their inclusion in the study protocol.

Clinical characteristics of RA patients

The mean (SD) duration of RA was 8.8 (5.4) years. There was a median of 9 months (range: 4-48 months) since the last use of GC. Nineteen (86.4%) patients were positive for rheumatoid factor and displayed erosions on the X-rays of hands and feet. Three patients (14.3%) used three or more disease-modifying anti-rheumatic drugs (DMARDs), 10 (47.6%) used 2 DMARDs and 8 (38.1%) used only one DMARD.

To assess disease activity, we used the DAS28 (van Gestel et al., 1998; Smolen et al., 2003). The mean DAS28 (SD) was 4.45 (1.35) (range: 2.33-7.37), which characterized the group of RA patients as having moderate disease activity. Mean (SD) erythrocyte sedimentation rate (ESR) was 29.5 (20.8) mm (range: 6-89 mm).

Thirteen patients (61.9%) did not show morning stiffness. In patients showing this characteristic, the mean (SD) duration was 25 (27.5) min. The median number of tender and swollen joints was 6 (ranges of 0-49 and 0-30, respectively). The mean (SD) visual pain scale (Carlsson, 1983) was 3.05 (2.92). Patient and medical global scales had means (SD) of 3.87 (2.37) and 4.16 (2.42), respectively.

The functional status reflecting disability was measured by the HAQ (Health Assessment Questionnaire) (Fries et al., 1982; Ramey et al., 1992) and showed a median score of 0.67 (range: 0-2.13).

Very low dose intravenous dexamethasone suppression test (VLD IV-DST)

All patients and controls were submitted to the very low dose (20 μg/m^2) intravenous dexamethasone suppression test. We recently described how this new test can be used to evaluate individual glucocorticoid sensitivity in different age groups (Faria et al., 2008). Briefly, after a fasting period of 10-12 h and 30 min of resting, a blood sample was taken for cortisol measurement (and also for separation of peripheral mononuclear cells). Afterward, individuals of both groups received disodium phosphate dexamethasone intravenously (Decadron® 2 mg/mL - Prodome Chemical and Pharmaceutical, Brazil) at a dose of 20 μg/m^2 BSA as a bolus. A subsequent blood sample was taken 120 min later for cortisol measurement by RIA (BRIDGE, Adaltis, Casalecchio di Remo, Italy). The *in vivo* assessment of pituitary glucocorticoid sensitivity was thus expressed as the percentage of cortisol reduction (F%) after VLD IV-DST.

NF-κB, IKK, c-Fos, and *GRα* mRNA determination by quantitative RT-PCR

Mononuclear cells were obtained from a 20-mL venous blood sample collected in tubes containing sodium heparin (Vacutainer, Becton-Dickinson) after the addition of 20 mL Histopaque® - 1077 (Sigma, USA) and centrifugation for 30 min at 800 *g*, according to manufacturer recommendations. Total RNA was isolated from cells using guanidinium thiocyanate-chloroform extraction (Trizol, Gibco, USA). Complementary DNA (cDNA) was synthesized from 1 µg total RNA using a reverse transcription reaction (TaqMan Reverse Transcription Reagents, Applied Biosystems).

NF-kB, IKK, c-Fos, and *GRα* mRNA were determined according to the protocol previously described by our group (Silva et al., 2010). Briefly, real-time polymerase chain reaction (PCR) was performed for these genes and *BCR* (breakpoint cluster region) as a normalizing gene. Primers and probes (when used) were as follows: NF-κB sense primer: AAACACTGTGAGGATGGGATCTG; NF-κB anti-sense: CGAAGCCGACCACCATGT; IKK sense primer: AAGAACTTCTTTCAGAGACAGGAATTTC; IKK anti-sense: GCTATC ACAGCCTCTAACTCCATCT; c-Fos sense primer: AGGAGAATCCGAAGGGAAAGG; c-Fos anti-sense: TCCGCTTGGAGTGTATCAGTCA; *GRα* sense primer: GAAGGAAACT CCAGCCAGAA; *GRα* anti-sense primer: CAGCTAACATCTCGGGGAAT; *GRα* probe: 6-FAM-GCTTCCAAACATTTTTGGATAAGACCAT-TAMRA; *BCR* sense primer: CCTTCG ACGTCAATAACAAGGAT; *BCR* anti-sense primer: CCTGCGATGGCGTTCAC; *BCR* probe: 6-FAM-TCCATCTCGCTCATCATCACCGACA-TAMRA.

