Regeneration of O6-Alkylguanine-DNA Alkyltransferase in Human Lymphocytes after Nitrosourea Exposure

Stanton L. Gerson

Hematology-Oncology Division, Department of Medicine, and the R. L. Ireland Cancer Center, University Hospitals of Cleveland, and Case Western Reserve University Medical School, Cleveland, Ohio 44106

ABSTRACT

Mitogen-stimulated human lymphocytes have an increased capacity to repair many forms of DNA damage caused by UV, ionizing radiation, and chemical carcinogens. Human lymphocytes rely on a particular DNA repair protein, O6-alkylguanine-DNA alkyltransferase (alkyltransferase) to repair efficiently O6-alkylguanine, an important mutagenic adduct formed by nitrosoureas and other N-nitroso compounds. The alkyltransferase is a "suicide" protein which becomes inactivated during the repair process. Thus, basal activity and the ability to synthesize new protein activity are important compounds of O6-alkylguanine repair. We compared basal and regenerated alkyltransferase activity in resting and mitogen-stimulated human lymphocytes. During stimulation with L-phytohemagglutinin, alkyltransferase activity increased by a mean of 70% over resting cells. Following exposure to N-nitroso-N-methyleurea (MNU), alkyltransferase activity was consumed in a dose-dependent manner in both resting and L-phytohemagglutinin-stimulated cells by the repair of MNU-induced O6-methylguanine-DNA adducts. Recovery of alkyltransferase activity began within 1 day of MNU exposure in the L-phytohemagglutinin-stimulated lymphocytes but did not occur in resting cells. Enzyme induction was not observed. When the alkyltransferase was only partially inactivated by low dose MNU, resting lymphocytes still failed to recover alkyltransferase activity. The rate of recovery of alkyltransferase activity in proliferating cells was dependent on the basal level of activity, which varied about 3-fold among donors. These data indicate that mitogen-stimulated lymphocytes develop an increased capacity to repair nitrosourea-induced DNA damage and are able to regenerate activity following nitrosourea exposure. In contrast, resting lymphocytes do not rapidly synthesize new alkyltransferase molecules after nitrosourea exposure and appear susceptible to DNA damage caused by persistent O6-alkylguanine adducts. Thus, both basal alkyltransferase activity and the proliferative state of normal lymphocytes influence the response to nitrosourea exposure.

INTRODUCTION

During mitogen stimulation, proliferating human lymphocytes have an increased capacity to repair the DNA damage produced by UV, ionizing radiation, and certain chemical carcinogens (1, 2). Many DNA repair systems are induced during mitogen stimulation, including the excision repair system (1, 2), polymerase α (2, 3), polymerase β (3), DNA ligase (3, 4), poly(ADP-ribose) polymerase (5, 6) and uracil-DNA glycosylase (7). These enzymes are induced in a coordinate fashion with the induction of the alkyltransferase activity requiring synthesis of new molecules (16).

One mechanism of repair of nitrosourea-induced DNA damage involves the protein called O6-alkylguanine-DNA alkyltransferase (alkyltransferase) (9, 10). Previous studies have found that this DNA repair protein is also induced in PHA-stimulated lymphocytes (11–13). The alkyltransferase is important because it repairs O6-alkylguanine, the major mutagenic adduct formed by methylating agents and the nitrosoureas (9, 10, 14). Cells containing adequate levels of the alkyltransferase are more resistant to the mutagenic effects of nitrosoureas (15). The alkyltransferase is unique among DNA repair proteins because it transfers the alkyl group from the adduct onto a cysteine moiety in the active site of the protein causing the protein to become inactivated (9, 14). Regeneration of alkyltransferase activity requires synthesis of new molecules (16) and occurs at a rate of 700–4500 molecules/h in human tumor cell lines (17). Because of its stoichiometric mode of action, the alkyltransferase is commonly referred to as a "suicide" protein rather than as an enzyme (9, 14). The mechanism of action of the alkyltransferase also means that there is a threshold level of exposure to nitrosoureas at which there are sufficient O6-alkylguanine adducts formed to inactivate the alkyltransferase. Once the alkyltransferase is consumed, the mutagenic effects of nitrosoureas occur (18, 19).

