Oncology Research, Vol. 13, pp. 147–160
Printed in the USA.

0965-0407/02 $20.00 + .0

Embryonal Mass and Hormone-Associated Effects of Pregnancy Inducing a Differential Growth of Four Murine Tumors

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(Submitted February 7, 2002; sent for revision July 8; received August 5; accepted August 9, 2002)

A differential effect of pregnancy on the growth of subcutaneous implants of four murine tumors has been observed. Two tumors lacking receptors for progesterone and estrogen [methylcholanthrene-induced fibrosarcoma (MC-C) and spontaneous lymphoid leukemia (LB)] exhibited slow kinetics throughout the course of pregnancy, although inhibition was stronger beyond day 10. On the other hand, one of two tumors bearing receptors for progesterone and estrogen [medroxyprogesterone (MPA)-induced mammary adenocarcinoma (C7HI)] exhibited three phases: up to days 8–10 of gestation the tumor grew faster than in virgins, between days 8–10 and 15 it reached a plateau, and beyond day 15 a sharp reduction in tumor mass was observed. The other tumor [mouse mammary tumor virus (MMTV)-induced mammary carcinoma (T2280)] behaved as a typical pregnancy-dependent tumor (i.e., it grew in pregnant but not in virgin mice, regressed soon after delivery, and reassumed its growth at the middle of a second round of pregnancy). Neither MPA nor estrogen affected MC-C and LB tumor growth. On the other hand, MPA-treated mice enhanced C7HI tumor and reciprocally C7HI tumor-bearing mice treated with estrogen strongly inhibited tumor growth. As for T2280, neither MPA nor estrogen alone could promote tumor growth and, in consequence, no tumor developed. However, when MPA plus estrogen was administered in a schedule simulating the successive appearance of these hormones in pregnancy, T2280 grew even faster than in pregnant mice. When the four tumors were implanted in mice bearing grafts of embryonal tissues (teratomas), all of them were inhibited. This antitumor effect was similar to that observed in pregnancy when tumors unresponsive to progesterone and estrogen were tested. On the other hand, with tumors bearing progesterone and estrogen receptors, differences in tumor growth were detected in pregnant and teratoma-bearing mice. This suggested the existence during pregnancy of two factors potentially acting on tumor growth. First, a progesterone and estrogen-mediated hormonal component, which would exert either inhibitory or stimulatory effects only evidenced with tumors bearing hormonal receptors. Secondly, an antitumor effect proportional to the growing embryonal mass, inhibiting all tumors independently of their origin or hormone responsiveness. This antitumor effect could be attributed to a heat-resistant serum factor (1000–1200 Da molecular weight) presumably associated with the pathway of the arachidonic acid metabolism. The interplay between the hormonal component and the serum factor associated with embryonal mass could account for some of the largely heterogeneous and otherwise unexplained effects of pregnancy on tumor growth reported in the literature and illustrated by the four tumors studied here.

Key words: Murine tumors; Pregnancy; Tumor dormancy; Embryonal mass; Hormone regulation

The influence of pregnancy on the development of clinical and experimental cancer is a matter of a long controversy. A large number of reports have claimed that the course of the neoplastic disease is aggravated during pregnancy. Some authors have suggested that this would be due to a state of tolerance to fetal antigens associated with tumors (oncofetal antigens) (1,2). Others have postulated that hormonal changes induced during pregnancy could play a main role. Supporting the latter are the pregnancy-dependent mammary tumors induced by mouse mammary tumor virus (MMTV[3]) in mice. These tumors appear in the middle of pregnancy when serum levels of progesterone and estrogen are high and continue to grow until the end of gestation; they regress soon after delivery and reappear at subsequent pregnancies when hormone serum levels are high again, suggesting that progesterone and/or estrogen play a main role (3–5). A hormone-mediated mechanism has also been postulated to explain the accelerated course of dimethyl-benzanthracene (DMBA)-induced mammary tumors in pregnant rats (6) and the increased rate or the worsened prognosis of some breast, pancreas, and brain tumors detected in women during pregnancy (7–9).

On the other hand, many studies (as many as the ones claiming a tumor-promotion effect of pregnancy) have demonstrated that pregnancy has no influence, or even has an inhibitory effect, on both the incidence and development of some human and animal tumors, including mammary cancer, lymphomas, and other hematologic malignancies (10–16). In addition, experiments performed by Moroson and Ioachim (17,18) revealed that rats bearing grafts of embryonal tissues (teratomas) were

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[3]Abbreviations used: MMTV, mouse mammary tumor virus; MPA, medroxiprogesterone acetate; TD_{50}, number of tumor cells able to grow SC in 50% of mice; LH, luteinizing hormone; FSH, follicle stimulating hormone.

protected against the induction of mammary carcinomas by nitroso-methylurea (NMU) and against the transplantation of carcinomas and lymphomas, suggesting that the embryonal tissues were a source of tumor-inhibitory factors.

Such contradictory data could be attributed to the use of different tumors and heterogeneous types of hosts, but the question why pregnancy displays such different and even opposite effects on tumor growth remains unanswered. We have studied four murine tumors widely differing in histological appearance, etiology, immunogenicity, and hormone responsiveness made to grow in pregnant, teratoma-bearing and virgin mice. Our goal was to try to conciliate in a coherent picture the previously reported and seemingly paradoxical observations concerning the effects of pregnancy on tumor growth.

MATERIALS AND METHODS

Animals

BALB/c mice of both sexes, 2–4 months old, were used. They were raised in our colony and maintained on Cooperación pellets (San Nicolás, Buenos Aires, Argentina) and water ad libitum. Animals were sex and age matched within each experiment.

Tumors

MC-C. The MC-C tumor is a fibrosarcoma induced in a 5-month-old BALB/c male after the SC implantation of a methylcholanthrene pellet. It was used within SC passages 5 and 20.

LB. The LB tumor is a T-lymphoid leukemia that arose spontaneously in a 6-month-old BALB/c male. It has been maintained by SC serial passages in syngeneic mice and was used between passages 68 and 88. More detailed description of this tumor is given elsewhere (19,20).

C7HI. The C7HI tumor is a highly metastatic mammary adenocarcinoma originated in a BALB/c female treated with 40 mg of medroxiprogesterone acetate (MPA) every 3 months for 1 year and maintained by syngeneic SC transplantation. It was used between passages 18 and 25. More detailed description of this tumor is given elsewhere (21).

T2280. The T2280 is a mammary tumor induced by a novel exogenous MMTV in a pregnant female mouse of BALB/c LA strain, a new variant of BALB/c mice (22). The tumor has been SC passaged in BALB/c mice and was used between passages 2 and 3.

All tumors were kept biofrozen and were thawed and SC passaged. Tumor volume was calculated according to the formula of Attia and Weiss (23): volume = $0.4(ab^2)$, where a and b are the larger and smaller diameters, respectively. TD_{50} is defined as the number of tumor cells able to grow SC in 50% of the mice.

Teratoma

After trophoblast remotion, mouse embryos of 10–14 days of gestation were finely minced and homogenized in medium. The homogenized embryonal tissues were implanted SC through a small skin incision in the dorsal side at a dose of one homogenized embryo for each male and female recipient mouse. Nodules of embryonal tissues (teratomas) were first palpable 10–15 days after implantation, attaining their maximum size at 30–45 days, and remained unchanged thereafter. Histological analysis revealed that teratomas were a complex mixture of cysts and well-differentiated tissues derived from the three germ layers; in our hands none of the mouse teratomas showed malignant transformation.

Hormonal Treatments

MPA-Treated Mice. Mice received a single injection of 40 mg of MPA of the depot type (Farlutale 500, Montedison, Milan, Italy). Vaginal smears showed that females were in a state of pseudopregnancy; after 3 months they began to cycle again. Serum levels of MPA, 30–60 days after MPA injection, were measured as described previously (24), by high pressure liquid chromatography, in five samples with a mean value of 41.4 ± 3.6 ng/ml. This value was similar to that measured in pregnant mice between days 3 and 16 of gestation (25,26).

Estrogen-Treated Mice. Mice received a SC implant of silastic pellet containing 0.5 mg estradiol benzoate (Sigma, St. Louis, MO, USA). Serum levels of estrogen were measured as previously described (21) by radioimmunoanalysis (RIA), 20 and 50 days after pellet implantation, with values of 58.7 ± 15.4 ($n = 3$) and 52.3 ± 14.8 ($n = 3$) pg/ml, respectively. These values were similar to that observed in pregnant mice between days 16 and 21 (day of delivery) (25).

Immunization Assays

Tumor Implantation and Excision. SC tumors were surgically excised when their volume reached 400–600 mm^3. Two weeks later, tumor challenge was carried out in the contralateral flank of the mice that had not relapsed.