In each PCR run, we used a standard curve of serial dilutions of cDNA obtained from a standardized Jurkat (E6-1 clone, ATCC) cell culture (Melo et al., 2004). PCR conditions were the same for both *GRα* and *BCR* genes, as previously described by our group (Melo et al., 2004), using TaqMan PCR Core kit (Applied Biosystems, USA). Briefly, 1X TaqMan buffer A, 500 µM each dNTP, 4.5 mM $MgCl_2$, 200 nM of each primer, 100 nM probe, 0.025 U/µL AmpliTaq Gold, 2 µL cDNA and water were incubated in a total volume of 25 µL.

We used SYBR Green detection for NF-κB, IKK and c-Fos amplification, which is an intercalating, non-specific dye. Quantitative RT-PCR preparation of c-Fos reactions consisted of using 1X (12.5 µL) Platinum SYBR mix, 0.4 µL of each primer (final concentration of 160 nM), 9.7 µL water and 2 µL cDNA in each reaction. For NF-κB and IKK, primer concentrations were adjusted to 0.3 µL of each primer (final concentration of 120 nM), with corresponding increase in water (9.9 mL) in each reaction.

Cycle conditions on an ABI 7500 (Applied Biosystems) were equal for all genes: 95°C for 10 min (AmpliTaq Gold or Taq Platinun activation) followed by 45 cycles of 95°C for 15 s (denaturation) and 60°C for 90 s (annealing and extension). When SYBR Green detection was used, a final dissociation stage was included and melting curves were analyzed to ensure specific amplification.

Statistical analysis

Calculations of expression units for *GRα*, NF-κB, IKK, and c-Fos, with *BCR* normalization, were performed in MS-Excel 2000 for Windows (Microsoft).

Statistical analysis was carried out using SigmaStat for Windows v.3.05 (SPSS, Chicago, IL, USA) with the level of significance set at $P < 0.05$. For comparison of quantitative

variables, we used the Student *t*-test or Mann-Whitney rank sum test, when appropriate. Linear regression analysis was used to compare DAS28 with mRNA levels of all genes, as well as F%. Linear regression analysis was also performed to compare mRNA level of gene with that of the other. We compared proportions of qualitative variables using the chi-square test.

RESULTS

The groups of patients and controls were matched not only for age (median of 54 years for patients *vs* 61.5 years for controls) and gender (all females) but also had similar characteristics regarding weight, height, body surface area, body mass index, and waist and hip circumferences (Table 1).

Table 1. General characteristics of groups with rheumatoid arthritis (RA) and controls.

	RA	Controls	P
Age (years)**	54 (46-66)	61.5 (53-68)	0.584
Weight (kg)*	62.8 (10.4)	63.3 (10.8)	0.886
Height (m)*	1.56 (0.06)	1.56 (0.08)	0.964
Body surface area (m^2)*	1.61 (0.13)	1.62 (0.14)	0.878
Body mass index (kg/m^2) **	24.68 (22.7-28.9)	24.52 (23.3-28.4)	0.903
Waist circumference (cm)*	93.83 (17.75)	90.18 (11.89)	0.431
Hip circumference (cm) **	97.0 (93-107)	98.0 (96-104)	0.233

*t-test - data as means (SD). **Mann-Whitney rank sum test - data as medians (P25-P75).

Comparison of sensitivity to glucocorticoids in the two groups

Analysis of basal cortisol in RA patients and controls showed similar medians (interquartile range), 15.9 (12.75-19.52) mg/dL and 13.5 (10.8-17.0) mg/dL, respectively (P = 0.132).

After performing the suppression test with VLD IV-DST, we observed that the extent of cortisol suppression at 120 min was similar in the two groups with a median (interquartile range) of 40.3% (29.8-52.3) for patients and 38.4% (28.6-48.5) for controls (P = 0.697). The proportion of patients (6/21, 28.6%) who had more than 50% cortisol suppression was also similar to that of controls (5/22, 22.7%, P = 0.928).

