Glucocorticoid Receptors and the Effect of Glucocorticoids on the Growth of B16 Melanoma

Hemlata S. Bhakoo, Norman S. Paolini, Richard J. Milholland, Reynold E. Lopez, and Fred Rosen

ABSTRACT

The glucocorticoid receptors were measured and characterized in the transplantable B16 murine melanoma using [3H]-dexamethasone by a charcoal adsorption technique. In the tumor cytosols assayed, the levels of receptors ranged from 44 to 200 fmol/mg protein, and the corresponding Ke's ranged from 2 to 43 nM. Sucrose density gradient analysis showed a peak sedimenting at 7.1S under low-ionic-strength buffer which was completely eliminated with a 100-fold molar excess of unlabeled triamcinolone acetonide in the incubation mixture. This peak of bound radioactivity shifted to the 4.4S region under high-ionic-strength buffer (0.4 M KCl) conditions. Competition experiments, using [3H]dexamethasone and various unlabeled steroids at a 100-fold molar excess, showed characteristics typical of glucocorticoid receptors seen in other tissues.

Administration of various glucocorticoids, e.g., dexamethasone, hydrocortisone acetate, and prednisolone, in different doses and regimens showed a marked and significant inhibition of tumor growth as measured by mean tumor diameter and weight. Although glucocorticoid treatment does not seem to affect the incidence of pulmonary metastases, the number of pulmonary nodules appears to be significantly greater in some groups treated with higher doses of these drugs. In survival experiments, administration of hydrocortisone acetate in various doses and regimens also resulted in a significant increase in the median survival of mice compared to 0.9% NaCl solution-treated controls. These results indicate that the growth inhibition of B16 melanoma by glucocorticoids may be a direct effect mediated by interaction with the glucocorticoid receptor.

INTRODUCTION

Recent studies have reiterated the importance of the interaction between the ligand and specific cytoplasmic receptor molecules as the initial step in the regulation of phenotypic expression by steroid hormones. We have reported previously the presence and characterization of high-affinity glucocorticoid receptors in 86% of melanoma biopsies obtained from a group of patients undergoing treatment at Roswell Park Memorial Institute (4). Although the role of glucocorticoid receptors in melanoma is far from clear, the presence of these receptors indicates that this neoplasm may be glucocorticoid responsive. Furthermore, the hormone responsiveness of some melanomas has been suggested previously in clinical studies (21) and animal models (11, 13, 17, 20, 25) and recently in tissue culture (31).

In this study, we chose B16 malignant melanoma in mice as a model system to investigate the presence and the nature of glucocorticoid receptors and study the effect of pharmacological doses of glucocorticoids on the growth of this neoplasm. Our studies show that B16 melanoma contains high-affinity limited-capacity receptors for glucocorticoids demonstrable by the charcoal dextran adsorption technique and sucrose density gradient analyses. Also, the growth of this tumor is inhibited by the treatment with pharmacological doses of glucocorticoids.

MATERIALS AND METHODS

Male and female C57Bl/6J mice were obtained from West Seneca Laboratory, Buffalo, N. Y. Injectable hydrocortisone acetate [Hydrocortone Acetate; 21-(acetyloxy)-11β,17-dihydroxy pregn-4-ene-3,20-dione] was purchased from Merck Sharp & Dohme, West Point, Pa.; dexamethasone phosphate [Hexadrol Phosphate; pregn-4-ene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(phosphonoxy)-disodium salt (11β,18a)] from Organon Inc., West Orange, N. J.; and prednisolone acetate (Meticortelone Acetate; 11β,17,21-trihydroxyprogren-1,4-diene-3,20-dione-21-acetate) from Schering Corp., Kenilworth, N. J. Trizma base, EDTA, dithiothreitol, and dextran were of reagent grade and purchased from Sigma Chemical Co., St. Louis, Mo. as were dexamethasone (9α-fluoro-11β,17a,21-trihydroxy-16α-methylpregna-1,4-dione-3,20-dione) and TA.4 Norit A, purchased from Fisher Scientific Co., Pittsburgh, Pa., was activated by washing with 1 N HCl, followed by a saturated solution of sodium bicarbonate to a final pH of 7.4. Dextran-coated charcoal consisted of a suspension of 5% activated charcoal and 0.5% dextran in a buffer containing 10 mm Tris, 1.5 mMDTA, and 0.5 mm dithiothreitol (pH 7.4) at 4°. [1,2-3H]Dexamethasone (20.9 Ci/mmol) and [6,7-3H]TA (33.7 Ci/mmol) were purchased from New England Nuclear, Boston, Mass. RNase-free crystalline sucrose was obtained from Schwarz/Mann, Orangeburg, N. J. ACS was a product of Amersham/Searle Corp., Arlington Heights, Ill.

