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Effects of Antineoplastic and Immunosuppressant Drugs on Deoxyribonucleic Acid-Polymerizing Enzymes of Rat Thymus and Spleen

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SUMMARY

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Most drugs used in cancer chemotherapy have both antineoplastic and immunosuppressant activities. Inhibition of nucleic acid synthesis and cell division is a common mechansim of action of such drugs, but their effects on DNA polymerases and DNA synthesis in lymphoid cells have not been extensively described. Thymus and spleen are major sites of replication of lymphoid cells and play important roles in cellular and humoral immunity, respectively. We report the time course of changes in the activities per cell of DNA-dependent DNA polymerases α and β (DNA nucleotidyltransferase, EC 2.7.7.7), terminal deoxynucleotidyltransferase (nucleoside triphosphate:DNA nucleotidylexotransferase, EC 2.7.7.31), and adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) in rat thymus and spleen following administration of vincristine, 5-fluorouracil, actinomycin D, cyclophosphamide, cytosine arabinoside, or dexamethasone. Ten hours after administration of drug, only dexamethasone treatment was associated with a significant ($p < 0.001$) loss of thymic weight and enzyme activity. Terminal deoxynucleotidyltransferase and adenosine deaminase activities per cell in thymus had declined to less than half their pretreatment values. By 24 hr after drug, the weights of the thymus and spleen had significantly ($p < 0.001$) decreased in all animals. As calculated on the basis of activity units per cell, thymocytes remaining after vincristine or dexamethasone treatment contained less than 10% of the normal activities of terminal deoxynucleotidyltransferase and less than 30% of the normal activities of DNA polymerase α. Treatment with vincristine, 5-fluorouracil, actinomycin D, or cyclophosphamide reduced DNA polymerase α activity per spleen cell to less than 30% of normal. Terminal deoxynucleotidyltransferase activity was never found in spleen, whether drug-treated or not. Thymic lymphocytes were separated into density classes on bovine serum albumin gradients, and changes in enzyme activities are reported for cells of different densities. DNA-metabolizing enzymes in spleen and thymus responded differently to drug treatment. These differences may be related to differences among drugs in their relative suppression of humoral as opposed to cellular immunity.

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INTRODUCTION

Inhibition of DNA synthesis and cell division is a common mechanism of action of antineoplastic drugs. Unusual types of DNA synthesis and a unique DNA polymerase, terminal deoxynucleotidyltransferase (nucleoside triphosphate:DNA nucleotidylexotransferase, EC 2.7.7.31), play essential roles in the differentiation of lymphoid cells. It seemed possible that inhibition of unique types of nucleic acid synthesis in lymphoid cells could account in part for the immunosuppression produced by many drugs used in cancer chemotherapy. Terminal deoxynucleotidyltransferase is a DNA polymerase which normally is present only in thymus and bone marrow (1, 2). It is not present in mature T or B lymphocytes. It differs from the two major eukaryotic, DNA-dependent DNA polymerases in that it is a terminal addition enzyme which does not utilize template instruction. Because of its unique location and reaction mechanism *in vitro,* terminal deoxynucleotidyltransferase has been postulated to act as a somatic mutator and to play a role in programming cells of the immunological system. Two models have been proposed for the function of this enzyme. The first model states that DNA coding for the variable amino acid sequences in the V region of immunoglobulins or comparable regions on surfaces of T cells could be generated in the following manner (3). One strand of the DNA duplex could be nicked by a specific endonuclease, and a small DNA segment excised by an exonuclease. The result would be a single-stranded gap in the V region of the immunoglobulin gene. Terminal deoxynucleotidyltransferase could then extend the DNA across the gap, thus creating a random sequence of bases. Finally, DNA ligase could rejoin the DNA segments to produce double-stranded DNA containing a small segment of random sequence. Each lymphoid precursor in which this process occurred would by chance be programmed for a different immunoglobulin. The second model proposed that a small segment of DNA is synthesized by terminal deoxynucleotidyltransferase extrachromosomally and integrated into the V region of the immunoglobulin gene by a specific recombination system (4). Terminal deoxynucleotidyltransferase is not present at equal activity in all cells from thymus (5) or bone marrow (6), but rather is concentrated in a subpopulation of cells which may play a special role in immunological programming.