Comparison of gene expression between the groups

Median mRNA values of NF-κB, IKK, c-Fos, and *GRα* were similar in the two groups (Table 2). We performed linear regression analysis of all gene pairs both in patients and controls (Table 3). There was a positive correlation between NF-κB and IKK mRNA levels in both patients and controls, but the expression of c-Fos did not correlate with that of other genes in either group.

Table 2. Comparison of mRNA expression of NF-κB, IKK, c-Fos, and GRα between rheumatoid arthritis patients and controls.

Gene	Patients	Controls	P
NF-κB	8.73 (5.8-18.0)	8.25 (5.6-16.0)	0.761
IKK	3.44 (1.5-5.2)	2.94 (1.8-4.3)	0.99
c-Fos	2.41 (1.0-10.5)	2.29 (0.7-9.4)	0.86
GRα	38.41 (16.3-107.0)	41.02 (3.2-78.7)	0.208

Data are reported as median (P25-P75). P = significance level (Mann-Whitney test).

Table 3. Correlation coefficients of gene expression in rheumatoid arthritis (RA) patients and controls (C).

Gene	Group	NF-κB	IKK	c-Fos
IKK	RA	P < 0.001	-	-
		r = 0.950	-	-
	C	P < 0.001	-	-
		r = 0.886	-	-
c-Fos	RA	P = 0.712	P = 0.756	-
		r = 0.085	r = 0.072	-
	C	P = 0.214	P = 0.531	-
		r = 0.283	r = 0.145	-
GRα	RA	P < 0.001	P < 0.001	P = 0.990
		r = 0.869	r = 0.740	r = 0.003
	C	P = 0.790	P = 0.147	P = 0.776
		r = 0.062	r = 0.327	r = 0.066

The quantity of *GRα* mRNA correlated with NF-κB and IKK mRNA levels only in the group of patients (r = 0.869 and r = 0.740, respectively) but not in controls (r = 0.062 and r = 0.327, respectively) (Figure 1).

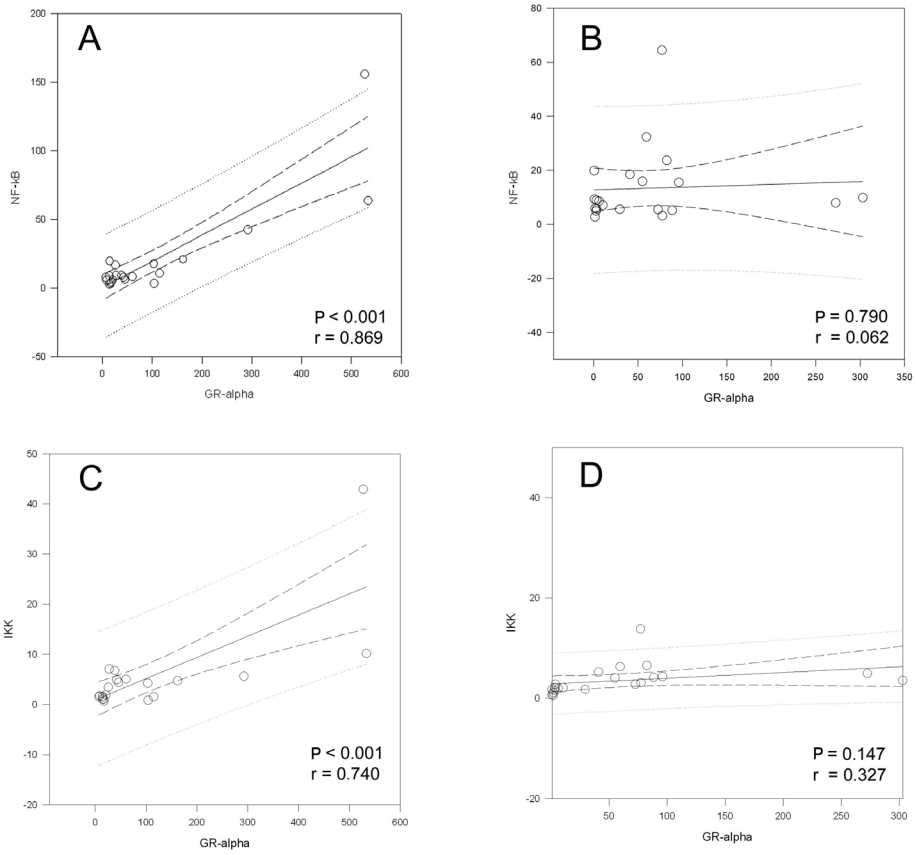

Figure 1. Correlation of mRNA levels of NF-κB and IKK with GRα mRNA in patients and controls. A and C - patients, B and D - controls.

