Role of Corticosteroid Hormones in the Control of Cell Proliferation in Residual Tumor after Surgical Cytoreduction

Paul G. Braunschweiger, Han L. Ting, and Lewis M. Schiffer


ABSTRACT

Changes in tumor cell proliferation in local and distant residual tumor were studied after subtotal surgical cytoreduction in three experimental tumor models varying in corticosteroid receptor content, cell proliferation, and animal host. In residual s.c. RIF-1 and R3230AC tumors, proliferation was inhibited within 24 hr after 75% resection. Subsequently, intervals of increased proliferation, characterized by increases in tritiated thymidine ([\(3^H\)]dThd) labeling index, primer-dependent DNA polymerase labeling indices, and S-phase clonogenic fractions, were observed. In RIF-1 "artificial" lung metastases, [\(3^H\)]dThd uptake in tumor-bearing lungs increased by about 70% at 3 days after amputation of "primary" tumor-bearing legs. When dexamethasone was given every 12 hr during the postsurgical recovery interval, changes in [\(3^H\)]dThd labeling indices and [\(3^H\)]dThd uptake per lung indicated that the proliferative recovery was delayed until after cessation of dexamethasone treatments. Other studies with RIF-1 tumors indicated that postsurgical tumors indicated that postsurgical proliferation inhibition was dependent on intact adrenal function and that the initiation of postsurgical proliferative recovery was preceded by reestablishment of normal serum corticosterone levels and presurgical levels of saturable glucocorticosteroid receptor. The effectiveness of cyclophosphamide 5-fluorouracil after surgery was time dependent in residual local and distant tumor, with the most efficacious intervals being coincident with postsurgical proliferative recovery. Our results indicate that, in these experimental tumor models, changes in endogenous corticosteroid hormones resulting from the surgical trauma, cellular corticosteroid hormone receptor levels and cytoreduction may influence the time course of the proliferative response in residual tumor after surgical cytoreduction.

INTRODUCTION

Postsurgical chemotherapy has become a routine strategy for the management of nonresectable malignant disease. In animal tumor models, we and others have observed that, following subtotal resection, cell proliferation in residual tumor tissue (6, 17, 22, 30) or proliferation of tumor tissue distant to the primary site (17, 23, 33) is often transiently increased. It was concluded from these studies that chemotherapy for existing disease, and perhaps adjuvant chemotherapy, could be more beneficial if initiated during these intervals of increased proliferation. Subsequent studies to test this hypothesis in both the laboratory (6, 15) and in the clinic (11, 25) have often yielded conflicting results.

In C3H/He mammary tumors, the results from regrowth delay experiments indicated that better local control could be achieved when chemotherapy treatments were administered at intervals coincident with intervals of increased proliferation in the residual tumor tissue (6). Although a survival advantage was observed for appropriately sequenced adjuvant therapy in Lewis lung tumor (5, 9), no such survival advantage was observed in a transplantable mammary tumor line (15).

In the present studies, changes in cell proliferation of residual tumor after surgical cytoreduction were studied in RIF-1, SaD2, and R3230AC tumor models. These tumor models were chosen because pilot studies indicated that these models varied considerably with respect to their corticosteroid receptor content and cell proliferation parameters. Other studies were conducted to assess the effect of intact adrenal function and the influence of corticosteroid hormones on the postsurgical proliferative response in RIF-1 tumors.

MATERIALS AND METHODS

Tumor Models

RIF-1 tumors were produced in female retired breeder C3H/HeJ mice by s.c. or i.m. inoculation of 1 x 10^6 or 1 x 10^6 tissue culture cells (originally obtained from Dr. R. Kallman, Stanford University, Palo Alto, Calif.), respectively. So that we might evaluate the effect of primary tumor resection on cell proliferation and chemosensitivity in distant tumor deposits, both primary RIF-1 tumors and "artificial" lung metastases were produced by i.m. and i.v. inoculation of 1 x 10^6 and 1 x 10^5 RIF-1 tumor cells, respectively. Mice having both a "primary" leg tumor and "artificial" lung metastases were initiated into studies 14 days later. Base-line cell kinetic parameters for 14-day s.c. tumors were: [\(3^H\)]dThd LI, 17%; Ts, 7.6 hr; GF by the PDP assay, 41.3%; and Tc, 16 hr. (7).

