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Androgen Binding Sites in Peripheral Human Mononuclear Leukocytes of Healthy Males and Females

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Androgen binding sites have been identified in circulating human mononuclear leukocytes of healthy donors of both sexes. Cells were separated from blood samples on a Ficoll gradient and incubated with different concentrations of [^{3}H]testosterone in the presence or absence of a 400-fold excess of unlabelled testosterone. Binding data were derived from Scatchard analysis. The binding sites fulfil the required criteria for specific steroid binding sites however differ somewhat from the classic androgen receptors from genital skin fibroblast: in fertile adult males ($n = 20$) the binding sites showed (1) a high affinity for testosterone (1.32 ± 0.49 nM; mean ± SD), (2) a saturable capacity (184 ± 52 binding sites per cell; mean ± SD), and (3) a characteristic competitive binding profile for other steroid hormones (relative binding affinities: testosterone = dihydrotestosterone > 17β-estradiol > progesterone, whereas aldosterone, 17-hydroxy-progesterone and cortisol did not compete appreciably). Furthermore the number of binding sites determined using [^{3}H]dihydrotestosterone, [^{3}H]RU-1881, or [^{3}H]testosterone were comparable. This raises the possibility that androgen receptors in peripheral mononuclear leukocytes differ from those in genital skin fibroblasts. There was no apparent correlation between serum testosterone concentrations and androgen binding sites. In fertile women remarkable changes in androgen binding sites were seen in the course of the menstrual cycle, with a significant increase in the immediate preovulatory period. The presence of androgen receptors in peripheral mononuclear leukocytes provides for the first time the experimental basis for an hypothesis of direct, receptor-mediated effects of androgens on mature immunocompetent cells. The immunological implications of these results are discussed.

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INTRODUCTION

Androgen binding sites have been extensively studied in classical androgen target cells such as genital skin fibroblasts [1, 2] and in tissues of the male accessory sex organs [3–5]. More recently, androgen binding sites were identified in human blood cells i.e. in erythroblasts [6] and leukaemic cells [7].

Several lines of evidence from experimental investigations in animals and humans suggest that sex hormones considerably influence cellular and humoral immunity (for detailed reviews see Refs [8–10]), causing gender differences in the immune responses [11, 12]. Females show higher immunoglobulin titres than males [13, 14], and estrogens enhance immunoglobulin synthesis *in vitro*, whereas androgens show the opposite effect [15, 16]. In untreated hypogonadal males with low testosterone levels the concentration of T-helper cells is significantly higher than in androgen treated patients [17]. This may in part explain why the incidence of autoimmune diseases like systemic lupus erythematosus (SLE) [18, 19], myasthenia gravis [20] and autoimmune thyroiditis [21] shows a female predominance. With regard to cellular immunity, androgens seem to influence immunity via an enhancement of suppressor activity [22, 23]. This is supported by clinical studies which have shown, that the helper T/suppressor T cell ratio decreases following androgen treatment [24, 25], conversely to estrogens [26].

The underlying mechanisms whereby sex steroids modulate immunological responses are still not

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completely understood. It is widely accepted that steroid hormones interact with the target cell via specific nuclear receptors. Therefore it would be anticipated that hormone binding sites exist in immunological cells and tissues. Indeed, in recent years, steroid receptors in peripheral mononuclear leukocytes (PML) have been identified for estrogens [27–29], progesterone [30], and mineralocorticoids [31]. Corresponding attempts, however, to establish the presence of androgen binding sites in PML have failed in previous studies [27, 29, 31], whereas the existence of specific androgen binding sites has already been widely demonstrated in mammalian [33–35] as well as in both human [36, 37] thymic tissue and maturing human thymocytes [32].

We have been able to identify and characterize high-affinity androgen binding sites in PML in humans, indicating that circulating immunocompetent cells are androgen target cells. Furthermore, this method provides a test for repeated quantitative receptor measurements in intact circulating cells which are easily available from blood samples, so that this whole-cell assay can determine the androgen binding capacity under various physiological and clinical situations. In the present study we have evaluated the binding sites for androgens in PML of healthy, fertile males and females. In addition we describe apparent cycle-dependent changes of androgen binding sites. The immunological implications of our findings are discussed.

MATERIALS AND METHODS

Fasting healthy male and female adult volunteers were recruited from the hospital staff. Care was taken to study PML only from fertile subjects. Blood samples (10–15 ml) for receptor and hormone determinations were drawn at 9 a.m. and heparinized. Serial hormone and receptor determinations were performed in women during a single menstrual cycle while they were participating in a study to evaluate natural birth control. In every case, ovulation was determined by ultrasound.

