to 20×10^6 cells of line D1 acquired resistance to active AA. The reactivity of line A2 to collagen type II may be explained by some cross-reactivity between antigens of *M. tuberculosis* and collagen. However, this hypothesis can only be tested with cloned A2 cells. In any case, our results indicate that lines of autoimmune effector T lymphocytes can be isolated by their response to bacterial antigens and that such cells can vaccinate against experimental arthritis. Rheumatoid arthritis, although it seems to arise spontaneously, may be triggered by an environmental agent—possibly infective (*15*)—that initiates a self-perpetuating autoimmune process.

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Human Endometrial Adenocarcinoma Transplanted into Nude Mice: Growth Regulation by Estradiol

Abstract. *A model for studying the growth of primary tumors of human endometrium and its regulation by 17β-estradiol has been developed in which ovariectomized nude mice are used as recipients. The receptors for sex steroids are maintained during serial transplantation of the tumor in this system. Although the rate of growth of receptor-negative endometrial tumors transplanted into ovariectomized nude mice is unaffected by the sustained presence or absence of estradiol, the growth of receptor-positive tumors is significantly increased by estradiol. Receptor-positive tumors treated with estradiol produced elevated concentrations of progesterone receptor. That the progesterone receptor is functional in this tumor is evident from the induction of estradiol 17β-dehydrogenase activity upon progestin administration. These findings are consistent with receptor-mediated regulation of growth of endometrial carcinoma.*

Steroid actions on target tissues are thought to be mediated through steroid-specific, saturable, high-affinity receptor proteins (*1*). Accordingly, the growth of tumors that are positive for the estrogen receptor may be modulated by 17β-estradiol (E_2), whereas the growth of estrogen receptor–negative tumors may be steroid-independent. However, studies of a breast tumor cell line (MCF-7) in nude mice, of breast cancer tissue, and of other E_2 target tissues have suggested that some other host factors may be involved in E_2-mediated growth (*2, 3*). We report here our studies with primary adenocarcinomas of human endometrium transplanted into ovariectomized athymic nude mice (*4*). Our results provide evidence that the steroid-receptor mechanism participates in the accelerated growth of receptor-containing tumors exposed to estrogen, but has no such effect on tumors lacking receptor. We also report on the maintenance of receptors during serial transplantation and the induction of a progestin-sensitive enzyme, estradiol 17β-dehydrogenase in the ovariectomized nude mouse.

Two primary endometrial adenocarcinomas, one well differentiated and one poorly differentiated, were transplanted subcutaneously in the infrascapular region of ovariectomized Balb/c (nu/nu) nude mice. Pallets of E_2 were implanted in half of the animals, while the others served as controls. The rate of growth of the well-differentiated tumor was dramatically increased by the sustained presence of E_2 (Fig. 1, A and B). The average weight of tumors in E_2-exposed mice at 6 weeks was more than ten times that of controls (controls, 0.087 g; E_2-treated, 1.03 g). In contrast, the growth rate of the poorly differentiated adenocarcinoma was unaffected by administration of E_2 (Fig. 1, C and D). The average tumor weights at the time the mice were killed were 1.65 g for controls and 1.56 g for the E_2-treated mice.

The estradiol and progesterone receptor concentrations were determined in freshly obtained primary tumors and after each passage into nude mice by Scatchard plot analysis (*5*) of data for specific tritiated ligand binding (Table 1). Significant numbers of E_2- and progesterone-specific binding sites were found in the freshly obtained well-differentiated adenocarcinoma (estrogen receptor, 43 fmole per milligram of dissociation constant protein, (K_d), $3.4 \times 10^{-10} M$; progesterone receptor, 1090 fmole per milligram of protein, K_d, $3.9 \times 10^{-9} M$). Both estrogen and progesterone receptors were maintained in tumors transplanted into ovariectomized nude mice (controls). However, when the tumors were retransplanted, the estrogen receptor levels were maintained but there was no detectable progesterone binding activity, presumably because of the absence of circulating E_2. That mechanisms for the synthesis of progesterone receptors were still intact, and that sensitivity to E_2 was retained, was confirmed by the ability of the tumor to maintain high concentrations of cytoplasmic progesterone receptors in nude mice with implanted pellets of E_2 (serum concentrations of 200 to 300 pg/ml for 60 days). The induction of progesterone receptors by E_2 implants was reproducible during the second passage of the tumor into nude mice. The lower cytoplasmic estrogen receptor concentrations in these animals may reflect the nuclear translocation of the receptor in the presence of circulating E_2.

