



Neuroendocrine Immune Perturbations in Rheumatoid Arthritis: Causes, Consequences, or Confounders in the Disease Process?

Neuroendocrine immune (NEI) mechanisms in rheumatoid arthritis (RA) and rheumatic diseases are receiving considerable attention, and interest is growing¹⁻³. Each system is profoundly complex, and moreso for their bidirectional reciprocal interactions. Notwithstanding, basic research on mechanisms and controlled clinical or correlative studies are yielding penetrating insights into the physiopathogenesis of these diseases¹⁻³.

Technological Advances Do Not Obviate Limitations or Requisites of Protocol Design

Neuroendocrine and immune or inflammatory factors can now be comprehensively assayed in small aliquots (e.g., a few cc) of serum or plasma at not-exorbitant costs. Despite technical advances, serum levels of any one hormone or cytokine within the milieu cannot be simply interpreted as indicators of tissue activities¹. Also, selection of proper patients and controls is rigorous in NEI studies. Thus, multiple confounding host variables and technical errors can reduce validity of such research¹⁻³.

Generalizability of Results Depends upon the Selected Study Subjects

The research community acknowledges the necessity for representative subject sampling. However, research implications of the stage of disease deserve comment. Early patients (e.g., diagnosed within one year of clinical onset) are preferred in NEI studies, since pre-illness, baseline status is less likely to be altered by inflammatory disease processes,

than in patients at later progressive or disabling stages. Less bias occurs when comparing healthy subjects to early- than late-course patients. Even greater protocol validity may be achieved in prospective, controlled studies, particularly when subjects are selected in a representative manner. Still, generalizations can only be made validly to the extent permitted by selected study subjects and their particular sampling frames³.

Are NEI Findings in RA Cause, Effect, or Confounder Relations?

Interpretations of hormonal deviations observed in patients with active RA versus healthy controls (CN) are challenging¹⁻³. Do the findings reflect true differences of inherent susceptibility between the study subjects, as opposed to alternative explanations, e.g.: (1) biases from demographic discrepancies; (2) influences of unrecognized confounding factors; or (3) secondary disease effects? Such enigmas may be diminished in controlled prospective study designs, since differences found at baseline, i.e., before clinical onsets, are not usually considered secondary. Also, more accurate data can be obtained at baseline for stricter selection of controls and more valid assessments of risk factors³. Observational studies require replication for confidence in the results, regardless of the protocol design quality. Also, judgmental criteria should be applied when interpreting results, in order to assess credibility of the observed relations based upon a particular disease model³.

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Serum Levels of DHEAS, Estrone (E1), and 17 β -estradiol (E2) in Patients with Active RA

In this issue of *The Journal*, Tengstrand and colleagues⁴ found that serum dehydroepiandrosterone sulfate (DHEAS) and estrone (E1) levels were lower, but 17 β -estradiol (E2) was higher, in their series of active male RA patients compared to healthy controls. Estradiol, but not estrone, levels correlated strongly and positively with various clinical and blood indices of inflammation in the patients. This group recently reported⁵ a high frequency of hypogonadism, as indicated by significantly lower serum levels of bioavailable testosterone (BT), in the same male RA series compared to healthy CN men. The current report⁴ is an extension of those analyses to include new data on serum DHEAS and the estrogens (i.e., E1 and E2), not previously described in RA patients.

Serum DHEAS Levels Were Lower in Active RA versus Male Controls

The previous literature is not conclusive regarding serum DHEAS levels in male glucocorticoid-naïve RA patients versus healthy subjects¹. In the current study⁴, an approximately one-third lower mean serum DHEAS concentration was observed in the RA versus CN men 40 to 69 years of age. Adjusting for age, the mean level was 2.65 μ mol/l in 58 RA versus 3.93 μ mol/l in 72 CN subjects ($p < 0.01$).

Such notable difference warrants attention and investigation of possible mechanisms. One essential question is whether or not the deficiency observed in patients with RA had pre-existed or predisposed to the onset of clinical disease? Our prospective study of 18 pre-RA males versus 72 matched CN (1 RA:4 CN) can help address the latter question³. The pre-RA males entered the cohort a mean of 12.8 years prior to clinical onset (range 3 to 20) and their mean entry age was 41.6 versus 41.8 years for CN subjects. The mean (\pm SEM) baseline serum DHEAS levels (μ mol/l) of the somewhat younger males in our study were 7.5 (\pm 1.1) for the pre-RA versus 7.3 (\pm 0.4) for the CN subjects ($p = 0.797$). Serum DHEAS levels do not seem to be low before clinical onset of RA in males, according to these data. Only premenopausal women had low DHEAS levels prior to clinical onset of disease before age 50 years in our controlled prospective studies^{3,6}.