Androgen binding studies

Cell preparation. Human mononuclear leukocytes were isolated from peripheral blood according to the method described previously [38]. Briefly, platelet-rich plasma was removed by low-speed centrifugation; the remaining blood was diluted 1:10 with platelet-poor plasma, layered on a Ficoll gradient (Pharmacia, Uppsala, Sweden), and centrifuged at 400 *g* for 20 min. The mononuclear cell layer was removed and the cells in the interphase were washed three times with saline (0.15 M NaCl, pH 7.2) at room temperature. The resulting cell suspension was free of granulocytes. The ratio of PML to thrombocytes is 2:1. This dilution does not interfere with PML-specific binding, inasmuch as platelets have been shown not to bind androgens. The ratio of lymphocytes to monocytes was about 10:1. Cell viability checked by Trypan blue exclusion was 98% both prior to and after incubation.

Binding studies. After isolation of mononuclear leukocytes by gradient centrifugation, the cells were resuspended in RPMI-1640 incubation medium [purchased from Serva, Heidelberg, Germany, supplemented with 700 mg/l L-glutamine (Bio-Mérieux, Lyon, France) and 20 nM HEPES, pH 7.4] at a concentration of 5–8 $\times$ 10^6 cells/ml. A 500 μl aliquot of the cells (3×10^6) was incubated in the presence of increasing amounts of [^{3}H]testosterone, (from New England Nuclear Corp., Boston, MA, U.S.A., sp. act. 85 Ci/mmol) for 90 min at a constant temperature of 37°C. Specificity of the androgen binding was assessed by the inhibition of labelling in samples containing the same number of cells incubated in the presence of a 400-fold excess of radio-inert testosterone (obtained from Sigma, St Louis, MO, U.S.A.). At the end of the incubation period, the reaction was stopped by the addition of 2 ml cold phosphate buffered saline (0.15 M NaCl and 6.7 mM phosphate, pH 7.2). The suspension was then centrifuged at 600 *g* for 3 min at 4°C and the supernatant discarded.

Thereafter cells were washed three times in 2 ml of the same buffer, resuspended in 200 μl 0.15 NaCl, transferred into scintillation vials and counted in a Beckmann LS 1801 scintillation counter at 60% efficiency using the scintillation cocktail "Qicksafe A" of Zinser Analytic Ltd, Maidenhead, England. The binding characteristics, binding capacity (B_{max}) and binding affinity (K_d) of the androgen binding sites were derived from Scatchard plots [39]. The results were calculated as number of binding sites per cells, by determining the amount of bound [^{3}H]testosterone per aliquot containing a known number of cells. In addition binding sites were determined using [^{3}H]dihydrotestosterone and [^{3}H]RU-1881 as ligand (kindly supplied by Roussel-UCLAF, Paris, France).

To determine the specificity of androgen binding sites in PML, competition assays with various steroid hormones were performed. Cells were incubated with [^{3}H]testosterone at a concentration of 5 nM under the same conditions described above, in the presence or absence of three different concentrations (5, 25 or 50 nM) of dihydrotestosterone or 50, 250 or 500 nM of 17β-estradiol, progesterone, aldosterone, 17-hydroxy-progesterone and cortisol, respectively (Table 1).

Hormonal measurements

Serum testosterone (T), dihydrotesterone (DHT), 17β-estradiol (E_2), progesterone (P), aldosterone (Aldo), 17-hydroxy-progesterone (17-OH-P) cortisol (F) and gonadotropin concentrations were measured with commercially available radioimmunoassay kits. Sex hormone binding globulin (SHBG) has been measured in the incubation volume of the radioreceptor assay using a commercially available IRMA-assay. (We would like to thank Dr Lupa, Clinical Chemistry

Laboratory, Klinikum Grosshadern, for performing this assay for us.)

RESULTS

Mononuclear cell preparations were investigated for specific androgen binding sites in a whole cell assay, and binding data represented as Scatchard plots. A representative plot of [^{3}H]testosterone binding to androgen binding sites in PML is shown in Fig. 1: a rectilinear Scatchard plot was obtained in all cases, indicating that [^{3}H]testosterone binds to a single class of receptor with high affinity and limited capacity.

In healthy adult men ($n = 20$) the binding capacity, expressed as number of saturable binding sites per PML, ranged from 124 to 262 sites per cell, with a mean of 184 ± 52 (SD). These results were obtained after a 90 min incubation; prolonged incubation period did not increase binding. In individual men the androgen binding sites varied only slightly. The interassay coefficient of variation (5 consecutive receptor assays in 1 male subject, over the course of 4 months) was 12.5%. There was no apparent correlation between androgen binding sites in PML and baseline total serum testosterone concentration ($r = -0.02$) or binding affinity ($r = -0.22$).