If cells which are depleted of alkyltransferase activity during the repair process can rapidly regenerate alkyltransferase, the DNA-damaging effects of nitrosoureas can be limited even further. The rate of regeneration after DNA damage varies between cells and with the agent used (17, 20–23) and in some cells, regeneration of alkyltransferase activity reaches 1.5–3 times basal level (17, 23). In normal tissues, rat liver alkyltransferase is induced following exposure to dimethylnitrosamine (19, 24); however we have noted that the alkyltransferase is slowly regenerated in murine bone marrow and kidney following MNU exposure (25). Thus, it is not clear whether all normal tissues regenerate alkyltransferase activity quickly following nitrosourea damage and whether this regeneration is dependent on DNA synthesis.

In humans, lymphocytes provide an ideal source of cells in which to evaluate the effect of cell proliferation on regeneration of alkyltransferase activity following nitrosourea exposure. Not only is it possible to induce cell proliferation by PHA stimulation, but it is also possible to evaluate the importance of interindividual variations in basal alkyltransferase activity and whether this influences the level or rate of regeneration observed following DNA repair-induced inactivation of the protein. If the lymphocyte alkyltransferase is inactivated in humans exposed to chemotherapeutic nitrosoureas, mutagenicity as well as cytotoxicity may occur. The balance between the ability of the lymphocyte alkyltransferase to be inactivated during repair of O6-alkylguanine adducts and its ability to be induced during DNA proliferation and possibly after methylating agent exposure are important parameters with which to gauge individual susceptibility to nitrosoureas.

We investigated changes in alkyltransferase activity in resting and in mitogen-stimulated human lymphocytes following MNU exposure. When the alkyltransferase was consumed by repair of O6-alkylguanine adducts, regeneration of alkyltransferase activity occurred mainly in proliferating lymphocytes rather than in resting cells. The range in responses among donors received 2/11/88; revised 5/16/88; accepted 6/17/88. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. 1 Supported in part by the American Cancer Society, Ohio Division, Cuyahoga County Unit, Grant ESCA 00134 from the National Institute of Environmental Health Sciences, and Grants CA 07912 and P30-CA 47303 from the National Cancer Institute. Recipient of a Physician Scientist Award and a Mallinckrodt Foundation Scholar. 2 The abbreviations used are: PHA, L-phytohemagglutinin; PBS, phosphate-buffered saline; MNU, N-nitroso-N-methyleurea.
suggests that there may be a range in individual susceptibility to nitrosoureas.

MATERIALS AND METHODS

Chemicals and Reagents. Lyophilized PHA (HA-15; Burroughs-Wellcome) was reconstituted in sterile PBS as described by the manufacturer and was stored at -20°C. MNU (Sigma Chemical Co., St Louis, MO) was stored desiccated at -20°C and suspended in 1% acetic acid just before use. Other chemicals and reagents unless specified were obtained from Sigma. Tissue culture reagents were obtained from Hazleton Research Products (Lenexa, KS) and fetal bovine serum was obtained from HyClone Laboratories (Logan, UT).

Isolation and Drug Exposure of Peripheral Blood Lymphocytes. Freshly obtained peripheral blood lymphocytes were isolated from normal donors, as previously described (26), washed in 50 ml PBS, and resuspended at 5 x 10⁸ cells/ml in RPMI 1640 supplemented with 15% fetal bovine serum, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 100 units/ml penicillin, and 100 μg/ml streptomycin (tissue culture medium) (26). One-half of the suspensions were mitogen stimulated by addition of 1% (v/v) PHA. The two suspensions were incubated at 37°C, 5% CO₂ for 4 to 5 days, and were collected for measurement of either [³H]thymidine incorporation or alkyltransferase activity as described below. To measure the cell concentration of the cultures, cells were diluted 1 to 20 into counting buffer (0.082 M hexadeclyltrimethylammonium bromide (Eastman Kodak Co., Rochester, NY) 0.15 M NaCl, 2 mM EDTA, pH 5) to disperse aggregates and were passed through a Model 2 M Coulter Counter.

