ABSTRACT

Nuclear glucocorticoid receptor number, glucocorticoid effects on protein synthesis, and concanavalin A effects on protein synthesis were examined in rat splenic and thymic lymphocytes on Days 1 to 7 following exposure to a single 300-mg/kg injection of cyclophosphamide in vivo. Significant decreases in nuclear glucocorticoid binding occurred within 1 day after cyclophosphamide treatment. Binding continues to decrease until Day 3 and recovers partially by Day 7. Glucocorticoid receptor subcellular distribution studies demonstrate that receptor binding of glucocorticoids is decreased in both cytoplasmic and nuclear subcellular compartments when whole cells are incubated at 0 and 37°, respectively. No inhibitory effects of glucocorticoids on leucine incorporation into trichloroacetic acid-precipitable material were observed in both spleen and thymic lymphocytes for at least 3 days after the initial cyclophosphamide treatment. Maximal stimulation of leucine incorporation into protein by concanavalin A was suppressed during the entire experimental time course in both tissues, although partial recovery of the response was observed in splenic lymphocytes on Day 7. Unlike thymocytes, which responded to glucocorticoids with an inhibition of protein synthesis by Day 4, spleen cells were unresponsive for at least 7 days. The magnitude of the decrease in nuclear glucocorticoid-receptor binding decreased glucocorticoid responsiveness, and response to concanavalin A was dependent on the dose of cyclophosphamide administered in vivo. Drug concentrations as low as 25 mg/kg were sufficient to reduce receptor binding, glucocorticoid effects, and mitogen effects on protein synthesis. These observations suggest that the administration of cyclophosphamide in vivo destroys the capability of remaining splenic and thymic lymphocytes to respond to glucocorticoids.

INTRODUCTION

Combination therapy with 2 or more drugs with different modes of action has frequently proven useful in the chemotherapeutic treatment of cancer. One example of such a combined drug regimen commonly used in the treatment of lymphosarcoma is CY, 2 an alkylating agent; vincristine, a spindle arrestor; and prednisone, a synthetic glucocorticoid (7). The biochemical and pharmacological complications that may arise as a result of combined therapy have not yet been fully researched and understood. This study examines the sensitivity of splenic and thymic lymphocytes to glucocorticoids and mitogens in vitro following in vivo treatment of animals with CY. CY is used in immunosuppression as well as cancer therapy (6). It has found application particularly for acute and chronic lymphocytic leukemia, multiple myeloma, and lymphomas. CY is metabolized to the active phosphoramide mustard by hepatic microsomal enzymes (2), and most of its cytotoxic effects are believed to result from disruption of DNA synthesis that is due to the formation of guanine cross-bridges (6). CY is therefore most efficient in inhibiting the growth of rapidly dividing cells. Lymphoid cells are particularly responsive to CY with a 60 to 70% depletion of lymphoid organ lymphocytes observed within 1 day after CY and 95% depletion observed within 3 days in the mouse (17). Recovery of lymphoid cells in the spleen was observed within 7 days after 1 CY injection (17). Turk and Poultier (16) observed that a selective depletion of B-cells occurs following CY. Recent reports, however, suggest effects on T-cells specifically, with selective cytotoxicity towards T-suppressor and T-helper subpopulations (1, 17). Milton et al. (10) reported decreased T-cell cytotoxicity with prolonged skin graft survival in mice. In addition to a depletion in B- and T-cells, CY in vivo diminishes the ability of cells to synthesize DNA in response to T-cell mitogens such as concanavalin A and phytohemagglutinin (5, 10). Dumont and Barrois (5) ascribe this decreased responsiveness to a damaged mitotic apparatus or a less responsive subpopulation to T-cells.

Glucocorticoids are used as antiinflammatory; antitumor, and immunosuppressive agents and their applicability in cancer treatment corresponds to the presence of specific steroid hormone receptors in the cancerous cells (6). Glucocorticoids also protect against thrombocytopenia, hemolytic anemia, and bone marrow depletion that are caused by other methods of chemotherapy. Glucocorticoids interact initially with cytoplasmic receptors and subsequently undergo a temperature- and energy-dependent activation and translocation of the steroid:receptor complex to the nucleus. Supposedly, the nuclear accumulation of steroid:receptor complexes results in mRNA synthesis which allows for the production of proteins and hormonal effects (8, 9, 12, 13). In lymphoid cells these effects include inhibition of glucose, decreased RNA synthesis, decreased protein synthesis, increased nuclear fragility, and cell death. The data in this study show that treatment of animals with CY diminishes the ability of the remaining splenic and thymic lymphocytes to respond to glucocorticoids in vitro. This effect appears to be mediated by loss in both cytoplasmic and nuclear hormone receptor binding capacity.
MATERIALS AND METHODS

