Hormonal pattern in women affected by rheumatoid arthritis

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ABSTRACT. Gonadal sex hormones may account for the sexual dimorphism in the immune response and for the greater incidence of autoimmune disease in females. We have previously reported the presence of progesterone (P) deficiency in female patients with thyroid and ovarian autoimmune disease. In this context, the hormonal profile in 9 women with rheumatoid arthritis (RA) and in 9 age-matched ealthy women, were evaluated to verify the presence of a steroid hormone secretion impairment in a systemic autoimmune disease, further supporting our hypothesis of P deficiency involvement. P and androgen plasma levels, in the luteal phase, were significantly lower ($p<0.05$ and 0.005, respectively) in RA patients than in the control group, with a consequent decrease of the free androgen index. Moreover, despite normal cortisol values, corticosterone (B) plasma levels were significantly higher in the RA patients ($p<0.01$ and 0.05 in follicular and luteal phase, respectively). Therefore, our present data confirm the androgen deficiency in patients with a systemic autoimmune disease, such as RA and support the immunomodulator effect of P. Finally, the higher B plasma levels in RA patients may suggest the presence of a slight impairment of the immune hypothalamic-pituitary-adrenal axis (HPAA), supporting its role in certain phases of RA pathogenesis. In conclusion, in addition to androgens, the immunomodulator role of P should also be taken into account in the pathogenesis of the systemic autoimmune disease.

INTRODUCTION

It is well known that autoimmune diseases are more commonly reported in females than in males, the different sex steroid environment being most likely due to a sexual dimorphism in the immune response (1,2). Moreover, autoimmune diseases are more frequently reported during the post-partum period or in menopause (3), while pregnancy (4) or exogenous sex steroid treatment (3, 5) generally exerts beneficial effects on the disease activity.

The predominance of rheumatoid arthritis (RA) in women over men also appears to be related to the influence of sex hormones on the immune response (6). In fact, increased estrogen and low androgen plasma levels with increased estrogen/androgen ratio, especially in the acute phase of the disease, have been reported either in RA or in other autoimmune diseases, such as systemic lupus erithematosus (7, 8).

In this study, the hormonal pattern in females with RA was determined in both the follicular and luteal phases, to evaluate the presence of a disorder of steroid hormone secretion and its possible involvement in RA pathogenesis.

MATERIALS AND METHODS

Nine women (aged 25-42 yr) with RA in the active phase and nine age-matched, healthy women were studied in the precocious follicular phase (day 4-6) and in the luteal phase (day 20-21). All the patients were without glucocorticoid therapy and received nonsteroidal antiinflammatory drugs. The diagnosis of RA was based on the diagnostic criteria of the American College of Rheumatism (ACR). The Steinbroker stage was used for the stadiation of the disease and the activity was evaluated with the erythrocyte sedimentation rate (ESR), protein electrophoresis (PE) and rheumatoid factor (RF) titer.

All the plasma samples were stored at -20C until processed for FSH, LH, prolactin (PRL), testosterone (T), 17-OH-progesterone (17OHP), delta-4 androstenedione (D4), dehydroepiandrosterone sulphate (DHEA-S), cortisol (F), corticosterone (B), 17-ß estradiol (E2), progesterone (P), 11-deoxycortisol (S), total estrogens (TE) and sex binding globulin

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Table 1 - *The lower limit of detection (sensitivity) and the intra- and interassay coefficient of variations (CV) for all the different hormones assayed.*

Hormones	Sensitivity	% intraassay CV	% Interassay CV
FSH	0.2 mUI/ml	4.7	7.4
LH	0.15 mUI/ml	1.8	4.8
PRL	0.8 ng/ml	5.1	6.2
E2	7.0 pg/ml	3.5	4.9
T.E.	15.0 pg/ml	5.7	10.8
P	0.05 ng/ml	4.4	4.0
T	0.1 ng/ml	7.9	8.4
17OHP	10.0 ng/dl	5	6.2
D4	10.0 ng/dl	4.7	7.6
DHEA-S	2.0 µg/ml	6	8.5
F	2.5 ng/ml	3.6	6.8
B	0.1 ng/ml	4.4	8.6
S	10.0 ng/dl	4.3	11.5
SHBG	2.5 ng/ml	4.6	3.3

(SHBG), using commercial RIA kits. The lower limit of detection and the % of intra- and interassay coefficients of variation (CV) for each hormone assayed are shown in Table 1. Moreover, the free androgens index (FAI=T*100/SHBG) and the free estrogens index (FEI=E2/SHBG) were calculated.
Statistical analysis was performed using analysis of variance. The results were considered significant when the *p* value was below 0.05.