Recently reported studies indicate that an immunosuppressive drug, hydrocortisone, when given in large doses either inhibits the synthesis of terminal deoxynucleotidyltransferase or selectively destroys cells containing high levels of this enzyme (4, 7). A variety of antineoplastic drugs have immunosuppressive side effects which are similar to those produced by hydrocortisone and its derivatives. The major question in the present experiments is whether these agents have similar effects on DNA-polymerizing activities in cells from the immunological system. For several reasons our interest was in acute effects of the drugs. Hydrocortisone-induced changes in many enzyme systems are evident within a few hours after drug treatment (8–14). Practical considerations of choice of dose, drug toxicity, and susceptibility of animals to infection rendered long-term studies at low doses of antineoplastic agents less attractive for initial studies of polymerases. Doses of drugs used in our experiments were chosen to cause significant involution by weight of thymus and spleen within 24 hr.

Cells from thymus and spleen were chosen as representative of replication sites for immunocompetent T and B lymphocytes or their precursors. While results from studies *in vivo* are often difficult to interpret, several factors dictated their use. Activation and destruction of many commonly used antineoplastic agents are complicated processes involving a whole animal system. For example, cyclophosphamide must be metabolized in the liver to yield active metabolites. Effects of these compounds cannot be studied in cells in culture.

The enzymes measured in addition to terminal deoxynucleotidyltransferase were (a) DNA-dependent DNA polymerases

α and β (DNA nucleotidyltransferase, EC 2.7.7.7), which are thought to be associated with normal DNA synthesis and repair, and (b) adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4), a purine salvage enzyme which may also play a special role in immunocompetent cells, since its absence is associated with a severe hereditary immunodeficiency disease of children (15, 16). Chemotherapeutic agents were of the following clinically important classes: alkylating agents, cyclophosphamide; antimetabolites, 5-fluorouracil and cytosine arabinoside; intercalating, actinomycin D; microtubule inhibition, vincristine; lympholytic, dexamethasone.

MATERIALS AND METHODS

Aqueous solutions of the drugs were injected intraperitoneally into male Wistar rats weighing 50–100 g. Because of the difficulties associated with development of precise criteria for equivalent dosages of drugs alone or in combination, we limited this initial study to single drug dosages which resulted in significant involutions of thymus and spleen within 24 hr. Dexamethasone and cytosine arabinoside are not acutely lethal. A dexamethasone dose-response curve for involution of rat thymus and decrease in terminal deoxynucleotidyltransferase has been reported by Bollum (4). Acute toxicity data were provided by the manufacturers of each drug. Doses administered (milligrams per 100 g of body weight) were: vincristine (Lilly; LD_{50} after 5 days, 0.13), 1.0; 5-fluorouracil (Roche Laboratories; LD_{50} after 5 days, 23), 100; actinomycin D (Merck Sharp & Dohme; LD_{50} after 7 days, 0.8), 0.5; cyclophosphamide (Mead Johnson; LD_{50} after 48 hr, 72), 50; cytosine arabinoside (Upjohn; no acute toxicity at 200), 100; dexamethasone sodium phosphate (Merck Sharp & Dohme; no acute toxicity at 2000), 1.0. Control rats were injected with 0.9% NaCl in a volume equivalent to that used for drugs. Rats were killed by decapitation 2, 4, 10, and 24 hr after injection, and the thymus and spleen were removed. Weighed tissues were teased through a No. 40 wire mesh screen to form a cell suspension in RPMI-1640 tissue culture medium (Gibco). Cells were washed twice in RPMI-1640, counted using a Coulter ZBI electronic cell counter, and pelleted by centrifugation. If not used immediately in experiments, cells were frozen at −70°.

Enzyme assays. Radioactive deoxynucleoside triphosphates and radioactive adenosine were obtained from New England Nuclear Corporation, and unlabeled deoxynucleoside triphosphates, from P-L Biochemicals. Oligo $d(pA)_{\overline{50}}$ was a generous gift of Dr. F. J. Bollum. All other chemicals were of reagent grade.

Pelleted cells were suspended in 0.25 M potassium phosphate buffer, pH 7.2, at 10^8 cells/ml and sonicated four times in 15-sec bursts with cooling. The sonic extracts were centrifuged at 100,000 × *g* for 60 min. Supernatant fractions were collected and assayed for terminal deoxynucleotidyltransferase (17) and adenosine deaminase (18). DNA-dependent DNA polymerases α and β were measured separately in the crude extract, utilizing differences between the two in pH optima and sensitivity to *N*-ethylmaleimide (19). DNA polymerase α has a pH optimum of 7.2 and is completely inhibited by *N*-ethylmaleimide, whereas DNA polymerase β has a pH optimum of 8.6 and is resistant to *N*-ethylmaleimide. For all enzyme activity measurements, 125-μl reaction mixtures were used and aliquots were taken at 5, 10, 15, and 20 min to obtain initial rates. Rates for all enzyme assays were linear for at least 60 min. One unit of terminal deoxynucleotidyltransferase or DNA polymerase activity represents 1 nmole of nucleotide polymerized per hour. One unit of adenosine deaminase equals 1 nmole of inosine produced per minute. Enzyme activities are expressed as units per 10^8 nucleated cells, so that comparison among different tissues following drug treatment is directly related to the number of nucleated cells present.

