MOLECULAR AND CELLULAR BIOLOGY, Aug. 1982, p. 904–913
0270-7306/82/080904-10$02.00/0

Vol. 2, No. 8

Regulation of the Capacity for O^6-Methylguanine Removal from DNA in Human Lymphoblastoid Cells Studied by Cell Hybridization

KATHLEEN AYRES, ROBERT SKLAR, KAREN LARSON, VALERIE LINDGREN, AND BERNARD STRAUSS*

Department of Microbiology and Committee on Genetics, The University of Chicago, Chicago, Illinois 60637

Received 20 January 1982/Accepted 5 March 1982

Hybrids were made between a ouabain-resistant, thioguanine-resistant human lymphoma line able to remove O^6-methylguanine from its DNA (Mex^+) and human lymphoblastoid lines deficient in this capability (Mex^-). The formation of hybrids was confirmed by chromosomal analysis. Hybrid cells had an O^6-methylguanine removal capacity per mole of guanine about one third to one half that of the Mex^+ parents, i.e., about the same per cell. Cell hybrids removed the same amount of the alkylation adduct 3-methyladenine as did their parents per mole of guanine, i.e., about twice as much per cell. Although the cell hybrids had intermediate resistance to the cytotoxic action of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine used to induce O^6-methylguanine and 3-methyladenine, there is evidence that the ability to remove O^6-methylguanine and resistance to the cytotoxic effect of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine are dissociable characteristics.

The DNA alkylation product O^6-methylguanine (O^6MeG) plays a central role in carcinogenesis and mutagenesis induced by S_N1 methylating agents such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and *N*-methylnitrosourea (20, 25). The O^6MeG adduct is lost from both bacterial and mammalian cells by a special reaction mechanism in which the methyl group of the O^6MeG is transferred to the cysteine of an acceptor protein (3, 21, 24). As a result of this transfer, the acceptor protein is inactivated. Repair is therefore a stoichiometric reaction in which the amount of removal is limited by the amount of acceptor protein present (4). In *Escherichia coli*, cells respond to the presence of O^6MeG by the production of acceptor protein (29). No such inducible process for O^6MeG has yet been definitively observed in mammalian cells, although some suggestive observations have been published showing an increased removal capacity in animals pretreated with *N*-methylnitrosourea (22) and an increased survival in cells pretreated with MNNG (27).

The O^6MeG removal capacity of different organs varies. Rodent kidney and brain, for example, are relatively deficient in acceptor capacity relative to liver (10, 23). Even different cells within an organ may have different removal capacity. For example, parenchymal cells of the liver have greater capacity for removal than do their nonparenchymal neighbors (17). Human lymphoblastoid cells may be either removal competent (Mex^+) or incompetent (Mex^-), and this characteristic is independent of other DNA repair capabilities: lymphoblastoid lines derived from different xeroderma pigmentosum complementation groups deficient in their ability to remove pyrimidine dimers may be either Mex^+ or Mex^- (32).

The human fibroblast lines examined to date have invariably been O^6MeG removal competent (8). However, some (but not all) human fibroblast lines transformed with simian virus 40 have turned out to be removal negative (8). Day and Ziolkowski first described this phenomenon showing that removal competent lines (called Mer^+ by Day) were able to reactivate MNNG-treated adenovirus in contrast to the lack of reactivation in removal-deficient (Mer^-) lines (7). Some, but not all, tumor lines are Mer^-; all normal human fibroblast lines so far tested are Mer^+ (Mex^+).

The difference between Mex^+ and Mex^- strains could be due to some difference in the structural gene for the methyl acceptor protein, but it could also be due to a regulatory difference between the Mex^+ and Mex^- lines so that Mex^- lines shut off acceptor protein by means of some diffusible inhibitor. We therefore decided to attempt a genetic analysis by producing cell hybrids between Mex^+ and Mex^- lines. If a regulatory difference were involved, dominance of one characteristic over the other would be expected since many cell lines with capacity for specialized functions lose this capacity in cell hybrids, indicating a regulatory turn-off of func-

tion (14). In this study we report on the formation of hybrids of $Mex^+ \times Mex^-$ lymphoblastoid lines and on their ability to remove the O^6MeG methyl group.

