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Estrogen-Dependent Leydig Cell Protein Recognized by Monoclonal Antibody to MCF-7 Cell Line

Abstract. *A protein (27,000 molecular weight) was previously found in rat Leydig cells after treatment with estradiol (E_2) and human chorionic gonadotropin (hCG) in vitro. The effect of hCG occurred through increased E_2 production. This hormone-regulated rat testicular protein was compared to an estrogen-regulated protein of similar physical characteristics isolated from a human mammary cancer cell line (MCF-7) and present in normal human estrogen target organs. The Leydig cells from rat and human tissue showed specific immunofluorescence and immunoperoxidase staining in the cytoplasm upon incubation with a monoclonal antibody (C11) to the estrogen-regulated protein from MCF-7 cells. Leydig cells after exposure to E_2 or hCG showed the highest fluorescence intensity; this intensity was reduced by treatment with Tamoxifen. No reaction was associated with other testicular cells. The estrogen-regulated protein from human cell lines is therefore immunologically similar to that from the rat Leydig cell. The monoclonal antibody should be useful for further characterization of the Leydig cell protein.*

The distribution of an estrogen-regulated protein detected by monoclonal antibodies (*1*) has been described in human estrogen target organs, tumors, and cell lines (*2*). This protein was first found in the MCF-7 human breast cancer cell line (*3*), and its function is not known. The protein is interesting because it is present only in cell lines positive for estrogen and progesterone receptors (*2*) and because there is evidence that it is a marker of hormonal events in the human endometrium (*2*). Also, an estrogen-regulated protein of similar molecular weight (27,000) was found in cultured rat Leydig cells treated with estradiol (E_2) and human chorionic gonadotropin (hCG) (*4*). The effect of hCG was dependent on increased E_2 production derived from androgen that was stimulated by the trophic hormone, and it preceded the appearance of the estrogen-induced distal steroidogenic lesion of the androgen pathway (*4*). It was therefore of interest to explore the cross-immunological identity of these proteins and to determine whether the protein isolated from human cancer cells was also produced by human and rat Leydig cells.

For these studies we used Leydig cells isolated from adult rat testes and from two normal human testes prepared as described (*4*), and small pieces of rat and human testes fixed in Bouin's solution and routinely processed to obtain paraffin sections. Leydig cell preparations were evaluated histochemically for their content of Δ_4-3β-hydroxysteroid dehydrogenase by staining with nitro blue tetrazolium (*4*). The Leydig cells isolated from rat and human tissue showed marked immunofluorescence (indirect technique) (*5*) when incubated with a mouse monoclonal antibody (C11) to the estrogen-regulated protein from MCF-7 human breast cancer cells (*1*). Comparison of the histochemical reaction with the immunocytochemical reaction revealed that the rat Leydig cells were positive for immunofluorescence (Fig. 1, a and b). In some of the Leydig cells the fluorescence was clearly seen in the cytoplasm (Fig. 1c). No reaction was observed when the isolated Leydig cells were incubated with a negative control, that is, with C11 that had been absorbed with the purified protein from MCF-7 cells (Fig. 1d). To establish that the Leydig cell protein (27,000 molecular weight) was the source of the cross-reacting determinant, we absorbed the monoclonal antiserum with ruptured Leydig cells. For this experiment, isolated Leydig cells from adult (60-day-old) and immature (5-day-old) normal rats were used. After being frozen and thawed three times, the cells were resuspended in distilled water (8×10^5 cells per milliliter), and cell rupture was verified under phase-contrast microscopy. The immunostaining was attenuated when C11 was absorbed separately with the supernatant (100,000*g*) and the pellet for 72 hours at 4°C. The final concentration of C11 was 5 μg/ml; this concentration has given specific immunostaining in the avidin-biotin peroxidase complex (ABC) technique (Vector Laboratories) (*2*). No attenuation was observed when C11 was absorbed with the pellet or supernatant of ruptured Leydig cells from immature rats (Fig. 1, e and f).