Irradiated Cells. Cell suspensions were irradiated with 90 Gy in a plastic irradiation chamber; X-rays were generated in a Phillips 250/15 radiotherapy apparatus at 220 kV, 14 mA and filtered with 1 mm Al. The dose rate was 3.13 Gy min^{-1} at a focus–target distance of 29 cm. Animals were pretreated with two SC doses of 2–4 × 10^6 irradiated tumor cells, 7 and 14 days before tumor challenge.

Progesterone and Estrogen Receptors Studies

Progesterone (PR) and estrogen (ER) receptors were detected by immunohistochemical analysis, performed on formalin-fixed paraffin-embedded tissues. Anti-PR and anti-ER (Santa Cruz Biotechnology Inc., CA, USA) polyclonal antibodies were diluted 1:100. Staining was achieved using a biotin-conjugated secondary antibody and the ABC horseradish peroxidase detection reagent (Vector Lab) incubated for 30 min each. Chromogen was diaminobenzidine. Content was expressed as the percentage of the number of PR- or ER-positive cells over the number of total cells. PR and ER were also

measured by the charcoal technique as previously described (27,28). The hormone receptor levels were expressed as fentomoles per milligram cytosol protein. Chemicals used for these assays were [17 α-methyl-^{3}H]R5020 (87 μCi/mmol) and [2,4,6,7-^{3}H]estradiol (169 μCi/mmol) and were purchased from NEN (Boston, MA, USA).

Anti-Inflammatory Drugs

Indomethacin (Sigma) was diluted in 0.015 M NaCl to obtain a dose of 0.5 mg/kg in 0.2 ml, and it was administered daily by the IP route for 2 consecutive days.

Lung Nodule Evaluation

C7HI tumor-bearing mice were anesthetized with ether and killed by cervical dislocation. The lungs were removed and rinsed in distilled water, and surface metastatic nodules (diameter ≥0.1 mm) present in both lungs were counted under a dissecting microscope at 10× magnification. The lungs were then fixed in 15% formaldehyde, 5% acetic acid, and 80% methanol and processed for tissue sectioning. Serial thin sections of 3–4 μm were stained with hematoxylin and eosin. Microscopic lung metastases (diameter <0.1 mm) were counted under a microscope at 125× magnification in fields with 0.32 mm diameter.

Mitosis and Apoptosis Counting

Mitotic number was evaluated per high power field (HPF) at 600× magnification in well-preserved areas (nonnecrotic) with similar cell densities, while taking into account metaphase and anaphase. Apoptotic cells were detected morphologically on hematoxylin and eosin-stained slices: nuclear condensation and picnosis increase, cell shrinkage, membrane blebbing, chromatin marginalization, and apoptotic bodies were considered as features of apoptotic cells.

Angiogenesis Assay

Pregnant (10–12 days of gestation) and virgin female mice received a SC implant of murine syngeneic placenta or neonatal testis (1 mm^3), 10^6 peritoneal elicited macrophages (PEM), or 2.5×10^5 MC-C, LB, or C7HI tumor cells. Five to 7 days later, mice were sacrificed and dermis at the site of implant was examined. Macroscopic vessels were evaluated under a dissecting microscope at 10× magnification. In order to quantify the neovascularization, photographs of the skin at the site of implant were projected on a screen and vessel density by cm^2 was counted. Microscopic vessels were evaluated in thin sections as previously described (29) using both hematoxylin-eosin stain and antibody against von Willebrand factor at 125× and 250× magnification, respectively.

Medium

The medium used was RPMI-1640 without phenol red (Gibco, USA), with penicillin G sodium (10 μg ml^{-1}), streptomycin sulphate (25 μg ml^{-1}), and amphotericin B as fungizone (25 μg ml^{-1}). When necessary, medium was supplemented with 5–10% fetal calf serum.

Serum

Mice were bled through the retroorbital plexus. The blood was kept at room temperature for 1 h for clotting. Serum obtained after centrifugation was stored at –20°C until used. For in vitro assays, serum was decomplemented at 56°C for 30 min.

Hormone Levels in Serum

Serum samples were measured in duplicate for progesterone, estrogen, prolactin, luteinizing hormone (LH), and follicle stimulating hormone (FSH) by radioimmunoanalysis (RIA) using specific anti-hormone antiserum. For progesterone, the DSL-500 active 17 α-OH progesterone-coated tube RIA kit was used; for estrogen the coat-A count estradiol-6 kit was used; for prolactin, LH, and FSH, RIA kits provided by the NIDDK were utilized. For progesterone, prolactin, LH, and FSH the values were expressed in ng/ml; for estrogen the values were expressed in pg/ml.

[^{3}H]Thymidine Uptake Assay

Proliferation of tumor cells in 0.1 ml of medium was determined in 96-well microtiter plates (Corning, NY, USA) in the presence of 0.1 ml of several twofold dilutions of serum from virgin, pregnant, or teratoma-bearing mice. Immediately after adding the serum, the cultures were pulsed with [^{3}H]thymidine (Dupont, NEN, Boston, USA) at a final concentration of 1 μCi ml^{-1} and the mixture was incubated at 37°C for 18–24 h in a 5% carbon dioxide humidified atmosphere and harvested with an automated cell harvester. The radioactivity incorporated into the cells was counted in a liquid scintillation Beta counter (Beckman). The assays were usually carried out in triplicate or quadruplicate and the percentage of inhibition for each serum dilution was determined as: % inhibition = 1 – (cpm of serum – background)/(cpm of medium – background) × 100.

The titer of growth inhibitory activity was defined as the reciprocal of the serum dilution producing 50% inhibition of [^{3}H]thymidine uptake by cells compared with medium only, and was expressed as growth inhibitory units 50 per ml (GIU_{50}/ml).

^{51}Cr Released Assay

Spleen cell or peritoneal macrophage suspensions were mixed in triplicate with 10^4 ^{51}Cr-labeled tumor or YAC-1 cells at a effector/target ratio of 100:1. The mixture was incubated for 4 h at 37°C in a 5% CO_2 humidified atmosphere. After this period, the cells were centrifuged and radioactivity of the supernatant was considered as a measure of antitumor activity of the effector cells tested. The percentage of cytotoxicity was calculated by the formula: % specific cytotoxicity = (experimental counts – spontaneous release counts)/(maximal counts – spontaneous release counts) × 100. Spontaneous release was always less than 5%.

Complement-dependent cytotoxic antibodies against

tumor cells labeled with ^{51}Cr were evaluated according to the method of Rao et al. (30). Titer of cytotoxic antibodies was defined as the reciprocal of the serum dilution producing 50% of specific lysis.

Enzymes

Enzymatic treatment was performed as described previously (31). Enzymes used were: pronase, trypsin, DNase, RNase, α and β glucosidases, and lipase (Sigma Co.).

Statistical Analysis

Student's *t*-test and Mann-Whitney *U*-test were used. Differences were considered significant when the value was $P \leq 0.05$.

RESULTS

Immunogenicity of Murine Tumors

Immunogenicity of MC-C, LB, C7HI, and T2280 tumors was evaluated using two classical immunization assays: tumor implantation and excision, and pretreatment with irradiated tumor cells. As shown in Table 1, MC-C exhibited a strong immunogenicity because its TD_{50} markedly increased after immunization procedures. On the other hand, LB, C7HI, and T2280 behaved as nonimmunogenic tumors.

Progesterone and Estrogen Receptors

Taking into account that progesterone and estrogen are known to be important hormones affecting both incidence and growth of transplantable tumors in the mouse model (3), progesterone and estrogen receptors were evaluated on MC-C, LB, C7HI, and T2280 tumors. As shown in Table 2, progesterone and estrogen receptors were consistently demonstrated on C7HI and T2280 tumor cells; on the other hand, minimal or nondetectable values were found on MC-C and LB tumor cells.

Table 1. Immunogenicity of Four Murine Tumors Expressed as the Increase in TD_{50} in Immunized Compared With Control Mice

		Immunized	
Tumors	Control (TD_{50})	Implantation and Excision (TD_{50})	Pretreatment With Irradiated Cells (TD_{50})
MC-C	41,600 ($n = 5$)	>2,000,000 ($n = 4$)	>2,000,000 ($n = 1$)
LB	1,400 ($n = 3$)	<3,000 ($n = 2$)	1,200 ($n = 3$)
C7HI	5,500 ($n = 2$)	6,200 ($n = 2$)	NT
T2280	38,800 ($n = 2$)	21,700 ($n = 2$)	NT

TD_{50} (tumor dose 50) is the number of tumor cells able to grow in 50% of the mice. Mice were immunized against the tumors by using classical immunization assays: tumor implantation and excision, and pretreatment with irradiated tumor cells. *n* is the number of experiments in which TD_{50} was calculated. Standard error never exceeded 15% of mean value. For experiments with T2280 tumor, mice had received a SC dorsal implant of progesterone plus estrogen. NT: nontested.