Relationship between glucocorticoid sensitivity or gene expression and disease activity (DAS28)

We did not observe a significant correlation between *in vivo* GC sensitivity, as assessed by suppression of cortisol after VLD IV-DST, and disease activity assessed by DAS28 (P = 0.166, r = 0.314). There was a weak positive correlation between DAS28 and NF-κB (r = 0.568, P = 0.007), IKK (r = 0.553, P = 0.009) and *GRα* (r = 0.466, P = 0.033), but not with c-Fos (r = 0.256, P = 0.262). In a multivariate analysis, only NF-κB mRNA quantity remained as an independent variable in predicting disease activity.

The suppression of cortisol after VLD IV-DST, reflecting the pituitary GC sensitivity, did not correlate significantly with peripheral mononuclear cell expression of *GRα* (r = 0.13, P = 0.574) or with the expression of c-Fos (r = 0.004, P = 0.985), NF-κB (r = 0.197, P = 0.393) and IKK (r = 0.143, P = 0.536). A multivariate analysis indicated ESR as the only independent variable for F% determination (r = 0.524, P = 0.015).

NF-κB and IKK mRNA levels correlated significantly with ESR (r = 0.628, P = 0.002) and (r = 0.639, P = 0.002), respectively. However, there was no correlation between the quantity of c-Fos or *GRα* mRNA and ESR. In a multivariate regression analysis, only NF-κB mRNA quantity remained as an independent variable.

There was no correlation between the expression levels of the genes studied and the HAQ score.

DISCUSSION

We studied longstanding RA patients with no glucocorticoid therapy for 120 days or more, regarding their *in vivo* glucocorticoid sensitivity using VLD IV-DST and the mRNA levels of *GRα* isoform and other inflammatory genes (NF-κB, IKK and c-Fos). Although there was no restriction in relation to gender, duration and intensity of disease in the inclusion criteria, patients were all females with longstanding RA and with a disease activity estimated as moderate by the DAS28 score system. These characteristics are similar to those found in the Brazilian literature for most patients with this disease (Louzada-Junior et al., 2007).

The majority of selected patients (86.4%) had characteristics of poor prognosis RA, such as positive rheumatoid factor and the presence of articular erosions in X-rays of hands (Bértolo et al., 2007).

Basal cortisol concentrations were greater than 10 mg/dL suggesting that the HPA axis was not suppressed in either group at the time of sample collection, so it can be assumed that the expression of *GRα* and the results of cortisol suppression tests were not influenced by a negative feedback mechanism or down-regulation of glucocorticoid receptors.

Analysis of basal cortisol in RA patients and controls showed similar results, in agreement with several studies that addressed the HPA axis in rheumatoid arthritis and showed cortisol concentrations similar to or higher than in the control group (Crofford et al., 1997; Boss and Neeck, 2000).

Glucocorticoid sensitivity *in vivo* was similar in patients and controls, as assessed by suppression of cortisol after VLD IV-DST, contrary to previous studies (Onda et al., 2004; Cobra et al., 2009). There was no correlation of cortisol suppression with disease activity assessed by DAS28, which should be evaluated in conjunction with clinical findings to allow

comparisons. Comparing the clinical profile of patients in our previous and current studies, we note that despite being similar with respect to DAS28 values (4.49 and 4.45, respectively), a higher proportion of patients in the first study (Cobra et al., 2009) used 3 or more DMARDs (53.1 *vs* 14.3%), had higher HAQ score (1.7 *vs* 0.67), and showed a significant worsening of RA symptoms 60 days after GC discontinuation. We also observed that almost all of those patients resumed the use of GC as part of their regimen in a few months. Since the patients of the current study were off GC for at least 120 days, there was no need for discontinuation of medication. All these features demonstrate greater clinical resistance to GC and disease severity in the patients included in our first study.

Like other studies (Onda et al., 2004; Cobra et al., 2009), we found no difference in the *GRα* expression in peripheral blood lymphocytes between RA and control groups. There was also no correlation between *GRα* expression and cortisol suppression after VLD IV-DST in patients or controls. Although there was a weak negative correlation between these variables in patients with RA in the previous study conducted by our group (Cobra et al., 2009), there was also no correlation between these variables in the controls. The difference in these results can be explained again by the difficulty of GC removal in the first study or differences in disease severity.