Male Sprague-Dawley rats (50 to 125 g) were adrenalectomized and then maintained on 0.9% NaCl solution and rat chow for 1 to 2 weeks prior to use. CY (Mead Johnson and Co., Evansville, Ind.) was injected i.p. in 1 ml of sterile 0.9% NaCl solution 1 to 7 days before sacrifice.

On the day of the experiment, the animals were decapitated and the spleen and thymus were removed and weighed. The spleen was inflated with Krebs-Ringer bicarbonate buffer, pH 7.4 by repeatedly injecting the tissue with buffer through a syringe. This procedure effectively removes ~80% of the RBC. The thymus and spleen tissue was minced with scissors, homogenized in a silicone-coated Kontes No. 22 glass homogenizer, and filtered through nylon mesh as described (11). The cells were pelleted by a 3- to 4-min centrifugation (500 × g), and the supernatant was discarded. After the cells were washed with an additional 40 to 50 ml of buffer, they were again centrifuged, resuspended, and filtered through the nylon mesh in the final desired volume of MEM without leucine. The cytocrit (cell concentration per ml of packed cell suspension) was determined on a microhematocrit apparatus. Viability as judged by trypan blue exclusion was determined on all preparations.

Nuclear and cytoplasmic glucocorticoid receptors were determined by the hypotonic shock and dextran-coated charcoal procedures described by Munck and Wira (14). Cell suspensions in Krebs-Ringer bicarbonate buffer were incubated with [3H]TA specific activity, 33.7 Ci/mmol, New England Nuclear, Boston, Mass. at concentrations ranging from 1.25 × 10^-7 to 5.6 × 10^-11 M. Incubations were carried out for 30 min in a shaking water bath at 37°, while the cells were maintained in a 95% O2:5% CO2 environment. A 20-μl pulse of 1 to 2 μCi/ml [14C]leucine or [3H]leucine (New England Nuclear) was added to each well of the microtiter plate, and the reaction was stopped by cooling the cells on ice and precipitating the protein with 20 μl of 50% TCA per well at 0°. The protein was pelleted by a 10-min centrifugation at 1500 × g at 4° in a Sorvall RC-3 centrifuge. The TCA pellets were washed twice with 100 μl 10% TCA and recentrifuged; and subsequently, 100 μl sodium hydroxide were added to dissolve the protein. Fifty-μl aliquots of the supernatant were removed, placed in scintillation vials, and counted as described.

In one series of experiments, the TCA-soluble fraction was measured in order to assess the influence of CY on leucine transport into both thymic and splenic lymphocytes. In these experiments the cells were incubated with [3H]leucine as described previously, cooled on ice, washed twice with ice-cold MEM containing leucine, and precipitated with 100 μl of 10% TCA for 30 min at 0°. After centrifugation 50 μl of the TCA supernatant were sampled for determination of radioactivity.

RESULTS

Chart 1 shows that spleen and thymus weights decrease for 4 days after 1 injection of CY (300 mg/kg). Spleen weight decreased by 43% within 1 day of CY, remained at about 50% of control weight until Day 5, and returned to control values by Day 7. Thymus weight decreased to 20% of its control weight within 2 days following CY and returned to control values by the seventh day after CY. Similar lymphocyte depletion replenishment patterns have been observed in the mouse after CY treatment (17).

Chart 2A shows that the number of nuclear receptors for [3H]leucine incorporation into TCA-precipitable material. Eighty-μl aliquots of cell suspension that were prepared in MEM without leucine at cell concentrations of 1.70 to 3.60 × 10^7/ml were incubated in microtiter plates with varying concentrations of concanavalin A or dexamethasone for 5 hr in a 95%O2:5% CO2 environment. A 20-μl pulse of 1 to 2 μCi/ml [14C]leucine or [3H]leucine was added to each well of the microtiter plate, and the incubation was continued for 30 min. The reaction was stopped by cooling the cells on ice and precipitating the protein with 20 μl of 50% TCA per well at 0°. The protein was pelleted by a 10-min centrifugation at 1500 × g at 4° in a Beckman LS-150 liquid scintillation counter with a 40% efficiency for tritium. Determination of [3H]TA was made by centrifuging the remaining whole cells for 1 min in a Beckman microfuge, sampling a 10-μl aliquot of the supernatant, and determining the radioactivity as above. The data from these saturation curves were analyzed by the method of Scatchard (15).