Table 2. Progesterone and Estrogen Receptors in MC-C, LB, C7HI, and T2280 Murine Tumors

Tumors	Progesterone Receptor	Estrogen Receptor
MC-C	1.69 ± 0.31 ($n = 4$)	0.43 ± 0.09 ($n = 5$)
LB	1.29 ± 0.22 ($n = 3$)	0.32 ± 0.15 ($n = 4$)
C7HI	25.00 ± 1.16* ($n = 4$)	19.47 ± 3.96† ($n = 4$)
T2280	52.10 ± 5.74* ($n = 4$)	59.33 ± 9.03* ($n = 4$)

Hormone receptors were measured by immunohistochemical analysis; content was expressed as the percentage of the number of progesterone or estrogen positive cells over the number of total cells. Each value represents the mean ± SE of *n* individual tumors. For each tumor 10–20 fields were examined in thin sections at 800× magnification. On C7HI and LB tumor cells, hormone receptors were also measured by the charcoal technique: on C7HI, values of progesterone and estrogen were 167.7 ± 48.4 ($n = 4$) and 34.8 ± 8.8 ($n = 4$) fentomoles per milligram of cytosol protein, respectively; on LB, values were under the limit of detection of the method ($n = 3$).
*$P < 0.001$ compared with both MC-C and LB tumors.
†$P < 0.001$ compared with MC-C and $P < 0.01$ compared with LB.

Effect of Pregnancy on Tumor Growth

Female mice received 1×10^5 MC-C, LB, C7HI, and T2280 tumor cells SC, 3–6 days before the onset of pregnancy. The growth of MC-C and LB tumors was significantly retarded in pregnant compared with control virgin mice (Fig. 1A, B). When survival of tumor-bearing mice exceeded 40 days (as in the case of MC-C tumor bearers), inhibition was also observed in a second round of pregnancy. For mice bearing MC-C tumor, survival time (mean ± SE of three experiments) was 98.6 ± 7.6 days in pregnant and 66.9 ± 5.5 days in virgin mice ($P < 0.01$); for mice bearing LB tumor, survival (mean ± SE of four experiments) was 24.7 ± 0.4 days in pregnant and 21.8 ± 0.7 days in virgin mice ($P < 0.02$).

As for C7HI tumor, its growth exhibited three phases. Up to days 8–10 of pregnancy, the tumor grew faster than in the controls; between days 8–10 and 15 it reached a plateau; and beyond day 15, a dramatic reduction in tumor mass was observed, which was maximal at the time of delivery. On successive rounds of pregnancy a similar pattern was observed (Fig. 1C). As a whole, after 80 days of tumor growth the size of C7HI tumor was significantly smaller and its lung metastases were inhibited in pregnant mice compared with the controls (Table 3). A sample of C7HI tumor-bearing mice subjected to three rounds of pregnancy exhibited a largely prolonged survival time (196.0 ± 10.0 days, $n = 3$ mice) compared with that of virgin mice (113.0 ± 4.7 days, $n = 6$ mice), even though beyond day 80 no new

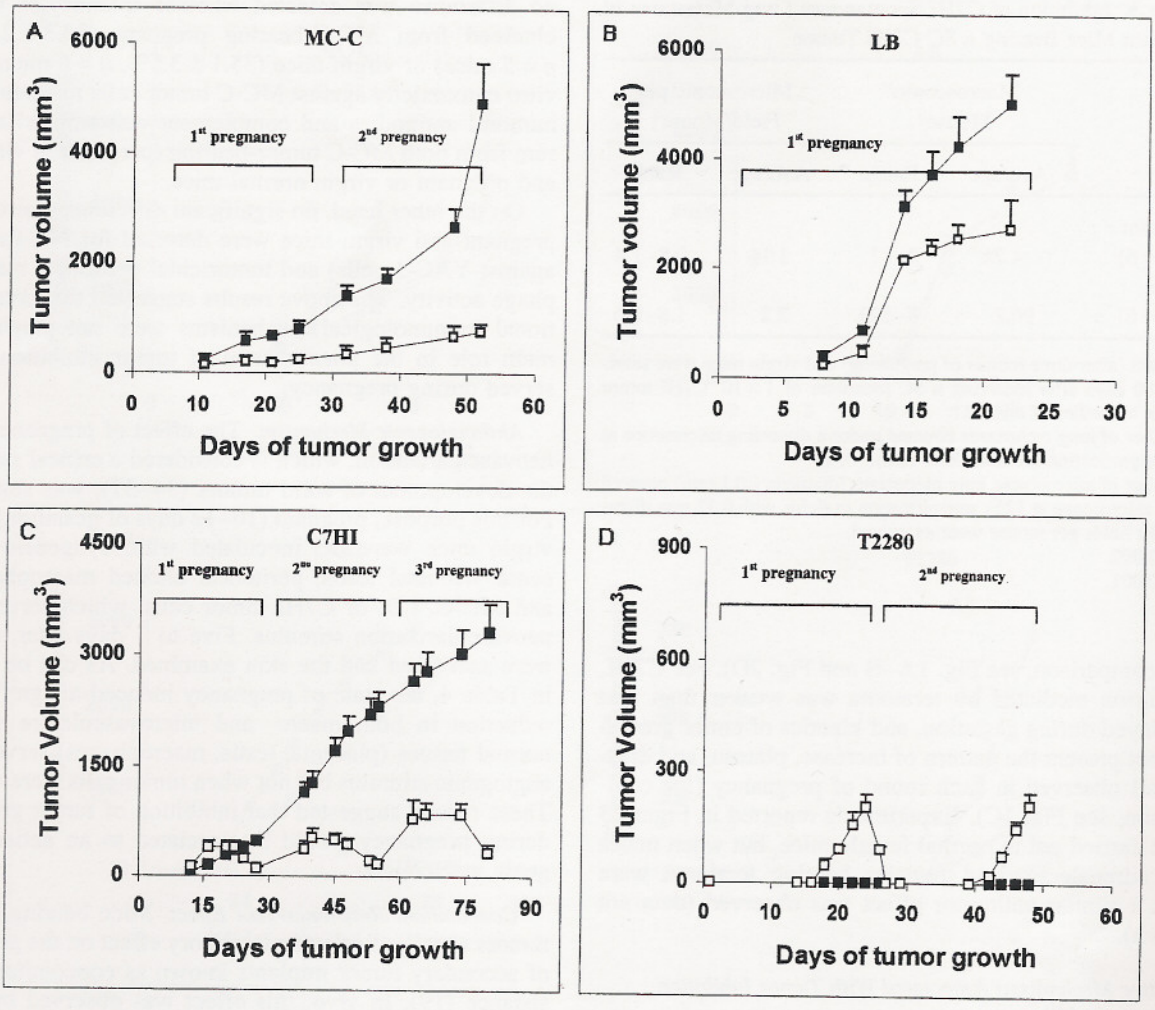

Figure 1. Effect of pregnancy on tumor growth. Comparative growth of MC-C (A), LB (B), C7HI (C), and T2280 (D) tumors in both pregnant (□) and virgin (■) mice. Tumor growth was initiated with a SC inoculum of 1×10^5 tumor cells, 3–6 days before the onset of pregnancy. (A) $P < 0.01$ day 25 of tumor growth, $P < 0.001$ day 21 and day 32 onwards. Mean of three experiments including 19 pregnant and 36 virgin mice. (B) $P < 0.05$ day 11, $P < 0.01$ day 14 onwards. Mean of four experiments including 56 pregnant and 41 virgin mice. (C) $P < 0.05$ days 12–16, $P < 0.001$ day 27 onwards. Mean of 24 pregnant and 24 virgin mice. (D) $P < 0.02$ days 18 and 42, $P < 0.001$ days 20–26 and 44 onwards. Mean of 14 pregnant and 12 virgin mice.

round of pregnancy was allowed; the difference was highly significant ($P < 0.001$).

On the other hand, T2280 tumor grew in pregnant but not in virgin mice; it regressed soon after delivery and reassumed growth at the middle of a second round of pregnancy (Fig. 1D), indicating that it is a pregnant-dependent mammary tumor (PDMT).

Tumor Growth in Progesterone- and Estrogen-Treated Mice

When MC-C and LB tumors were studied, no differences in kinetics nor in survival time were detected between MPA-treated, estrogen-treated, and control virgin mice (Fig. 2A, B). As for C7HI tumor, MPA-treated mice exhibited enhanced tumor development compared with that of virgins and reciprocally C7HI tumor-bearing mice only treated with estrogen showed a dramatic inhibition of tumor growth (Fig. 2C). As for mice bearing T2280 tumor cells, treatment with either MPA or estrogen alone did not promote tumor growth and, in consequence, as occurred in control virgins, no tumor developed. However, when MPA plus estrogen was administered in a schedule simulating the successive appearance of these hormones in pregnant serum, T2280 grew even faster than in pregnant mice (Fig. 2D).

