Relationships between Glucocorticoids and Gonadal Steroids in Rheumatoid Arthritis

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ABSTRACT: **Gender and sex hormones are strongly related to the incidence and progression of autoimmune rheumatic diseases. Although sex steroids have been shown to have direct effects on the immune system, their influence *in vivo* may be mediated via interactions with third party systems including the hypothalamic–pituitary–adrenal axis. Such interactions are well demonstrated in experimental animals. In humans, there is increasing, although indirect, evidence that these interactions also occur. Possible interactions at the cell and gene level, with mutual antagonism or synergy between cortico- and gonadal steroids, open new exciting hypotheses that await clarification.**

KEYWORDS: **glucocorticoids; sex hormones; estrogens; androgens, rheumatoid arthritis; hypothalamic–pituitary–adrenal axis**

SEX HORMONE CORRELATIONS WITH RHEUMATOID ARTHRITIS

There is a wealth of epidemiological and clinical evidence supporting the concept that sex hormones have a major influence on the onset and progression of rheumatoid arthritis (RA). Incidence of RA is two- to three-fold higher in adult women than in men. Interestingly, this ratio is greater during child-bearing age, when it can reach 5:1, and decreases after the menopause, as the gonadal hormone environment becomes more similar between the sexes, reaching values close to parity after the seventh decade.[1,2] Age has also been identified as an important determinant of disease progression: most studies on this matter conclude that older onset of RA (after age 65) is associated with more aggressive and destructive disease.[3,4]

Recently, we have tried to clarify whether this effect of age might be a reflection of the menopause, by studying the influence of gender, age, and the menopause in disease activity, radiographic destruction, and physical disability in a longitudinal cohort of early RA. We concluded that female gender is associated with more severe disease and that the menopause is responsible for the major part of these gender differences.[5]

Evidence from experimental models of arthritis is also strongly supportive of a role of sex hormones in the pathogenesis of these diseases, as we, and others, have shown similar gender differences in animals and significant effects of sex hormone manipulation in the onset and progression of experimental arthritis.[6,7] Overall, analysis

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of experimental evidence led to the concept that estrogens depress T-cell-mediated diseases and enhance B-cell-mediated processes, while androgens depress both types of disease processes.[8]

Bearing in mind that RA seems to be essentially mediated by T-cell-dependent pathways, we may note that the epidemiological data in human disease is in striking agreement with this latter interpretation of experimental evidence. Although the higher F:M ratios in adults might suggest that estrogens promote the disease, Masi[2] has shown that the actual incidence of RA increases throughout the aging process in females, instead of decreasing after the menopause. The incidence in males increases more markedly after age 50, approaching the incidence in females. Both estrogens and androgens would seem, in this light, to have beneficial effects upon the risk of RA.

We might expect this trend to be mirrored by differences in sex hormone levels between age-matched RA patients and controls. However, estrogen levels have been consistently found to be similar in both male and female RA patients and controls. Two differences, however, emerge quite consistently from the published reports. Female RA patients present lower levels of serum of dehydroepiandrosterone sulfate (DHEAS) than osteoarthritis and normal controls. Male RA is associated with low serum-free and total testosterone levels.[9,10]

The possibility that these findings translate a cause-effect relationship needs to be considered with caution, as similar changes have been identified in a variety of chronic inflammatory conditions, raising the hypothesis that these differences are a non-specific consequence of chronic disease.[11]

Masi *et al.*[12] have presented data demonstrating that significantly low serum DHEAS levels were present in women who later developed RA in the premenopausal years. The authors inferred that low androgenicity might be a predisposing factor to the development of the disease. A more recent study, also prospective, questioned these observations. However, their control levels of DHEAS were outside the usual range, raising doubt about possible assay error.[13] On the other hand, a recent study found a remarkably high prevalence of auto-immune and rheumatic diseases in a small group of untreated hypogonadal men in comparison to age-matched normal controls.[14]