B16 Tumor Transplantation. The B16 mouse melanoma used in these studies was obtained from Dr. Goldrosen of the Department of Surgical Oncology at this institute. Six- to 8-week-old inbred C57BL/6J mice were each given s.c. injections of 1 x 10⁵ viable cells in the medial thigh. Thereafter, each mouse was examined twice a week, and the day of appearance of the tumors was noted. When the tumors became palpable, they were measured in 2 dimensions with calipers. After 28 to 32 days, the animals were killed, the tumors were

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4 The abbreviations used are: TA, triamcinolone acetonide; 9α-fluoro-11β,16α,17α,21-tetrahydroxy-pregna-1,4-diene-3,20-dione-16,17-acetonide; ACS, aqueous counting scintillant.
excised and weighed, and the macroscopic lung metastases were counted under a dissecting microscope. Neither ulcerated nor necrotic tumors were included in the study. Significance of differences in both tumor measurements and pulmonary metastases between treatments was tested by Student’s t test.

In another experiment, the animals were adrenalectomized or sham operated under Nembutal anesthesia 1 week before tumor implantation. These animals were maintained throughout the experiment ad libitum on 0.9% NaCl solution.

Administration of Drugs. Various doses of hydrocortisone acetate were made immediately before injection by appropriately diluting the stock suspension (25 mg/ml) into 0.9% NaCl solution. Dexamethasone and prednisolone were similarly diluted from their stock solutions with 0.9% NaCl solution. Each mouse received the designated dose of the drugs s.c. in a total of 0.1 ml of solvent. In most of the experiments, mice were treated 1 day after the tumor transplantation while, in others, the treatment started 2 weeks after tumor transplant. The exact regimens of drug administration are specified in the chart legends.

Glucocorticoid Receptor Assay. B16 melanomas excised from tumor-bearing mice usually 2.5 to 3 weeks after transplantation were used for receptor studies. The tumor was weighed and placed in 5 volumes of ice-cold buffer containing 10 mM Tris, 1.5 mM EDTA, 0.5 mM dithiothreitol, and 10% (v/v) glycerol (pH 7.4) at 4° and a cell-free preparation made by using a Polytron PT 10 ST homogenizer (Brinkmann Instruments, Inc., Westbury, N. Y.) set at 5. The tissue was homogenized 4 times for 15 sec each, allowing a 1-min interval between each exposure for cooling.

The 108,000 × g supernatant (cytosol) was prepared and pretreated with dextran-coated charcoal prior to incubation exactly as described earlier (4). The soluble cytoplasmic protein concentration ranged from 6 to 10 mg/ml and was determined by the method of Lowry et al. (14). The binding of glucocorticoid to melanoma cytosol was measured by incubation of 200 μl cytosoI with 100 μl of [3H]dexamethasone or 100 μl of [3H]dexamethasone containing 100-fold molar excess of unlabeled dexamethasone for 4 hr at 4° in 12-x 75-mm culture tubes. Incubations were carried out at a minimum of 5 concentrations of [3H]dexamethasone with final concentrations varying from 5 to 100 nM. The actual concentration of [3H]dexamethasone was determined by counting 10-μl aliquots of the incubation mixture. At the end of the incubation, the amount of dexamethasone bound was determined by treating 75 μl of incubation mixture with 25 μl of dextran-coated charcoal for 10 min at 4° and then centrifuging at 850 × g for 10 min. The supernatant was counted in 5 ml ACS in a Beckman LS-100C or LS-150 liquid scintillation counter at 40 to 45% efficiency.

Specific binding was determined by the difference between total binding and binding in the presence of a 100-fold molar excess of unlabeled dexamethasone. Total binding capacity and Kd were calculated according to the method of Scatchard (22).