The poorly differentiated tumor also contained estrogen and progesterone receptors initially. We believe that the high concentrations of progesterone receptor in the original tumor may be due to heterogeneity, since areas of well-differentiated tumor were observed in the original surgical specimen. We have recently reported on the problem of tumor and tissue heterogeneity and how this might interfere with the interpretation of

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receptor data in endometrial carcinoma (*6*). Transplantation of this tumor into nude mice resulted in the disappearance of estrogen and progesterone receptors in control animals as well as in E_2-exposed animals. Histologic examination of tumors obtained after transplantation revealed a homogeneous, anaplastic tumor.

The direct action of progestins on the induction of estradiol 17β-dehydrogenase activity in human endometrium has been well established (*7, 8*). To test the progestin sensitivity of the receptor-positive tumor, we gave each animal, during the first passage, 1 mg of Depo-Provera (intramuscularly) 10 days before we removed the tumors for determination of the enzyme activity. The enzyme activity in tumors from control animals remained unchanged after progestin administration [controls, 1.35 nmole of E_1 (estrone) formed per milligram of protein per hour; progestin-treated, 1.12 nmole of E_1 formed per milligram of protein per hour]. However, there was a significant amount of progesterone receptor in one control tumor in which this was assayed, possibly because of a low concentration of circulating E_2 due to incomplete ovariectomy or to extraovarian sources. The tumor weight in this animal was not significantly different from the tumor weights of animals in the same group. In the tumors of animals exposed to E_2 there was a more than twofold increase

Table 1. Concentrations of estrogen and progesterone receptors in endometrial carcinomas transplanted into nude mice. Cytosolic receptors were assayed in supernatants obtained by centrifugation at 105,000*g* of tumor homogenates in TED buffer (20 m*M* tris-Hcl containing 3 m*M* EDTA, 1 m*M* dithiothreiotol, and 0.01 percent sodium azide, *p*H 7.8). The radioactive ligands, [^{3}H]estradiol and [^{3}H]progesterone, were used for determining the estrogen and progesterone receptors, respectively. For determination of total bound [^{3}H]E_2, portions (200 μl) of cytosol were incubated at 30°C for 3 hours with increasing concentrations of [^{3}H]E_2 (0.2 to 2 n*M*) and 100-fold concentrations of dihydrotestosterone. Identical samples containing a 100-fold excess of unlabeled E_2 served for determination of nonspecific binding. The cytosol progesterone receptor concentrations were determined as described (*8*). For determination of total [^{3}H]progesterone binding, 200 μl portions of cytosol preparation were incubated at 0° to 4°C for 3 hours with increasing concentrations of [^{3}H]progesterone (1 to 10 n*M*) and 100-fold concentrations of cortisol. Identical samples containing a 100-fold excess of unlabeled progesterone served as nonspecific controls. Free labeled ligands were separated from bound radioactivity by treatment with Dextran-coated charcoal (10 minutes at 0°C for estrogen receptors and 5 minutes at 0°C for progesterone receptors). Receptor concentrations were estimated by Scatchard plot analysis (*5*) of specific labeled ligand binding data. Receptor concentrations were determined in only two tumors from each group (control mice and mice with E_2 implants) at transplantation No. 1 (A). The other two animals in each group received progestin for studies of estradiol 17β-dehydrogenase induction. Receptor values represent determinations in an individual tumor specimen. The apparent dissociation constant ranged from 0.3 to 0.9 n*M* for [^{3}H]estrogen-specific binding sites and from 0.1 to 0.5 n*M* for [^{3}H]progesterone-specific binding sites, and did not appear to vary upon serial transplantation. NP, assay not performed for lack of sufficient sample.