Tumor Growth in Teratoma-Bearing Mice

In order to determine the effect of growing embryonal tissues on tumor development, SC growth of MC-C, LB, C7HI, and T2280 tumors was evaluated in teratoma-bearing and control virgin mice (for T2280, MPA plus estrogen had to be injected to permit tumor growth). Growth of the four tumors was restricted by the presence of a growing large teratoma (Fig. 3A–D) but not when the teratoma was regressing or incipient (data not shown). For MC-C, LB, and T2280, this tumor inhibition was similar to that observed in pregnant mice

Table 3. Inhibition of C7HI Spontaneous Lung Metastases in Pregnant Mice Bearing a SC C7HI Tumor

	Macroscopic/ Mouse*		Microscopic per Field/Mouse†	
Group	Average	Range	Average	Range
Pregnant ($n = 6$)	4.2‡	0–17	1.1§	0.8–1.4
Virgin ($n = 6$)	96.2	8–173	2.2	1.8–2.6

Pregnant (after three rounds of pregnancy) and virgin mice were sacrificed 80 days after receiving a SC inoculum of 1×10^5 C7HI tumor cells; n = number of mice.
*Number of lung metastases counted under a dissecting microscope at 10× magnification (nodules ≥0.1 mm).
†Number of microscopic lung metastases (diameter <0.1 mm) counted under microscope at 125× magnification in fields with 0.32 mm diameter; 30 fields per mouse were examined.
‡$P < 0.002$.
§$P < 0.001$.

(for comparison, see Fig. 1A–B and Fig. 2D). For C7HI, inhibition mediated by teratoma was weaker than that displayed during gestation, and kinetics of tumor growth did not present the pattern of increase, plateau, and drastic fall observed in each round of pregnancy (for comparison, see Fig. 1C). Experiments reported in Figure 3 were carried out in normal female mice, but when males and adrenalectomized females bearing teratoma were used a similar antitumor effect was observed (data not shown).

Putative Mechanisms Associated With Tumor Inhibition in Pregnant Mice

The above experiments suggested that the promotion effect of pregnancy on C7HI tumor growth up to days 8–10 of pregnancy and on T2280 tumor growth from day 14 until delivery could be attributed to high levels of serum progesterone or to a combined effect of progesterone plus estrogen, respectively. On the other hand, a high level of circulating estrogen could explain, at least in part, the inhibition of C7HI tumor growth beyond day 15 of gestation. However, mechanisms underlying the inhibitory effect on the growth of all tumors displayed by an embryonal growing mass were more elusive. In order to investigate this antitumor effect, supposedly shared by both pregnant and teratoma-bearing mice, immunological, antiangiogenic, and other putative mechanisms were explored.

Immunological Mechanisms. As mentioned above, three out of four tumors used were not immunogenic (LB, C7HI, and T2280) and, as previously demonstrated, they did not induce either humoral or cellular-specific immune responses (19,32,33). As for the strong immunogenic MC-C, in vitro cytotoxicity against MC-C tumor cells mediated by splenocytes from MC-C tumor-bearing mice was $34.3 \pm 2.5\%$ ($n = 11$ mice), significantly higher ($P < 0.05$) than that induced by splenocytes from normal virgin mice ($26.2 \pm 1.8\%$, $n = 6$ mice). However, no difference was detected whether splenocytes were obtained from MC-C-bearing pregnant ($33.3 \pm 2.4\%$, $n = 5$ mice) or virgin mice ($35.1 \pm 3.5\%$, $n = 6$ mice). In vitro cytotoxicity against MC-C tumor cells mediated by humoral antibodies and complement was similar in serum from both MC-C tumor-bearing (pregnant or virgin) and pregnant or virgin normal mice.

On the other hand, no significant differences between pregnant and virgin mice were detected for NK (tested against YAC-1 cells) and tumoricidal peritoneal macrophage activity. The above results suggested that conventional immunological mechanisms were not playing a main role in the phenomenon of tumor inhibition observed during pregnancy.

Antiangiogenic Mechanism. The effect of pregnancy on neovascularization, which is considered a critical step in the development of solid tumors (34–37), was studied. For this purpose, pregnant (10–12 days of gestation) and virgin mice were SC inoculated with: syngeneic placenta, neonatal testis, peritoneal elicited macrophages, and MC-C, LB, or C7HI tumor cells, which served as neovascularization stimulus. Five to 7 days later, mice were sacrificed and the skin examined. As can be seen in Table 4, the state of pregnancy induced a significant reduction in both macro- and microvasculature when normal tissues (placenta, testis, macrophages) served as angiogenic stimulus but not when tumor cells were used. These results suggested that inhibition of tumor growth during pregnancy would be unrelated to an antiangiogenic mechanism.

Concomitant Resistance-Like Effect. Mice bearing large tumors usually display an inhibitory effect on the growth of secondary tumor implants known as concomitant resistance (19). In vivo, this effect was observed by the increase of apoptotic nuclei and the decrease of mitotic figures in the secondary tumor. In vitro, serum from tumor-bearing mice caused [^{3}H]thymidine uptake reduction by tumor cells (31,33). We tested whether growing embryonic mass in the form of teratoma and pregnancy could mimic the presence of a large tumor by inducing a concomitant resistance-like effect. We found that tumor growing in pregnant and teratoma-bearing mice showed a significant increase of apoptotic cells per HPF (2.74 ± 0.44) compared with virgins (1.40 ± 0.20, mean ± SE of 8 experiments, $P < 0.02$) and a significant lower number of mitosis per HPF (0.50 ± 0.16 vs. 1.18 ± 0.21, mean ± SE of 10 experiments, $P < 0.02$). It is worth noting that these data were obtained from parenchymal, nonstromal, MC-C and LB tumor cells. In addition, serum from pregnant and teratoma-bearing mice was assayed on the in vitro proliferation of tumor cells. As can be seen in Table 5, serum from early pregnancy exhibited an effect on MC-C, LB, and C7HI tumor cell proliferation similar to that observed in serum from control virgin mice, but afterward, as pregnancy proceeded, the serum displayed an inhibitory activity that increased progressively, reaching its maximal value near the end of gestation. Rapidly after delivery, inhibitory activity dropped to control values. No natural cytotoxic antibodies could be responsible for this effect because, as shown above, in pregnant