The SaD2 fibrosarcoma tumor model was originally obtained from Dr. A. Bogden (Mason Research Institute, Worcester, Mass.) and was maintained in 6- to 8-week-old male DBA/2J mice (The Jackson Laboratory, Bar Harbor, Maine) by s.c. implantation of 1 x 10^6 enzyme-dispersed lung metastases were initiated into studies 14 days later. Base-line cell kinetic parameters for 14-day s.c. tumors were: [\(3^H\)]dThd LI, 17%; Ts, 7.6 hr; GF by the PDP assay, 63.4%; and Tc, 16 hr. (7).

The R3230AC rat mammary tumor model was also obtained from Dr. Bogden and maintained by trocar implantation of 1- to 2-cm fragments on the flank of 8- to 10-week-old female Fischer 344 rats (Harlan Sprague Dawley, Inc., Indianapolis, Ind.). Tumor-bearing rats were initiated into studies 21 days later, when tumor volumes were approximately 2.5 cu cm.
cm. Base-line cell proliferation parameters in this tumor model were: \([^{3}H]dThd\) LI, 13.3%; \(T_{S}, 10.9\) hr; GF by PDP assay, 55.9%; and \(T_{G_{1}}\), 41 hr (10).

All animals were housed (10 to 12 mice/cage; 2 to 3 rats/cage) in animal facilities with a 12-hr dark cycle. Standard mouse/rat chow (Purina, Evanston, Ill.) and water were provided ad libitum.

Cell Kinetic Assays

\([^{3}H]dThd\) LI. Lls were determined by in vitro \([^{3}H]dThd\) labeling of mechanically dissociated tumor tissue (2). Tumor fragments were minced with scissors in Eagle's minimal essential medium with 10% fetal bovine serum (Grand Island Biological Co., Grand Island, N. Y.). Three-mI aliquots of the minced cell suspension were incubated with \([^{3}H]dThd\) (3 \(\mu\)Ci/ml; 20 to 40 Ci/mmol; New England Nuclear, Boston, Mass.) for 1 hr at 37°. Labeling was stopped on ice, and 95% trypan-negative cell suspensions were obtained by density gradient centrifugation on Lymphoprep (Litton Bionetics). Cells obtained from the gradient-media interface were washed, diluted, applied to microscope slides with a cyto-centrifuge (Cytospin; Shandon Southern, Swickley, Pa.), air-dried, and fixed in methanol.

\(T_{S}\) cell suspensions prepared and labeled with \([^{3}H]dThd\), as above, were incubated for an additional 30 min with \([^{14}C]dThd\) (0.25 Ci/ml). Double-labeled cell suspensions were then treated as described above for \([^{3}H]dThd\)-only labeled cells (2).

PDP Index. Aliquots of the minced cell suspension were applied to microscope slides with a cyto-centrifuge and were rapidly air dried. Estimation of the tumor GF by the PDP assay has been described in detail previously (3, 31). Briefly, this autoradiographic assay measures the simultaneous presence of DNA polymerase \(\alpha\) and available primer template activity in the nuclei of the cells by the incorporation of \([^{3}H]d\)TPP. Previous studies have indicated that the LI obtained (PDP index) is a good estimate of the tumor GF for a wide variety of experimental tumor models including RIF-1 fibrosarcoma (7, 31).

Autoradiography. NTB-2 liquid photographic emulsion (Eastman Kodak Co., Rochester, N. Y.) was applied in the dark (54°) by dipping. The autoradiograms were exposed in tight light boxes (4°) for up to 7 days. Autoradiograms were developed with amido (Eastman Kodak Co.) developer after intensification of the latent image by KClAuO4 as described previously (2). \([^{3}H]dThd\) LI's and PDP indices were determined by counting at least 500 cells. DNA synthesis times were determined as described previously by counting at least 200 labeled cells in double-emulsion autoradiograms (2). Local background rarely exceeded one grain per equivalent cell area, and cells with 3 or more grains over the nucleus were considered labeled.

\([^{3}H]dThd\) Uptake. RIF-1 lung tumor nodule-bearing mice were given i.p. injections of \([^{3}H]dThd\) (1 \(\mu\)Ci/body wt; 20 Ci/mmol; New England Nuclear), killed 30 min later by cervical exsection, and chilled on ice. Lungs were removed, rinsed in cold 0.9% NaCl solution, weighed, and fixed in Clarke's fixative for 48 hr. The tissue was then rinsed in 85% ethanol and stored at 4°. The suspension was homogenized in 105,000 x g in an L8-55 ultracentrifuge (Beckman Instruments, Palo Alto, Calif.). The supernatant was carefully removed, so as not to disturb the fat layer, and diluted with buffer to a protein concentration of 2 to 4 mg/ml. Total protein measurements on the cytosol were made by the method of Lowry et al. (23).