Tumor passage No.	Treatment	Estradiol receptor (fmole/mg protein)	Progesterone receptor (fmole/mg protein)
		Tumor grade I	
Fresh		43	1090
1	Control	NP; 260	NP; 280
	E_2	11; 84	545; 740
2	Control	344; NP	< 10*; <10
	E_2	< 10; 36	490; 1000
		Tumor grade II	
Fresh		15	1050
1	Control	< 10; < 10	50; < 10
	E_2	< 10; < 10	< 10; < 10
2	Control	< 10; < 10	< 10; < 10
	E_2	< 10; < 10	< 10; < 10

*The limit of detectability of receptor in our assay system is 10 fmole per milligram of protein.

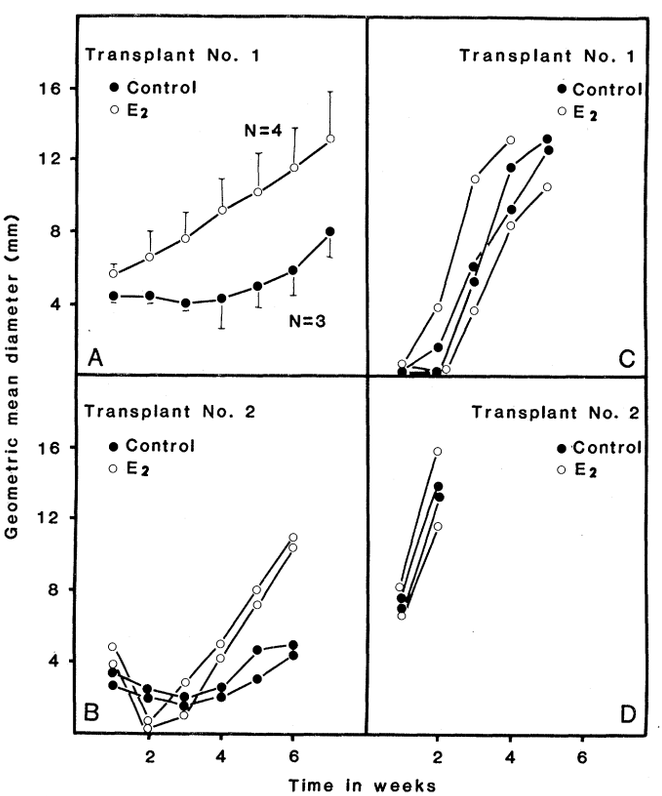

Fig. 1. Effect of 17β-estradiol on the growth of human endometrial adenocarcinoma transplanted into nude mice. About 100 to 200 mg of tissue from two primary endometrial adenocarcinomas (A) well differentiated and (C) poorly differentiated, were subcutaneously transplanted in the infrascapular region of ovariectomized nude mice. Half of the animals served as controls while the other half received subcutaneous implants of E_2 pellets (Innovative Research, Maryland). The pellets release E_2 at a rate that results in plasma E_2 levels of 200 to 300 pg/ml for up to 60 days. The tumor volume was determined at weekly intervals with vernier calipers. In (A), the results are shown as means ± standard deviation. There was no tumor growth in one of the controls at transplantation No. 1, and this result was not included in the figure. (B) Tumors from E_2-treated animals were used for transplantation No. 2. (D) A control tumor was used for transplantation. Average weights of tumors were: (A) and (B), controls 87 mg and E_2 implant 1.03 g; (C) and (D), controls 1.65 g and E_2 implant 1.56 g.

in estradiol 17β-dehydrogenase activity upon progestin administration (in E_2 controls, 4 nmole of E_1 was formed, but in progestin-treated animals, 10.1 nmole of E_1 was formed per milligram of protein per hour). A progestin-induced change in subnuclear vacuolization was observed only in histological sections of tumors of E_2-exposed animals treated with Depo-Provera.