Unlike previous reports, in the present study, the levels of NF-κB, IKK, c-Fos, and *GRα* mRNA were similar in the two groups. Expression of NF-κB is reported to be increased in RA and could be one of the factors involved in disease pathogenesis. Other transcription factors related to the inflammatory process, such as protein AP-1 and NF-κB are more expressed in various tissues and mainly in cells involved in immune and inflammatory responses (Baeuerle and Baltimore, 1996; Fujisawa et al., 1996; Mckay and Cidlowski, 1999; Eggert et al., 2001, 2002). Onda et al. (2004) showed greater c-Fos expression in peripheral blood mononuclear cells in RA patients than in control groups.

The importance of NF-κB is reinforced in our study, since in a multivariate analysis it was the only gene that correlated with disease activity as expressed by DAS28. Although IKK and *GRα* mRNA amount were also correlated with DAS28, both were removed from the model in the multivariate analysis, reflecting the importance of NF-κB in disease activity and possibly in the mechanism of inflammation, at least in RA patients with these clinical characteristics.

The degree of inflammation in our patients was possibly not sufficient for the detection of changes in mRNA expression from peripheral blood mononuclear cells, but other possibilities include the interference of previous chronic use of GC and DMARDs. In some previous studies, there was the exclusion of patients using methotrexate in order to minimize this possible interference (Onda et al., 2004).

It seems that NF-κB and IKK have a greater influence on *GRα* expression in the group of RA patients than in controls (Figure 1). This apparently paradoxical relationship, with higher amounts of NF-κB in patients with higher *GRα*, could be explained by the chronicity of inflammation in our patients with RA. The expected phenomenon of down-regulation is well described in the literature only in acute situations. However, it is unclear how the chronic regulation of this process occurs, especially if there are counter-regulatory mechanisms that favor heterodimerization of *GRα*/NF-κB molecules and consequently a greater amount of *GRα* in the presence of increased NF-κB levels.

It is surprising that only c-Fos did not correlate with other genes, since its pattern is supposed to be similar to that of NF-κB and IKK (Trabandt et al., 1992; Asahara et al., 1997; Shiozawa et al., 1997; Firestein, 2004; Onda et al., 2004). It is possible that some DMARDs

showed a greater influence on the regulation of c-Fos than of other genes. Our patients had a relatively low and chronic inflammatory component that might not have been sufficient to trigger the activation of c-Fos/AP-1, since they were able to withstand GC-free therapeutic regimens, even with elevated DAS28. Alternatively, there may be a rapid degradation of c-Fos mRNA in these cases. To elucidate these issues, additional studies are needed with newly diagnosed and untreated patients, allowing the assessment of these genes' mRNA and protein at the time of diagnosis and during follow-up after starting treatment with GC.

Expression of NF-κB and IKK correlated moderately with ESR, which reinforces the pro-inflammatory character of these genes (Baldwin Jr., 1996; Baeuerle and Baltimore, 1996; Ghosh et al., 1998). There was no correlation between the expression of c-Fos or *GRα* and ESR. A previous study showed a negative relationship between the expression of *GRα* and ESR, but disease severity and duration were not fully described to allow further comparisons (Onda et al., 2004).

There was no correlation between the expression of genes studied and HAQ, which is regarded as a score particularly suited for determining changes with treatment and for identifying functional disability in patients with RA (Callahan et al., 1997; Wolfe, 2001).

The experience of our group in the current and previous studies (Cobra et al., 2009) clearly showed the impact of disease activity and stringency ensuring the complete discontinuation of GC. It should be noted that patients with RA, with chronic joint pain, know the benefits of using GC from personal experience and can often use GC without informing the physician. Thus, laboratory monitoring and low cortisol levels as exclusion criteria should be rigid. The clinical characterization of our group of RA patients is also a strong aspect of this study. Most molecular studies often present very limited data regarding disease severity and other clinical features, which are very important in interpreting the findings.

Another strong aspect of this study is the adequate matching of the control group. Our group already described the influence of age (Faria et al., 2008) and obesity (Faria et al., 2010) on glucocorticoid sensitivity and *GRα* quantitation.