Bovine serum albumin density gradients. Cell suspensions from thymus (2–3 × 10^9 cells) were layered on top of discontinuous BSA[1] gradients with 10 layers

[1] The abbreviations used are: BSA, bovine serum albumin; TCA, trichloracetic acid.

ranging from 16% to 34% BSA (w/w) in phosphate-buffered NaCl (5, 20, 21). The gradients were centrifuged at 1000 × *g* for 35 min at 4°. Seven distinct cell layers formed in the gradient and were removed with a 1-ml syringe fitted with a long 15-gauge needle. Cells were washed in RPMI-1640 tissue culture medium and counted with a Coulter counter. Washed, pelleted cells were sonicated as before and centrifuged to obtain a supernatant extract. The extracts were assayed for DNA polymerases α and β, terminal deoxynucleotidyltransferase, and adenosine deaminase activities.

Thymidine pulse. Thymic lymphocytes fractionated on BSA gradients were pulsed with [^{3}H]thymidine in one of two ways. (a) Washed cells from the gradient layers were resuspended in RPMI-1640 tissue culture medium at a cell concentration of 5 × 10^6/ml and incubated with [^{3}H]thymidine (5 μCi of thymidine per 5 × 10^6 cells, 95 nM, 7.8 × 10^5 cpm/pmole, New England Nuclear) at 37° for 30, 60, or 90 min. Rates of incorporation of [^{3}H]thymidine were linear for at least 90 min. After incubation, the cells were cooled to 4°, precipitated with cold 5% TCA, and filtered onto Whatman GF/C discs. The discs were washed three times with 5% TCA and twice with 95% ethanol, and then air-dried. Dried discs were suspended in a mixture of toluene and 0.4% 2,5-bis[2′-(5′-*tert*-butylbenzoxazolyl)]thiophene and counted in a liquid scintillation counter. (b) In certain experiments cells were pulsed with [^{3}H]-thymidine before separation on a gradient. Suspended thymus cells (10^9 cells) were incubated at a cell concentration of 5 × 10^6 /ml with [^{3}H]thymidine (5 μCi/5 × 10^6 cells) in RPMI-1640 tissue culture medium at 37° for 60 min. Labeled cells were washed with RPMI-1640, pelleted, and suspended in 16% BSA prior to layering on the discontinuous gradient. Following centrifugation of the gradients, labeled cells were removed, washed, and counted. From each gradient fraction 5 × 10^6 cells were filtered onto GF/C discs, washed with 5% TCA and 95% ethanol, dried, and then counted in a liquid scintillation counter.

Statistics. Statistical analyses were performed by Mr. James Hawke, Department of Statistics, University of Kentucky. The data were tested both directly, using a two-tailed *t*-test, and indirectly, after a variety of transformations. Independent standard deviations for treatment groups were not found to be significantly different, so that pooled standard deviation values were used for all treatment groups as described in the tables. For the purposes of the data presented here, three levels of significance are indicated in the tables. Stringent levels of significance were applied because of the large number of comparisons. For the purposes of this study, the minimum level of significance is set at $p \leqq 0.001$.

RESULTS

Organ weight and cell yield. Organ weight and cell yield from thymus and spleen are presented in Table 1. At the early time points (2 and 4 hr) enzyme activity data from thymus and spleen were highly variable, possibly because of variations in onset of absorption and metabolism of the drugs. Therefore only the 10- and 24-hr time intervals were investigated in detail.

At 10 hr the dexamethasone-treated rat thymus had begun to involute, so that there was a decrease in organ weight as well as a significant decrease ($p < 0.001$) in a number of cells obtained from each gland. However, cell yield (cells per unit of weight) was not significantly different in treated and control rats at 10 hr. Actinomycin D administration resulted in a transient, but significant, increase in cell yield after 10 hr. The effects of the other drugs were not significant at $p < 0.001$. By 24 hr dexamethasone- and vincristine-treated animals exhibited significant ($p < 0.00001$) loss in thymic weight as well as a dramatic decrease in cell yield ($p < 0.00001$). Although all the other drugs caused significant weight loss at 24 hr, the loss is not reflected in the yield of cells per gram of tissue.