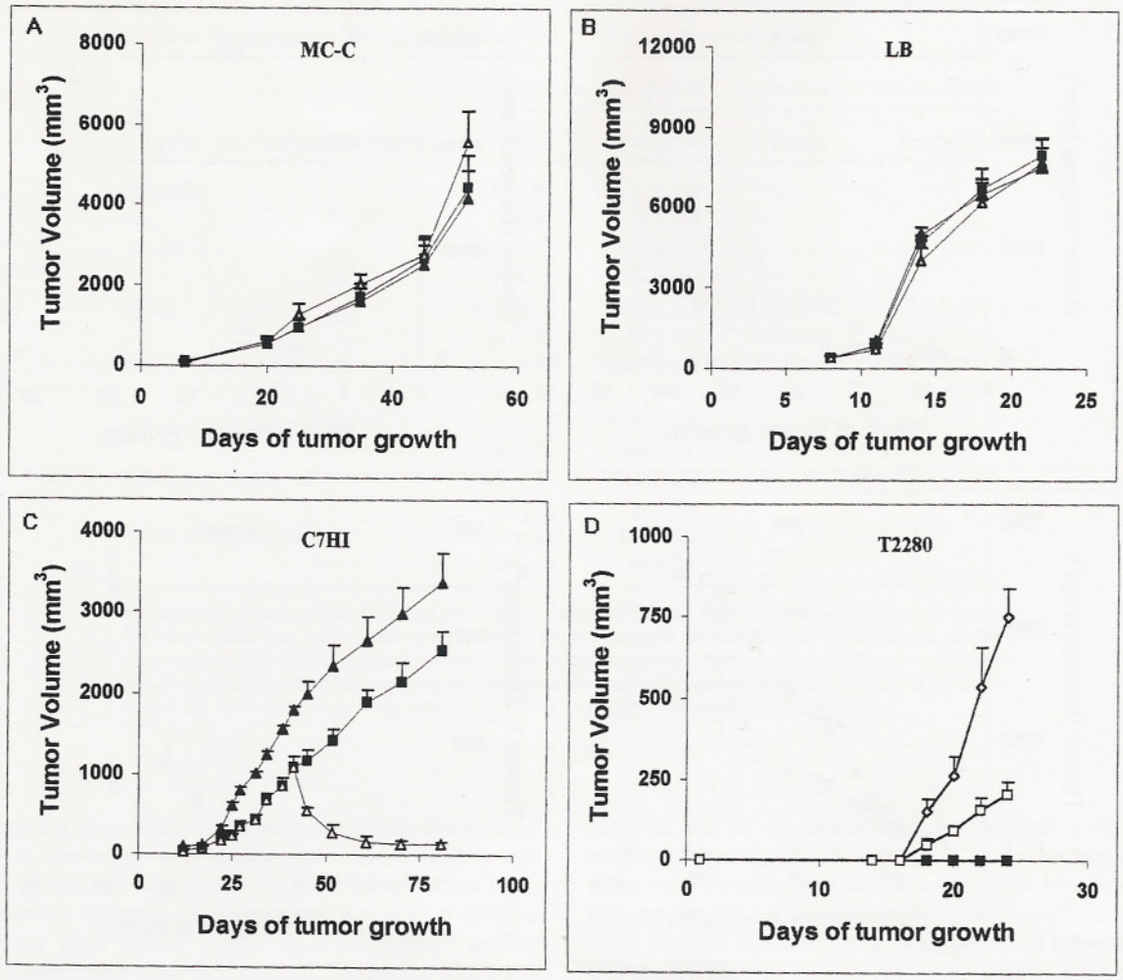

Figure 2. Tumor growth in progesterone- and estrogen-treated mice. Comparative growth of MC-C (A), LB (B), and C7HI (C) tumors in medroxiprogesterone acetate (MPA)-treated (▲), estrogen-treated (△), and control virgin (■) mice. For T2280 tumor (D), data from mice receiving MPA plus estrogen (◇), virgin (■), and pregnant mice as control of tumor growth (□) are shown. Data from mice receiving MPA alone or estrogen alone were identical to that of virgin and for simplicity are not shown. Tumor growth was initiated with a SC inoculum of 1×10^5 tumor cells. MPA was administered simultaneously with tumor inoculum. Estrogen was administered at day 7 of MC-C and LB tumor growth, at day 41 of C7HI tumor growth, and at day 14 after T2280 implantation. Comparison between tumor growth in MPA-treated versus virgins: (A) and (B) NS at all days of tumor growth; (C) $P < 0.05$ days 20–22 and day 61 onwards, $P < 0.01$ days 34–52, and $P < 0.001$ days 24–31. Comparison between tumor growth in estrogen-treated versus virgins: (A) and (B) NS at all days; (C) $P < 0.01$ day 45, $P < 0.001$ day 52 onwards. Comparison between tumor growth in MPA plus estrogen-treated versus virgins and pregnant: (D) $P < 0.001$ day 18 onwards versus virgins; $P < 0.02$ day 18, $P < 0.01$ days 20–22, $P < 0.001$ day 24 vs. pregnant. (A) 4 MPA-treated, 4 estrogen-treated, and 8 control virgin mice bearing MC-C tumor. (B) 4 MPA-treated, 4 estrogen-treated, and 12 control virgin mice bearing LB tumor. (C) 4 MPA-treated, 4 estrogen-treated, and 10 control virgin mice bearing C7HI tumor. (D) 6 MPA-treated, 14 estrogen-treated, 10 MPA plus estrogen-treated, 12 virgin, and 14 pregnant mice receiving a SC implant of T2280 tumor.

serum, no complement-dependent cytotoxicity on tumor cells was observed. Circulating estrogen could be responsible, at least in part, for the inhibitory effect of pregnant serum on C7HI, because control serum supplemented with estrogen, but not with progesterone, showed an inhibitory activity on in vitro C7HI tumor cell proliferation. On the contrary, neither MC-C nor LB tumor cells were affected by a high concentration of estrogen or progesterone, suggesting that the inhibitory effect of pregnant serum on MC-C and LB was unrelated to the presence of these hormones. Serum from teratoma-bearing mice also exhibited an inhibitory activity on in vitro proliferation of MC-C, LB, and C7HI tumor cells; this activity was slightly lower than that found in serum from advanced pregnancy. On the other hand, serum from advanced pregnancy (>15 days of gestation) permitted the in vitro proliferation of T2280 but not medium or serum from normal, early pregnant, or teratoma-bearing mice. However, when cultures were supplemented with MPA plus estrogen to permit T2280 tumor cell proliferation, serum from advanced pregnancy and from teratoma-bearing mice displayed an inhibitory ef-

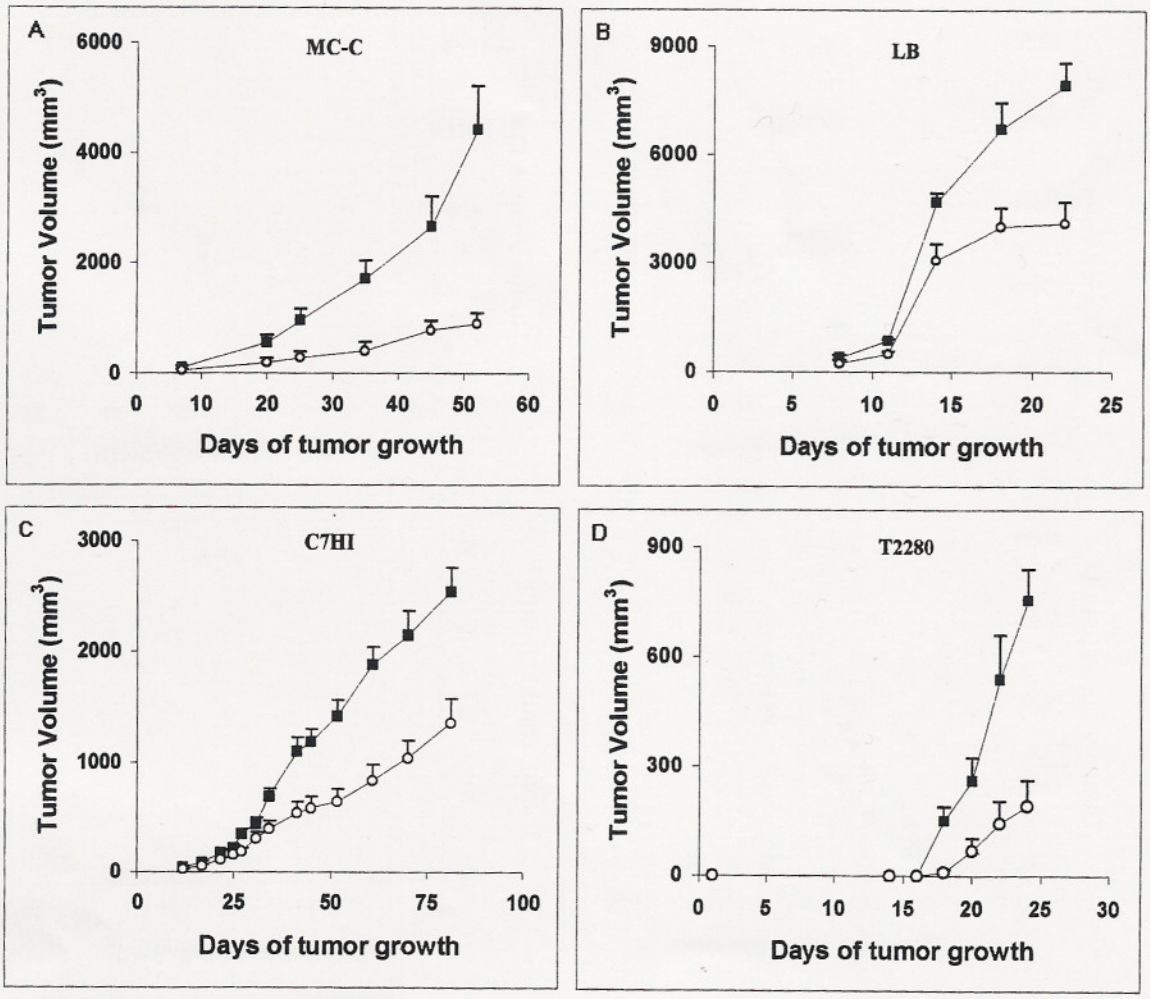

Figure 3. Effect of teratoma on tumor growth. Comparative growth of MC-C (A), LB (B), C7HI (C), and T2280 (D) tumors in teratoma-bearing (○) and virgin (■) mice. Tumor growth was initiated with a SC inoculum of 1×10^5 tumor cells carried out contralaterally to a SC growing teratoma, volume 1600–1700 mm^3 at day of tumor inoculum. For T2280, both teratoma-bearing and virgin mice were pretreated with MPA plus estrogen to permit tumor growth. (A) $P < 0.05$ day 20 of tumor growth, $P < 0.02$ days 25–45, $P < 0.01$ day 52. Mean of 6 teratoma-bearing and 8 virgin mice bearing MC-C tumor. (B) $P < 0.05$ day 18, $P < 0.01$ days 14 and 22, $P < 0.001$ day 11. Mean of 6 teratoma-bearing and 12 virgin mice bearing LB tumor. (C) $P < 0.05$ days 27–42, $P < 0.01$ day 45 onwards. Mean of 6 teratoma-bearing and 10 virgin mice bearing C7HI tumor. (D) $P < 0.05$ days 20–22, $P < 0.02$ day 18, $P < 0.001$ day 24. Mean of 6 teratoma-bearing and 12 virgin mice bearing T2280 tumor.

fect on this tumor significantly higher than that exhibited by control serum (i.e., when basic hormonal conditions for T2280 tumor growth were provided to the culture, the existence of inhibitory activity in serum from advanced pregnancy and from teratoma-bearing mice was revealed).