MATERIALS AND METHODS

Chemicals. Fetal calf serum, premium horse serum, and powdered RPMI 1640 (Roswell Park Memorial Institute medium 1640) were obtained from Kansas City Biological Inc. Glutamine was obtained from GIBCO Laboratories, and Sea Plaque Agarose was from Marine Colloids. Hypoxanthine, thymidine, 6-thioguanine, ouabain, alpha-ketoglutarate, vinblastine sulfate, quinacrine mustard dihydrochloride, and 7-methylguanine were from Sigma Chemical Co. Aminopterin was obtained from Nutritional Biochemical Corp. 3-Methyladenine was obtained from Cyclo Chemical Co., and O^6MeG was obtained from David Ludlum and hydrolyzed to the free base. MNNG was obtained from Aldrich Chemical Co., and *N-[methyl-*^{3}H] MNNG (112 or 117.6 mCi/mmol) was obtained from Amersham Corp. MN 300 cellulose plates were from Analtech, and polyethylene glycol 6000 was obtained from the J. T. Baker Co.

Cells. The cell lines used are described in Table 1. Raji is a Mex^+ line able to remove O^6MeG; L33-6-1 is Mex^- (32). Bioassay Systems found these cultures to be free from mycoplasma contamination. They were routinely grown as suspension cultures in RPMI 1640 medium without antibiotics, supplemented with 17% fetal calf serum and 6 mM glutamine. All sera used for cloning and general stock maintenance were heat inactivated at 56°C for 30 min.

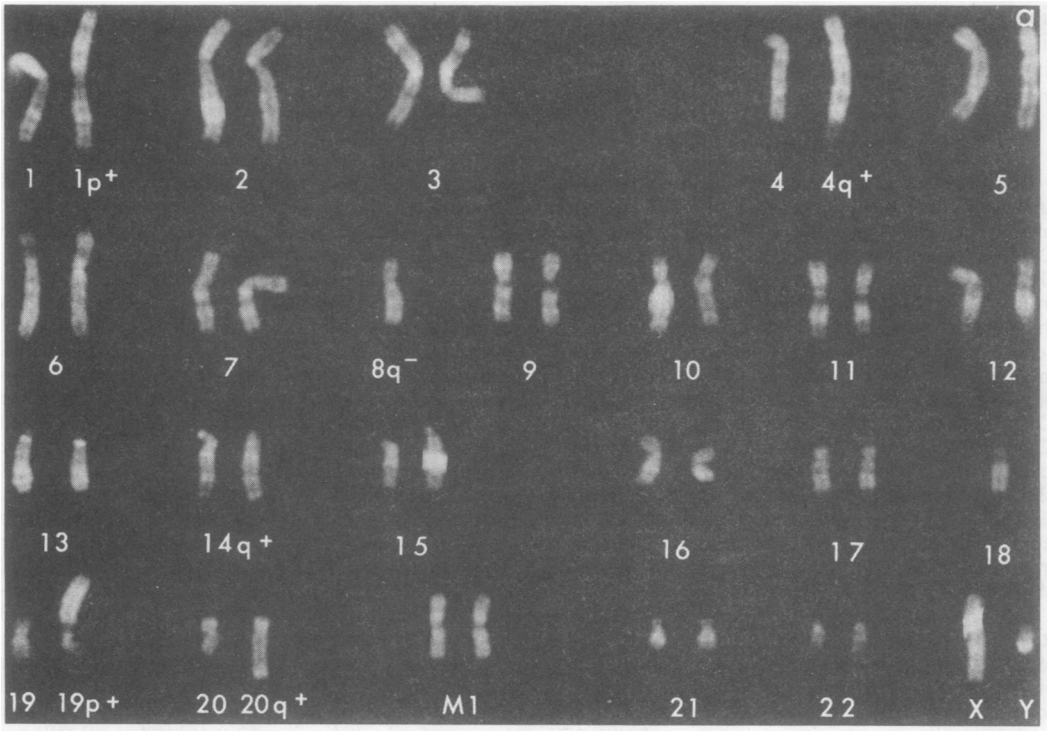
Cell growth. Cells were cloned by a modification of the method of Sato et al. (28) with the omission of feeder layers in a medium consisting of 5% fetal calf serum, 10% horse serum, 1 mM alpha-ketoglutarate, and 6 mM glutamine in a base of RPMI 1640 (1). HAT medium was RPMI cloning medium containing 10^{-4} M hypoxanthine, 4.0×10^{-7} M aminopterin, and 1.6×10^{-5} thymidine (19). HOT medium was made by the addition of ouabain to HAT medium (5).