Aliquots of the cytosol preparation were incubated with at least 4 and usually 5 concentrations of \([^{3}H]dexamethasone with and without 200-fold molar excess of unlabeled ligand in V-vial microtiter plates for 4 hr. Autoradiograms were developed with amido (Eastman Kodak Co.) developer after intensification of the latent image by KClAuO4 as described previously (2). \([^{3}H]dThd\) LI's and PDP indices were determined by counting at least 500 cells. DNA synthesis times were determined as described previously by counting at least 200 labeled cells in double-emulsion autoradiograms (2). Local background rarely exceeded one grain per equivalent cell area, and cells with 3 or more grains over the nucleus were considered labeled.


derived from the acetabulum after ligation of the femoral artery. The wound was closed at a wide margin with wound clips so that, in

\(1 - \left(\frac{PE_{\text{Hu}}}{PE_{\text{control}}}\right)\)

for primary tumors, where PE is plating efficiency, and:

\(1 - \left(\frac{\text{clonogenic cells/lung}_{\text{Hu}}}{\text{clonogenic cells/lung}_{\text{control}}}\right)\)

for lung nodules. This technique was used in previous studies to study changes in RIF-1 clonogenic cell proliferation after dexamethasone treatments in vivo (7).

GR Assay. GR assays were done using the dextran-coated charcoal competitive binding assay described previously (4, 7, 10). Tumor tissue was finely minced in 4 to 5 volumes of 10 mM Tris, 1.5 mM EDTA, and 0.5 mM dithiothreitol plus 10% glycerol, pH 7.4, at 4°. The suspension was homogenized (Polytron; Brinkman Instruments, Inc., Westbury, N. Y.) using four 15-sec bursts and 30-sec cooling periods. The homogenate was centrifuged at 105,000 x g in an L8-55 ultracentrifuge (Beckman Instruments, Palo Alto, Calif.). The supernatant was carefully removed, so as not to disturb the fat layer, and diluted with buffer to a protein concentration of 2 to 4 mg/ml. Total protein measurements on the cytosol were made by the method of Lowry et al. (23).

Aliquots of the cytosol preparation were incubated with at least 4 and usually 5 concentrations of \([^{3}H]dexamethasone with and without 200-fold molar excess of unlabeled ligand in V-vial microtiter plates for 4 hr. The suspension was homogenized in 105,000 x g in an L8-55 ultracentrifuge (Beckman Instruments, Palo Alto, Calif.). The supernatant was carefully removed, so as not to disturb the fat layer, and diluted with buffer to a protein concentration of 2 to 4 mg/ml. Total protein measurements on the cytosol were made by the method of Lowry et al. (23).

Serum Corticosterone Assay. At various intervals after surgery, animals were killed by cervical exsection, and cardiac blood samples were obtained with a heparinized syringe. The serum was separated by centrifugation and stored at 4°. Samples from 2 to 3 mice were pooled per time point. Serum samples were shipped on ice to Endocrine Sciences Corp. (Tarzana, Calif.), where total corticosterone levels were determined by radioimmunoassay.

Surgical Procedures. Animals bearing s.c. RIF-1, SaD2, or R3230AC tumors were anesthetized with ether and immobilized on a dissecting board. Tumor volumes at the time of surgery were approximately 1.5 to 2.0, 1.5, and 2.5 cu cm for RIF-1, SaD2, and R3230AC tumors, respectively. The skin was disinfected with 85% ethanol, and incisions were made at wide margins to the tumor. The skin was reflected and, using a scalpel, a transverse incision was made so that approximately three-fourths of the tumor could be carefully removed without disturbing the orientation of the residual tumor tissue. Although tumor sizes within experimental groups varied by as much as 20% at the time of surgery, care was taken to leave approximately the same amount of tumor tissue in each animal. The skin was folded back over the residual tissue, and the incision was closed at a wide margin with wound clips so that, in regrowth delay experiments, residual tumor growth could be followed by caliper measurements (6). Mortality from this procedure was usually less than 5%, and invariable anesthesia related.