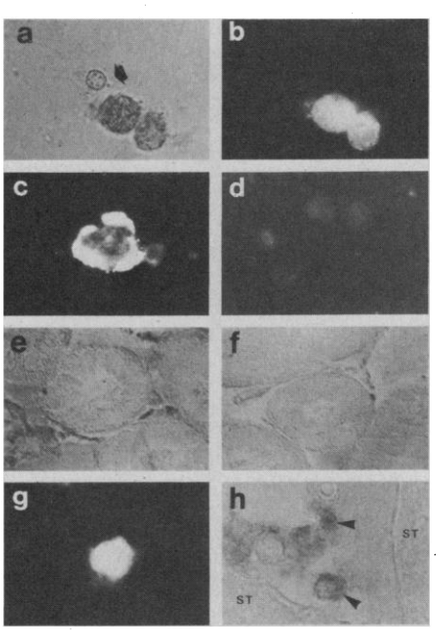

Fig. 1. Phase-contrast micrographs showing the reactivity of the monoclonal antibody (C11) to the estrogen-regulated protein from MCF-7 cells with rat and human Leydig cells. (a) Isolated rat Leydig cells from a normal testis stained with nitro blue tetrazolium (compare to b). The arrow points to a lymphocyte that remained unstained; this cell type is often associated with Leydig cells (*4*). (b) The cells stained with nitro blue tetrazolium showed positive fluorescence (×500). The immunofluorescence staining was strongest in the isolated rat Leydig cells obtained from hCG-treated testes (c); note that the reaction is mainly localized in the cytoplasm of the cell (×600). (d) Staining of the Leydig cells with a negative control (absorbed C11) (×450). Paraffin sections from a normal rat testis processed for the ABC technique (*2*) showed positive staining in the Leydig cells (e) when C11 was absorbed with the pellet of ruptured Leydig cells from immature rats. The adjacent serial section (f) shows attenuated reaction in the Leydig cells when C11 was absorbed with the particulate fraction of Leydig cells from adult rats (×150). (g) Positive fluorescence in the Leydig cells from a normal human testis maintained in short-term culture (*4*) (×500). (h) Paraffin sections from a normal human testis processed for ABC immunostaining (*2*) showing moderate positive reaction in the Leydig cells (arrows); tubule cells did not react (ST, seminiferous tubule) (×400).

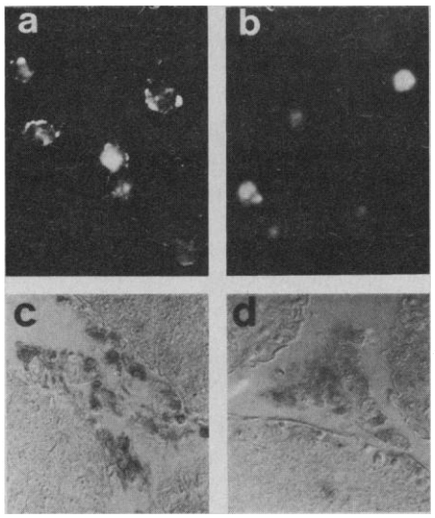

Fig. 2. Reactivity of C11 with rat Leydig cells from animals treated with hCG and HCG + Tamoxifen (see Table 1). (a) Isolated Leydig cells treated with hCG showing marked immunofluorescence. (b) Isolated Leydig cells treated with hCG + Tamoxifen showing weak immunofluorescence (×450). (c) Paraffin section from a testis treated with hCG showing marked immunoperoxidase staining in the cytoplasm of the Leydig cells. (d) Paraffin section from a testis treated with hCG + Tamoxifen showing weak immunoperoxidase staining in the Leydig cells (×300).

Fetal and neonatal rat Leydig cells are refractory to desensitization by gonadotropins and show low aromatase activity with consequently low or nearly undetectable amounts of E_2 receptors (*6*). These characteristics suggest that the 27,000 molecular weight estrogen-regulated protein would not be present in these immature cells; this is consistent with our results.

Leydig cells from normal human testis (biopsy specimens from 32- and 56-year-old men within 6 hours after death) also showed positive immunofluorescence when incubated with C11 (Fig. 1g). The histochemical reaction revealed that the steroidogenically functional human Leydig cells were positive for fluorescence. The Leydig cells isolated from rat and human testes also reacted with C11 when the ABC method was applied (data not shown).

The paraffin sections of the rat and human testes were stained by the ABC technique as described (*2*) with a minor modification: the slides with C11 were incubated for 72 hours at 4°C in a moist chamber. The estrogen-induced protein was detected in the Leydig cells of both rat and human testes (Fig. 1, e and h).