Serum Antitumor Activity in Pregnant and Teratoma-Bearing Mice. To test whether the antitumor activity found in serum of pregnant and teratoma-bearing mice was unrelated not only to progesterone and estrogen but also to other pregnancy-associated hormones, kinetics of progesterone, estrogen, prolactin, FSH, and LH in serum from both pregnant and teratoma-bearing mice was studied. As shown in Figure 4, the kinetics of serum antitumor activity during pregnancy was different from that of any hormone tested. In effect, while antitumor activity increased progressively throughout pregnancy and disappeared rapidly after delivery, serum levels of LH and FSH remained basal, progesterone decayed beyond day 15 of gestation, estrogen only increased near the end of pregnancy, and prolactin level remained high during the postpartum suckling period. Moreover, when partial remotion of embryos was carried out, no significant alteration in the pattern of serum hormones was observed while a sharp decrease in serum antitumor activity was induced. In the same way, the presence of teratoma did not induce changes in serum content of progesterone, estrogen, prolactin, FSH, and LH, although it displayed a high antitumor activity; this activity was observed independently of the presence of ovary and adrenal glands.

Serum from pregnant and teratoma-bearing mice did

Table 4. Neovascularization Induced by Syngeneic Placenta, Neonatal Testis, Peritoneal Elicited Macrophages (PEM), and Living Tumor Cells in Pregnant Mice

	Macroscopic Vessels/cm^2		Microscopic Vessels/Field	
Stimulus for Neovascularization	Virgin	Pregnant	Virgin	Pregnant
Placenta	13.0 ± 0.3 (n = 13)	4.6 ± 0.4* (n = 18)		
Testis	18.7 ± 1.0 (n = 15)	2.5 ± 0.4* (n = 16)		
PEM	10.3 ± 1.1 (n = 10)	2.9 ± 0.5* (n = 10)	12.6 ± 0.2 (n = 8)	5.7 ± 0.13* (n = 7)
MC-C living tumor cells	16.4 ± 3.0 (n = 9)	13.1 ± 3.0 (n = 7)	5.6 ± 0.6 (n = 4)	3.7 ± 0.4 (n = 4)
MC-C dead tumor cells	11.7 ± 1.5 (n = 3)	10.0 ± 1.8 (n = 3)		
LB living tumor cells	4.5 ± 5.0 (n = 8)	5.1 ± 1.0 (n = 7)		
C7HI living tumor cells	15.3 ± 6.0 (n = 6)	13.8 ± 6.0 (n = 3)		

Stimulus for neovascularization was given SC in pregnant (10–12 days of gestation) and virgin mice; 5–7 days later, skin was examined and macro- and microscopic vessels counted. Macroscopic vessels were evaluated at 10× magnification under a dissecting microscope. Microscopic vessels were evaluated at 150× and 250× magnification using both hematoxylin-eosin stain and antibody against von Willebrand factor; 20 fields per mouse were examined. Values represent the median ± SE of n mice.
*$P < 0.001$.

not contain urea (urease method), creatinine (method of Jaffe et al.), uric acid (method of Henry, Sobel, and Kim), or salt content higher than in control serum (data not shown). Content of polyamines was also evaluated by using high power liquid chromatography: neither spermidine, spermine, nor n-acetil putrescine displayed values higher than in control serum. Content of putrescine was significantly higher in pregnant (titer = 4100 ± 500 pmol/ml, $n = 2$) than in virgin mice (150 ± 50 pmol/ml, $n = 2$, $P < 0.02$); however, no inhibitory effect of putrescine on in vitro LB proliferation was detected even using a concentration of putrescine more than 2000 times higher than present in pregnant serum. Serum from pregnant and teratoma-bearing mice was subjected to dialysis (12,500 Da molecular weight cutoff). Antitumor effect was recovered only in the dialysable fraction and proved to be resistant to heating at 56°C for 30 min and at 100°C for 5 min. On Sephadex G-15 chromatography activity was located at fractions corresponding to 1000–1200 Da molecular weight. No effect on inhibitory activity was detected when the dialysable fraction of pregnant serum was incubated with pronase, trypsin, DNAse, RNAse, and glucosidases (data not shown). On the other hand, treatment with lipase (72 U/ml) sharply increased such inhibitory activity from 82.9 ± 16.6 to 208.7 ± 32.7 GIU_{50}/ml ($P < 0.02$, mean ± SE of 4 experiments), suggesting that a derivative of a lipid or phospholipid could be involved.

Effect of Anti-Inflammatory Drugs. In order to evaluate the effect of anti-inflammatory drugs on the antitumor activity present in serum during pregnancy, eight pregnant mice were divided into two groups, receiving on days 12 and 13 of gestation an IP inoculation of indomethacin ($n = 4$ mice) or none ($n = 4$ mice). On day 14, mice were bled and the antitumor activity present in serum was tested on the in vitro proliferation of LB tumor cells. Titer of this activity in pregnant mice treated with indomethacin was 31 ± 9 GIU_{50}/ml, significantly lower ($P < 0.05$) than that observed in nontreated pregnant mice (78 ± 2 GIU_{50}/ml), while titer of virgin mice was 33 ± 4 GIU_{50}/ml ($n = 3$). Treatment with indomethacin did not alter the development of embryos as tested in four nontreated and four indomethacin-treated pregnant mice.

Serum Transfer. Antitumor effect shared by serum from pregnant and teratoma-bearing mice was assayed in vivo on LB tumor growth. LB was used as target to bypass the effects of progesterone and estrogen present in pregnant serum, taking into account its lack of receptors for these hormones. The following experiment was carried out: 18 mice received in the right flank a SC implant of 5×10^4 LB cells (day 0) and were divided in three groups of six mice. First and second groups received, between days 0 and 15, a daily IV inoculation of 0.3–0.5 ml of serum from pregnant and teratoma-bearing mice, respectively, while the third group received serum from control virgin mice. A significant inhibition of LB tumor and prolonged survival were observed in the first and second groups compared with the controls (Fig. 5).

DISCUSSION

There has been in the literature a large number of apparently contradictory reports concerning the effect of

Table 5. Inhibitory Effect of Serum From Pregnant and Teratoma-Bearing Mice on the In Vitro Proliferation of MC-C, LB, C7HI, and T2280 Tumor Cells

	Inhibitory Activity of Serum on Tumor Cells (GIU_{50}/ml, mean ± SE)			
Serum Group	MC-C	LB	C7HI	T2280
Virgin	31 ± 9	23 ± 4	35 ± 7	25 ± 3
	(n = 6)	(n = 7)	(n = 4)	(n = 2)
Pregnant (day 5)	28 ± 11	29 ± 4	20 ± 0.3	
	(n = 2)	(n = 6)	(n = 2)	
Pregnant (days 10–15)	60 ± 6*	71 ± 9†	97 ± 13†	
	(n = 5)	(n = 16)	(n = 3)	
Pregnant (days >15)	84 ± 7‡	105 ± 9†	120 ± 12†	70 ± 2†
	(n = 2)	(n = 11)	(n = 2)	(n = 2)
After delivery	30 ± 8	24 ± 6	37± 8	
	(n = 4)	(n = 6)	(n = 2)	
Virgin plus estrogen	31 ± 6	27 ± 3	70 ± 5	
	(n = 2)	(n = 2)	(n = 2)	
Virgin plus progesterone	30 ± 3	23 ± 0.2	25 ± 2	
	(n = 2)	(n = 2)	(n = 2)	
Teratoma-bearing	65 ± 2	75 ± 17†	93 ± 19*	67 ± 5*
	(n = 2)	(n = 2)	(n = 2)	(n = 2)

GIU_{50}/ml: titer of growth inhibitory activity defined as the reciprocal of serum dilution producing 50% inhibition of [^{3}H]thymidine uptake by cells compared with medium and expressed per milliliter. n = number of individual assays. Groups were: virgin mice; pregnant mice at day 5 of gestation, pregnant mice at days 10–15; pregnant mice at days 15–21; mice 1–2 days after delivery; virgin mice supplemented with 17 β-estradiol 10^{-9} M (for MC-C and LB, concentrations of 10^{-8}–10^{-7} M were also noninhibitory); virgin mice supplemented with MPA 10^{-7} M; mice bearing growing teratoma 2000–2500 mm^3. Target cells: $1–3 \times 10^5$/well LB tumor cells were added to the plates simultaneously with serum and [^{3}H]thymidine; MC-C, C7HI, and T2280 tumor cells were added ($0.2–0.3 \times 10^5$/well) to the plates 24–48 h before serum and [^{3}H]thymidine. T2280 cells were cultured in the presence of MPA 10^{-7} M and 17 β-estradiol 10^{-9} M.