For 14-day i.m. RIF-1 tumors, the tumor-bearing leg was amputated, under ether anesthesia, by dislocation of the proximal end of the femur from the acetabulum after ligation of the femoral artery. The wound...
closed with wound clips. Surgical mortality was usually less than 10% and was due in most cases to uncontrolled bleeding, overanesthesia, or a combination of the 2. Animals exhibiting excessive bleeding at the amputation site were given 0.5 ml of warm 0.9% NaCl solution as fluid replacement.

Bilateral adrenalectomy was performed through dorsal incisions under ether anesthesia. Animals were subsequently maintained on 0.9% NaCl drinking water.

**Drug Treatments.** 5-FUra (Roche Laboratories, Nutley, N. J.), CP (Cytoxan; Mead Johnson, Evansville, Ind.), and dexamethasone sodium phosphate (Hexadrol; Organon, West Orange, N. J.) were always freshly prepared and administered by i.p. injection of 0.2 ml of diluent. 5-FUra and CP were administered at doses of 84 and 150 mg/kg, respectively.

Regrowth delay was used to assess the effectiveness of therapeutic treatments. Tumors were measured frequently in 3 diameters, and tumor volumes were estimated as described previously (4, 6, 7). The regrowth delay for individual tumors was determined by subtracting the time in days for control tumors to reach 4 times pretreatment size from the time required for treated tumors to reach the same endpoint. Regrowth delays were expressed as a multiple of the doubling time for that tumor, during the regrowth phase (4, 6, 7), so that the results from serial experiment could be more easily compared. In some studies, the effect of the drug treatment given in sequence after surgery was determined by subtracting the regrowth delays seen in the surgery-alone controls from those seen after surgery and drug treatment. In this way, the effectiveness of the chemotherapy treatment given at a particular sequence interval could be compared with effect of the drug alone in intact tumors (6).

Student’s t test was used to assess differences between group means, and \( p < 0.05 \) was assumed sufficient to reject the null hypothesis.

**RESULTS**

Chart 1 shows the results from a series of experiments to assess the effect of surgical cytoreduction on the proliferation of RIF-1 tumor cells in residual s.c. primary tumors. Within 24 hr after surgery (75% tumor removal), \(^{3}H\)dThd LIs were reduced by approximately 60%. Hydroxyurea killing in vitro also suggested relatively few S-phase clonogenic cells in the tumor at this time. Proliferation in both the clonogenic compartment and the total cell population began to recover at 72 hr after surgery. At 96 hr, S-phase clonogenic fractions and \(^{3}H\)dThd LIs were 60 to 70% greater than those observed prior to surgery. The PDP index remained unchanged during the first 48 hr but, by 72 hr after surgery, PDP index values were increased by approximately 80%. Dexamethasone treatments (10 mg/kg), every 12 hr for 4 doses beginning 36 hr after surgery, delayed the proliferative recovery in the residual tumor tissue until about 48 hr after the last dexamethasone treatment.

Changes in cell proliferation after partial tumor resection were also observed to correlate temporally with changes in the sensitivity of residual RIF-1 tumor to 5-FUra and CP (Chart 2). In these studies, mice with s.c. RIF-1 tumors were subjected to 75% primary tumor resection and then treated with either 5-FUra (84 mg/kg) or CP (150 mg/kg) at various intervals after surgery. When the regrowth delay for surgery alone was subtracted from the regrowth delay observed for surgery plus drug treatment, the results indicated that the residual tumor responded best at 48 to 72 hr after surgery. The regrowth delays with the 48- and 72-hr CP treatment were approximately twice those seen in the intact tumors. The response to 5-FUra was somewhat more sequence-dependent but, as in the CP study, the best sequence interval was 72 hr. 5-FUra-induced regrowth delays at this time were approximately 70% longer than after a similar dose in intact tumors. These results correlate well in time and in magnitude with the nearly 80% increase in the PDP index seen in residual RIF-1 tumors 3 days after surgery.

Chart 3 shows the changes in clonogenic cell proliferation and lung weights in RIF-1 “artificial” lung metastases after amputation of a “primary” RIF-1 tumor. The changes in the clonogenic S-phase fraction, assessed by HU killing in vitro, indicated that, in this “artificial” metastasis model, tumor cell proliferation was...
transiently increased at 72 hr after primary tumor surgery. Changes in lung weights for control and surgery groups indicated inhibition of tumor growth up to 72 hr after surgery. Increased proliferation seen at 72 and 96 hr was manifest by a doubling in lung weights between 4 and 5 days after surgery. When similarly prepared mice (n = 16 mice/group) were treated with a single i.p. dose of CP (150 mg/kg) at 24, 48, 72, 96, 120, or 188 hr after surgery, median survival, relative to the surgery-alone control was increased by 33, 15, 81, 11, 33, and 22%, respectively.