Sucrose Gradient Centrifugation. For sucrose density centrifugation analyses, the cytosols from the tumors were incubated with a final concentration of 20 nM [3H]TA or 20 nM [3H]TA plus 2 μM unlabeled TA, since receptor TA complex has been shown to be more stable than the dexamethasone receptor complex (10). After the incubation of the cytosol with TA as described above, the bound steroid was separated from free steroid by addition of 75 μl of dextran-coated charcoal to 225 μl of the incubation mixture. After centrifugation, 200 μl of the supernatant were layered on a 5-m linear gradient of 5 to 20% (w/v) sucrose in buffer containing 10 mM Tris, 1.5 mM EDTA, 0.5 mM dithiothreitol, and 10% (v/v) glycerol (pH 7.4) at 4°. The samples were centrifuged at 49,000 rpm for 17 hr at 4° in a SW 50.1 rotor, and then 8-drop fractions were collected by puncture of the gradient tube at the bottom. Radioactivity was determined by the addition of 5 ml of ACS and counted in a liquid scintillation counter. Bovine serum albumin with a sedimentation constant of 4.4S was used as a marker for the determination of the sedimentation coefficient according to the method of Martin and Ames (15).

RESULTS

Glucocorticoid Receptor and Its Characterization. Chart 1 shows saturable and specific binding of [3H]dexamethasone to melanoma cytosol. Scatchard analysis (22) of the binding data (inset) shows a single class of high-affinity low-capacity binding sites, with an equilibrium Kd of 19.5 nM and total receptor site concentration of 181 fmol/mg protein. In 7 different tumors assayed for glucocorticoid receptors, the values for total receptor sites ranged from 44 to 200 fmol/mg protein, and the corresponding Kd ranged from 2 to 43 nM.

Sucrose gradient analyses were performed to further characterize the glucocorticoid receptor, and the binding of [3H]TA showed the typical characteristic of a steroid hormone receptor. Chart 2 shows the results of the gradient analysis of [3H]TA binding to a representative melanoma cytosol. The binding of [3H]TA alone is characterized by a 7.1 S peak under low-salt conditions shown in Chart 2A; with a 100-fold molar excess of unlabeled TA in the incubation mixture, most of the binding in 7.1 S peak was eliminated. This peak of bound radioactivity shifted to 4.4S region under high-ionic-strength buffer (0.4 M KCl) conditions (Chart 2B). These results indicate a specific glucocorticoid receptor in B16 melanoma with typical characteristics of a steroid hormone receptor under high- and low-ionic-strength conditions.

![Chart 1](chart1.png)

Chart 1. A representative plot of specific binding of [3H]dexamethasone to B16 melanoma cytosol as a function of [3H]dexamethasone concentration. Inset, Scatchard plot of the binding data. In this tumor, the Kd was calculated to be 19.5 nM, and the total binding capacity was 181 fmol/mg protein.
Glucocorticoid Receptors and Chemotherapy of B16 Melanoma

Chart 2. Density gradient centrifugation of \[^{3}H\]TA receptor complex from B16 melanoma cytosol without 0.4 M KCl (A) or with 0.4 M KCl (B); O, \[^{3}H\]TA; \(\bullet\), \[^{3}H\]TA + cold TA; BSA, position of bovine serum albumin in the gradient which sedimented at 4.4S.

Competition experiments using \[^{3}H\]dexamethasone and various unlabeled steroids at a 100-fold molar excess showed 10 and 7% competition with estradiol and 5α-dihydrotestosterone, respectively, while progesterone, 17,21-dimethyl-19-nor-4,α-pregnadiene-3,20-dione (R5020), a synthetic progestin, and TA showed almost 100% competition compared to radioinert dexamethasone. This is typical of glucocorticoid receptors in other tissues (1, 8) as well.

Glucocorticoid Inhibition of Tumor Growth. The effect of various doses of dexamethasone on the growth of B16 melanoma in male mice is shown in Chart 3, and the average tumor weights and the number of pulmonary nodules from the same experiment are summarized in Table 1. Each mouse received either 0.05, 0.4, or 1.25 mg of dexamethasone or 0.9% NaCl solution 3 times a week starting the day after tumor transplant. Chart 3 shows the mean tumor diameters expressed as the percentage of control. While all 3 doses of dexamethasone tested showed a significant reduction in tumor size as well as tumor weight (Table 1), the 1.25-mg dose was the most effective. The number of pulmonary nodules, however, did not show any differences between the 50- and 400-μg dexamethasone treatment groups.