Rat spleen also showed a significant weight loss 10 hr following the injection of dexamethasone, vincristine, cyclophosphamide, or cytosine arabinoside. Cells ob-

TABLE 1

Organ weights and yield of cells from thymus and spleen of treated rats

The levels of significance indicated were determined by the two-tailed *t*-test. Values represent mean weight in grams of organ or cells per organ. The standard deviations for all treatment groups were not found to differ significantly and were therefore pooled. This value is shown in parentheses after value for sham-treated animals. Tissue was minced and forced through a wire mesh screen in order to obtain a homogeneous cell suspension. The numbers of animals in each experiment were as follows; 10 hr, no drug, five; vincristine, four; dexamethasone, six, cyclophosphamide, four; cytosine arabinoside, four; actinomycin D, four; 5-fluorouracil, four; 24 hr, no drug, 12; vincristine, six; dexamethasone, 15; cyclophosphamide, five; cytosine arabinoside, five; actinomycin D, four; 5-fluorouracil, five.

Drug	Organ weight		Cells per organ		Cell yield	
	10 hr	24 hr	10 hr	24 hr	10 hr	24 hr
	g	*g*			*cells/g tissue*	
Thymus						
None	0.384 (0.06)	0.417 (0.04)	6.5×10^8 (1.4)	8.4×10^8 (1.4)	16.9×10^8 (2.6)	20.1×10^8 (3.1)
Vincristine	0.317	0.257[c]	6.5×10^8	2.1×10^{8c}	20.5×10^8	8.1×10^{8c}
Dexamethasone	0.251	0.215[c]	3.5×10^{8a}	1.2×10^{8c}	13.8×10^8	5.5×10^{8c}
Cyclophosphamide	0.334	0.260[c]	7.6×10^8	3.9×10^{8c}	22.4×10^8	15.5×10^8
Cytosine arabinoside	0.358	0.301[c]	7.9×10^8	5.9×10^8	22.1×10^8	19.9×10^8
Actinomycin D	0.449	0.286[c]	10.9×10^{8b}	5.7×10^{8a}	24.8×10^{8a}	19.9×10^8
5-Fluorouracil	0.352	0.292[c]	7.9×10^8	7.0×10^8	22.8×10^8	23.8×10^8
Spleen						
None	0.526 (0.06)	0.674 (0.02)	5.2×10^8 (0.8)	6.6×10^8 (0.6)	10.0×10^8 (1.3)	9.8×10^8 (1.4)
Vincristine	0.356[a]	0.474[c]	4.5×10^8	5.1×10^{8b}	12.5×10^8	10.6×10^8
Dexamethasone	0.296[c]	0.441[c]	3.5×10^8	2.2×10^{8c}	11.7×10^8	5.1×10^{8b}
Cyclophosphamide	0.313[c]	0.387[c]	4.1×10^8	3.1×10^{8c}	13.1×10^8	7.9×10^8
Cytosine arabinoside	0.370[a]	0.595[c]	4.1×10^8	5.3×10^8	11.0×10^8	8.9×10^8
Actinomycin D	0.430	0.360[c]	4.1×10^8	4.2×10^{8c}	9.5×10^8	11.7×10^8
5-Fluorouracil	0.464	0.461[c]	4.5×10^8	6.8×10^8	9.8×10^8	14.9×10^{8c}

[a] $p < 0.001$.
[b] $p < 0.0001$.
[c] $p < 0.00001$.

tained per organ and cell yield were not significantly changed following injection of any of the drugs. At 24 hr all rats treated with drugs showed a decrease in the weight of the spleen ($p < 0.00001$). Cell yield decreased significantly ($p < 0.0001$) only in dexamethasone-treated animals. Vincristine, cyclophosphamide, and actinomycin D administration resulted in a loss of cells obtained per organ, but no appreciable decrease in cell yield. Contrary to the results obtained with thymus, only dexamethasone had a pronounced effect on organ weight, cells per organ, and cell yield.

Enzyme activities. Values for activities of DNA polymerase α, terminal deoxynucleotidyltransferase, and adenosine deaminase (units per 10^8 cells) in thymus and spleen cells 10 and 24 hr following drug administration are given in Table 2. None of these drugs at a concentration of 0.3 mg/ml inhibited the activities of the enzymes *in vitro*. Certain of the antineoplastic

drugs dramatically affected enzyme activities in thymus. Ten hours after injection of dexamethasone the activity of terminal deoxynucleotidyltransferase per cell was only 25% of that found in cells from normal thymus ($p < 0.00001$). Adenosine deaminase activity was slightly decreased, while the activity of DNA polymerase α per cell probably was increased.