Besides the strong influences of natural aging^{4,7,8}, chronic illness or stress can cause secondary lowering of serum DHEAS concentrations¹, although the biochemical mechanisms are not clearly defined. Cytokine [including tumor necrosis factor- α (TNF- α)] blunting of ACTH-stimulated sulfotransferase, which converts DHEA to DHEAS, is one possible explanation⁹. The inhibitory effects of TNF- α were more striking on sulfotransferase than on 17-hydroxylase (CYP 17) production⁹. Such relative inhibitions of ACTH-stimulated enzyme synthesis would preferentially enhance the glucocorticoid, rather than the adrenal-androgen, pathway in steroidogenesis. Another possible mechanism for

low DHEAS synthesis is inhibition of 17, 20 lyase activity by cytokines¹⁰ or other post-translational steroidogenic mechanisms¹¹. The 17, 20 lyase converts 17-hydroxypregnenolone (17-OH P5) to DHEA by the delta (Δ) 5 pathway, and 17-hydroxyprogesterone (17-OH P4) to androstenedione (Δ 4A) by the Δ 4 pathway. Teleologically, inhibition of the sex steroid precursors (e.g., DHEA and Δ 4A) would preserve greater amounts of substrates (e.g., 17-OH P5 and 17-OH P4) for more essential glucocorticoid synthesis.

Higher Serum E2, but Lower E1 Levels, in Active RA versus Male Controls

In 64 male glucocorticoid-naïve RA patients, the mean serum E2 level (pmol/l) was 91.5 compared to 73.5 in 49 CN of similar age ($p < 0.001$)⁴. The 25% higher mean E2 level in RA deserves further consideration, especially since BT (direct precursor of E2) levels were significantly ($p < 0.001$) lower in these RA compared to CN males⁵.

A central question is whether or not E2 levels were higher (or the E2:BT ratio was greater) before clinical onset of RA: our prospective study of 18 pre-RA males compared to 72 matched controls does not indicate a significant baseline difference in levels of E2 (pmol/l), total T (nmol/l), or the E2:T ratio (BT levels were not assayed) between the study groups. The respective mean (\pm SEM) levels were: 69.7 (\pm 6.0) versus 65.3 (\pm 2.9) for E2 ($p = 0.635$); 19.2 (\pm 1.8) versus 18.3 (\pm 0.8) for total T ($p = 0.643$); and 4.1 (\pm 0.47) versus 4.1 (\pm 0.25) for the E2:T ratio (pmol/nmol \times 1,000) ($p = 0.762$).

If E2 and T levels truly did not differ prior to clinical onset, how then might they have developed in active RA compared to normal CN subjects? In males, E2 is produced by aromatization of androgens^{12,13}, as depicted in the authors' Figure 2⁴. About 85% of E2 production occurs by such aromatization in peripheral adipose, muscle, and nervous tissues, and the remainder in Leydig cells of the testes^{12,13}. Estradiol is synthesized either by direct aromatization of the steroid A ring of T (immediate E2 precursor) or by indirect aromatization of Δ 4A to E1, and subsequent conversion of E1 to E2 by 17 β -hydroxysteroid dehydrogenase (17 β -HSD) activity^{12,13}.

Among males 40 to 69 years of age in the current study⁴, the median level (interquartile ranges) of E1 was lower in 51 glucocorticoid-naïve RA patients [150 (134–182)] versus 79 CN [212 (172–245)] ($p < 0.0001$). Increased conversion of E1 to E2 by the 17 β -HSD enzyme group in the RA versus CN males could have contributed to these estrogen findings⁴. Interestingly, a *positive* correlation ($r = 0.39$, $p < 0.001$) was also observed between E1 and E2 levels⁴. Further study is needed to better interpret the estrogen alterations (i.e., low E1 and high E2 levels) observed in active RA patients⁴, e.g., relative enzymatic activities of aromatization of Δ 4A to E1 vis-a-vis the subsequent 17 β -HSD conversion of E1 to E2¹³.

Serum DHEA and $\Delta 4A$ levels were not reported in the current study⁴. If sulfation of DHEA to DHEAS were inhibited in active RA, then enhanced conversion of DHEA to $\Delta 4A$ might have occurred. Relatively enhanced $\Delta 4A$ production could increase E1 as well as T (via 17 β -HSD) production. The reported E1 versus E2 alterations⁴ also depend upon the preceding relative enzyme activities and substrate concentrations¹³.

In our prospective study, steroidogenic pathways were similar in 18 pre-RA males versus 72 CN as well as in 25 pre-RA postmenopausal women versus 100 CN, as reflected by ratios of the molar concentrations of *product to precursor* steroids (i.e., P-P ratios)¹⁴. Such data suggest that alterations in steroidogenesis observed in active RA mainly occur following, rather than preceding, clinical onset of disease. However, further controlled research is needed before firm conclusions can be drawn. Among our male CN, serum E2 levels correlated with both $\Delta 4A$ ($r = 0.302$, $p = 0.021$) and T ($r = 0.260$, $p = 0.049$) concentrations, after adjusting for age and degree of cigarette smoking, since each correlated with E2 levels ($r = 0.381$, $p = 0.001$, and $r = 0.325$, $p = 0.005$, respectively). Such data support the physiological contribution of both serum $\Delta 4A$ and T to E2 levels¹³. Low $\Delta 4A$ substrate availability in the active RA patients may have contributed to their lower observed serum E1 levels than levels found in the healthy CN subjects⁴.

