# Adjuvant Arthritis in the Rat is Associated With Decreased Binding of Nuclear Receptors to Thyroid Hormone Responsive Element in Spleen Extracts

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**Abstract.** In vertebrates, thyroid hormone and its cognate nuclear receptors are involved in a complex arrangement of physiological and developmental function Since thyroid hormone has also been shown to affect immune responses we investigated the DNA binding status of $T_3$ receptors of spleen nuclear extracts in a) rats with adjuvant arthritis (AA) b) adrenalectomized rats (ADX), and c) animals with adjuvant arthritis followed by adrenalectomy (AA+ADX) A marked diminution in the functional binding of nuclear thyroid hormone receptors to DR4 thyroid hormone responsive DNA element was found in the spleens of AA and AA+ADX rats when compared to a control group or ADX rats The data based on *in vivo* experiments suggest that the nuclear receptor thyroid hormone responsive element complex status within the cell nucleus may be altered in adjuvant arthritis

**Key words:** Rat — Adjuvant arthritis — Adrenalectomy — Spleen — Nuclear receptors — Thyroid hormone responsive element

## Introduction

The biologically active thyroid hormone, 3,5,3′-triiodothyronine ($T_3$), is involved in the regulation of cell differentiation and is essential for normal development

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and homeostasis of vertebrates (Evans 1988) Thyroid hormones exert their action via nuclear receptors ligand inducible transcription factors One of the most important properties of $T_3$ nuclear receptor complex is its ability to stimulate or inhibit transcription of ligand-responsive genes (Glass et al 1989, Glass and Holloway 1990) Thyroid hormone nuclear receptors (TRs) bind as monomers or dimers to specific ligand response elements (thyroid hormone response elements, TREs) containing one or two half-sites related to the AGGTCA motif (Forman et al 1992) TR binding was found to be augmented by TR heterodimerization with 9-cis retinoic acid receptors (RXRs) or other not yet characterized auxiliary proteins (Berrodin et al 1992)

AA in rats is a chronic inflammatory joint disease resembling rheumatoid arthritis in humans It is accompanied by persistent pain hind paw edema local activation of monocytes producing a whole array of lymphokines which leads to cartilage destruction (Arend and Dayer 1990) During AA stimulated production of interleukin-1 $\beta$ was observed in the rat spleen which was further potentiated by adrenalectomy demonstrating direct involvement of glucocorticoids in the regulation of the inflammatory process (Stephanou et al 1992) Among the inflammatory lymphokines tumor necrosis factor-$\alpha$, interleukin-1 $\beta$ and interleukin-6 were found to decrease nuclear thyroid hormone capacity in a liver cell line, leaving the affinity of receptors unchanged (Wolf et al 1994) Recently, it has also been shown that members of the nuclear receptor family themselves may inhibit synthesis of collagenase-1 (matrix metalloproteinase-1), an enzyme produced in large quantities by rheumatoid tissue causing the connective tissue degradation (Schroen et al 1997) Therefore we focused our attention on the evaluation of the effect of AA and/or of adrenalectomy in rats on the status of functional TRE binding 3 5 3′-triiodothyronine receptors in the spleen

## Materials and Methods

In the study, we used 11 weeks old male Long Evans rats weighing 180 215 g They were housed 6 or 7 to a cage with free access to food and water, and were kept at 12 12 h light dark cycle On day 0, they were injected with 50 $\mu$l of heat-killed *Mycobacterium butyricum* (Difco Lab , USA) in mineral oil (5 mg/ml) into the tail base

The study consisted of four groups Controls (C), adrenalectomized (ADX) adjuvant injected (AA) animals, and adjuvant injected subsequently adrenalectomized (AA+ADX) animals Adrenalectomy was performed on day 12 after the induction of AA On day 24, the animals were killed by decapitation trunk blood was collected serum was separated and stored at −20°C Spleens were removed snap frozen in liquid nitrogen and stored at −70°C for subsequent extraction of

nuclear proteins. Hind paw edema was measured volumetrically on day 24 immediately after the decapitation. Serum albumin (ALB) levels were measured by spectrophotometric method as described earlier (Prokopova et al. 1992).