By 24 hr vincristine, dexamethasone, and cyclophosphamide had lowered terminal deoxynucleotidyltransferase activity per cell to 12%, 2%, and 40% of normal, respectively. DNA polymerase α activity was about 30% of normal in cells from vincristine- and cyclophosphamide-treated animals, and 14% of normal in dexamethasone-treated animals. None of the drug treatments except dexamethasone affected adenosine deaminase activity in thymus. Of the drugs tested, dexamethasone had the most immediate and profound effect on thymus cells, followed by vincristine.

All extracts of spleen were assayed for terminal deoxynucleotidyltransferase activity, but, as previously reported (1), this enzyme activity is not present in spleen. DNA polymerase α activity was not significantly reduced by any of the drugs 10 hr after injection. There was a transient loss of spleen adenosine deaminase activity after 10 hr which was not apparent at 24 hr. At 24 hr all drug-treated animals exhibited lower DNA polymerase α activity in cells from spleen. Adenosine deaminase activity was not significantly different from normal in any of the treatment groups after 24 hr.

Gradient analysis of cells from thymus of dexamethasone-treated animals. Suspensions of thymic lymphocytes were fractionated on BSA gradients in order to measure enzyme activities in specific cell types. Only the effects of dexamethasone were studied in detail. This drug was of primary interest since it caused the most

TABLE 2

Enzyme activity measurements in rat thymus and spleen cells following administration of drugs

The levels of significance indicated were determined by the two-tailed *t*-test. Values represent mean enzyme activity in the following units: terminal deoxynucleotidyltransferase and DNA polymerase α, nanomoles of nucleotide incorporated per 10^8 cells per hour; adenosine deaminase, nanomoles of inosine formed per 10^8 cells per minute. Assays of enzyme activities gave linear rates for periods up to 1 hr. The standard deviations for all treatment groups were not found to differ significantly and were therefore pooled. This value is given in parentheses after values for sham-treated animals. The number of animals in each experiment is indicated in Table 1. Terminal deoxynucleotidyltransferase is not present in spleen.

Drug	Terminal transferase		DNA polymerase α		Adenosine deaminase	
	10 hr	24 hr	10 hr	24 hr	10 hr	24 hr
Thymus						
None	29.1 (4.5)	28.7 (5.2)	13.9 (2.1)	14.7 (3.4)	800 (235)	665 (232)
Vincristine	31.7	3.7[c]	15.1	4.7[c]	930	497
Dexamethasone	7.9[c]	0.7[c]	17.9	1.9[c]	422	278[c]
Cyclophosphamide	24.3	10.1[c]	14.4	5.2[c]	789	651
Cytosine arabinoside	26.3	22.7	14.3	14.1	934	1076
Actinomycin D	34.6	22.8	14.9	16.7	872	463
5-Fluorouracil	37.6	23.5	16.6	7.4[a]	790	891
Spleen						
None			16.2 (4.3)	15.9 (1.7)	112 (33)	142 (47)
Vincristine			10.7	4.6[c]	80	108
Dexamethasone			15.9	8.8[c]	38[c]	181
Cyclophosphamide			6.0	3.7[c]	99	151
Cytosine arabinoside			6.6	10.9[a]	99	95
Actinomycin D			10.6	5.0[c]	46	77
5-Fluorouracil			6.4	5.2[c]	55	105

[a] $p < 0.001$.
[b] $p < 0.0001$.
[c] $p < 0.00001$.

rapid changes in enzyme activities and thymic weight. Previous data indicated that cells from thymus are biochemically heterogeneous (5), with a subpopulation that contains most of the DNA polymerase α and terminal deoxynucleotidyltransferase activities. More than 90% of lymphocytes loaded onto gradients were recovered after centrifugation. The control cells were distributed on the gradient (Fig. 1A) in a pattern identical with that seen in our previous experiments (5). The majority of cells from dexamethasone-treated rats banded at a slightly lower density than those from normal thymus.

The incorporation of thymidine into fractionated cells is shown in Fig. 1B. The pattern of thymidine incorporation into normal thymic lymphocytes is identical with previously observed patterns (5). The low incorporation in fractions 5 and 6 occurred in cells with low levels of thymidine kinase activity (5). In contrast, the thymic lymphocytes from dexamethasone-treated rats incorporated [^{3}H]thymidine very poorly across the entire gradient.