Therapeutic Effect of Sex Steroids

In an open study in male RA patients, Cutolo *et al.*[15] found that oral testosterone undecanoate, given for six months, resulted in a significant reduction in the number of affected joints and NSAID consumption and a decrease in CD4+/CD8+ cells. In a double-blind, placebo-controlled trial of 30 male RA patients, we found no significant effects of i.m. testosterone enanthate in disease activity.[16] However, the dosing regime may have been suboptimal, and parenteral administration of testosterone resulted in a significant rise of estradiol levels. Interestingly, a similar trial, employing i.m. administration of testosterone propionate with progesterone to 107 postmenopausal females with RA, demonstrated a significant improvement of pain scores, Health Assessment Questionnaire results, and ESR, with 21% of the treated patients satisfying the ACR improvement criteria after one year.[17]

The use of estrogens as adjuvant therapy in postmenopausal RA women has given conflicting results. In a double-blind, placebo-controlled trial, 40 patients were treated

ith either 2 mg of estradiol valerate or placebo: no significant differences were found the end of the study.[18] In a larger study, following a single-blind randomized, pla-ebo-controlled design, 200 postmenopausal RA patients were treated with transder-al estradiol for six months. Women showed a significant serum estradiol increase, nd they presented improved pain scores, general well-being assessments, and ESR.[19]

Although the contradictions and ambiguities of these results may shed scepticism n the potential influence of sex steroids in human RA, one should agree that ear-cut and easily identifiable differences are hardly to be expected in a disease ith such complex and multifactorial pathogenesis. Most probably, sex steroid influ-nces, if present, need to be considered as constituting one amongst a myriad of fac-rs whose combined fine-tuning is the actual determinant of the establishment and rogression of disease. Although the exact role of sex steroids is still elusive in this rchestration of factors, accumulated evidence is certainly strong enough to support ur continued efforts to unravel their participation in the complex play.

SEX HORMONES AND THE IMMUNE SYSTEM

Direct Effects

There is clear evidence that sex steroids can directly influence a large variety of ne putative mechanisms involved in RA pathogenesis. For example, estrogen and ndrogen receptors have been identified in virtually all organs and immunocompe-ent cells as well as in effector cells in rheumatoid synovium.[20] Studies in isolated n *vitro* systems demonstrate an array of sex hormone effects on mechanisms consid-red pivotal in RA. These mechanisms include leukocyte trafficking and maturation; eactive oxygen species production; nitric oxide synthesis; production and activity f IL-1, TNFα, IL-6, and TGFβ; lymphocyte apoptosis; and the synthesis of metal-oproteinases—to cite only a few (reviewed by Cutolo and Wilder[20]).

Given the importance of endogenous glucocorticoids and the hypothalamic–ituitary–adrenal (HPA) axis in the regulation of immune processes and autoimmune iseases,[21] potential interactions between the gonadal and the adrenal systems might rovide powerful opportunities for indirect sex hormone influence on inflammatory iseases, such as RA.

Sex Hormones and the Hypothalamic–Pituitary Axis

Glucocorticoids are the most powerful endogenous immunomodulators, interfering vith virtually every step of inflammatory and immune reactions. The hypothalamic–ituitary–adrenal (HPA) axis plays a crucial role in the control of inflammatory and mmune reactions. Cytokines released from inflammatory sites, especially IL-6, IL-1, nd TNFα, interfere with the central nervous system to induce the production and elease of corticotropin-releasing hormone (CRH) at the hypothalamus. This will, in urn, increase the production of ACTH by the anterior pituitary. ACTH results in ncreased glucocorticoid synthesis and release by the adrenals, which close the circle y inhibiting the inflammatory process. Circulating glucocorticoids exert negative eedback control upon CRH release from the hypothalamus.[21]

Accumulated evidence clearly demonstrates that sex hormones exert powerful modulating effects upon the HPA axis responsiveness to a variety of stimuli, including inflammatory and immune conditions and mediators. The type of influence is overtly sexually dimorphic and also depends on the type of stress stimulation and species.[22,23] This is not surprising, given the variety and complexity of the mechanisms involved in HPA axis activation as well as the multitude of factors influencing its response. We will focus on studies regarding inflammatory conditions.