SHBG was never measurable in the incubation volume of the radioreceptor assay with and without sonification of PML ($n = 5$).

The affinity of testosterone binding to the androgen binding sites was found to be 1.3 ± 0.5 nM (mean $\pm$ SD; $n = 20$). The relative binding affinity of various steroid hormones for androgen binding sites is depicted in Table 1: T and DHT bound almost equally (97%) followed by E_2 (12%) and P (6%); other hormones examined as competitors (Aldo, 17-OH-P, and F) bound with affinities that were <0.001% of T.

Table 1. Relative affinity of various steroids to the androgen binding sites in peripheral human mononuclear cells

Steroid	Relative affinity (%)
T	100
DHT	97.3 ± 15.9
E_2	14.4 ± 9.3
P	4.6 ± 2.3
Aldo	<0.001
17-OH-P	<0.001
F	<0.001

Steroids measured for binding affinity in this assay were besides testosterone (T): dihydrotestosterone (DHT), 17β-estradiol (E_2), progesterone (P), aldosterone (Aldo), 17-hydroxy-progesterone (17-OH-P) and cortisol (F). Aliquots of PML (3×10^6 cells) were incubated with [^{3}H]T (in the presence of a 400-fold excess of unlabelled T) with increasing concentrations (5, 25, and 50 nM) of a nonradioactive competitor at 37°C for 90 min. Specific binding of [^{3}H]T is set at 100%. A total of 5 studies were done and results are given as mean $\pm$ SD.

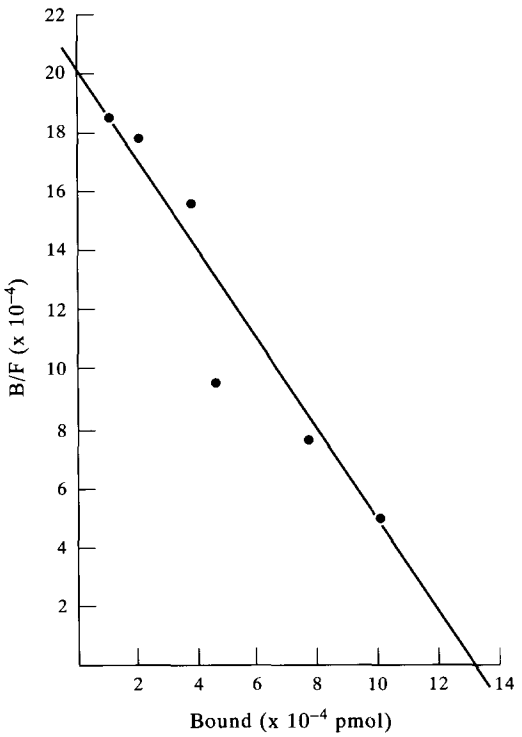

Fig. 1. Scatchard analysis of binding data of [^{3}H]T to binding sites in PML in a whole cell assay on mononuclear cells obtained from a healthy 28-year-old, male. After a 90 min incubation of PML (3×10^6 cells in 500 μl incubation medium at 37°C, pH 7.4) in the presence of increasing concentrations of [^{3}H]T (0.5–15 nM) with or without a 400-fold excess of unlabelled T, cells were washed and radioactivity determined. Maximum binding capacity and affinity were calculated from the Scatchard plot. On the abscissa: concentration of receptor-bound [^{3}H]T; on the ordinate: the bound [^{3}H]T to free [^{3}H]T ratio (B/F).

Using [^{3}H]DMT or [^{3}H]RU 1881 as ligands, binding sites in PML were similar to the amount measured with [^{3}H]T.

In healthy adult females the number of binding sites for T varies considerably over the course of the menstrual cycle, with a characteristic pattern (Fig. 2). T receptor levels during the preovulatory and late luteal phase are comparable to those found in men, and just before ovulation these binding sites are markedly increased. This cyclic change is highlighted by serial hormone measurements over a single menstrual cycle (Fig. 3) which show increased androgen binding sites prior to the gonadotropin rise, and the subsequent estrogen and progesterone elevation. Over the whole study period plasma T concentrations remained at or below assay sensitivity.