Measurements of activity of DNA polymerase α, DNA polymerase β, and adenosine deaminase (units per 10^8 cells) are shown in Fig. 2A, B, and C. As shown in assays of extracts from whole thymus, DNA polymerase α activity was slightly higher in dexamethasone-treated cells than in control cells. The activities of DNA polymerase β and adenosine deaminase were relatively invariant across the gradient. In extracts of whole thymus there was a slight but insignificant decrease in adenosine deaminase activity. This was not reflected in assays of cells from the gradient (compare adenosine deaminase activities in Fig. 2C with those in Table 2).

Figure 3 illustrates terminal deoxynucleotidyltransferase activities in thymic lymphocytes separated on a BSA gradient. Enzyme activity per cell from dexametha-

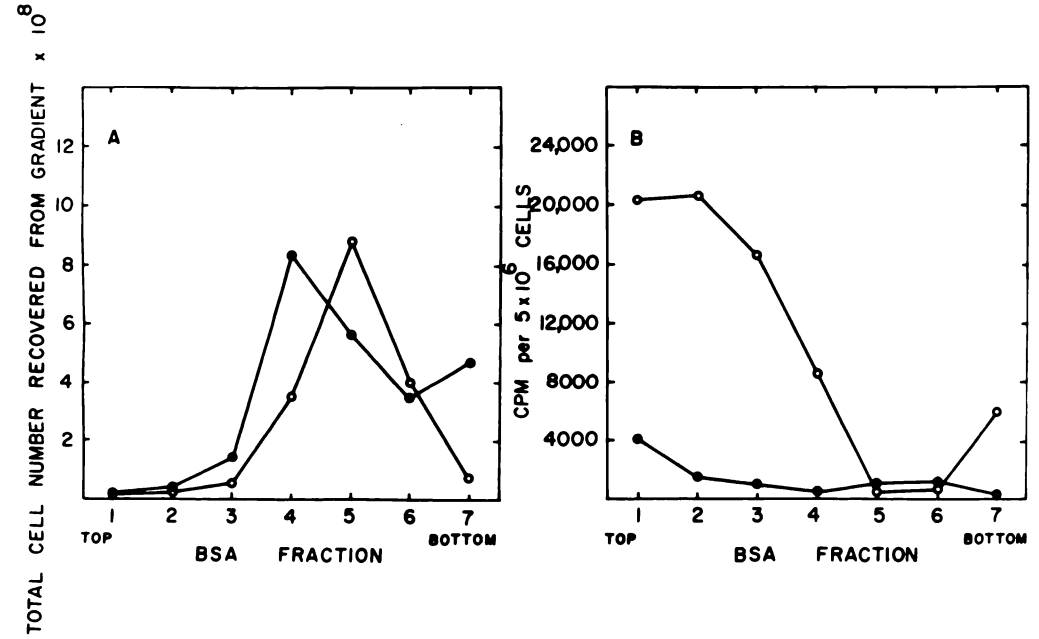

FIG. 1.

A. Numerical distribution of thymus cells fractionated on BSA gradients. Suspensions of cells from thymus were layered on top of discontinuous BSA gradients and centrifuged at 1500 × g for 30 min. Fractionated cells were removed, washed, and counted. Rats had been treated 10 hr earlier with NaCl (○——○) or dexamethasone (●——●).

B. Incorporation of thymidine into thymus cells fractionated on BSA gradients. These cells were labeled with [^{3}H]thymidine prior to gradient fractionation. Rats had been treated 10 hr earlier with NaCl (○——○) or dexamethasone (●——●).