Overall, females mount stronger glucocorticoid responses to immune challenge than males. This is dependent on sex hormones, as gonadectomy tends to diminish or abrogate these differences.[22]

Most studies agree that, in males, gonadectomy enhances and testosterone inhibits the HPA response to stress, even though gender differences may not be found in resting states.[22] The mechanisms involved are complex but seem to depend on a potentiating effect of androgens on the negative feedback exerted by glucocorticoids on the hypothalamic secretion of CRH.[24] A recent study actually suggests that, at least in rats, the negative feedback by glucocorticoids is dependent on the presence of functional testis or replacement testosterone.[25] Conversely, androgens have no effect on the HPA axis in the absence of circulating corticosterone. Previous research had shown that androgens increase the expression of glucocorticoid receptors in key areas of the central nervous system involved in the regulation of the HPA.[26] Similar interactions upon the immune system could have a decisive role in understanding the potent immunosuppressive effects of androgens *in vivo*.[24]

The influence of estrogens seems to be more variable, depending on gender, species, and the model of stress used. In our studies, using Balb/c mice, we have demonstrated that oophorectomy diminishes the HPA axis responses both to chronic inflammation (cotton-induced granulomatous reaction) and to IL-1β.[27] Similar results have been presented with non-inflammatory stresses, such as restraint in rodents.[28] These results suggest that the influence of estrogen is focused on the HPA responsiveness and not on the production of activating mediators in the inflammatory site. In contrast, studies employing either endotoxin or IL-1 in rats and nonhuman primates show a decreased glucocorticoid response following estradiol.[29,30] In human studies, estradiol has been reported to increase the HPA response to psychological stress in men, and to have the opposite effect in women.[31,32] More recent studies have helped clarify this issue. Investigations with oophorectomized rhesus monkeys performed by Xiao *et al.*[33] showed that estradiol increases ACTH response to i.v. IL-6 but decreases ACTH production following i.v. IL-1. Using i.v. endotoxin to activate the HPA axis in postmenopausal women, Puder *et al.*[23] found that estradiol replacement diminishes the production of ACTH and cortisol, probably due a marked inhibition of the serum levels of IL-6 and TNFα.

These results underline the need to take into account the complex nature of the models used, given that estrogens may have divergent effects on different steps of the biological mechanisms involved in the final response, which may vary according to the disease.

The mechanisms involved in these interactions are complex. Estrogens have a direct stimulating effect on the production of the key regulator of the HPA axis: corticotropin-releasing hormone, through estrogen responsive elements present in the CRH gene.[34] Estrogens have also been shown to diminish the expression of

glucocorticoid receptors in rat hypothalamus, thus resulting in an inhibition of the negative feedback of glucocorticoids.[35] It is not known if these actions of estrogens are intrinsically related, but they influence the system in the same direction, that is, increased HPA responsiveness.

Overall, the influences of estrogens and androgens upon glucocorticoid action on the CNS amount to antagonistic and potentiating effects, respectively. The possibility that similar interactions occur peripherally would have far-reaching implications in the understanding of the gender dimorphism of the immune system and in the pathophysiology of RA and other inflammatory arthritis. However, this hypothesis remains to be explored. CRH is also expressed peripherally in inflammatory sites, including the rheumatoid synovium, where it exerts a potent pro-inflammatory role. The possibility that estrogens (and androgens) may affect this expression warrants investigation. Equally, potential effects of sex hormones on the expression of glucocorticoid receptors in immunocompetent cells, similar to those observed in the SNC, have not been studied.

Sex hormones have also been shown to affect other levels of the HPA axis. In a study by Gallucci *et al.*,[36] women presented a stronger response to ovine CRH then men, translated in higher levels of ACTH and a prolonged glucocorticoid rise, suggesting an influence of sex steroids upon the hypophyseal production and/or release of ACTH. The adrenal may also be affected by sex steroids. Recent studies have demonstrated that contrary to classical belief, women mount a stronger glucocorticoid response to the Synacthen test than men.[37] In our studies of the HPA axis response to intravenous IL-6, we have found that males and females show similar cortisol responses, despite higher levels of ACTH in males, suggesting a higher sensitivity of the female adrenal to ACTH.[38] The cortisol/ACTH ratio was significantly higher in females, both at baseline and after IL-6 infusion. Similar gender differences and sex hormone influences on these ratios have been found in the general population and can be derived from published data on the ACTH and cortisol response to endotoxin in humans.[23]