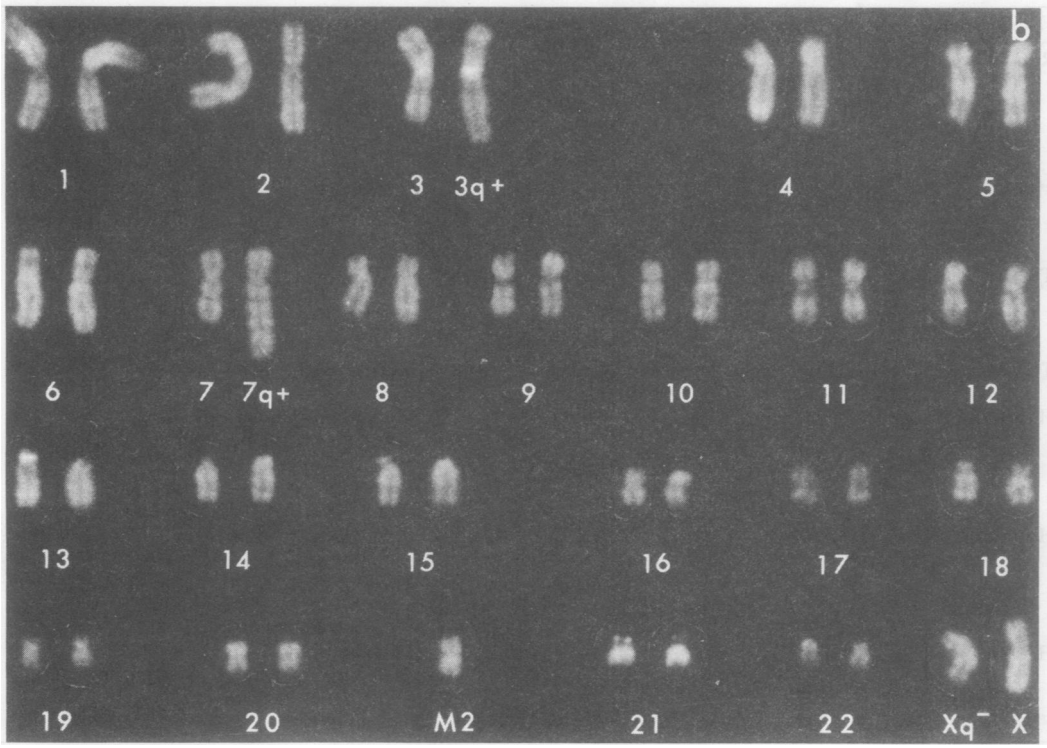
MNNG dose responses were determined by a modification of the microwell test method (15). Samples containing 2×10^7 logarithmic cells were pelleted at 800 rpm in the HL-4 rotor of the Sorvall GLC-2 clinical centrifuge, washed once with phosphate-buffered saline, and then suspended in phosphate-buffered saline in a 37°C water bath for 5 min. The cells were treated with MNNG (made up as a 1-mg/ml stock in 100% ethanol) at appropriate dilutions for 15 min at 37°C. After treatment, the cells were pelleted at 800 rpm, suspended in cloning medium, counted, and diluted to 10^5 cells per ml. Serial dilutions were made, and the cells were plated in Linbro microtiter plates at 2, 20, and 200 cells per well. The Linbro plates were incubated at 37°C in humidified incubators and scored after 3 weeks of growth.