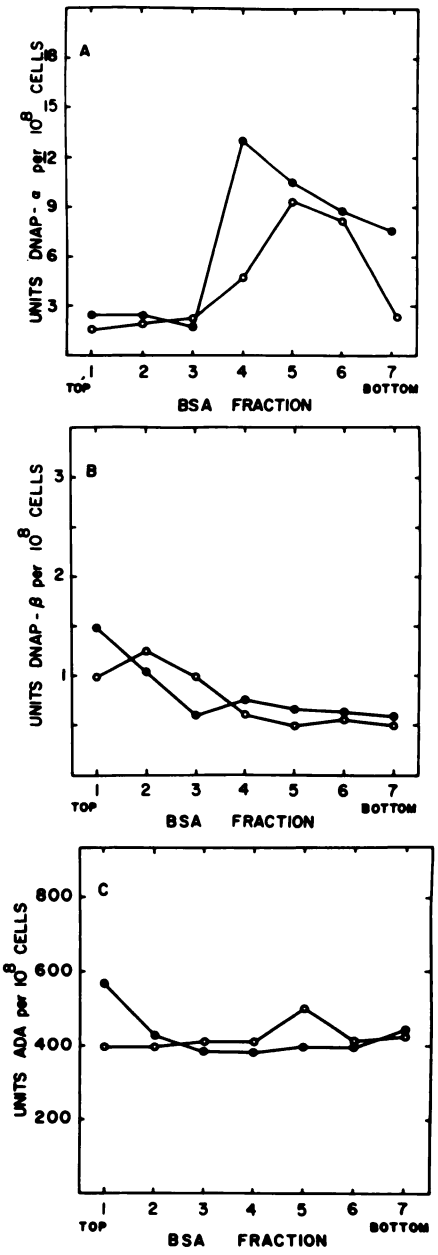

FIG. 2. *Enzyme activities associated with rat thymus cells separated on BSA gradients*

Rats had been treated 10 hr before with NaCl (○——○) or dexamethasone (●——●). Enzymes measured were DNA polymerase α (DNAP-α) (A), DNA polymerase β (DNAP-β) (B), and adenosine deaminase (ADA) (C).

sone-treated animals was decreased in every fraction except the one with lowest density (fraction 1). In that fraction terminal deoxynucleotidyltransferase activity was barely detectable, and the difference between treated and untreated cells was not significant. Generally the activity of this enzyme in cells from dexamethasone-treated thymus was approximately 25% of that present in cells from untreated animals.

DISCUSSION

Thymic lymphocytes contain an unusual, non-template-directed DNA polymerase which *in vitro* can elongate one strand of duplex DNA that contains a gap produced by the concerted action of endo- and exonucleases. This enzyme, terminal deoxynucleotidyltransferase, is capable of creating diversity in cellular DNA. Except for thymus and marrow, terminal deoxynucleotidyltransferase has not been detected in normal cells (1, 2). Since the enzyme does not copy a template, there have been suggestions that it may act as a generator of somatic mutations in lymphoid cells so that they become programmed for immunological functions (3, 4, 22). DNA polymerases α and β are the two primary template-directed enzymes in mammalian cells. Levels of DNA polymerase α increase in cells stimulated to proliferate (23-25). This enzyme has been implicated

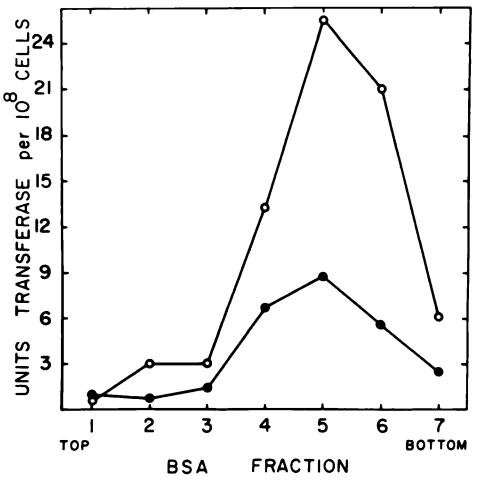

FIG. 3. *Terminal deoxynucleotidyltransferase activity associated with thymus cells separated on BSA gradients*

Rats had been treated 10 hr before with NaCl (○——○) or dexamethasone (●——●).

as the primary replication enzyme during cell division. DNA polymerase β appears to be a constitutive enzyme in mammalian cells and may be associated with a DNA repair mechanism. DNA polymerases α and β have been detected in all animal cells, in contrast to terminal deoxynucleotidyltransferase. Adenosine deaminase is a ubiquitous purine salvage enzyme which appears to play some critical role in lymphoid cells. Its absence has been associated with a severe hereditary immunodeficiency disease of children (15, 16). In addition, adenosine deaminase activities in lymphocytes of peripheral blood change from normal when certain types of antineoplastic chemotherapy are administered to humans (26).

When hydrocortisone, one type of immunosuppressive agent, is administered in large doses to animals, it causes a rapid decrease in terminal deoxynucleotidyltransferase activity in thymus (6). It has been postulated that this effect could be related to the immunosuppressive activity of hydrocortisone and its derivatives. The hypothesis that antineoplastic agents which are immunosuppressive generally cause loss of terminal deoxynucleotidyltransferase can be tested. The mechanism of action could be either destruction of cells containing this enzyme or interference with maintenance of normal levels of active enzyme. In either case, administration of the drug should cause a decrease in terminal deoxynucleotidyltransferase activity in thymus. The drugs chosen for our study were antineoplastic agents of several types: alkylating agents, intercalating agents, lympholytic agents, mitotic inhibitors, and antimetabolites. All have been associated with varying degrees of immunosuppression as well as with inhibition of tumor growth. These experiments were limited to effects of the drugs which were produced in short time intervals.