Lymphocytes were exposed to MNU as follows. Suspensions of PHA-stimulated lymphocytes were incubated in the presence of PHA for 72 h, whereas resting lymphocytes were freshly prepared from the same donor. To determine the inactivation of alkyltransferase activity by MNU, 40-ml aliquots of lymphocytes resuspended at 5 x 10⁸ cells/ml were incubated at 37°C for 2 h in the presence of 0.005 to 1 μM MNU. To determine the time course of regeneration of alkyltransferase after inactivation by MNU, lymphocytes were exposed to 0.075-0.5 mM MNU for 2 h at 37°C, centrifuged at 400 x g for 10 min, resuspended in tissue culture medium with or without 1% PHA, and incubated at 37°C in 5% CO₂ for 0-72 h. In certain experiments, cells were incubated in cycloheximide (0.2 mM) or hydroxyurea (10 mM) to inhibit protein or DNA synthesis, respectively. At various time points, 20 x 10⁶ cells were removed, washed twice in 40 ml PBS, 1 mM EDTA, resuspended at 4 x 10⁹ cells/ml in cell extract buffer (70 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.8, 0.1 mM EDTA, 5% glycerol, and 1 mM dithiothreitol) and frozen at -80°C (23). Cell suspensions were sonicated 3 times for 5 s at 4°C to complete cell disruption by using a microsonicator (Heat Systems Ultrasound, Farmingdale, NY), followed by centrifugation at 10,000 x g for 2 min to remove cellular debris (26), and stored at -80°C. Samples of extracts were taken for determination of protein and DNA content by using previously described procedures (26, 27).

DNA Synthesis. DNA synthesis was measured by [³H]thymidine incorporation. Triplicate aliquots of 1 x 10⁶ lymphocytes were suspended in 2 ml tissue culture medium and incubated at 37°C for 30 min; 1 μCi [³H]thymidine (82.3 Ci/mmol) (New England Nuclear, Boston, MA), was added to the cell suspension and the cells were incubated for 2 h at 37°C (28). The incorporation was stopped by addition of 5 ml 0.9% NaCl solution at 4°C and the cells were recovered following centrifugation at 1000 x g for 7 min and then resuspended in 5 ml 5% trichloroacetic acid at 4°C. The suspension was sonicated, collected on Whatman GF/C glass fiber filters, and processed for scintillation counting as previously described (26).

O⁵-Alkylguanine-DNA Alkyltransferase Assay. O⁵-Alkylguanine-DNA alkyltransferase activity in cell extracts was measured as removal of the ³H-methyl adduct from O⁴⁺[³H]methylguanine in [methyl-³H]-DNA alkylated with N⁰⁴[H]methylnitrosourea as previously described (26, 27). The reaction mixture was incubated for 60 min at 37°C, the DNA was precipitated with 7.5% trichloroacetic acid, and the methyl purines were liberated by acid hydrolysis and separated by high performance liquid chromatography as previously described (26, 27). One unit of alkyltransferase activity was defined as removal of 1 fmol of O⁵-methylguanine/μg cellular DNA. This expression adjusts for changes in DNA content of the cell that occur during blastogenesis of proliferating lymphocytes. We have chosen this unit because it can be accurately measured in the cell extract as opposed to cell number, which is subject to experimental error and loss during processing. In certain experiments activity is also expressed relative to the protein content of the cell extract.

RESULTS

Induction of Alkyltransferase Activity during Mitogen Stimulation of Lymphocytes. The relationship between the induction of DNA synthesis and alkyltransferase activity in resting and mitogen-stimulated lymphocytes is shown in Fig. 1. After 36 h there was a significant rise in DNA synthesis and cell proliferation in the PHA-stimulated cells. Alkyltransferase activity rose in PHA-stimulated cells from 8.2 ± 0.6 (SE) units to a maximum of 13.5 ± 0.6 units on the fourth day of culture (P < 0.001). This increase in alkyltransferase activity occurred at the same time as the induction of DNA synthesis but plateaued after 2 days, compared to the maximal increase in DNA synthesis which occurred at day 5. Thus, mitogen-stimulated lymphocytes undergo a significant increase in alkyltransferase activity concurrent with the increase in DNA synthesis which occurs 36-48 h following mitogen stimulation. Induction of alkyltransferase activity occurred within 48 h in all donors and was maximum between 48 and 96 h.