TRs functional binding to TRE was evaluated by electrophoretic mobility shift analysis (EMSA). Nuclear proteins were extracted from rat spleenocytes in a buffer containing 20 mmol/l HEPES (pH 7.9), 25% glycerol, 0.42 mol/l NaCl, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l EDTA, 0.2 mmol/l phenylmethylsulfonylfluoride (PMSF), and 0.5 mmol/l dithiothreitol (DTT) (Andrews and Faller 1991). The oligonucleotide used for EMSA was derived from hormone response element found at position −697 in the 5′ regulatory region of human type I iodothyronine 5′ deiodinase which represents an ideal DR4 thyroid hormone response element (Jakobs et al. 1997). A 5′ labelling reaction with the T4 polynucleotide kinase (Promega, USA) and [$\gamma$ $^{32}$P] ATP (Amersham, England) was used for the preparation of a labelled double stranded oligonucleotide (Maniatis et al. 1982). Nuclear proteins were then incubated in a binding buffer containing 10 mmol/l Tris-HCl (pH 7.5), 50 mmol/l NaCl, 1 mmol/l EDTA, 5% (v/v) glycerol, 0.2 mmol/l DTT, 0.75 mg/ml bovine serum albumin with 25 ng/$\mu$l poly[d(I-C)] (Pharmacia Biotech, USA), 250 pg of labelled oligonucleotide with or without a 100 fold excess of unlabelled oligonucleotide (as competitor) on ice for 1 h. Then 20 $\mu$l of the samples containing 20 $\mu$g nuclear proteins was loaded into individual wells. Specific nuclear protein DNA complexes formed during the reaction were separated on 7% non-denaturing polyacrylamide gels in a 1 × TBE (0.089 mol/l Tris base, 0.089 mol/l boric acid, 0.002 mol/l EDTA) as an electrophoresis buffer.

Statistical analysis was performed by one way ANOVA followed by Dunn's test.

## Results

First clinical symptoms of hind paw edema occurred on day 11 after induction of adjuvant arthritis in the experimental rats. By day 24, severe polyarthritis developed in both AA and AA+ADX animals as measured by hind paw volume and serum albumin levels (ALB). Arthritic animals exhibited a marked body weight reduction as compared to control rats. Adrenalectomy resulted in body weight retardation to the same extent as adrenalectomy in combination with AA. The spleen mass was enhanced in AA and AA+ADX groups as an indicator of excessive immune reaction (Table 1).

As shown in Fig. 1, a marked diminution of the $^{32}$P signal in retarded bands indicating the binding of TRs to labelled oligonucleotide which represents DR4 TRE was found in the spleen of rats with adjuvant arthritis in comparison with the control group of rats. A similar diminution of the signal of retarded bands indicating

**Table 1.** Effect of adjuvant arthritis (AA) and/or adrenalectomy (ADX) on selected parameters in male Long Evans rats

| Group of rats ($n$) | Hind paw volume [ml] | Serum albumin [g/l] | Body mass [g] | Spleen mass [mg] |
|---|---|---|---|---|
| (7) Control | 1 16 ± 0 03 | 32 37 ± 0 61 | 343 ± 5 75 | 862 5 ± 45 3 |
| (5) ADX | 1 23 ± 0 08 | 29 50 ± 0 95 | 219 ± 9 53** | 779 4 ± 48 6 |
| (7) AA | 2 40 ± 0 10** | 25 60 ± 0 63** | 229 ± 5 73** | 1118 1 ± 73 2 |
| (4) AA+ADX | 2 05 ± 0 23†† | 25 00 ± 0 76†† | 230 ± 14 80 | 1297 0 ± 189 1†† |

Results are expressed as means ± S E M ** indicates $p < 0\ 01$ vs control group †† indicates $p < 0\ 01$ vs ADX group