Chart 4 shows the results from 2 studies to assess the influence of the systemic surgical stress on cell proliferation in s.c. RIF-1 tumors. In s.c. RIF-1 tumors, amputation of a non-tumor-bearing leg (surgical controls) resulted in a transient decrease in the [3H]dThd LI which returned to normal, without an overshoot, by 72 hr. In animals bilaterally adrenalectomized 24 hr prior to partial tumor resection, [3H]dThd LI in the residual s.c. tumor were increased by nearly 50% at 24 hr after surgery, remaining at or near this level for 96 hr. HU killing indicated that clonogenic cell proliferation was significantly increased 24 hr after adrenalectomy (Chart 5) and, at 24 hr after surgery, the clonogenic S-phase fraction was nearly twice that in untreated controls. The decrease in clonogenic cell proliferation observed 48 hr after surgery in adrenalectomized animals is difficult to explain, but such a decrease was observed in 2 replicate experiments. Clonogenic cell proliferation was also increased at 72 and 96 hr after surgery.

In "artificial" lung metastases, decreased [3H]dThd uptake per mg of lung also suggested decreased proliferation after amputation of the contralateral non-tumor-bearing leg, (Chart 6). The time course of the response in these surgery controls was not...
different from that in the tumor amputation groups; however, in
the latter, \[^{[3}H\]dThd uptake remained elevated for at least an
additional 24 hr relative to that in the surgery controls. Changes
in \[^{[3}H\]dThd uptake per mg after surgery and dexamethasone
treatments indicated that, as in residual primary tumors (Chart
1), dexamethasone treatments during the expected recovery
interval delayed proliferative recovery until approximately 48 hr
after dexamethasone treatments.

In previous studies, we documented the presence of high-
affinity, low-capacity binding sites for dexamethasone in RIF-1
cystosol preparations (7), and it seemed reasonable to hypoth-
thesize that the transient decrease in cell proliferation in the
residual RIF-1 tumor after surgical cytoreduction could have been
mediated by changing levels of endogenous corticosterone after
surgery. Chart 7 shows the results from 2 replicate studies to
assess changes in total serum corticosterone after 75% resec-
tion of s.c. RIF-1 tumors. Corticosterone levels increased nearly
5-fold within 2 hr after the surgical procedure (9 a.m.). Total
corticosterone levels were within normal range by 96 hr after
surgery, with transient increases observed at 18 and 48 hr. As
might be expected, few saturable (4°) dexamethasone binding
sites were detected in samples collected 6 hr after surgery.
Recovery or desaturation of the dexamethasone receptors cor-
responded to the reduction in serum corticosterone levels. Re-
sumption of clonogenic cell proliferation occurred at a time when
corticosterone levels and dexamethasone receptor site satura-
tion returned to nearly presurgical levels.

Corticosterone levels were also observed to increase after leg
amputation in surgical controls (Chart 8). In general, the response
was similar to that seen after 75% tumor resection. Peak serum
levels, noted within 2 hr, rapidly decayed to near control by 12
hr. As after 75% tumor resection, a slight rise in serum cortico-
sterone levels was noted at 48 hr, but the increase at 18 hr,
observed in the tumor surgery group, was not seen in the leg
amputation controls. Resolution of cell proliferation in the
tumors from these surgery controls was observed 24 to 48 hr after
surgery.

Since previous studies indicated that the timing for resumption
of cell proliferation after dexamethasone treatments could be
directly correlated with the level of GR (7, 8, 11), we thought it
reasonable to postulate that postsurgical proliferative recovery
should occur sooner in tumor models with lower GR content.
Charts 9 and 10 show changes in cell proliferation in residual
s.c. SaD2 and R3230AC tumors at various intervals after three-
fourths tumor resection. In the SaD2 mouse model (GR, 75 fmol/
mg of protein), although peak \[^{[3}H\]dThd LI values were not
observed until 36 hr after surgery, increases in the PDP index
suggestive of increases in the fraction of actively cycling cells
were observed within 12 hr after surgery. In the R3230AC
mammary tumor model (GR, 125 fmol/mg of protein), \[^{[3}H\]dThd
LIs were subnormal up to 36 hr after surgery. At 48 hr, \[^{[3}H\]dThd
LIs were increased nearly 60% above those in presurgical con-
trols.