In one series of experiments, a different experimental protocol was used to determine the subcellular distribution of glucocorticoid receptors following incubation of both thymus and spleen cells at 3° and 37° from CY-treated and control animals. In these experiments cell suspensions were incubated with 1.66 × 10^-6 M [3H]TA alone or with 2 × 10^-6 M unlabeled dexamethasone. The cells were incubated either at 3° for 2 hr or 37° for 30 min. Cytoplasmic and nuclear binding were then measured as described (14).

The effects of concanavalin A and dexamethasone on protein synthesis in both thymic and splenic lymphocytes of the CY-treated rats were measured by [14C]leucine or [3H]leucine incorporation into TCA-precipitable material.
Chart 2. The influence of 1 injection of CY on nuclear glucocorticoid receptor binding and nuclear steroid-receptor dissociation constants in thymus and spleen cells. Nuclear [3H]TA binding sites per cell and dissociation constants were calculated following Scatchard analysis of saturation curves performed on tissue from animals sacrificed on the days after CY indicated. All Scatchard plots were linear, indicating 1 class of binding sites. The dissociation constants were calculated from the slopes of the lines. A, spleen cells; B, thymus cells.

[3H]TA per spleen cell decreases following CY treatment. Control animals had an average of 2906 sites/cell with a K_d of 1 x 10^-8 M. Within 3 days after injection of CY, nuclear [3H]TA binding sites were reduced by 98% with each cell containing only 51 sites/cell with a K_d of 1.1 x 10^-8 M. The dissociation constants for steroid-receptor interaction were relatively constant at each time interval after CY. No increase in nuclear [3H]TA-binding sites per cell occurred after Day 4 with only 32% of control values replenished by Day 7.

Thymocytes from control animals (Chart 2B) have an average of 2000 nuclear [3H]TA-binding sites per cell with K_d of 1.6 x 10^-8 M. The number of nuclear binding sites decreased by 71% to 500 sites/cell within 2 days after CY treatment. These receptors appeared to have a slight decrease in receptor-binding affinity (K_d of 3.3 x 10^-8 M). Nuclear binding receptor site number was replenished to 1131 sites/cell, 65% of control values by the seventh day after CY treatment. The dissociation constant of these cells was 2 x 10^-8 M, approximately equal to that found in control animals. The affinity of the thymocyte glucocorticoid receptor was consistently higher than was that of the spleen receptor.

The data from the experiments presented in Table 1 show the results of studies designed to assess the subcellular distribution of glucocorticoid receptors in thymic and splenic lymphocyte from both CY-treated and control animals. In these experiments animals were sacrificed 2 days after a single 300-mg/kg CY injection. As can be seen in both spleen and thymus from control animals, the majority of glucocorticoid-receptor complexes are found in the cytoplasmic fractions following a 2-hr incubation at 3°. We consistently observe some nuclear receptor binding even at this low temperature. When control cells are incubated at 37°, the majority of the receptors are localized in or on the nucleus. In concert with our previous studies (Chart 2), thymic and splenic lymphocytes from CY-treated animals have considerably fewer total cellular receptors (cytoplasmic and nuclear) than do control animals when receptor binding is measured at either 0° or 37°. The appearance of nuclear receptors in splenic lymphocytes from CY-treated
animals suggests that these receptors at least have the capability to nuclear translocate. The few receptors remaining in the thymus cells of CY-treated rats also appear to nuclear translocate at 3°. The zero binding levels that we consistently observe in the thymus subcellular fraction probably reflect our inability to detect the few remaining glucocorticoid receptors at the concentrations of [3H]TA used in these assays.