One limitation of this study was the exclusion of patients with very severe RA, which could yield quite different results. However, to include these patients we would need to change the assessment of *in vivo* GC sensitivity and the inclusion criteria regarding the use of GC, since its removal is difficult in patients with a more active disease and involves important ethical issues.

Some groups have addressed this problem by including only treatment naive patients, a practice that is difficult in our country due to the peculiarities of our health care system, in which RA patients take too long to get an appointment with a rheumatologist and frequently have already received high doses of GC before seeing a specialist. This approach also has the advantage of eliminating the influence of DMARDs on the evaluation of these patients.

We must also consider that gene expression may be different from protein functional activity, so the results of mRNA quantitation must be interpreted with some caution (Longui and Faria, 2009). Many groups prefer to quantify mRNA due to the currently superior technical aspects, since there is some lack of specificity of antibodies directed to those proteins. Another interfering factor is the use of many drugs, such as methotrexate, which has the potential to alter the expression of the genes studied.

Despite these limitations, we demonstrated for the first time in patients with longstanding moderate RA that there is no change *in vivo* or *in vitro* of GC sensitivity and that there is a direct correlation between increased expression of NF-κB (and to a minor extent also of *GRα* and IKK) and disease activity, according to DAS28, but without the same pattern of correlation for c-Fos.

ACKNOWLEDGMENTS

We are grateful to the Support Center for Scientific Publications of Santa Casa de São Paulo, Faculty of Medical Sciences, for editorial assistance.

REFERENCES

American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines (2002). Guidelines for the management of rheumatoid arthritis. *Arthitis Rheum.* 46: 328-346.

Asahara H, Fujisawa K, Kobata T, Hasunuma T, et al. (1997). Direct evidence of high DNA binding activity of transcription factor AP-1 in rheumatoid arthritis synovium. *Arthritis Rheum.* 40: 912-918.

Baeuerle PA and Baltimore D (1996). NF-kappa B: ten years after. *Cell* 87: 13-20.

Baldwin AS Jr (1996). The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu. Rev. Immunol.* 14: 649-683.

Bértolo MB, Brenol CV, Schainberg CG, Neubarth F, et al. (2007). Atualização do consenso brasileiro no diagnóstico e tratamento da artrite reumatóide. *Rev. Bras. Reumatol.* 47: 151-159.

Boss B and Neeck G (2000). Correlation of IL-6 with the classical humoral disease activity parameters ESR and CRP and with serum cortisol, reflecting the activity of the HPA axis in active rheumatoid arthritis. *Z. Rheumatol.* (Suppl 2) 59: II-62-II-64.

Brenol CV, Monticielo OA, Xavier RM and Brenol JCT (2007). Artrite reumatóide e aterosclerose. *Rev. Assoc. Med. Bras.* 53: 465-470.

Callahan LF, Pincus T, Huston JW III, Brooks RH, et al. (1997). Measures of activity and damage in rheumatoid arthritis: depiction of changes and prediction of mortality over five years. *Arthritis Care Res.* 10: 381-394.

Carlsson AM (1983). Assessment of chronic pain. I. Aspects of the reliability and validity of the visual analogue scale. *Pain* 16: 87-101.

Cobra JF, Melo MR, Faria CD, Longui CA, et al. (2009). Simultaneous evaluation of *in vivo* glucocorticoid sensitivity and expression of glucocorticoid receptor alpha-isoform in rheumatoid arthritis patients. *Arq. Bras. Endocrinol. Metabol.* 53: 24-30.

Crofford LJ, Kalogeras KT, Mastorakos G, Magiakou MA, et al. (1997). Circadian relationships between interleukin (IL)-6 and hypothalamic-pituitary-adrenal axis hormones: failure of IL-6 to cause sustained hypercortisolism in patients with early untreated rheumatoid arthritis. *J. Clin. Endocrinol. Metab.* 82: 1279-1283.

De Bosscher K, Vanden Berghe W and Haegeman G (2001). Glucocorticoid repression of AP-1 is not mediated by competition for nuclear coactivators. *Mol. Endocrinol.* 15: 219-227.

Eggert M, Schulz M and Neeck G (2001). Molecular mechanisms of glucocorticoid action in rheumatic autoimmune diseases. *J. Steroid Biochem. Mol. Biol.* 77: 185-191.