Cells for alkylation studies were grown in Spinner culture. Four logarithmically growing cultures of 60 ml each were pooled, diluted to 300 to 400 ml of culture having a final cell density of 2×10^5 to 4×10^5 cells per ml, and transferred to a 1-liter Spinner flask. Cultures

TABLE 1. Lymphoblastoid lines used

Designation	Origin	Genetic markers	Chromosome composition	Reference
ROT-5	Raji (male Burkitt's lymphoma) from Pulvertaft provided by P. Gerber	Oua^r Thg^r	45, XY, $1p^+$, $4q^+$, -8, $8q^-$, $14q^+$, $14q^+$, -18, $19p^+$, $20q^+$, +M1/46, XY, $1p^+$, $4q^+$, -8, $8q^-$, $14q^+$, $14q^+$, -18, $19p^+$, $20q^+$, +M1, +M1	26
L6	L33-6-1 (female infectious mononucleosis) from P. Glade, subcloned by Sato, provided by J. Littlefield		47, X, Xq^-, $3q^+$, $7q^+$, +M2	28
Tk −/−	Tk^{6-}/BMS/9 from HH4 from WI-L2 (male spherocytosis) from Skopek provided by H. Liber	Thymidine $kinase^-$	47, XY, +13, $21p^+$	33
TGL2	From L33-6-1 by selection in thioguanine	$6\text{-}Thg^r$	Not done	
Hybrid no. 1.1	ROT-5 × L6	$Oua^{r\pm}$	Modal chromosome no. 88, + minute or fragment including $1p^+$, $4q^+$, $8q^-$, $14q^+$, $19p^+$, $20q^+$, Y, 2-M1 from ROT-5 and Xq^-, $7q^+$, M2 from L6	
Hybrid no. 2	ROT-5 × L6	$Oua^{r\pm}$	Modal chromosome no. 92 including $1p^+$, $4q^+$, 1 or $2\text{-}8q^-$, $2\text{-}14q^+$, $19p^+$, $20q^+$, Y, 2-M1 from ROT-5 and Xq^-, $3q^+$, $7q^+$, M2 from L6	
HAT-1	TGL2 × Tk −/−		Modal chromosome no. 94 including $7q^+$ from L33-6-1 (L6) and $21p^+$, Y from Tk −/−	
F1-2	ROT-5 × Tk −/−		Not done	


a
1 1p+ 2 3 4 4q+ 5
6 7 8q− 9 10 11 12
13 14q+ 15 16 17 18
19 19p+ 20 20q+ M1 21 22 X Y