RESULTS

Table 2 shows the clinical aspects of the 9 women with RA with a graduation of the disease.

Sex hormones and related hormone concentrations in the follicular and luteal phases, in both controls and RA patients, are reported in Table 3 and are the mean of three determinations.
Neither the FSH, LH, PRL, E2, T.E., DHEA-S, 17OHP, F, S, and SHBG plasma values nor the FEI were significantly different in the two groups, both in the follicular and luteal phases. D4 and P plasma values were not significantly different in the two groups during the follicular phase; a significant decrease ($p<0.025$ and $p<0.05$, respectively), however, was evident in RA patients during the luteal phase. Moreover, T plasma levels were significantly lower in both the follicular and luteal phases

Table 2 - *Age, clinical stage and main laboratory findings of the 9 women with rheumatoid arthritis. The rheumatoid factor (RF) was considered positive with a titer >1:20.*

Name	Age (yr)	Clinical stage (Steinbroker staging)	RF	ESR* (I h)	PE**
P.R.	36	II	-	30	NR***
D.G.P.	27	II	±	5	NR
T.G.	25	I	±	8	NR
M.G.	42	III	-	12	NR
M.C.	30	II	±	10	NR
L.C.	20	II	±	15	NR
C.A.M.	39	II	±	24	↑ α-2gl.^
I.S.	32	II	-	11	NR
C.M.R.	27	II	-	56	↑ α-2gl.^

*ESR=Erythrocyte sedimentation rate

**PE=Protein electrophoresis

***NR=Values in the normal range

^α-2gl.=α2-globulins.

Table 3 - *FSH, LH, Prolactin (PRL), 17ß-Estradiol (E2), Total Estrogens (T.E.), Progesteron (P), Testosterone (T), 17-Hydroxyprogesterone sulphate (DHEA-S), Cortisol (F), Corticosterone (B), 11-deoxycortisol (S), Sex Hormone Binding Globulin (SHBG), values in normal controls and in RA patients expressed as Mean±SD, in follicular (f) and in luteal (l) phases.*

Hormones		Controls (9)	RA patients (9)	
FSH	(f) (mUI/ml)	4.4 ± 1.6	4.8 ± 1.6	NS
FSH	(l) (mUI/ml)	3.8 ± 1.0	3.5 ± 1.7	NS
LH	(f) (mUI/ml)	6.4 ± 2.3	4.0 ± 3.6	NS
LH	(l)	6.3 ± 1.6	5.7 ± 2.9	NS
PRL	(f) (ng/ml)	5.3 ± 1.4	5.7 ± 2.4	NS
PRL	(l)	6.1 ± 1.4	6.9 ± 3.1	NS
E2	(f) (pg/ml)	105.0 ± 28.7	95.0 ± 67.5	NS
E2	(l)	135.2 ± 40.7	158.9 ± 87.8	NS
T.E.	(f) (pg/ml)	228.8 ± 68.4	199.6 ± 83.3	NS
T.E.	(l)	199.4 ± 64.5	286.1 ± 66.5	NS
P	(f) (ng/ml)	0.7 ± 0.2	1.2 ± 0.8	NS
P	(l)	11.8 ± 5.2	7.7 ± 6.1	$p<0.05$
T	(f) (ng/ml)	0.4 ± 0.2	0.2 ± 0.1	$p<0.025$
T	(l)	0.6 ± 0.1	0.4 ± 0.1	$p<0.005$
17OHP	(f) (ng/dl)	69 ± 12.4	59.3 ± 16.7	NS
17OHP	(l)	104.7 ± 54.2	175.7 ± 108	NS
D4	(f) (ng/dl)	133.3 ± 51.1	105.5 ± 26.2	NS
D4	(l)	180.4 ± 49.2	119.3 ± 62.9	$p<0.025$
DHEA-S	(f) (µg/dl)	119.1 ± 48.4	112.4 ± 68.9	NS
DHEA-S	(l)	156.0 ± 53.7	104.9 ± 60.3	NS
F	(f) (ng/ml)	111.0 ± 24.8	115.6 ± 34.3	NS
F	(l)	110.5 ± 39.3	109.9 ± 23.0	NS
B	(f) (ng/ml)	1.7 ± 0.6	2.9 ± 1.0	$p<0.01$
B	(l)	1.6 ± 0.5	2.4 ± 0.9	$p<0.05$
S	(f) (ng/dl)	143.9 ± 42.1	180.2 ± 77.1	NS
S	(l)	156.5 ± 35.2	192.8 ± 72.5	NS
SHBG	(f) (ng/ml)	38.5 ± 7.4	54.2 ± 28.3	NS
SHBG	(l)	49.8 ± 19.1	58.6 ± 25.3	NS

($p<0.025$ and $p<0.005$, respectively) while B plasma levels increased significantly ($p<0.01$ and $p<0.05$ in the follicular and luteal phase, respectively) in RA patients. Although the FAI in RA patients was not significantly different from the control group in the follicular phase, it became significantly lower in the luteal phase ($p<0.025$).