To determine the extent to which the induction of alkyltransferase activity varied among donors, we examined lymphocytes from 17 donors before and 72 h after mitogen stimulation (Fig. 2). An increase in lymphocyte alkyltransferase activity following PHA stimulation was observed in all donors and ranged from 1.1 to 2.3-fold. Donors whose resting lymphocytes had low alkyltransferase activity tended to have lower levels of activity in their cells following PHA stimulation than donors with a high level in their resting cells. However, this was not dependent on the induction of DNA synthesis, as all cells cultures had at least a 20-fold increase in [³H]thymidine incorporation and a rise in cell number between days 1 and 3 of PHA treatment. On repeat testing, donors with low levels of activity maintained these levels and had lower levels of activity in proliferating cells than donors with high levels of activity in their resting cells (data not shown). Variation in donor lymphocyte alkyltransferase activity was observed when donors were repeatedly tested over a 9- to 12-month period. In those sampled 3-8 times (n = 7), the mean standard deviation of sample alkyltransferase activity was 28% of the absolute activity, and range was 13-42%. These intrindividual variations were small relative to the interindividual variations shown in Fig. 2. The degree of induction of activity was independent of the age or sex of the donors.

The data reported are based on the alkyltransferase activity per μg cellular DNA rather than per mg cellular protein, because this gives a better index of the DNA repair capacity in the cell relative to the target for DNA damage than does reporting the alkyltransferase activity based on the amount of protein present in the cell. It is more accurate in our laboratory than reporting activity as molecules/cell because the number of cells present in the pellet used to make the extract is difficult to determine in each experiment. In addition, the changes in both the protein and DNA content of the cell which occur during PHA stimulation can be controlled for by reporting alkyltransferase activity/μg DNA. In the experiments reported here, the approximate conversion between μg DNA and cell number is 1.56 ± 0.40 x
Fig. 1. Induction of Alkyltransferase Activity in PHA-stimulated Lymphocytes. Peripheral blood lymphocytes were isolated from normal donors and incubated in culture medium at 5 x 10^6 cells/ml. Mitogen-stimulated cells were cultured in the presence of 1% PHA. Cells were collected at the time indicated, counted in counting buffer, and incubated for an additional 2 h in 1 μCi [3H]thymidine. A separate aliquot was assayed for alkyltransferase activity as described in "Materials and Methods." A, Cell counts; B, [3H]thymidine incorporation; C, alkyltransferase activity measured as fmol O6-methylguanine (O6mG) removed/μg cellular DNA. Points, mean of 8–14 donors; bars, SEM.

Fig. 2. Variation among Donors in Induction of Alkyltransferase Activity following PHA Stimulation. Peripheral blood lymphocytes were collected from 17 donors. The alkyltransferase activity in freshly harvested lymphocytes or lymphocytes exposed to PHA for 3 days in tissue culture medium at 37°C was measured. Alkyltransferase activity is recorded as described in Fig. 1. Points, average of 2–3 determinations of each sample. Variations between determinations was less than 12%. Circles with bars, mean ± SE of each group. P < 0.001 by the paired t test analysis. O6-mG, O6-methylguanine.

10^6 cells/μg DNA for control cultures and 1.22 ± 0.17 x 10^6 cells/μg DNA for PHA-stimulated cultures.

To determine whether the induction of alkyltransferase activity was linked to DNA synthesis, PHA-stimulated lymphocytes were incubated in the presence of either hydroxyurea to prevent DNA synthesis, or cycloheximide to inhibit protein synthesis. During hydroxyurea exposure, PHA-induced [3H]thymidine incorporation was inhibited by 96%. The effect of these inhibitors on alkyltransferase activity is shown in Table 1. Because of differences between cultures in the protein and DNA content of cells, alkyltransferase activity is expressed relative to cellular protein and DNA content. Alkyltransferase activity was induced 48 h after PHA stimulation in cells treated with hydroxyurea but was not induced in cycloheximide-treated cells. Thus, although mitogen-stimulated lymphocytes undergo a significant increase in alkyltransferase activity concurrent with the increase in DNA synthesis, the induction of activity requires protein synthesis and takes place even when DNA replication is inhibited.

Table 1 Induction of alkyltransferase during inhibition of protein or DNA synthesis in PHA-stimulated lymphocytes

<table>
<thead>
<tr>
<th>Culture condition</th>
<th>Time (h)</th>
<th>Alkyltransferase activity (fmol O6mG removed/μg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>251 ± 18</td>
</tr>
<tr>
<td>PHA</td>
<td>24</td>
<td>203 ± 35</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>444 ± 16</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>380 ± 34</td>
</tr>
<tr>
<td>PHA + cycloheximide (0.2 mM)</td>
<td>24</td>
<td>155 ± 12</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>204 ± 6</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>117 ± 47</td>
</tr>
<tr>
<td>PHA + hydroxyurea (10 mM)</td>
<td>24</td>
<td>268 ± 12</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>437 ± 14</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>268 ± 32</td>
</tr>
</tbody>
</table>

*Mean ± SE of average values obtained from 3 experiments.