b
1 2 3 3q+ 4 5
6 7 7q+ 8 9 10 11 12
13 14 15 16 17 18
19 20 M2 21 22 Xq− X

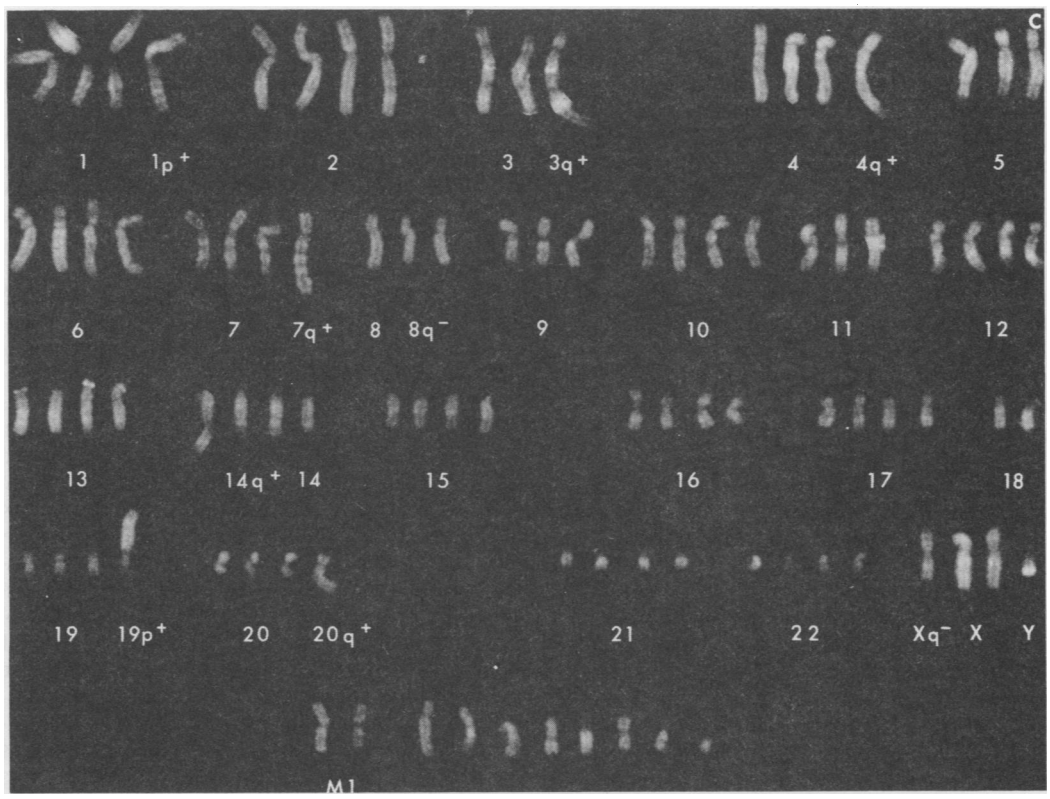


FIG. 1. Karyotypes of two parental lines and of one of their hybrid clones. (a) Karyotype of ROT-5, a Raji subclone with a bimodal chromosome distribution. The cell illustrated, with 46 chromosomes, is representative of about 55% of the cells. The remaining 45% have 45 chromosomes and lack a second M1 chromosome. (b) Karyotype of L6, a derivative of L33-6-1 with a modal chromosome number of 47. (c) Karyotype of hybrid clone no. 2, the product of fusion of ROT-5 and L6. Marker chromosomes $1p^+$, $4q^+$, $8q^-$, $14q^+$, $19p^+$, $20q^+$, and M1 of undetermined origin and the Y chromosome of ROT-5 and marker chromosomes $3q^+$, $7q^+$, and Xq^- of L6 are present in this hybrid clone. The clone, with a modal number of 92, also contains new markers of undetermined origin (see bottom row of chromosomes) and a new marker $14q^+$.

were diluted after doubling to give 2 liters of rapidly growing cells and were then assayed for O^6MeG removal.

Chromosome preparation and analysis. Chromosome preparation and identification methods were very similar to those of Farber and Davidson (9). Cells were seeded at 10^5 cells per ml and harvested on day 3 after a 2-h treatment with Velban (vinblastine sulfate) at 0.08 μg/ml. Cells were treated with 0.075 M KCl for 15 min at 37°C, fixed at least three times with Carnoy fixative, and air dried on slides. Preparations were soaked in pH 4.1 McIlvaine buffer for 4 min, stained in 50 μg of quinacrine mustard dihydrochloride per ml for 8 to 10 min, and soaked for 4 min in pH 4.1 McIlvaine buffer. After rinsing in water, slides were mounted in pH 7.0 McIlvaine buffer and photographed. At least 10 cells were karyotyped, and the chromosomes of 40 additional cells were counted for each parent and hybrid clone analyzed.

Removal of O^6MeG. Two liters of cells grown in Spinner culture was centrifuged, washed, and suspended in 100 ml of phosphate-buffered saline. Cells were treated with various amounts of [*methyl*-^{3}H]MNNG at 37°C for 10 min. The cells were then suspended in 100 ml of prewarmed RPMI 1640 plus 10% fetal calf serum and 4 mM L-glutamine and incubated for 2 h at 37°C. Cells were collected, washed, and suspended in SSC (0.15 M NaCl plus 0.015 M sodium citrate), and the DNA was isolated as previously described (31). This DNA was depurinated, and the purines were chromatographed along with a mixture of methylated markers on a 0.5-mm cellulose MN 300 thin layer chromatography plate prechromatographed with solvent and developed in 2-propanol–28% NH_4OH–water (7:2:1) (16). UV-absorbing bands corresponding to the markers and guanine were circled, removed from the plate with a razor blade, placed in scintillation vials, eluted, and counted as described elsewhere (31). The amount of guanine in the eluted band was determined spectrophotometrically. The amount of O^6MeG removed was calculated as previously reported (31) and is expressed per mole of guanine since both O^6MeG and guanine can be determined from the same chromatogram.