The production of IL-6, the major activator of the HPA axis in inflammatory conditions, is also affected by sex hormones.[23] Furthermore, our studies also suggest that some individuals present a relative resistance to the activation of the HPA by IL-6, a phenomenon we found more frequently in females than in males.[39]

CONCLUSIONS

Epidemiological correlation between significant life events regarding gonadal physiology (such as menarche, menopause, and aging) and the incidence and severity of RA establish a strong suggestion that sex hormones have potent influences on the pathophysiology of this disease. Although sex hormones have important direct effects on the immune system, their influence upon immunological processes may be essentially indirect, mediated through interactions with third-party mechanisms, as part of a complex network of regulatory mechanisms, including, but not limited to, the HPA axis. This perspective may help clarify the contradictory and inconclusive results of studies trying to establish a direct correlation between sex hormone or even glucocorticoid levels or manipulations and the course of immune-mediated diseases, such

as RA. This may well remain the case until we devise studies that try to integrate a large array of related effectors in a common algorithm whose combined result would, most probably, be more revealing than the consideration of individual factors. The enormous difficulties involved in such studies need no underlining, but the extreme complexity of the system we are trying to understand may preclude the reconstruction of the whole picture based solely on the fragments of reality that detail-focused studies offer. The experimental evidence that sex hormones influence the HPA axis and its response to immunological challenge is overwhelming. Evidence from human studies is fragmented but fits a similar pattern. One must recognize that the direct evidence to support relevant interactions between sex hormones and glucocorticoids in RA is limited. However, the opportunities for such interactions are numerous, and several pieces of the puzzle suggest that this is an important part of the overall picture. Certainly, these opportunities are worthy of our continued efforts to unravel the intertwined pathways of the neuro–endocrine–immune basis of RA.

REFERENCES

1. MASI, A.T. 1994. Incidence of rheumatoid arthritis: do the observed age–sex interaction patterns support a role of androgenic-anabolic (AA) steroid deficiency in its pathogenesis? Br. J. Rheumatol. **33:** 697–699.
2. MASI, A.T. 1994. Incidence of rheumatoid arthritis: do the observed age-sex interaction patterns support a role of androgenic-anabolic (AA) steroid deficiency in its pathogenesis? Br. J Rheumatol. **33:** 697–699.
3. SHERRER, Y.S., D.A. BLOCH, D.M. MITCHELL, *et al.* 1986. The development of disability in rheumatoid arthritis. Arthritis Rheum. **29:** 494–500.
4. VAN DER HEIJDE, D.M.F.M., P.L.C.M. VAN RIEL, M.A. LEEWEN, *et al.* 1991. Older versus younger onset rheumatoid arthritis: results at onset and after 2 years of a prospective follow-up study of early rheumatoid arthritis. J. Rheumatol. **18:** 1285–1293.
5. KUIPER, S., H.L. SWINKELS, A.M. VAN GESTEL, *et al.* 2001. The influence of gender, age and particularly the menopausal state on the course of early rheumatoid arthritis. J. Rheumatol. **28:** 1809–1816.
6. DA SILVA, J.A.P., J.-P. LARBRE, M.P. SEED, *et al.* 1994. Sex differences in inflammation-induced cartilage degradation in rodents: the influence of sex steroids. J. Rheumatol. **21:** 330–337.
7. WILDER, R.L. 1996. Hormones and autoimmunity: animal models of arthritis. Baillière's Clin. Rheumatol. **10:** 259–271.
8. HOLMDAHL, R., H. CARLSTEN, L. JANSSON, *et al.* 1989. Oestrogen is a potent immunomodulator of murine experimental rheumatoid disease. Br. J. Rheumatol. **28**(Suppl. 1): 54–58.
9. DA SILVA, J.A.P. & G.M. HALL. 1992. The effects of gender and sex hormones on outcome in rheumatoid arthritis. Baillière's Clin. Rheum. **6**(1): 193–219.
10. MASI, A.T. 2000. Hormonal and immunologic risk factors for the development of rheumatoid arthritis: an integrative physiopathogenetic perspective. Rheum. Dis. Clin. North Am. **26:** 775–803.
11. MASI, A.T., J.A.P. DA SILVA & M. CUTOLO. 1996. Perturbations of hypothalamic–pituitary–gonadal (HPG) axis and adrenal androgen (AA) functions in rheumatoid arthritis. Baillière's Clin. Rheum. **10:** 295–332.
12. MASI, A.T., R.T. CHATTERTON, G.W. COMSTOCK, *et al.* 1994. Decreased serum dehydroepiandrosterone sulfate (DHAS) before onset of RA in younger, premenopausal women: a controlled, prospective study. Arthritis Rheum. **37**(Suppl.): S315.
13. KEIKKILA, R., K. AHO, M. HELIOVAARA, *et al.* 1998. Serum androgen-anabolic hormones and the risk of rheumatoid arthritis. Ann. Rheum. Dis. **57:** 281–285.