Inactivation and Recovery of Alkyltransferase Activity in Lymphocytes after Exposure to MNU. To determine the dose-dependent inactivation of alkyltransferase by MNU exposure in human lymphocytes, resting and PHA-stimulated cells were exposed to MNU for 2 h and were assayed for residual alkyltransferase activity (Fig. 3). The dose-dependent inactivation of the alkyltransferase by MNU in PHA-stimulated and resting lymphocytes followed parallel curves, although a higher concentration of MNU was required to inactivate the alkyltransferase in PHA-stimulated lymphocytes. This was due to a higher level of activity in the stimulated cells prior to MNU treatment.

The regeneration of alkyltransferase activity following exposure to 0.5 mM MNU in resting (5 donors) and PHA-stimulated (8 donors) lymphocytes is shown in Fig. 4. This dose of MNU was chosen because it was the minimal dose required to inactivate maximally the alkyltransferase in both cell types. In PHA-stimulated lymphocytes, alkyltransferase activity had recovered to between 34 and 81% of baseline within 2 days of exposure, whereas recovery did not occur in resting lymphocytes. In resting cells, the level of alkyltransferase activity was only 8–23% of control at 3 days after MNU exposure. The differences between recovery of alkyltransferase activity in mitogen-stimulated and resting cells is summarized in Fig. 4C, which shows...
the ratio of activity in the two cell cultures. Although alkyltransferase activity is reduced to approximately 1 unit in both cultures following MNU exposure, recovery of activity in stimulated cells reaches a maximum of 4.7 times that of resting cells 2–3 days following MNU exposure. This indicates that PHA-stimulated cells not only have higher alkyltransferase activity but also recovery activity much more readily than resting cells.

Fig. 4 also shows the marked divergence among donors in their ability to recover alkyltransferase activity following nitrosourea exposure. Differences among donors were most marked 18–24 h postexposure. Five of 8 donors still had less than 2 units of alkyltransferase activity, whereas the other three had 4.0, 5.2, and 6.8 units of activity, respectively. Viability (>90%) and rate of DNA synthesis (1–3 × 10⁶ dpm/10⁶ cells/h, 24 h after MNU exposure) were similar in cell cultures from all donors. In addition, individuals with the highest basal alkyltransferase activity had the greatest degree of alkyltransferase recovery in their PHA-stimulated cells and to a lesser extent in their resting lymphocytes. These results suggested that resting lymphocyte cultures did not synthesize new functional alkyltransferase molecules in the 72 h following MNU exposure. However, the possibility remained that resting lymphocytes synthesize alkyltransferase molecules at the same rate as PHA-stimulated cells but that this activity is consumed by repair of persistent O⁶-methylguanine adducts. To address these issues, the dose of MNU was titrated so that an equivalent amount of residual alkyltransferase activity remained in both resting and PHA-stimulated lymphocytes from the same donor (Fig. 5). Alkyltransferase was reduced to either 2 units/µg DNA or 0.5 units/µg DNA in both resting and proliferating cells with doses of MNU between 75 and 200 µM. As can be seen, resting lymphocytes again failed to express a functional increase in alkyltransferase activity within 48 h. In addition, donors with low levels of alkyltransferase maintained similar levels of activity during recovery from MNU damage. Thus, it appears that, even at doses of MNU which have a similar effect on the alkyltransferase, resting lymphocytes fail to recover activity whereas proliferating cells do.

DISCUSSION

The important finding of this study is the demonstration that recovery of alkyltransferase activity following nitrosourea exposure is much greater in PHA-stimulated normal lymphocytes than in resting lymphocytes. This results in a level of repair capacity 2–4 days after exposure that was almost 5 times greater in stimulated than in resting cells. Under the conditions of our experiments, both proliferating and resting cells were incubated in a concentration of MNU that was sufficient to produce enough O⁶-methylguanine adducts to inactivate the alkyltransferase through the suicide repair mechanism of the protein (9, 14). Once the alkyltransferase was inactivated, persistent O⁶-methylguanine adducts should have been repaired by newly synthesized alkyltransferase molecules, leading to further consumption of repair capacity (29, 30). This may explain why, at the same concentration of MNU, lymphocytes with high initial levels of alkyltransferase activity recovered their activity more quickly than did lymphocytes with lower levels of activity.