Eggert M, Kluter A, Rusch D, Schmidt KL, et al. (2002). Expression analysis of the glucocorticoid receptor and the nuclear factor-kB subunit p50 in lymphocytes from patients with rheumatoid arthritis. *J. Rheumatol.* 29: 2500-2506.

Faria CD, Cobra JF, Sousa E Silva, Melo MR, et al. (2008). A very low dose intravenous dexamethasone suppression test as an index of glucocorticoid sensitivity. *Horm. Res.* 69: 357-362.

Faria CDC, Castro RB, Longui CA, Kochi C, et al. (2010). Impact of prolonged low-grade physical training on the *in vivo* glucocorticoid sensitivity and on glucocorticoid receptor mRNA levels of obese adolescents. *Horm. Res. Paediatr.* 73: 458-464.

Firestein GS (2004). NF-kappaB: Holy Grail for rheumatoid arthritis? *Arthritis Rheum.* 50: 2381-2386.

Fries JF, Spitz PW and Young DY (1982). The dimensions of health outcomes: the health assessment questionnaire, disability and pain scales. *J. Rheumatol.* 9: 789-793.

Fujisawa K, Aono H, Hasunuma T, Yamamoto K, et al. (1996). Activation of transcription factor NF-kappa B in human synovial cells in response to tumor necrosis factor alpha. *Arthritis Rheum.* 39: 197-203.

Ghosh S, May MJ and Kopp EB (1998). NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* 16: 225-260.

Longui CA and Faria CD (2009). Evaluation of glucocorticoid sensitivity and its potential clinical applicability. *Horm. Res.* 71: 305-309.

Louzada-Junior P, Souza BDB, Toledo RA and Ciconelli RM (2007). Análise descritiva das características demográficas e clínicas de pacientes com artrite reumatóide no estado de São Paulo, Brasil. *Rev. Bras. Reumatol.* 47: 84-90.

McKay LI and Cidlowski JA (1999). Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. *Endocr. Rev.* 20: 435-459.

Melo MR, Faria CD, Melo KC, Reboucas NA, et al. (2004). Real-time PCR quantitation of glucocorticoid receptor alpha isoform. *B.M.C. Mol. Biol.* 5: 19.

Onda K, Rimbara E, Hirano T, Oka K, et al. (2004). Role of mRNA expression of transcription factors in glucocorticoid sensitivity of peripheral blood mononuclear cells and disease state in rheumatoid arthritis. *J. Rheumatol.* 31: 464-469.

Ramey DR, Raynauld JP and Fries JF (1992). The health assessment questionnaire 1992: status and review. *Arthritis Care Res.* 5: 119-129.

Senna ER, De Barros AL, Silva EO, Costa IF, et al. (2004). Prevalence of rheumatic diseases in Brazil: a study using the COPCORD approach. *J. Rheumatol.* 31: 594-597.

Shiozawa S, Shimizu K, Tanaka K and Hino K (1997). Studies on the contribution of c-fos/AP-1 to arthritic joint destruction. *J. Clin. Invest.* 99: 1210-1216.

Silva TS, Longui CA, Rocha MN, Faria CDC, et al. (2010). Prolonged physical training decreases mRNA levels of glucocorticoid receptor and inflammatory genes. *Horm. Res. Paediatr.* 74: 6-14.

Smolen JS, Breedveld FC, Schiff MH, Kalden JR, et al. (2003). A simplified disease activity index for rheumatoid arthritis for use in clinical practice. *Rheumatology* 42: 244-257.

Trabandt A, Aicher WK, Gay RE, Sukhatme VP, et al. (1992). Spontaneous expression of immediately-early response genes c-fos and egr-1 in collagenase-producing rheumatoid synovial fibroblasts. *Rheumatol. Int.* 12: 53-59.

van Gestel AM, Haagsma CJ and van Riel PL (1998). Validation of rheumatoid arthritis improvement criteria that include simplified joint counts. *Arthritis Rheum.* 41: 1845-1850.

Wolfe F (2001). Which HAQ is best? A comparison of the HAQ, MHAQ and RA-HAQ, a difficult 8 item HAQ (DHAQ), and a rescored 20 item HAQ (HAQ20): analyses in 2,491 rheumatoid arthritis patients following leflunomide initiation. *J. Rheumatol.* 28: 982-989.