DISCUSSION

A large number of clinical and experimental studies has provided a clear evidence of a sexual dimorphism in the immune response (1). Consequently, sex hormones are also likely to be strictly related to the pathogenesis of autoimmune diseases, providing a linkage with the greater prevalence of autoimmune diseases in women (1, 2).

In this context, the immunosuppressive role of androgens has been well documented (9, 10). Significantly lower androgen plasma levels have been reported in men with RA than in normal men, both basally and after the human chorionic gonadotropin stimulation test (11, 12). Moreover, the improvement of laboratory and clinical parameters after testosterone replacement therapy in men with RA (12, 13) further supports the hypothesis of a protective role played by androgens against RA manifestations. Conversely, the physiologic role of estrogens or that of contraceptive therapy in the etiology and natural course of the autoimmune disease is still uncertain (1-3, 10).

Furthermore, autoimmune diseases are frequently reported to flare up in conditions characterized by low progesterone plasma levels or by an increased estrogens/progesterone ratio, such as post-partum period or menopause; on the other hand, remis-

sions are often experienced in pregnancy, when a physiologic increase in progesterone secretion occurs (3). Moreover, estrogens have been reported to increase B-cell differentation and their polyclonal immunoglobulin response to nonspecific mitogen (14); finally, estrogen receptors have been described only on T-suppressor lymphocytes, consequently limiting the generic estrogen depression of the T-cell function only to the T-suppressor function (15-17). Thus, both clinical and experimental evidence suggests a direct involvement of either estrogens or progesterone in the immune system regulation and autoimmune disease pathogenesis. While estrogens are likely to exert immunostimulating effects, progesterone may be the physiologic immunodepressing agent, as also suggested by its role in the prevention of maternal-fetal allograft rejection (18).

In this context, we have previously reported the presence of P deficiency in women with autoimmune thyroid diseases and the positive effect of P therapy in the management of both thyroid and ovarian autoimmune diseases (19-23). We, therefore, evaluated the basal hormonal enviroment in RA women when verifying whether P deficiency is present also in autoimmune diseases different from ovarian and thyroid pathology. By doing so we extended our observation to adrenal and androgen secretion, thus bearing in mind the relevance of the hypothalamic-pituitary- adrenal axis in the normal function of the immune system (24-25).

As a matter of fact, P plasma levels in the luteal phase are significantly lower in the RA patients than in the controls, as is the expression of disorders in the ovarian secretory function. This further confirms our observations on the presence of P deficiency in organ-specific autoimmune diseases and supports the positive outcome of intraarticular P therapy in the treatment of RA patients (26).

In addition, in agreement with previous studies, our RA patients presented lower androgen plasma levels than the controls. In particular, T is lower in both the follicular and luteal phase, while D4 only in the luteal phase. Consequently, the FAI, expression of the peripheral androgen activity, is decreased.

Finally, it is interesting to report the presence of higher B plasma levels in RA patients with a consequent increase in the B/F ratio. The physiopathologic significance of this evidence, suggestive of an alteration in the 17-hydroxylase pathway, needs further proof considering that a disregulation in the hypothalamic-pituitary-adrenal-immune axis has already been described in an experimental animal model of spontaneous arthritis (27).

In this context, the intricate link between sex hormones and cytokines (28-31) might further account for the role of sex hormones in the pathogenesis of RA. The role of interleukin 1-ß (IL-1) (a monocyte and macrophage product) in fact is well-documented (32, 33) in RA intraarticular inflammatory damage. In addition, IL-1 activity is reported to increase during the luteal phase of the menstrual cycle, concomitantly with progesterone secretion; moreover, gonadal steroids are reported to modulate human monocyte IL-1 activity, while cytokines, such as IL-1 and tumor necrosis factor-α are likely to exert an autocrine and paracrine control on gonadal steroid secretion (29, 30). These data are suggestive of a bidirectional influence of cytokines and steroid biosynthesis. We, therefore, hypothesize that, as a result of P or androgen deficiency, different and multiple steroid hormone secretion impairments may be involved in the etiology of a systemic autoimmune disease such as RA.

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