We next wanted to test the functional responsiveness of thymic and splenic lymphocytes from CY-treated animals to dexamethasone and concanavalin A. The data in Table 2 show the influence of CY treatment of rats on the ability of thymic and splenic lymphocytes to take up and incorporate leucine in TCA-soluble and -precipitable pools. Thymus cells from CY-treated animals consistently show slightly lower values for both leucine uptake and incorporation into protein, as compared to cells from control animals. The ability of splenic lymphocytes from CY-treated rats both to take up and to incorporate leucine into protein is significantly decreased as compared to control animals. There is, however, a substantial amount of leucine both taken up and incorporated in protein in the CY-treated cells.

As a result of the differences in leucine uptake and incorporation into protein in cells from CY-treated animals, we have expressed our subsequent data on the effects of concanavalin A and dexamethasone on leucine incorporation into protein as a percentage of nonhormone or mitogen-treated cells. This method of expression of the data allows us to analyze hormone mitogen-induced effects over and above those of CY treatment on leucine incorporation, particularly with regard to splenic lymphocytes. We also show in Table 2 that concanavalin A can stimulate leucine incorporation into both TCA-soluble and TCA-insoluble material in splenic and thymocyte lymphocytes. This intracellular increase in leucine probably results from concanavalin A-stimulated amino acid transport. This increase in amino acid pool probably accounts for a portion of the effects that we have observed in analyzing TCA-insoluble material.

Chart 3 shows that in spleen cells from animals not treated with CY, concanavalin A (6.25 μg/ml) increased leucine incorporation into protein by 88%, whereas dexamethasone at 10⁻³ M inhibits leucine incorporation by 33% within 5 hr. Within 1 to 2 days after CY treatment, neither concanavalin A nor dexamethasone affected the level of leucine incorporation in spleen cells. This loss of response was not due to decreased viability, since spleen cells from CY-treated animals were greater than 80% viable over the entire time course of this experiment. Spleen cells taken from rats 4 days after CY seem to respond to both concanavalin A and dexamethasone with small effects on leucine incorporation into protein. By Day 7 only partial but significant responses are seen with both concanavalin A and dexamethasone. Thus, partial ability to respond to concanavalin A and dexamethasone seems to return between the third and seventh days after 1 injection of CY (300 mg/kg).

### Chart 3.
The effect of time after in vivo exposure to CY (300 mg/kg) on responsiveness of rat splenic and thymic lymphocytes to concanavalin A and dexamethasone in vitro. Leucine incorporation into TCA precipitates was measured over a 5-hr incubation at 37°, as described in “Materials and Methods.” These data shown are expressed as percentage of stimulation of concanavalin A or percentage of inhibition (dexamethasone) as compared to the appropriate controls. Each value is the mean of at least 4 separate determinations, and the S.E.’s of the means of these values do not exceed the size of the symbols.

### Table 2
The influence of CY treatment 2 days prior to sacrifice of animals, and concanavalin A (6.25 μg/ml) stimulation of control lymphocytes on leucine uptake, and incorporation into protein in rat splenic and thymic lymphocytes.

<table>
<thead>
<tr>
<th></th>
<th>TCA Soluble</th>
<th>TCA Precipitate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thymus</td>
<td>Spleen</td>
</tr>
<tr>
<td>Control</td>
<td>1,082 ± 163*</td>
<td>1,287 ± 201</td>
</tr>
<tr>
<td>CY</td>
<td>773 ± 321</td>
<td>460 ± 60</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>1,448 ± 187</td>
<td>1,555 ± 218</td>
</tr>
</tbody>
</table>

*Mean ± S.E. cpm/10⁷ cells from a minimum of 5 to 11 separate determinations. Values were determined after the same 5-hr in vitro incubation used in the other experiments.
The magnitude of this response in the Day 7 preparation is, however, less than one-half of that observed in untreated cells. This lack of complete response at least for dexamethasone correlates well with the reduced complement of nuclear glucocorticoid receptors (Chart 2A).

In T-cells concanavalin A (6.25 μg/kg) stimulates protein synthesis by over 300%, and dexamethasone inhibits protein synthesis by 28%. Within 1 day of CY treatment, concanavalin A stimulation of leucine incorporation in thymocytes was reduced to 88% stimulation above control values; by 2 days after CY treatment, thymocytes responded to concanavalin A with only 25% stimulation of leucine incorporation (Chart 3). On Day 7 thymocytes showed a slightly greater response to concanavalin A, which correlates to the increased cell mass. Dexamethasone at concentrations as high as 10^{-8} M had no inhibitory effect on T-cell leucine incorporation into protein on Days 1 and 2 after CY. On both Days 4 and 7, the higher concentration of dexamethasone (10^{-7} to 10^{-8} M) significantly inhibited leucine incorporation into protein. Cell viability at the end of each experiment was 80 to 85% in both control and CY-treated groups, suggesting that our data cannot be the result of increased cell death during our experiments.