RESULTS

Isolation of hybrids. We decided to select $Mex^+ \times Mex^-$ hybrids by introducing the markers 6-thioguanine resistance (Thg^r) and ouabain resistance (Oua^r) into a Mex^+ line. Since Thg^r is recessive and Oua^r is dominant, only hybrids of a Thg^r $Oua^r \times Thg^s$ Oua^s cross will grow when plated in medium with hypoxanthine, aminopterin, thymidine, and ouabain (HOT medium; 5). We first determined that Raji cells are growth inhibited at 6-thioguanine doses over 6×10^{-7} M (46% survival), with less than 0.01% survival at 6×10^{-5} M thioguanine. Similarly, both Raji and line L33-6-1 fail to grow at ouabain concentrations greater than 10^{-8} M, with about 0.003% survival at 5×10^{-8} M. A Thg^r Oua^r subline of the Raji lymphoma line (ROT-5) was obtained by plating 5×10^5 Raji cells per 60-mm petri dish in agarose medium containing 10^{-6} M ouabain and selecting resistant clones. The clones were picked with a Pasteur pipette and placed in blood dilution tubes containing 0.5 ml of medium plus 10^{-6} M ouabain. When the medium became turbid, the tubes were diluted with 2.5 ml of medium, transferred to Corning T-25 flasks, and grown in the presence of ouabain for 1 month. One ouabain-resistant Raji clone was then plated at 10^5 cells per 60-mm petri dish in medium containing 5×10^{-5} M 6-thioguanine. Spontaneously arising resistant clones were selected and passaged for 4 months in cloning medium containing 6×10^{-5} M thioguanine and 10^{-6} M ouabain. One Thg^r Oua^r clone (ROT-5) with a bimodal chromosome number of 45/46 was chosen for these experiments (Fig. 1).

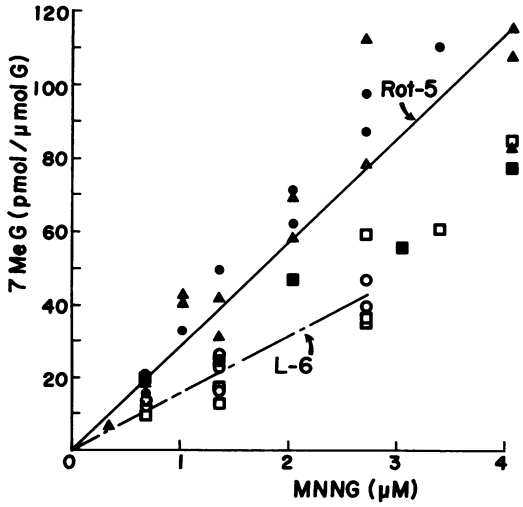


FIG. 2. Reactivity of cellular DNA as a function of external MNNG concentration. Cells were reacted with MNNG as described in the text. DNA was then isolated as described in the text, and analyzed for its content of 7-methylguanine. Results are expressed as picomoles of 7-methylguanine per micromole of guanine. Symbols: ○, L6; △, ROT-5; □, hybrid clone no. 2; ■, hybrid clone no. 1.1; ● Tk −/−.

The Mex^- parents were first screened for chromosome number. Since strain L33-6-1 was found to display a wide range of chromosome number with a high percentage of polyploids, it was recloned, and a clone (L6) with a modal chromosome number of 47 and with the least variation in number between cells was picked. A second derivative of L33-6-1 (TGL2) was isolated by selection in 6×10^{-5} M 6-thioguanine as described for Raji.