Thymus and spleen cells were chosen for study as easily obtainable representatives of T and B cells or their precursors. Earlier studies have indicated that lymphocytes from thymus and spleen have high levels of DNA polymerases, while contaminating red cells and epithelial and stromal elements have barely detectable levels of DNA polymerase (5, 25). Isolation of lymphocytes and expression of enzyme activities in terms of nucleated cell number eliminates problems of varying numbers of red cells in the determination of enzyme specific activity. Examination of two parameters, organ weight vs. cells obtained per organ, illustrates that involution by weight is not always associated with a proportional decrease in the number of lymphoid cells within the organ.

Vincristine is strongly lympholytic in both thymus and spleen. An antimitotic agent which brings about accumulation of cells at the metaphase stage (for a recent review, see ref. 27), vincristine drastically lowers the activity per cell of DNA polymerase α in both thymic and splenic lymphocytes at 24 hr. Similar effects on terminal deoxynucleotidyltransferase are observed in thymic lymphocytes. Vincristine does not directly inhibit either DNA polymerase α or terminal deoxynucleotidyltransferase, suggesting that the decrease in these two enzyme activities does not result from their direct inhibition. This drug may reduce the level of enzyme activity per cell by preventing cellular proliferation, especially if both enzymes are rapidly degraded *in vivo*.

Dexamethasone is lympholytic in both thymus and spleen. In thymic lymphocytes, dexamethasone causes a more rapid disappearance of terminal deoxynucleotidyltransferase activity than of DNA polymerase α. If dexamethasone administration results in cell lysis in a thymic population equally rich in DNA polymerase α and terminal deoxynucleotidyltransferase, both enzymes should be lost in the same time span. The finding that terminal deoxynucleotidyltransferase decreases before DNA polymerase α suggests that a subpopulation of thymic lymphocytes which is particularly sensitive to lysis by corticosteroids contains a high percentage of the total terminal deoxynucleotidyltransferase activity in thymus. The rapid disappearance of this activity following either dexamethasone or vincristine indicates that this enzyme as well as DNA polymerase α probably has a short half-life in thy-

mus. Dexamethasone does not cause the extensive disappearance of DNA polymerase α activity in spleen that it does in thymus at 24 hr. This may be reflected in the less drastic involution observed in spleen, as indicated by cell yield data (Table 1).

In thymus three of the six immunosuppressive drugs used (vincristine, dexamethasone, and cyclophosphamide) had effects on terminal deoxynucleotidyltransferase activity within 24 hr after drug administration. These same three drugs caused severe thymic involution in terms of lymphoid cells harvested per organ. It is interesting that vincristine and prednisone derivatives are extremely effective chemotherapeutic agents for acute lymphoblastic leukemia in man. Four of the drugs (vincristine, dexamethasone, cyclophosphamide, and 5-fluorouracil) had rapid effects on DNA polymerase α activity. In spleen all six immunosuppressive drugs inhibited DNA polymerase α 24 hr after drug administration. Although adenosine deaminase activity is affected in humans by chronic doses of antineoplastic agents (26), only dexamethasone affected this enzyme in thymus cells.

Prior work in our laboratory suggested that terminal deoxynucleotidyltransferase is found in high activity in small thymocytes (5). In thymic lymphocytes, 10 hr following dexamethasone administration, there was a differential loss of this enzyme activity relative to DNA polymerase activity. A possible explanation would be that a subpopulation of thymic lymphocytes was being preferentially lysed, but this could not be demonstrated in the density gradient cell separations. There was a shift in the mean density of thymic lymphocytes treated with dexamethasone, but there was a uniform loss in terminal deoxynucleotidyltransferase activity per cell across the entire gradient.

The antineoplastic agents used have different effects on thymic and splenic lymphocytes. It is possible that these differences are related to differences in clinical efficacy and side effects of the drugs. Lymphocytes in the peripheral blood rarely undergo spontaneous cell division, have low levels of DNA polymerase α, and display little or no DNA synthesis. That many antineoplastic agents interfere primarily with the action and quantity of DNA polymerases and the processes of cell division implies that the major effects of certain types of immunosuppressants are probably on early stages of lymphoid differentiation, when cells are replicating and terminal deoxynucleotidyltransferase is active.

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