*$P < 0.05$.

†$P < 0.01$ compared with virgin mice.

‡$P < 0.02$.

pregnancy on tumor growth. In effect, numerous clinical and experimental observations showing that pregnancy could enhance tumor growth (1–9) have been matched with other observations, in both human and animal models, pointing out that pregnancy may have a protective rather than an aggravating effect on neoplastic disease (10–16).

In this article, in an attempt to conciliate such paradoxical reports, the growth of four murine tumors, widely differing in histology, etiology, immunogenicity, and hormonal receptors, was comparatively studied in pregnant, teratoma-bearing, and virgin mice. Our study has clearly evidenced the differential effect of pregnancy on the growth of these four tumors. In effect, MC-C (strongly immunogenic methycholanthrene-induced fibrosarcoma) and LB (nonimmunogenic spontaneous lymphoid leukemia) exhibited slow kinetics throughout the course of pregnancy, although inhibition was stronger beyond day 10 of gestation. On the other hand, C7HI (nonimmunogenic MPA-induced mammary adenocarcinoma) exhibited three phases: up to days 8–10 of gestation, the tumor grew faster than in virgins; between days 8–10 and 15 it reached a plateau; and beyond day 15 a sharp reduction in tumor mass was observed. Lastly, T2280 (nonimmunogenic MMTV-induced mammary adenocarcinoma) behaved as a typical pregnancy-dependent tumor (i.e., it grew in pregnant but not in virgin mice, it regressed soon after delivery, and reassumed its growth in the middle of a second round of pregnancy).

Putative mechanisms underlying the different effects of pregnancy on tumor growth were analyzed. Neither adaptive T-dependent nor nonadaptive (NK and macrophages) antitumor immunological effectors were altered during pregnancy, meaning that conventional immunological mechanisms would not be involved. Similarly, even though in pregnant mice a significant antiangiogenic effect was observed when neovascularization was induced by implants of normal tissues, no significant effect was detected with tumor implants. This suggested that the induction of new vessel formation by normal and tumor tissues differed qualitative or quantitatively and in turn that the influence of pregnancy on tumor growth would be unrelated to an antiangiogenic mechanism.

The hormonal state might explain, in part, some of the effects of pregnancy on the growth of tumors carrying hormonal receptors (C7HI and T2280). In effect, in MPA-treated mice exhibiting serum levels of progesterone similar to those found up to day 16 of pregnancy, C7HI tumor development was enhanced, and reciprocally, in estrogen-treated mice exhibiting levels of serum estrogen similar to those observed in advanced preg-

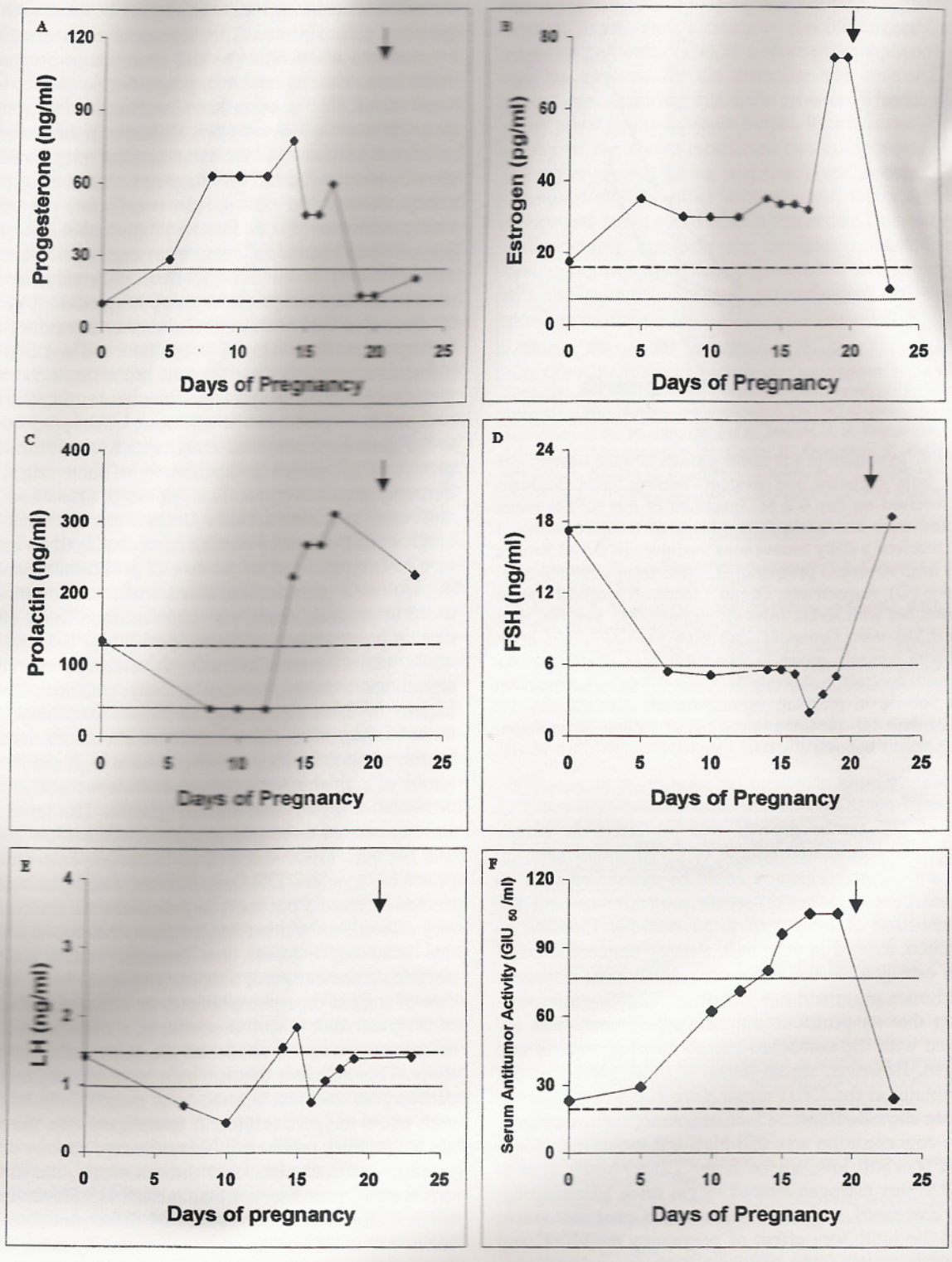

Figure 4. Serum concentration of progesterone (A), estrogen (B), prolactin (C), FSH (D), LH (E), and antitumor activity (F) during pregnancy and postpartum (◆). Antitumor activity was tested on in vitro proliferation of LB tumor cells. GIU_{50}/ml: titer of growth inhibitory activity defined as the reciprocal of serum dilution producing 50% inhibition of [^{3}H]thymidine uptake by LB tumor cells compared with medium and expressed per milliliter. Each point represents the mean of 2–4 mice. Standard errors were 7.2–13.2% of mean value in all determinations. Values in serum from virgin mice (broken line) and from teratoma-bearing mice (dotted line) are also indicated. Arrow indicates the day of delivery.