Significant Correlations Observed between E2 Levels and Indicators of RA Activity

Serum E2 levels strongly correlated ($r = 0.700$, $p < 0.0001$) with erythrocyte sedimentation rate (Figure 1⁴), C-reactive protein ($r = 0.663$, $p < 0.0001$), and other markers of disease activity in male RA patients (Table 3⁴). By contrast, significant correlations were not found between disease activity markers and serum levels of BT, DHEAS, or E1⁴. A strong correlation was reported between BT and DHEAS levels ($r = 0.5$, $p < 0.0001$), and less significantly between BT and serum E1 ($r = 0.27$, $p < 0.05$) as well as E2 ($r = 0.20$, $p < 0.05$) levels⁴. However, when the correlation of DHEAS and BT were analyzed in a multiple regression model, including age as a confounding factor in these hormone levels^{4,5,7,8,13-15}, significance was lost⁴.

Aromatase Activity Might Help Explain the Estrogen Findings

The reported lower mean serum BT⁵, but 25% higher E2⁴, levels in active RA versus CN males might seem inconsistent initially. However, in males, serum T or BT (nmol/l) concentrations are circa 100-fold greater than E2 (pmol/l) levels. In the presence of lowered BT, increased E2 levels in active RA could have resulted from increased aromatase activity, and yet not have appreciably reduced the BT substrate concentrations. Cytokines and inflammatory cells affect aromatization¹⁰, and may have contributed to the

current study findings⁴. Our prospective study did not indicate a difference in aromatase activity between pre-RA versus CN males, as indicated by the respective product-precursor (P-P) ratios of serum E2 to T¹⁴.

In addition, lowered BT is associated with increased sex hormone-binding globulin (SHBG) levels^{13,15,16}, which could increase serum total E2 (but not bioavailable E2), as was found in the RA patients versus CN subjects⁴. Normal aging and increased adiposity (especially "visceral fat") in males can show similar findings to those observed in the RA patients, i.e., lowered serum DHEAS and BT, but higher E2 levels^{4,5,7,8,13,17}. However, in non-obese, physically-conditioned healthy males, lower E2 and bioavailable E2 levels were also found in the elderly than in their younger counterparts¹⁶.

Inflammatory Mediators and Correlated Pathways Can Lower T in Active RA

The lower BT levels⁵ most likely resulted from decreased T production from its precursors. In our prospective study, baseline TNF- α levels of CN males correlated negatively ($r = -0.353$, $p = 0.007$) with the 17 β -HSD activity that converts $\Delta 4A$ to T, as indicated by their P-P ratios. Also, in mouse Leydig cells, TNF- α and interleukin-1 (IL-1) were reported to decrease 3 β -HSD activity, which contributes to T production¹⁸. Thus, TNF- α and other inflammatory mediators likely decrease T synthesis in active RA. Controlled therapy trials of new anticytokine biologic agents offer future opportunities to explore effects of TNF- α , IL-1 β , and other cytokine mediators on NEI relations.

Lower Serum Androgens in RA: Contributors as Well as Consequences of the Disease?

In premenopausal onset RA, markedly low baseline serum DHEAS levels ($< 0.7 \mu\text{mol/l}$) were observed to be a significant independent predictor of subsequent clinical disease onset before 50 years of age^{2,3,6}. By contrast, neither women with postmenopausal onset nor male pre-RA cases had mean baseline DHEAS levels lower than their matched controls. Interestingly, in the latter 2 host subsets, combined low baseline serum cortisol ($< 140 \text{ nmol/l}$) and low serum total T ($< 3.5 \text{ nmol/l}$ in women and $< 10.0 \text{ nmol/l}$ in men) were independent predictors of RA, albeit in a small minority of cases^{3,19}. In men, the combined low levels occurred in 2 (11%) of 18 pre-RA versus one (1.4%) of 72 matched CN³. In postmenopausal women, low combined levels occurred in 6 (33.3%) of 18 pre-RA, 4 to 17 (mean 11) years prior to clinical onset at age 50 years or older, compared to 6 (9.8%) of 61 matched CN¹⁹. Such findings suggest that glucocorticoid and androgenic hormonal insufficiency may predispose to clinical onset of RA in a minority of otherwise susceptible persons^{3,6,19}. Polymorphic adrenal and gonadal insufficiency was postulated, possibly involving glandular trophic deficiencies (i.e., cell hypocompetences or smaller cell masses),

rather than specific or genetically-controlled steroidogenic defects, as was previously schematized (Figure 10²⁰) and more recently reviewed^{19,21}.

Promising NEI Approaches to RA

The hormonal changes observed in RA are complex and bidirectionally intertwined with immune mechanisms. Nevertheless, basic research and well designed controlled clinical investigations are helping to unravel the profound intricacies as well as explain the significant NEI perturbations found in RA. NEI alterations or perturbations may soon be expected to be documented in persons susceptible to RA and appropriately normalized. In the future, onset risks of acquiring RA will hopefully be modified and its course ameliorated².

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