DISCUSSION

The results of the kinetic assays performed show the presence of androgen binding sites in circulating human mononuclear leukocytes recovered from peripheral blood by Ficoll gradient preparation. The PML preparation contains 70–80% T-lymphocytes, 5% B-lymphocytes, about 10–25% monocytes and some dendritic cells. The high affinity, saturable capacity and

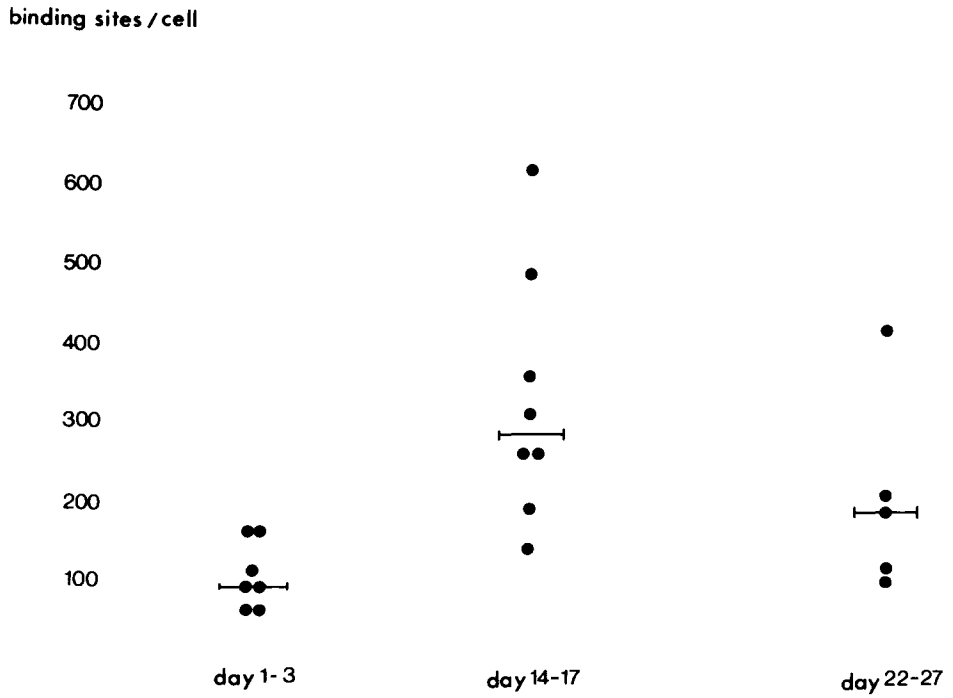

Fig. 2. Changes in the level of androgen binding sites in PML during menstrual cycles in healthy women. Ovulation was verified by ultrasound. Receptor assays were carried out at cycle day 1–3, 14–17 and 22–27. The mean value (horizontal bar) of binding sites in the immediate preovulatory period is significantly higher ($P < 0.05$) than the mean levels in the early preovulatory and late luteal phase as well as the mean levels measured in PML of healthy adult males ($n = 20$).

limited specificity of the androgen binding sites fulfil the criteria required for specific hormone receptors. It is, however, noteworthy that T binds with the same high affinity as DHT, although in other tissues DHT is found to have a higher affinity for androgen binding sites than T [40].

The affinity of T for androgen binding sites in PML as well as the hierarchy of relative binding affinities for various steroid hormones is comparable to the androgen binding sites in other human tissues [7, 32, 36, 41], though in particular in testicular fibroblasts [41] DHT shows a higher affinity for androgen binding sites than T. E_2 and P are less effective competitors, and glucocorticoids show negligible affinity.

There is no evidence for hormonal regulation of cellular androgen binding sites by T. In our study no correlation between peripheral T levels and cellular androgen binding sites of both sexes was seen. In females androgen binding sites corresponded during the first half of the menstrual cycles to those found in men, although over a wider range. In the second half of the ovulating cycle binding sites were double those