To determine whether the estrogen-induced protein present in human and rat Leydig cells undergoes modifications after administration of hCG, E_2, or Tamoxifen, we performed studies in vivo and in vitro (Table 1). The estrogen-regulated protein was detected in the Leydig cells obtained from the rat testes in vivo and in the human Leydig cells maintained in vitro (*4*). The Leydig cells exposed to hCG or E_2 showed the highest intensity (Fig. 1c and Fig. 2), while cells treated with Tamoxifen showed only low specific staining (Fig. 2), less than that observed in control cells. That a positive reaction was observed under control conditions was not surprising because detectable concentrations of estrogen are present in the basal state (*4*).

In previous studies with the double-labeling technique, both the rat Leydig cell protein and that from human MCF-7 cells were specifically stimulated by E_2 and inhibited by antiestrogens (*4*, *7*). By immunocytochemistry the positive reaction was not abolished in Tamoxifen-treated Leydig cells, but the staining intensity decreased. A similar effect was observed in nafoxidine-treated MCF-7 cells in culture (*8*). In addition, the proteins have similar molecular weights: 27,000 for the Leydig cell protein (*4*) and 28,000 for the MCF-7 cell line protein purified by monoclonal antibody affinity chromatography (*9*). Finally, C11 showed estrogen target-organ reactivity and not multiple-organ reactivity (*2*).

Our finding that C11 has cross-species specificity will facilitate studies of the estrogen-regulated protein with the use of animal models. Our results also indicate that this protein occurs in Leydig cells of the human testis. Therefore, C11 should prove useful for studies of the association between the Leydig cell protein and testicular abnormalities as well as for investigations of the structural and biological characteristics of the Leydig cell protein.

Table 1. Presence of the estrogen-regulated protein in rat and human Leydig cells. For the studies in vivo, adult male rats (n = 6 per group) were killed 16 hours after the first injection with vehicle or drug, and the testes were removed. Testes from each group were fixed in Bouin's solution, embedded in paraffin, and processed for the ABC technique (*2*). The remaining testes were processed to isolate the Leydig cells as described (*4*) and prepared for immunofluorescence (*5*) or the ABC technique. For the studies in vitro, Leydig cells from normal human testes obtained at autopsy were prepared for short-term culture as described (*4*). The immunostaining intensity was measured from photomicrographs of identical magnification and exposure by two observers in a blind-type study and was graded with an arbitrary three-point scale counting 200 cells per sample (see Figs. 1 and 2 for examples of enhanced or reduced staining intensity).

Treatment	Immunostaining intensity
Rat Leydig cells (in vivo)	
Control*	++
hCG†	+++
Tamoxifen‡	+
Tamoxifen + hCG§	+
Human Leydig cells (in vitro)	
Control*	++
hCG†	+++
Estradiol‖	+++
Estradiol + Tamoxifen¶	+

*In vivo, animals injected with vehicle; in vitro, untreated Leydig cells. †In vivo, hCG (5 μg) injected subcutaneously; in vitro, cultures exposed to hCG (2 μg) for 14 hours. ‡Tamoxifen citrate (Imperial Chemical Industries, Mecclesfield, England), 1 μg injected intramuscularly and 1 μg injected intraperitoneally 2 hours later. §Tamoxifen (1 μg) injected intramuscularly, 5 μg of hCG (Pregnyl, Organon) injected subcutaneously 1 hour after the first injection, and 1 μg of Tamoxifen injected intraperitoneally 2 hours after the first injection. ‖Cultures exposed to 100 ng of 17β-estradiol (Sigma) for 14 hours. ¶Cultures exposed to 100 ng of estradiol and 0.6 μg of Tamoxifen for 14 hours.

We thank W. L. McGuire, D. J. Adams, and D. P. Edwards, who provided the antibody to the estrogen-regulated protein from MCF-7 human cancer line used in this study. This antibody was raised in their laboratory at the University of Texas Health Science Center, San Antonio (NIH grant CA 11378).

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11. We thank W. L. McGuire, D. J. Adams, and D. P. Edwards, who provided the antibody to the estrogen-regulated protein from MCF-7 human cancer line used in this study. This antibody was raised in their laboratory at the University of Texas Health Science Center, San Antonio (NIH grant CA 11378).

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