Lack of recovery of alkyltransferase activity in resting lymphocytes may indicate that metabolically quiescent cells did not synthesize new alkyltransferase molecules rapidly or, less likely, that turnover is increased. Of note, we have found a similar difference between proliferating and resting lymphocytes when the alkyltransferase was inactivated with the non-DNA-damaging agent, O⁶-methylguanine (11). These results suggest that...
proliferating lymphocytes are more competent to repair mutagenic damage by nitrosoureas than are resting cells. Others have reported that following MNU and N-methyl-N'-nitro-N'-nitrosoguanidine depletion of the alkyltransferase, regeneration occurs in 4–48 h (17, 21, 22). These investigators have studied normal or transformed cell lines but none evaluated quiescent cells similar to resting human lymphocytes. Thus, it is possible that other resting human cells also fail to synthesize alkyltransferase activity rapidly following methylating agent damage. This may contribute to the limited clinical therapeutic index of nitrosoureas because proliferating malignant cells may have an increased capacity to regenerate the alkyltransferase compared to normal, resting cells.

In mammalian cells, the induction of alkyltransferase activity is limited to certain cells and tissues. In human and rodent cells, single or repeated exposure to low doses of certain methylating compounds such as dimethylnitrosamine can cause a 1.5- to 3-fold increase in alkyltransferase activity despite the fact that basal activity tends to be much higher in human cells (31, 32). In rat liver and in rat hepatoma cell lines, exposure to certain toxins, radiation, or bleomycin will also induce alkyltransferase activity (19, 33, 34). In synchronized human fibroblasts, alkyltransferase rises 2-fold preceding each wave of DNA synthesis (35), whereas in mouse C3H-10T½ cells, alkyltransferase-mediated repair decreases during DNA synthesis (31). Under the conditions of our study, proliferation but not MNU exposure caused an induction in alkyltransferase activity. Thus, we did not observe that DNA damage could induce the alkyltransferase activity in normal human cells. Whether this may be observed following other types of DNA damage remains to be determined. Morimoto et al. (36), however, have observed that repeated exposure to low doses of MNNG decrease sister chromatid exchange formation during subsequent exposure to higher dose MNNG in resting lymphocytes. If this “adaptive” response involves alkyltransferase repair of DNA damage, it suggests that under certain conditions, DNA damage could induce synthesis of alkyltransferase in human lymphocytes. These global measures of repair capacity (alkyltransferase activity and sister chromatid exchange formation) do not address the issue of DNA repair in regions of the DNA in which damage may correlate with cytotoxicity such as actively transcribed genes and regions undergoing active DNA replication (37).

Repair of O6-alkylguanine addsucts in these regions of DNA remains to be evaluated.

In addition to differences between resting and proliferating cells, we observed differences among donors in their lymphocyte alkyltransferase activity. This donor variability has been reported by us and others to occur in all human tissues surveyed (12, 26, 38, 39). In donors with low levels of alkyltransferase in their mitogen-stimulated lymphocytes, the enzyme was inactivated at lower concentrations of MNU and was regenerated much more slowly compared to donors with high levels of activity. The variation among donors was not due to ineffective mitogen stimulation, as all donors had evidence of significant increases in DNA synthesis and cell proliferation during PHA treatment. The differences among donors raise the possibility that turnover of the alkyltransferase protein may vary among individuals and may be an important determinant of basal and regenerating alkyltransferase activity (17). They may also explain why certain individuals appear to be more susceptible than others to the cytotoxic and mutagenic (in particular, leukemogenic) effects of nitrosourea exposure (40). For this reason, epidemiological studies, such as that recently reported (41), are needed to correlate the level of alkyltransferase activity and other DNA repair enzymes with the cytotoxicity, mutagenicity, and tumor response of alkylating agents in patients treated for various malignancies.

ACKNOWLEDGMENTS

The author thanks Dr. Nathan A. Berger and Dr. Joan E. Trey for helpful discussions, Kathleen Miller for excellent technical assistance, and Carol J. Tackett for preparation of the manuscript.

REFERENCES

ALKYLTRANSFERASE ACTIVITY IN LYMPHOCYTES

Regeneration of $O^6$-Alkylguanine-DNA Alkyltransferase in Human Lymphocytes after Nitrosourea Exposure

Stanton L. Gerson


**Updated version**
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/18/5368

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.