Chart 4 shows the influence of CY doses on spleen and thymus weights 2 days after 1 injection of CY. Spleen weight decreased to 40% of control weight after a 300-mg/kg dose, whereas thymus decreased to 23% of control values. On Day 2 the magnitude of the tissue depletion thus appears proportional to the dose of CY administered, with concentrations as low as 25 mg/kg being effective.

Chart 5A shows the effect of varying CY dosages on splenic lymphocyte nuclear glucocorticoid receptor number. Cells from untreated animals had 2900 sites/cell, \( K_d = 3 \times 10^{-8} \text{ M} \). The 25-mg/kg dose of CY reduced binding to 934 sites/cell with a \( K_d \) for the steroid-receptor interaction of 3.3 \times 10^{-8} \text{ M}. The 300-mg/kg dose decreased binding to 151 sites/cell, with a \( K_d \) of 1.9 \times 10^{-8} \text{ M}.

Chart 5B illustrates the influence of CY dosage on nuclear glucocorticoid receptor sites in T-cells. Glucocorticoid nuclear receptor numbers in thymocytes decreased in a dose-related manner in response to CY. Cells of control animals showed 2000 nuclear sites for [3H]TA per cells, with a \( K_d \) of 1.6 \times 10^{-8} \text{ M}. Two days after a 25-mg/kg dose, a 46% reduction to 905 sites/cell occurred. After a 300-mg/kg dose, nuclear binding sites were reduced to 500 sites/cell with a \( K_d \) of 1.10^{-8} \text{ M}, a 71% decrease from untreated cells.

As shown previously in Chart 3, concanavalin A is capable of increasing leucine incorporation by ~90% in spleen cells not exposed to CY (Chart 6). Splenic lymphocytes from animals given injection of 25, 50, or 200 mg/kg did respond
and glucocorticoid responsiveness appear not to be directly related. In previous studies high doses of concanavalin A significantly reduced glucocorticoid receptor number by only 40% in T-cell; however, cortisol did not have any inhibitory effect on glucose uptake in these cells. In this study we have observed that thymic responsiveness to glucocorticoids returned by Day 4 when only a few nuclear receptors were present. Spleen cells from the same animals had binding site depletion and replenishment patterns similar to those observed for the thymus, although the glucocorticoid response was suppressed longer. The discrepancy in the relationship between nuclear steroid receptors and response for the same type of receptor in 2 different lymphoid tissues may be due, at least in part, to a CY-resistant population of thymocytes that is not present in the spleen. In general we were unable to establish any good correlation between nuclear binding site number and glucocorticoid responsiveness in cells from animals that received less than a 300-mg/kg dose of CY. This may be due in part to an incomplete destruction of CY-sensitive cells in both thymus and spleen. Our time course studies with cells from animals that received a single 300-mg/kg injection indicate that a better correlation exists between nuclear hormone binding and glucocorticoid responsiveness. Only partial responsiveness to dexamethasone was observed at any time period when nuclear receptor number was below control values. Even in these animals, however, the return of some hormone responsiveness on Day 4 in the thymus and Day 7 in the spleen occurred when nuclear steroid receptor levels were below normal control values.

Although speculative the mechanisms by which CY reduces glucocorticoid receptor number in lymphoid cells may be related to its ability to interrupt the cell cycle at one particular stage in which the receptor number is low. Such a hypothesis could then be used to explain the CY actions on receptor number as an effect of cycle disruption rather than a direct interaction with the receptor or the gene responsible for its synthesis. Our previous observation which showed that glucocorticoid receptor in HeLa cells changes during the cell cycle supports this hypothesis (3). Alternately, our results may be accounted for by trafficking of cells in and out of the spleen and thymus following CY administration. This explanation seems unlikely since glucocorticoid receptors have been found in circulating lymphocytes (13), and our tissue preparation selects against epithelial cells and connective tissue.