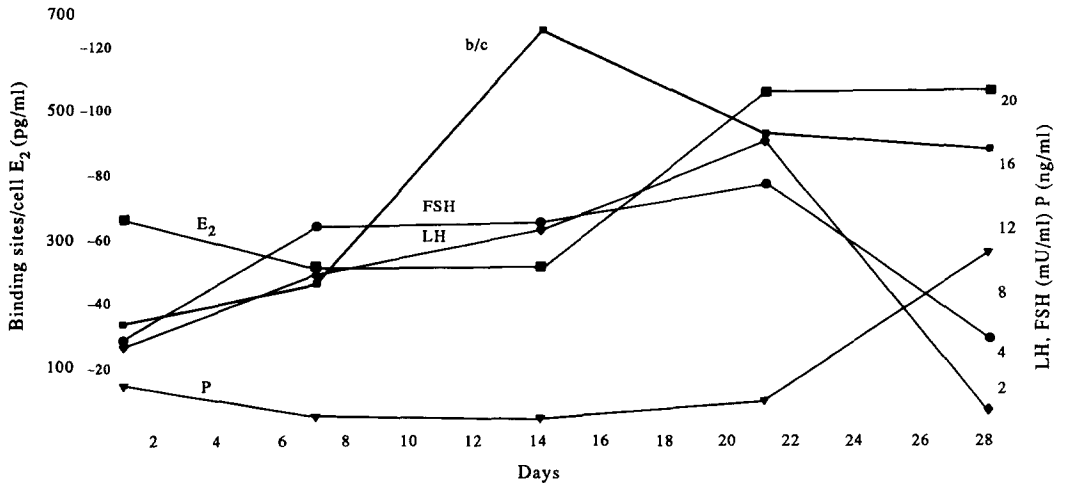

Fig. 3. Androgen binding sites per cell (b/c) and hormone values in the course of an individual menstrual cycle of a healthy female (E2 = 17β-estradiol, P = progesterone, FSH = follicle stimulating hormone, LH = luteinizing hormone). During the study period the T concentration remained at or below assay sensitivity.

in males. The obvious lack of relation between serum T and cellular androgen binding sites is consistent with findings in cells from human kidney [42] and skin [43]. However, other investigators found higher thymic androgen receptor concentrations in male than in female mice [43] and a significant decrease in androgen receptor levels after castration [44–46]. Moreover in early puberty the amount of androgen receptors in genital skin increases significantly, from low levels in childhood to a maximum in late puberty, in parallel with the rise of T concentration, though it should be noted that the total amount of androgen binding sites in genital skin fibroblasts is much higher than the numbers found in PML [47].

This study establishes the presence of androgen binding sites in intact PML of healthy adults of both sexes. This method provides a noninvasive technique for examining androgen binding sites in intact cells, thus minimizing the interference caused by tissue homogenization and prolonged cell culture. The inter-assay coefficient of variation is less than in genital skin fibroblasts [41]. In contrast to genital skin fibroblasts which are characterized by a higher affinity for DHT than for T the binding affinities for DHT and T are almost equal in PML. This raises the possibility that androgen receptors in PML differ from those in genital skin fibroblasts which further indicates that these receptors indeed do have different functions. While virilization of the external genitalia is dependent on the presence of DHT in the genital tract which originates from circulating T through the action of the enzyme 5α-reductase it seems to be that the immunological functions of androgens are mediated through T itself. Whether PML contain 5α-reductase activity at all, which would enable them to synthesize DHT from T, is not known (E. Stoner, personal communication). However it seems reasonable to speculate that, the immunological functions of androgens are mediated by T rather than DHT.

Until now it has not been clear whether androgen binding sites are present in all mononuclear leucocytes or are confined to a specific subpopulation. Analogous studies on PML have shown that estrogen receptors can be demonstrated only in OKT8-positive lymphocytes, a cellular subset which includes predominantly suppressor and cytotoxic lymphocytes [27].

The immunological implications of the identification of androgen binding sites in intact circulating PML are that androgens may interact directly with immunocompetent peripheral cells. In previous studies androgen binding sites were identified in thymic homogenates [36, 37] and [32] thymocytes, but not in circulating lymphocytes [27, 29, 32], suggesting that androgens might act on the immune system only during maturation of lymphocytes in the thymus. Androgen withdrawal certainly causes thymic hypertrophy and androgen substitution the opposite effect [48]. Besides possible modulatory effects on the maturation and differentiation of thymocytes [24, 49] and the release of interleukin 2 [32] in thymic reticular tissue, androgens may clearly act directly on mature lymphocytes if they contain androgen receptors. This is of special interest in the light of the frequently observed premenstrual aggravation of autoimmune diseases [50, 51]; whether the changes in androgen binding sites during the menstrual cycle provide an explanation for this phenomenon remains to be proven.

Hormonal investigation of patients with SLE shows altered androgen and estrogen metabolism, leading to a significantly lower androgen/estrogen ratio [19]. It has been shown that in patients with SLE androgen administration results in an increase of the lowered peripheral suppressor T-cell activity, and in clinical improvement. In this light it is of interest that in patients with Klinefelter's syndrome, often associated with subgonadal T levels [22], the incidence of SLE is increased [51, 52], possibly in part due to persistent estrogenic stimulation [53]. Interestingly androgen receptors are low in most patients with Klinefelter's syndrome [54]. To what extent androgen action via androgen receptors in immunologically relevant tissues such as PML is involved in these responses remains to be elucidated.

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