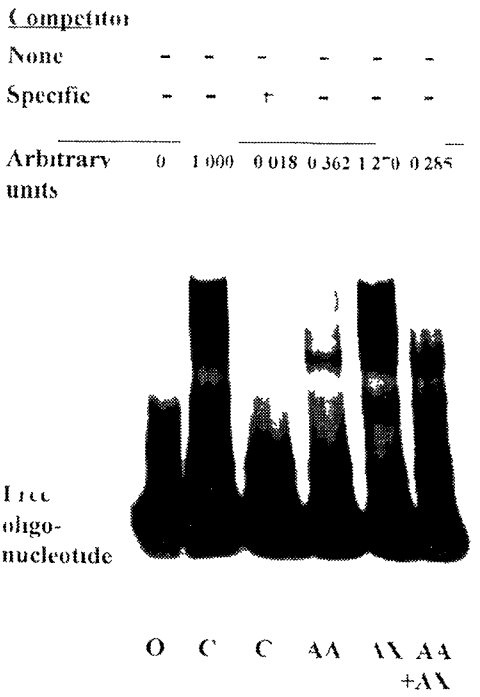


**Figure 1.** A representative autoradiogram pattern of the functional DNA binding of thyroid hormone receptors from nuclear extract of the spleen of rats with adjuvant arthritis (AA) adrenalectomy (ADX) and adrenalectomized rats with adjuvant arthritis (AA +ADX) when compared to non treated control group of rats (C) (0) Free oligonucleotide lacking nuclear proteins Quantitative evaluation of the autoradiogram from EMSA of TRs TRE complex formation was performed by laser densitometry (AU arbitrary units)

the formation of TR TRE complex was also found in the spleen of adrenalectomized rats after the treatment with the adjuvant in comparison with the control group or adrenalectomized animals The data were confirmed in two independent series

## Discussion

The adrenocortical steroids are known to be involved in protecting the organisms from immune challenges Harbuz et al (1993) showed that the absence of glucocorticoids potentiated the severity of AA in rats leading to high mortality In our experiments 50% of AA+ADX animals did not survive up to day 24 of arthritic condition suggesting an overexcessive immune reactions

An essential part of the acute phase reaction is a fall of serum ALB to prevent osmotic imbalance (Billingham and Gordon 1976) Under our experimental conditions AA and AA+ADX groups did not differ in the levels of ALB as well as other parameters We assume that only more resistant individuals having better host defense mechanisms survived the adrenalectomy which followed after the adjuvant treatment and therefore the expected more robust symptoms in AA+ADX rats were not present

The hypothalamo pituitary adrenocortical axis and thyroidal hormones are functionally interconnected Administration of thyroidal hormones attenuated streptococcal cell wall arthritis in rats by the mechanisms of activation of endogenous glucocorticoids (Rittenhouse and Redei 1997) Geister et al (1985) have suggested that hypothyroidism may induce destructive arthropathy of the finger joints and thyroid hormone may reverse the rheumatic complaints most probably via its cognate nuclear receptors It has been shown that thyroid hormone receptors bound to TRE can function either as transcriptional repressors in the absence of 3 5 3′ triiodothyronine ($T_3$) or as potent activators upon binding of thyroid hormone (Bamahmad et al 1992) The present results indicate a marked diminution in the TRs TRE complex formation in spleen extracts of rats with adjuvant arthritis or of adrenalectomized rats following the adjuvant treatment as compared to intact rats In comparison with the control group of rats adrenalectomy itself did not have any remarkable effect on the functional TR binding to TRE in the rat spleen A possible explanation for these results is that alteration in binding of thyroid hormone inducible transcription factors to thyroid hormone responsive element may represent one early event in thyroid hormone action on the immune system subsequently leading to alteration in target gene expression

In conclusion it is suggested that beside the thyroid hormone level in the organism its counterpart the status of the thyroid hormone receptor thyroid hormone responsive element complex within the cell nucleus, responsible for thyroid hormone action may also play a consequential role in adjuvant arthritis

**Acknowledgements.** The authors wish to acknowledge the technical assistance of Maria Danihelova Hana Šťastna and Helena Smetanova Supported in part by grants of VEGA 2 3015/96 and by GAČR, grant No 305/95/05712

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Final version accepted November 16, 1998