Chart 4 shows the effect of another synthetic glucocorticoid, prednisolone, at 2 different doses administered in male mice via different injection schedules. Prednisolone administered 2 days after tumor transplant at either a 0.6-mg dose for a total of 8 injections or at a 1.25-mg dose for a total of 5 injections was very effective in inhibiting the growth of this tumor as long as the administration of this drug was continued. The duration of treatment with the 1.25-mg dose of prednisolone in this experiment was reduced to 1.5 weeks because the toxicity of this drug at higher doses resulted in a lower number of mice surviving until the termination of the experiment. It is particularly noteworthy that the substantial inhibitory effect of prednisolone on the growth of B16 melanoma is obvious even when the compound is administered 2 weeks after tumor transplant and that it is not accompanied by any major toxicity. Table 2 summarizes the data on tumor weight and the incidence and number of pulmonary nodules from the experiment shown in Chart 4. Prednisolone appears to inhibit tumor growth as indicated by a reduction in the tumor weights at all of the doses tested. Although there were no substantial differences in the
incidence of lung metastases in various treatment groups, prednisolone treatment (1.25 mg 5 times) initiated 1 day after transplant showed a significant increase in the number of pulmonary nodules compared to 0.9% NaCl solution-injected controls at the end of the experiment. However, this dose and regimen of prednisolone appeared to be very toxic as seen by the reduction in the number of mice which survived the experimental period.

Prednisolone administered 2 weeks after transplant (Group 4; 1.25 mg 4 times) was also very effective in inhibiting the growth of the tumor as indicated by a significantly lower tumor weight (~70% below the controls).

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Dexamethasone (mg/mouse)</th>
<th>Tumor wt (g)</th>
<th>No. of pulmonary nodules</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>5.7 ± 0.600</td>
<td>5.6 ± 1.5 (1-14)</td>
</tr>
<tr>
<td>B</td>
<td>0.05</td>
<td>3.8 ± 0.400</td>
<td>4.10 ± 1.1 (0-12)</td>
</tr>
<tr>
<td>C</td>
<td>0.40</td>
<td>3.4 ± 0.300</td>
<td>3.6 ± 1.20 (0-12)</td>
</tr>
</tbody>
</table>

Effects of prednisolone administered s.c. on the growth of B16 melanoma in male mice. Injections were started on a Monday-Wednesday-Friday schedule either on the second day after transplantation (prednisolone, at 0.6 mg/mouse for a total of 8 injections, 0.9% NaCl solution, at 0.1 ml or 1.25 mg/mouse for 5 injections) or 15 days after transplantation (prednisolone, at 1.25 mg/mouse for a total of 4 injections). Each treatment group included 12 mice/group at the start of the experiment; numbers in parentheses, number of animals that survived until the termination of the experiment; points, mean tumor diameter; bars, S.E.

Table 2

<table>
<thead>
<tr>
<th>Prednisolone (mg/mouse x no. of injections)</th>
<th>Tumor wt (g)</th>
<th>Incidence*</th>
<th>Av. no./mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>4.0 ± 0.8</td>
<td>8/9</td>
</tr>
<tr>
<td>2</td>
<td>0.6 X 8</td>
<td>1.9 ± 0.4</td>
<td>5/7</td>
</tr>
<tr>
<td>3</td>
<td>1.25 X 5</td>
<td>1.9 ± 0.5</td>
<td>3/3</td>
</tr>
<tr>
<td>4</td>
<td>1.25 X 4</td>
<td>1.2 ± 0.2</td>
<td>8/10</td>
</tr>
</tbody>
</table>

* Indicates the number of animals showing pulmonary nodules among those that survived until the end of the experiment.

Table 3 summarizes the effect of different doses of hydrocortisone on the growth of B16 melanoma, the incidence and number of pulmonary metastases in female mice bearing B16 melanoma. Hydrocortisone, like the other glucocorticoids tested, inhibits the growth of this tumor as indicated by the mean tumor diameter on the 28th day following tumor transplant. This inhibition appears more significant in terms of the tumor weights, 80% inhibition in Group 2 receiving hydrocortisone starting 1 day after tumor transplant and 56% in Group 3 which received hydrocortisone 2 weeks after the transplant. Although the incidence of pulmonary metastases in either of the treatment groups does not seem to be markedly affected, the average number of pulmonary nodules is significantly higher only in the group which received greater number of hydrocortisone injections (Group 2).