Polyethylene glycol-mediated cell fusion was accomplished by slight modification of standard techniques (6, 11). Stationary cells were diluted to 2×10^5/ml and maintained at this concentration by daily dilution for 3 days. On day 3, 5×10^5 cells of each parental line were pelleted, washed in serum-free RPMI, mixed in serum-free RPMI, and pelleted. The pellet was suspended in 1 ml of 50% polyethylene glycol (molecular weight, 6,000 to 7,500), pH 6.5, for 1 min and then immediately diluted by dropwise addition of 1 ml of RPMI without serum. At 1-min intervals, 2, 4, 8, and 16 ml of warm serum-free RPMI were added, and the suspension was centrifuged gently. The final cell pellet was suspended in 20 ml of medium without ouabain or thioguanine, transferred to Corning T-75 flasks, and incubated at 37°C for at least 24 h. The cells were then plated in selective medium (HAT or HOT). Each HOT selection was screened at ouabain concentrations of 2×10^{-8}, 5×10^{-8}, 10^{-7}, 5×10^{-7}, and 10^{-6} M, but useful clones were obtained only after selection at 5×10^{-8} M ouabain. Sixteen clones were isolated and cultivated in HAT medium containing 5×10^{-8} ouabain. Chromosome analysis after 3 months of growth indicated that 8 of the 16 clones were close to tetraploid.

To make sure that these clones represented true hybrids and not, for example, spontaneous polyploids with a slightly increased ouabain resistance, the chromosomes of both parents and hybrids were analyzed (Table 1, Fig. 1). Given the variability in the chromosome constitution of cell hybrids, we karyotyped cells from the same source as those used for the analysis of O^6MeG removal capacity (see below). Easily recognizable marker chromosomes from both parents were present in each of the hybrids karyotyped (Table 1, Fig. 1c). Clones no. 2 and 1.1 resulting from a cross of ROT-5 × L6, and line HAT-1 resulting from an TGL2 × Tk −/− cross were analyzed and shown to be true fusion products. We infer that the other clones with high chromosome numbers growing on HAT plus ouabain are also hybrids.

Removal of alkylation products. The ability of the hybrid lines to remove O^6MeG was determined as a function of dose of MNNG. We found that the cellular reactivity with MNNG of the different lines used in these experiments varied (Fig. 2). Line L6, for example, showed significantly lower reactivity as measured by the amount of 7-methylguanine produced at any particular MNNG concentration than did either ROT-5 or the Tk −/− lymphoblastoid line. The reactivity of the L6 × ROT-5 hybrids appeared to be intermediate between the two parental lines, but there was much variability in the determinations. To compare O^6MeG removal at the same level of total alkylation, we carried out dose-response experiments with all strains. We express our results as picomoles of O^6MeG removed per micromole of guanine by using picomoles of 7-methylguanine produced per micromole of guanine as a measure of dose, since this relatively stable adduct indicates the amount of total alkylation. It should be pointed out that not all O^6MeG removal-deficient strains reacted poorly, since the Tk −/− strain had greater reactivity combined with low O^6MeG removal capacity as compared with the ROT-5 lymphoma line.

As expected, the ROT-5 parental line was able to remove O^6MeG, whereas the Mex^- L6 parent was deficient in this ability (Fig. 3). We also determined the amount of 3-methyladenine removed. This alkylation adduct is removed by a mechanism which differs from that used for the transfer of the methyl group of O^6MeG (18) and is controlled by a different mechanism since Mex^- strains show no deficiency in 3-methyladenine removal (32). O^6MeG removal capacity plateaus at the higher MNNG concentrations due to saturation of the methyl acceptor protein as discussed elsewhere (31). Per micromole of guanine, the hybrid clone no. 2 had a little less than half the capacity for O^6MeG removal of the Mex^+ parent. However, since the hybrid had twice the DNA content of the Mex^+ parent per cell, the total amount of O^6MeG removed per cell was about the same in hybrid and Mex^+ parental lines. The 3-methyladenine data indicate that per cell, the hybrid has twice the removal capacity of either parent, both of which are competent in this reaction. An independently obtained, recloned hybrid line (no. 1.1) had significant O^6MeG removal activity, although this seemed to be inhibited at higher MNNG concentrations (Fig. 3).