The results from the present studies in RIF-1, SaD2, and
R3230AC solid tumor models indicate that changes in endoge-
 nous adrenal hormones resulting from the systemic stress of the
surgical procedure may have mediated the postsurgical changes
in tumor cell proliferation. Further, the results would also seem

Cell Proliferation in Residual Tumors

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Corticosterone. In adrenalectomized tumor-bearing mice, proliferation inhibition after surgery was not observed, and proliferation in residual tumor was increased as early as 24 hr after surgery. The data obtained with the RIF-1 model also seem to indicate that postsurgical proliferation inhibition and the subsequent hyperplasia may be mediated through corticosteroid hormone receptors, since receptor site saturation and desaturation were found to have a close temporal correlation with the postsurgical "surge" in endogenous corticosterone and the subsequent decay to control levels. In both s.c. RIF-1 tumors and RIF-1 lung nodules, increased proliferation above that in the respective presurgery controls was noted only after tumor cytoreduction and not in the surgery controls.

Although, in our studies with the R3230AC mammary tumor, we did not measure serum corticosterone levels after tumor debulking, previous studies on rats (14) have indicated that the time course of changes in serum corticosterone levels after surgical trauma were similar to that seen in the C3H/HeJ mice. Previous studies in the R3230AC tumor model have also indicated that dexamethasone treatments result in a G, progression delay which can be correlated with the saturation and desaturation of the corticosteroid receptor (10). Thus, the results from the present studies would imply that, in the RIF-1 tumor and perhaps in the R3230AC tumor, changes in endogenous corticosterone levels after surgery may influence the time course of the postsurgical proliferative response. In C3H/HeJ mammary tumors (6), the timing of the postsurgical proliferative response was seen to correlate with the extent of primary tumor surgery. In retrospect, however, the observation could have been more directly related to the systemic stress of the various surgical procedures. Numerous clinical studies (18, 24, 26) have indicated that postsurgical plasma cortisol can be substantially increased in patients receiving general anesthesia, for up to 7 days after major surgery. In addition to a direct relationship between postsurgical plasma cortisol levels and the magnitude of surgical trauma, McIntosh et al. (24) demonstrated that, although the circadian rhythm of cortisol release was maintained during the postsurgical recovery interval, the periodicity of the cycle could be inversely correlated with the degree of surgical trauma.

Previous studies in numerous GR plus experimental tumor models, including the Ehrlich ascites tumor (22), C3H/HeJ mammary tumors (6, 15, 17), and Lewis lung tumors (32, 33) have indicated that, within a few days after surgical cytoreduction, proliferation of residual tumor, either at or distant to the surgical site, can be increased substantially over that seen prior to surgery. In C3H/He, spontaneous and first-generation mammary tumors (6), RIF-1, and R3230AC rat mammary tumors subjected to partial resection of s.c. tumor, the interval increased proliferation was preceded by a variable interval of reduced proliferation. A similar response pattern was observed in RIF-1 "artificial" lung metastases after surgical amputation of a "primary" tumor-bearing leg. In primary RIF-1 tumors, the interval of increased cell proliferation in the residual tumor was characterized by increases in [3H]dThd LIs, clonogenic S-phase fractions, and PDP indices. Although Tc estimates could not be made from the data, the observations are not inconsistent with a postsurgical increase in the fraction of actively proliferating cells in the residual tumor. A similar conclusion was made from studies in a transplantable mammary tumor model (17). The increase in sensitivity of residual tumor to 5-FUra and CP in the present studies was similar in magnitude to the increase in the PDP index and would be consistent with this conclusion.