In order to evaluate the influence of endogenous glucocorticoids on the growth of B16 melanoma, we studied the effect of surgical ablation of adrenals or sham operation on the growth of this neoplasm. Adrenalectomy 1 week before tumor transplant resulted in tumor diameters somewhat greater than in sham-operated controls although this difference was not statistically significant as measured up to 4 weeks after transplantation (data not shown).

Effect of Glucocorticoid on Survival. In this experiment, mice were treated with 2 different doses of hydrocortisone (0.3 and 0.6 mg) starting either the day after or 2 weeks after transplant for a period of 3 weeks and compared to 0.9% NaCl solution-injected controls. Chart 5 illustrates animal survival rates plotted versus time for various treatment groups. The median survival time for control animals was 32 days; hydrocortisone treatment administered the day after the transplant for 3 weeks prolonged the median survival to 123 and 130% of 0.9% NaCl solution-treated controls for the 0.3- and 0.6-mg doses, respectively. Similar enhancement of median survival of mice was observed at 0.3- and 0.6-mg doses of hydrocortisone given for 2 weeks when initiated 2 weeks following the transplant (123 and 140%, respectively). Thus, administration of hydrocortisone at both these doses and regimens resulted in a significant increase in the median survival of the animals (p < 0.01) as determined by one-way analysis of variance test.
Table 3
Effect of hydrocortisone on the growth of B16 melanoma and incidence and number of pulmonary metastases in C57BL/6J mice

Female mice (12/group), 7 to 8 weeks old, were given s.c. injections of \(1 \times 10^5\) B16 melanoma cells. Starting 1 day (Group 2) or 15 days (Group 3) after transplant, a suspension of hydrocortisone in 0.9% NaCl solution was injected s.c. on a Monday-Wednesday-Friday schedule. Drug dose and total number of injections were indicated in the table. Tumor diameters were measured on Days 8, 16, and 28 after transplant. All mice were killed 4 weeks after transplant, tumors were weighed, and the number of pulmonary metastases was determined with the aid of a dissecting microscope.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>8th day</th>
<th>16th day</th>
<th>28th day</th>
<th>Tumor wt (g)</th>
<th>Incidence (^a)</th>
<th>Av. no./mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9% NaCl solution</td>
<td>4.0 ± 0.1(^b)</td>
<td>9.10 ± 0.7(^b)</td>
<td>20.2 ± 1.3(^b)</td>
<td>4.1 ± 0.6(^b)</td>
<td>4/6</td>
<td>1.5 ± 0.7(^d) (0-4)(^e)</td>
</tr>
<tr>
<td>2</td>
<td>Hydrocortisone ((1.25 \text{ mg} \times 10))</td>
<td>3.5 ± 0.1</td>
<td>6.8 ± 0.3</td>
<td>14.1 ± 0.3</td>
<td>0.8 ± 0.3(^d)</td>
<td>10/10</td>
<td>6.3 ± 1.6 (1-15)(^e)</td>
</tr>
<tr>
<td>3</td>
<td>Hydrocortisone ((1.25 \text{ mg} \times 6))</td>
<td>3.9 ± 0.2</td>
<td>8.1 ± 0.6</td>
<td>15.9 ± 1.0</td>
<td>1.8 ± 0.3(^d)</td>
<td>6/9</td>
<td>3.8 ± 1.8 (0-14)(^e)</td>
</tr>
</tbody>
</table>

\(^a\) Indicates the number of animals showing pulmonary nodules among those that survived until the termination of the experiment.

\(^b\) Mean ± S.E.

\(^c\) Numbers in parentheses, range.

\(^d\) Group 1 versus 2 and 3 (\(p < 0.005\)).

\(^e\) Group 1 versus 2 (\(p < 0.05\)); Group 1 versus 3 (not significant).

In another experiment, hydrocortisone was administered s.c. at a dose of 0.5 mg twice a week until animal death in order to determine if chronic administration of hydrocortisone would maintain a slow growth of this tumor and result in a greater increase in the median survival time. The results of this experiment are illustrated in Chart 6. The median survival time for the control group in this experiment was 39 days; hydrocortisone treatment, initiated the day after the tumor transplant, resulted in a median survival of 46 days (\(p < 0.01\)). In the mice that received the same dose of hydrocortisone twice a week, starting 2 weeks after the tumor transplant, there was further increase in the median survival to 53 days (\(p < 0.01\)). Therefore, it appears that chronic administration of hydrocortisone starting 2 weeks are tumor transplant can further enhance the median survival time to 136% of control.