14. JIMÉNEZ-BALDERAS, F.J., R. TÁPIA-SERRANO, M.E. FONSECA, *et al.* 2001. High frequency of association of rheumatic/autoimmune diseases and untreated male hypogonadism with severe testicular dysfunction. Arthritris Res. **3:** 362–367.
15. CUTOLO, M., E. BALLEARI, M. GIUSTI, *et al.* 1991. Androgen replacement therapy in male patients with rheumatoid arthritis. Arthritis Rheum. **34:** 1–5.
16. HALL, G.M., J.-P. LARBRE, T.D. SPECTOR, *et al.* 1996. A randomised trial of testosterone therapy in males with rheumatoid arthritis. Br. J. Rheumatol. **35:** 568–573.
17. BOOIJ, A., C.M. BIEWENGA-BOOIJ, O. HUBER-BRUNING, *et al.* 1996. Androgens as adjuvant treatment in postmenopausal female patients with rheumatoid arthritis. Ann. Rheum. Dis. **55:** 811–815.
18. VAN DER BRINK, H.R., A.A. VAN EVERDINGEN, M.J.G. VAN WIJK, *et al.* 1993. Adjuvant estrogen therapy does not influence disease activity in postmenopausal female patients with RA. Ann. Rheum. Dis. **52:** 862–865.
19. HALL, G.M., M. DANIELS, E.C. HUSKISSON, *et al.* 1994. A randomised controlled trial of the effect of hormone replacement therapy on disease activity in postmenopausal RA. Ann. Rheum. Dis. **53:** 112–116.
20. CUTOLO, M. & R.L. WILDER. 2000. Different roles of androgens and estrogens in the susceptibility to autoimmune rheumatic diseases. Rheum. Dis. Clin. North Am. **26:** 825–839.
21. STERNBERG, E.M. 2001. Neuroendocrine regulation of autoimmune/inflammatory disease. J. Endocrinol. **169:** 429–435.
22. DA SILVA, J.A.P. 1995. Sex hormones, glucocorticoids and autoimmunity: facts and hypotheses. Ann. Rheum. Dis. **54:** 6–16.
23. PUDER, J.J., P.U. FREDA, R.S. GOLAND, *et al.* 2001. Estrogen modulates the hypothalamic-pituitary-adrenal and inflammatory cytokine responses to endotoxin in women. J. Clin. Endocrinol. Metab. **86:** 2403–2408.
24. DA SILVA, J.A.P. 1999. Sex hormones and glucocorticoids: interactions with the immune system. Ann. N.Y. Acad. Sci. **876:** 102–118.
25. VIAU, V, A. CHU, L. SORIANO, *et al.* 1999. Independent and overlapping effects of corticosterone and testosterone on corticotropin-releasing hormone and arginin vasopressin mRNA expression in the paraventricular nucleus of the hypothalamus and stress-induced adrenocorticotropic hormone release. J. Neurosci. **19:** 6684–6693.
26. VIAU, V & M.J. MEANEY. 1996. The inhibitory effect of testosterone on hypothalamic-pituitary-adrenal responses to stress is mediated by the medial pre-optic area. J. Neurosci. **16:** 1866–1876.
27. DA SILVA, J.A.P., S.H. PEERS, M. PERRETTI, *et al.* 1993. Sex steroids affect glucocorticoid response to chronic inflammation and to interleukin-1. J. Endocrinol. **136:** 389–397.
28. VIAU, V. & M.J. MEANEY. 1991. Variations in the hypothalamic–pituitary–adrenal response to stress during the estrous cycle. Endocrinology **129:** 2503–2511.
29. SPINEDI, E., M.O. SUESCUN, R. HADID, *et al.* 1992. Effects of gonadectomy and sex hormone therapy on the endotoxin-stimulated hypothalamo–pituitary–adrenal axis: evidence for a neuroendocrine-immunological sexual dimorphism. Endocrinology **131:** 2430–2436.
30. XIA-ZHANG, L., E. XIAO & M. FERIN. 1995. A 5-day estradiol therapy, in amounts reproducing concentrations of the early-midfollicular phase, prevents the activation of the hypothalamo-pituitary-adrenal axis by interleukin-1 alpha in the ovariectomized rhesus monkey. J. Neuroendocrinol. **7:** 387–392.
31. KIRSCHBAUM, C., N. SCHOMMER, I. FEDERENKO, *et al.* 1996. Short-term estradiol treatment enhances pituitary-adrenal axis and sympathetic responses to psychosocial stress in healthy young men. J. Clin. Endocrinol. Metab. **81:** 3639–3643.
32. KOMESAROFF, P.A., K. SUDHIR & M.D. ESLER. 1999. Effects of estrogen on stress responses in women. J. Clin. Endocrinol. Metab. **84:** 4292–4293.
33. XIAO, E., L. XIA-ZHANG, M. FERIN & S.L. WARDLAW. 2001. Differential effects of estradiol on the adrenocorticotropin responses to interleukin-6 and interleukin-1 in the monkey. Endocrinology **142:** 2736–2741.