In support of previous studies by others (5, 10), the ability of lymphocytes to respond to mitogens in vitro following CY in vivo sharply diminishes. We have shown at least in the spleen that the decreased responsiveness after CY treatment can in part be accounted for by decreased precursor transport into these lymphocytes in vitro. As a result of this observation, we have expressed our data as a percentage of control values to allow us to monitor any superimposed glucocorticoid and mitogen effects in these compromised lymphocytes. CY treatment of animals at dosages under 300 mg/kg leaves a population of lymphoid cells in which protein synthesis was stimulated by concanavalin A, whereas, following the 300-mg/kg injection, both splenic and thymic lymphocytes responded to concanavalin A with only small effects. The time course of the loss of the mitogen response is different in spleen and in thymus.

**DISCUSSION**

The cytotoxic effect of CY on both B-cells and T-cells is clearly demonstrated by the decreases in spleen and thymus weights observed after administration of CY to adrenalectomized male rats. Regeneration in both spleen and thymus (in terms of weight) began on Day 4 with control values reached by Days 5 and 7, respectively. Although initially CY was thought to have considerable B-cell specificity, our observations, in agreement with those of others (5, 10), suggest that the effect of CY may be more closely related to cellular mitotic activity, although the different time course of regeneration in spleen and thymus following 1 injection of CY may be indicative of some differential tissue sensitivity.

The decrease of glucocorticoid receptor sites following administration of CY (300 mg/kg) continued for 3 days postinjection in the spleen and 2 days in the thymus. The smaller reduction in nuclear receptor site number that we observed in the thymus may have resulted from either more rapid turnover of tissue during the time course of the experiment or relative resistance to the thymocytes to the cytotoxic effects of CY administration. Receptor subcellular distribution studies indicate that the decrease in nuclear 
[3H]TA binding which we observed following CY treatment, reflects a decrease in total cell receptor number rather than an inhibition of cytoplasmic to nuclear translocation.

As we have observed before (4) cellular receptor numbers and glucocorticoid responsiveness appear not to be directly related. In previous studies high doses of concanavalin A significantly reduced glucocorticoid receptor number by only 40% in T-cell; however, cortisol did not have any inhibitory effect on glucose uptake in these cells. In this study we have observed that thymic responsiveness to glucocorticoids returned by Day 4 when only a few nuclear receptors were present. Spleen cells from the same animals had binding site depletion and replenishment patterns similar to those observed for the thymus, although the glucocorticoid response was suppressed longer. The discrepancy in the relationship between nuclear steroid receptors and response for the same type of receptor in 2 different lymphoid tissues may be due, at least in part, to a CY-resistant population of thymocytes that is not present in the spleen. In general we were unable to establish any good correlation between nuclear binding site number and glucocorticoid responsiveness in cells from animals that received less than a 300-mg/kg dose of CY. This may be due in part to an incomplete destruction of CY-sensitive cells in both thymus and spleen. Our time course studies with cells from animals that received a single 300-mg/kg injection indicate that a better correlation exists between nuclear hormone binding and glucocorticoid responsiveness. Only partial responsiveness to dexamethasone was observed at any time period when nuclear receptor number was below control values. Even in these animals, however, the return of some hormone responsiveness on Day 4 in the thymus and Day 7 in the spleen occurred when nuclear steroid receptor levels were below normal control values.

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These data correlate to the different rate of reduction of tissue size following CY (Chart 2). Seven days after CY both splenic and thymic lymphocytes respond to concanavalin A with a 50% increase in protein synthesis. We speculate that CY treatment may alter the number or affinity of lymphocyte concanavalin A receptors in a manner analogous to that which we observed for glucocorticoids.

In light of these findings, combined use of CY and glucocorticoids should be examined more extensively in terms of the therapeutic efficacy of glucocorticoids. A CY induced depletion of glucocorticoid receptors could severely limit the beneficial functions of these hormones and reduce the overall effectiveness of therapy. The effect of such a depression of receptor numbers might be partially alleviated by an alternating schedule of administration of these agents. Thus, with minimized harmful drug interactions due to optimal scheduling, the synergism currently seen clinically in combined chemotherapeutic treatment of cancer with CY and glucocorticoids should be increased.

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Glucocorticoid and Mitogen Sensitivity of Rat Splenic and Thymic Lymphocytes in Vitro after in Vivo Cyclophosphamide Treatment

Susan F. Burroughs and John A. Cidlowski


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