**DISCUSSION**

These studies document the presence of a specific high-affinity glucocorticoid receptor in the cytosol of B16 melanoma. The binding of dexamethasone to glucocorticoid receptor has been shown to be saturable with apparently a single class of high-affinity binding sites. The characteristics of this receptor appear similar to those described in other normal (19) and neoplastic tissues (6, 12, 27). The sedimentation profiles of this receptor under low- and high-ionic-strength buffers also appear similar to those of other steroid hormone receptors (9, 32). Competition with various steroids in a 100-fold molar excess for the binding to glucocorticoid receptor show characteristics similar to those reported in biopsies from melanoma patients (4) and in human breast cancer (30).

Our studies indicate that administration of glucocorticoids effectively inhibits tumor growth, based on the criteria evaluated, e.g., tumor size and weight. Since each of the growth parameters measured is subject to some variation, this conclusion is based on a consistent observation made in at least 2 or 3 separate experiments. At all doses of prednisolone or hydrocortisone tested, treatment for 2 weeks beginning 2 weeks after tumor transplant was as effective in inhibiting tumor weight as 3 weeks of treatment beginning 1 to 2 days after tumor transplant (Tables 2 and 3). Both compounds when administered chronically for 3 weeks, starting 1 to 2 days after tumor transplantation, showed a significant increase in the number of pulmonary nodules. However, when the compounds were administered for 2 weeks starting 2 weeks after tumor transplant, there was no significant increase in the number of lung metas-
tases. Similar enhancing effects of even short-term glucocorticoid administration on the number of metastases have been reported previously for i.v.-injected B16 cells (7) and other tumors as well (3, 16, 34). Zeidman (34) has suggested that cortisone may increase metastases by promoting embolic arrest, since arrest of tumor emboli is necessary for the development of metastases.

Since administration of exogenous glucocorticoids inhibited the growth of B16 melanoma, it was of interest to determine if removal of endogenous corticosteroids, i.e., adrenalectomy prior to tumor transplant, would influence the growth of this neoplasm. Our results indicate that adrenalectomy did not have any significant affect on the growth of this tumor. In contrast, in MMI hamster melanoma possessing glucocorticoid receptor, Stanberry et al. (26) reported that adrenalectomy prior to tumor cell inoculation decreased tumor growth compared to sham-operated controls; furthermore, cortisol implants produced a dose-related increase in tumor growth. The contrast observed in the response of these 2 melanomas to cortisol treatment and adrenalectomy may be due to species differences as well as constitutive differences in the melanomas. Earlier studies by Adachi et al. (2) also reported cortisone suppression of B16 melanoma growth and suggested it to be a consequence of a decrease in enzymes limiting glucose utilization, namely, glucose-6-phosphate dehydrogenase and hexokinase activities, and subsequent production of energy.

Although glucocorticoids have been shown to inhibit effectively the growth of mammary tumors (29) and other murine experimental tumors, e.g., Lewis lung carcinoma (23), and to promote the remission of certain lymphoid cancers (18, 24), the precise mechanisms by which these compounds act are not known. Conceivably, the growth inhibition of B16 melanoma by glucocorticoids could take place directly on tumor cells mediated by the receptor mechanism or indirectly via the pituitary or an effect on the immune system (5, 33). Although glucocorticoids are known to be immunosuppressants, there are indications that this immunosuppression may be rather selective. For example, Schechter et al. (23) have reported that hydrocortisone treatment of mice bearing the Lewis lung carcinoma results in an inactivation of the precursor cells of suppressor cells with a resultant delay in tumor appearance. This report, coupled with the more recent finding of Stelzer et al. (28) of the appearance of an antigen-nonspecific suppressor cell along with a generalized immunosuppression in mice bearing the B16 melanoma, has suggested to us that hydrocortisone treatment of mice bearing the B16 melanoma might lead to a selective decrease in suppressor cells, thus allowing for a more marked antitumor immune response.

Further work is in progress to determine if immune modulations, e.g., thymectomy, irradiation, and treatment with thymosine of mice bearing B16 melanoma in combination with cortisol, influence the growth and metastases of this transplantable solid tumor.

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