34. VAMVAKOPOULOS, N.C. & G.P. CHROUSOS. 1993. Evidence of direct estrogenic regulation of human corticotropin-releasing hormone gene expression: potential implications for the sexual dimorphism of the stress response and immune/inflammatory reaction. J. Clin. Invest. **92:** 1896–1902.
35. BURGESS, L.H. & R.J. HANDA. 1993. Estrogen-induced alterations in the regulation of mineralocorticoid and glucocorticoid messenger RNA expression in the female rat anterior pituitary gland and brain. Mol. Cell. Neurosci. **4:** 191–198.
36. GALLUCCI, W.T., A. BAUM, L. LAUE, *et al.* 1993. Sex differences in sensitivity of the hypothalamus-pituitary-adrenal axis. Health Psychol. **12:** 420–425.
37. CLARK, P.M., I. NEYLON, P.R. RAGGATT, *et al.* 1998. Defining the normal cortisol response to the short Synacthen test: implications for the investigation of hypothalamic-pituitary disorders. Clin. Endocrinol. **49:** 287–292.
38. SILVA, C., L.S. INÊS, D. NOUR, *et al.* 2002. Ann. N.Y. Acad. Sci. **966:** this volume.
39. SILVA, C., L.S. INÊS, D. NOUR, *et al.* 1999. The influence of gender and sex steroids on glucocorticoid response to interleukin-6 in humans. Arthritis Rheum. **42**(Suppl.): S93.

Discussion

Cutolo: You explained the protective role of estrogens in osteoporosis. As you know, in obese women, and especially in postmenopausal women that are obese, there is aromatization leading to higher levels of estrogens. Are there any epidemiological data to support a connection between body mass index and the occurrence of rheumatoid arthritis?

da Silva: This is an interesting question. Probably more factors are involved in the relation between body mass index and the occurrence of osteoporosis. Data on body mass index and rheumatoid arthritis are not sufficient to give an evidence-based answer to your question.