Desser-Weist (14) showed that, in rats, the timing for initiation of the hyperplastic response after partial hepatectomy could be temporally correlated with changes in endogenous corticosterone levels. Changes in endogenous corticosteroid hormones have also been shown to affect cell proliferation in other normal tissues. For example, cell proliferation in the kidney was shown to increase after bilateral adrenalectomy (27). In immature lung, corticosteroid receptors (16) and proliferation of type II alveolar cells decrease (21), while surfactant production increases (13) as corticosteroid levels increase during the neonatal period or after corticosteroid treatments (12, 13, 20). In the present experiments with the RIF-1 tumor model proliferation, inhibition after surgery correlated temporally with increased levels of serum corticosterone. In adrenalectomized tumor-bearing mice, proliferation inhibition after surgery was not observed, and proliferation in residual tumor was increased as early as 24 hr after surgery. The data obtained with the RIF-1 model also seem to indicate that postsurgical proliferation inhibition and the subsequent hyperplasia may be mediated through corticosteroid hormone receptors, since receptor site saturation and desaturation were found to have a close temporal correlation with the postsurgical "surge" in endogenous corticosterone and the subsequent decay to control levels. In both s.c. RIF-1 tumors and RIF-1 lung nodules, increased proliferation above that in the respective presurgery controls was noted only after tumor cytoreduction and not in the surgery controls.

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Previous studies in numerous GR plus experimental tumor models, including RIF-1 (7), SaD2, and R3230AC (8, 10), have indicated that the antiproliferative effect of dexamethasone, a synthetic corticosteroid, is both dose and GR dependent (4, 5, 7–10), that the antiproliferative effect is a manifestation of a reversible G, block in cell cycle progression, and that the dose-dependent timing for cell cycle progression after dexamethasone treatments can be directly correlated with the pretreatment GR level (10).

In the present studies, maximal postsurgical proliferation was observed at 4 days in s.c. RIF-1 tumors (GR, 285 fmol/mg), at 3 days after tumor amputation in RIF-1 lung nodules (GR, 225 fmol/mg in RIF-1 primary), at 2 days in residual R3230AC tumors (GR, 125 fmol/mg), and at 1.5 days in residual SaD2 tumors (GR, 75 fmol/mg). That, in both the RIF-1 and SaD2 models, the PDP index increased prior to increases in S-phase cellularity suggests that, as in residual liver after partial hepatectomy (14), recruitment per se was not affected by high endogenous corticosteroids, but that cell cycle progression of newly recruited cells was inhibited until after corticosterone levels returned to nearly normal levels.

That changing hormonal levels may play a role in controlling...
the dynamics of proliferation in residual tumor tissue after surgery suggested a strategy by which proliferative recovery in the residual cell population could be manipulated. In residual s.c. RIF-1 tumors as well as in "artificial" lung metastases, the postsurgical proliferative response could be delayed with dexamethasone when treatments were initiated prior to the expected recovery interval. This is not unlike the results of studies with C3H/HeJ mammary tumors (6). In these studies, dexamethasone treatments also delayed postsurgical proliferative recovery. Further, chemotherapy was observed to be more effective after surgery and dexamethasone than after surgery alone.

Studies with the RIF-1, Lewis lung (5, 9), and C3H mammary tumor (6) models indicated that the effectiveness of postsurgery chemotherapy with vincristine, 5-FUra, vincristine plus 5-FUra, or CP was schedule dependent. In RIF-1 and C3H mammary tumors, increased effectiveness was concluded from regrowth delay studies while, in RIF-1 "artificial" metastases and Lewis lung metastases (5, 9), appropriately scheduled treatments provided a survival advantage.

In a highly transplanted rapidly proliferating mammary tumor model, "adjuvant" CP provided a survival advantage; however, no additional survival advantage was observed when a single CP dose was administered when cell proliferation in i.m. tumors was increased after amputation of contralateral tumor-bearing leg (15). Inasmuch as proliferation inhibition after surgery was not observed in those studies, it may be possible that this tumor model either did not have GR or that it is more like the low-GR-content, rapidly proliferating SaDu model studied in the present experiments.

These observations in experimental tumor models prompt a number of questions regarding whether similar responses occur in human tumors and, if so, could they be exploited to increase the effectiveness of adjuvant therapy? Although Buzdar et al. (11), in a retrospective analysis, found no advantage to "early" versus "late" initiation of adjuvant chemotherapy for disease-free interval, chemotherapy initiated at the time of surgery has, in other studies (25), been found to be more effective than when treatment was delayed. Although GR has been documented in many types of human tumors (1, 19, 29, 34), it is uncertain if cortisol changes in response to the physical and emotional stress associated with cancer surgery in humans are comparable to the surgical stress in the rodent systems. Nonetheless, sufficient rationale may exist to initiate a controlled clinical trial in GR plus tumors to determine if sequential perioperative adjuvant chemotherapy might offer a therapeutic advantage.

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