Shafie (*2*) suggested that estrogen had an indirect action on tumor growth since the growth of MCF-7 cells was entirely dependent on E_2 in nude mice but was independent of estrogen in culture. Several other investigators (*3*) have previously proposed similar indirect mechanisms in tumor growth in other estrogen target tissues and have attributed this to the involvement of host-related factors, such as estromedins. 17β-Estradiol has also been shown to reduce natural killer cells in mice (*9*). If host factors, rather than receptor-mediated events, are solely responsible for the differential rate of growth of tumors in E_2-exposed nude mice, then one would expect a similar effect of E_2 on the growth of estrogen receptor negative as well as positive endometrial tumors. However, the present studies show a distinct difference in regard to E_2-dependent growth only in receptor-containing endometrial adenocarcinoma. As far as we are aware, this is the first direct demonstration of accelerated growth of primary tumors of human endometrium by E_2. We have recently suggested that endometrial carcinoma may result from aberrant endometrial cell differentiation (*10*). According to our proposed scheme the growth rate of only the receptor-positive endometrial tumors could be influenced by E_2. The results presented here confirm this prediction. Both of these tumors have been serially transplanted into nude mice five times with maintenance of their morphologic and biochemical characteristics.

Several epidemiologic studies have indicated a relation between the use of exogenous estrogens and an increased incidence of endometrial carcinoma. Our results suggest that E_2, by virtue of its ability to accelerate the growth rate of an already existing tumor, might lead to increased detection. The observation that estrogen use in postmenopausal women primarily increases the risk of development of endometrial cancers of early stage and low grade (*11*) supports such an interpretation.

Currently, there are no other model systems available for studying endometrial carcinoma and its hormonal regulation. The established endometrial cancer cell lines or organ culture systems either do not contain receptors for steroid hormones or are unresponsive to steroids added to the culture medium. Recently, we demonstrated that the lack of progestin sensitivity in cultured endometrial carcinoma explants in vitro is due to progesterone receptor instability under culture conditions (*8*). Since the steroid receptors are preserved in human endometrial carcinoma during serial transplantation with the maintenance of progesterone receptor synthetic machinery as well as sensitivity to progestin, the ovariectomized nude mice system appears to be an ideal model for studying steroidal and antisteroidal regulation of endometrial growth.

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Dimethyl Sulfoxide Stimulates Tyrosine Residue Phosphorylation of Rat Liver Epidermal Growth Factor Receptor

Abstract. *Epidermal growth factor, a potent mitogen, stimulates phosphorylation of its 170,000-dalton plasma membrane receptor. Dimethyl sulfoxide selectively increased phosphorylation of the epidermal growth factor receptor in rat liver microsomal fraction. Maximal stimulation occurred at 15 to 25 percent dimethyl sulfoxide and resembled the effect of epidermal growth factor in magnitude and rapidity. Like epidermal growth factor, dimethyl sulfoxide selectively stimulated tyrosine residue phosphorylation of this protein.*

A newly described class of protein kinases that selectively phosphorylate tyrosine residues has been linked to the regulation of normal and neoplastic growth because of the association of these kinases with viral transformation and with growth factor receptors (*1–6*). The transforming gene products of the Rous sarcoma virus and other RNA tumor viruses are protein kinases that phosphorylate tyrosine residues in cellular fractions (*2*) and in cultured cells (*3*). The activity of the enzyme is correlated with the appearance of the transformed phenotype. Nucleic acid hybridization studies have shown that the transforming retrovirus genes that code for these kinases are homologs of normal cellular genes (*4*).

Tyrosine residue kinase activity is also associated with growth factor receptors (*5, 6*). Epidermal growth factor (EGF), a mitogen in many tissues and cell lines (*7*), stimulates the phosphorylation of tyrosine residues in membrane proteins of a human epidermoid carcinoma line, A-431, and of several other cell types and tissues (*5, 8, 9*). One or two EGF-stimulated phosphoprotein bands of molecular weight 150,000 to 180,000 have been observed in each instance. The EGF-stimulated kinase and its substrate purify with the A-431 cell EGF receptor on affinity chromatography; the kinase and substrate are immunoprecipitated along with the EGF receptor by an antibody to the receptor (*10*). These findings indicate that the kinase and substrate are intrinsic to the EGF receptor. This complex could mediate EGF action by phosphorylating cellular proteins on tyrosine residues (*11*).

Hepatic DNA synthesis is stimulated by EGF in vitro and in vivo (*12*). We

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