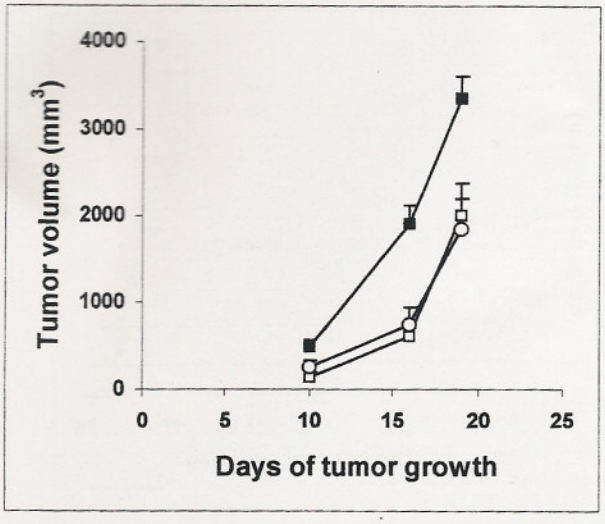

Figure 5. Inhibition of LB tumor growth in mice treated with serum from pregnant and teratoma-bearing mice. Eighteen mice received on day 0 a SC inoculum of 5×10^4 LB tumor cells and were divided in three groups of 6 mice. Groups 1 and 2 received a daily intravenous inoculum of 0.3–0.5 ml of serum from advanced pregnancy (□) and from teratoma-bearing mice (○), respectively. Group 3 (control) received similar treatment but with serum from virgin mice (■). Survival time (mean ± SE) was: Group 1: 23.2 ± 0.6 days ($P < 0.01$ compared with control), group 2: 24.1 ± 1.0 days ($P < 0.01$ compared with control), and group 3: 20.5 ± 0.4 days. Comparison tumor volume in pregnant versus control: $P < 0.02$ day 19, $P < 0.01$ days 10–16. Comparison tumor volume in teratoma-bearing versus control: $P < 0.02$ day 10, $P < 0.01$ days 16–19.

nancy, C7HI tumor growth was inhibited. In consequence, the accelerated course of C7HI tumor seen up to days 8–10 of pregnancy could be associated with an elevated concentration of serum progesterone and the dramatic drop of C7HI growth beyond day 15 could be associated, at least in part, with a rising concentration of serum estrogen. In the same way, MPA plus estrogen-treated mice permitted the growth of T2280 tumor, suggesting that its proliferation during pregnancy was associated with the combined effects of progesterone and estrogen. However, serum levels of these hormones did not explain: a) the C7HI tumor growth plateau observed between days 8–10 and 15 of pregnancy, when progesterone concentration was still high and estrogen concentration was still low; b) the faster T2280 tumor growth in MPA plus estrogen-treated virgin mice (pseudopregnant) compared with that observed in pregnant mice; and c) the inhibitory effect of pregnancy on MC-C and LB tumor growth, both of which have few or undetectable progesterone and estrogen receptors.

In order to understand more thoroughly the effects of pregnancy on tumor growth, mechanisms related to growing embryonal mass were investigated by using teratoma-bearing mice. Our experiments showed that the growth of the four tumors was retarded in the presence of a teratoma and that this effect was a function of the proliferation of the embryonal mass because it was observed with growing large teratomas but not with regressing or small ones. This antitumor effect mediated by teratoma was similar to that observed in pregnancy when tumors unresponsive to progesterone and estrogen were tested. On the other hand, with tumors responsive to progesterone and estrogen, differences between the effects of pregnant and teratoma-bearing mice on tumor growth were evident. This suggested that, during pregnancy, there would exist at least two factors potentially acting on tumor growth. First, a progesterone and estrogen-mediated hormonal component that would exert either inhibitory or stimulatory effects only evidenced on tumors bearing receptors for these hormones. It cannot be discarded that in other models other hormones may be important; for example, in spontaneous and DMBA-induced rat mammary carcinomas, prolactin or chorionic gonadotropin may play a significant role (38,39). Secondly, there would exist an antitumor effect proportional to the growing embryonal mass, which would limit the growth of all tumors independently of their origin and hormone responsiveness. This antitumor effect could be attributed to a serum factor because serum from both pregnant and teratoma-bearing mice displayed an inhibitory activity on in vitro tumor cell proliferation and on in vivo tumor growth. The kinetics of this antitumor activity in pregnant mice was different than those exhibited by progesterone, estrogen, prolactin, FSH, and LH, while partial removal of embryos, although inducing a significant reduction in that activity, did not alter the pattern of these circulating hormones. Similarly, teratoma-bearing mice did not present alterations in those hormonal serum levels but displayed a high serum antitumor effect, which was observed independently of the presence of ovary and adrenal glands. This antitumor activity proved to be due to a heat-resistant low molecular weight (1000–1200 Da) factor(s). Interestingly, pronase-, trypsin-, DNAse-, RNAse-, and glucosidase-treated serum did not show any reduction in their inhibitory effect. On the other hand, lipase treatment enhanced this activity. Therefore, this factor(s) would not be a peptide, a nuclear acid, or a carbohydrate but a derivative of a lipid or a phospholipid. In addition, treatment of pregnant mice with indomethacin, a nonsteroidal anti-inflammatory agent, abolished the serum antitumor activity. This indicates that it would be associated with the pathway of the arachidonic acid metabolism initiated with cyclo-oxygenase (40). It is unlikely that the well-known primary products of this pathway, namely prostaglandins, prostacyclins, and thromboxanes, are directly involved because they are heat labile (41). However, putative heat-resistant derivatives of these modified fatty acids, which we are now trying to detect by using different eicosanoids affinity columns, could account for this antitumor effect. This serum factor(s) would not be related to known small toxic molecules nor antitumor polypeptides (42–47). Similarly, it is highly unlikely that chrorionic gonadotropin could be involved taking into account its protein nature and its high molecular weight (near 30000 Da) (48), both features being sharply different from those of the serum factor described in this article. As for other articles reporting an antitumor activ-

ity in the serum of pregnant and teratoma-bearing rats and pregnant women (49,50), its relationship with that reported herein, needs further clarification.

The interplay between the progesterone- and estrogen-mediated hormonal component and the serum factor associated with embryonal mass could account for some of the largely heterogeneous and otherwise unexplained effects of pregnancy on tumor growth reported by the literature and illustrated by the four tumors studied herein. In effect, at the onset of pregnancy, the enhancing effect of progesterone on C7HI tumor growth could have overcome the low antitumor effect associated with embryonal mass, but afterwards, as gestation proceeded and embryonal mass became progressively larger, this antitumor effect must have exceeded that of progesterone, resulting in the characteristic plateau displayed by C7HI tumor growth between days 8–10 and 15 of gestation; beyond day 15, antitumor and estrogen effects must have reinforced each other to produce the drastic shrinking of tumor tissue occurring near the end of pregnancy. In the same way, when basic hormonal conditions for T2280 tumor development, characteristic of pregnancy, were matched between virgin and pregnant mice, the tumor not only grew in pseudopregnant virgins but actually grew faster than in pregnant mice. This could be easily explained by assuming that, in pregnancy, the tumor promotion effect induced by progesterone plus estrogen was tempered by the antitumor effect associated with embryonal mass. Lastly, as progesterone and estrogen were unable to affect the growth of MC-C and LB tumors, the factor associated with embryonal mass remained, in this case, the only one affecting tumor growth during pregnancy, making it understandable why inhibition of these tumors was increased beyond day 10 of gestation (i.e., when embryonal mass was larger).

Even though hormonal effects on tumor growth have been carefully investigated (3,21,32,51), mechanisms underlying the antitumor effect of pregnancy associated with embryonal mass have received little attention. This effect is intriguingly similar to the effect generated by a primary tumor against secondary implants of its own tumor, the so-called concomitant resistance phenomenon (19,31,33,52–55). In effect, both pregnant and primary tumor-bearing mice would share an inhibitory mechanism on the growth of all tumors and there is a direct correlation between the primary tumor volume and the intensity of concomitant resistance (19,55) in the same way that the more advanced the course of gestation, the more its ability to inhibit tumor growth. This inhibition was correlated in both pregnant and tumor-bearing mice (53), with a decreased number of mitosis and an increased number of apoptotic cells. Moreover, excision of a primary tumor induces the growth of previously dormant secondary tumors (33,55), just as removal of embryos or even delivery stimulates the growth of previously inhibited tumor implants. Lastly, as we have previously described, concomitant resistance is associated with the presence of a circulating factor of low molecular weight (31,33,53) with similar properties to that found in the serum of pregnant mice. It would be rather surprising that these analogies were only casual. Instead, the antitumor effect of pregnancy associated with embryonal mass and antitumor concomitant resistance might be particular cases of a more general phenomenon that has evolved for the detection and control of cell aberrations, ensuring that cells grow only in the appropriate place and to a proper extent. In conclusion, careful survey of the above-mentioned two components affecting tumor growth during pregnancy, one associated with hormones and the other with a serum factor related to embryonal mass, could offer a theoretical framework for understanding not only the complex relationship between pregnancy and tumor growth but also some of the still elusive mechanisms of physiological and even tumor homeostasis.

ACKNOWLEDGMENTS: This study was supported by national grants from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) and FUNDALEU (Fundación para combatir la leucemia), Argentina. The authors are grateful to Dr. M. A. Isturiz for critical discussion of the manuscript and to Messrs. J. J. Portaluppi, A. Morales, A. J. Oscoppiantini, H. Costa, V. Francisco, P. Bekinschtein, and Drs. V. Buggiano, G. Dran, C. Lanari, M. I. Piazzon, and I. Nepomnaschy for helpful suggestions and expert technical assistance.

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