To make sure that the increase in chromosome number or some other factor associated with hybridization did not activate the O^6MeG removal system, we also selected hybrids of a Mex^- × Mex^- cross made by fusing a Thg^r L33-6-1 derivative with a thymidine kinase-negative

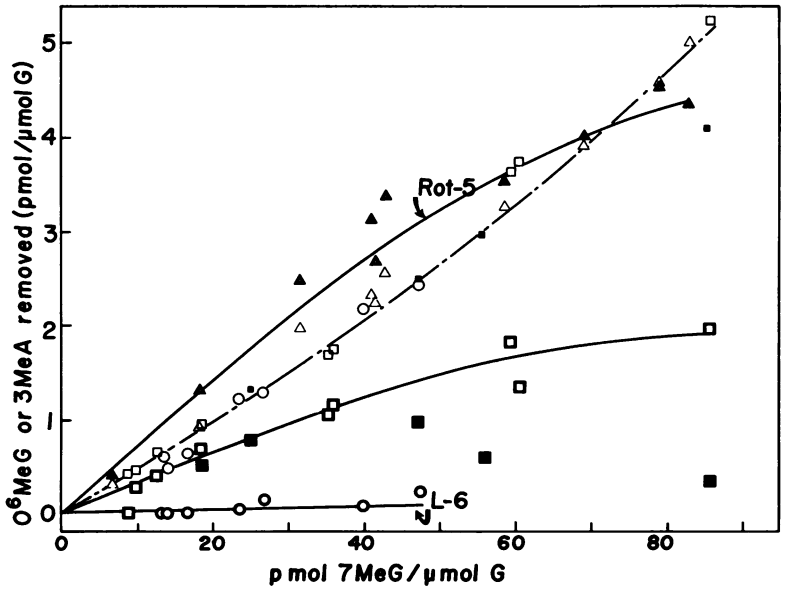


FIG. 3. Removal of O^6MeG and 3-methyladenine from the DNA of lymphoblastoid lines and their hybrids. Removal was measured as described in the text. Solid lines and boldface symbols, O^6MeG removal; dashed line and lighter symbols, 3-methyladenine. Adduct removal in picomoles per mole of guanine is plotted as a function of picomoles of 7-methylguanine produced per mole of guanine at particular MNNG concentrations. Symbols: O and ○, L6; Δ and △, ROT-5; □ and □, hybrid clone no. 2; ■ and ▪, hybrid clone no. 1.1.

line (Tk −/−) and selecting in HAT medium. Tk −/− and the line from which it was derived, strain HH-4 (32), appeared to have an O^6MeG removal capacity intermediate between L6 and ROT-5 (Fig. 4). We found barely detectable removal of O^6MeG at higher MNNG concentrations in the Tk −/− × TGL2 hybrid (Fig. 4). A hybrid line from the cross ROT-5 × Tk −/− had removal activity intermediate between these two parental lines. The 3-methyladenine removal capacity of the different cell lines and hybrids was the same per mole of guanine.

Lethal effects of alkylation. There is some evidence indicating that O^6MeG is a lethal lesion in mammalian cells or, more precisely, that the ability to remove the O^6MeG lesion is correlated with resistance to MNNG (7, 8, 30). We therefore tested the cytotoxicity of MNNG for both parental and some hybrid clones. Survival in these experiments was determined by plating in microtiter plates (15). The results (Fig. 5) are in accord with the hypothesis that O^6MeG is lethal: cells of the ROT-5 × L6 hybrids had much greater resistance to MNNG than did cells of the Mex^- parent, but were significantly more sensitive to killing by MNNG than were cells of their Mex^+ parent, even when the data are corrected for the different reactivities of the strains (Fig. 2). The effective dose of MNNG required to reduce ROT-5 survival to 37% was 3 μM; for L6 the 37% dose was 0.04 μM, and for the ROT-5 × L6 hybrid it was 1.5 μM (Fig. 5). The hybrid had an O^6MeG removal capacity per cell 80 to 100% that of the ROT-5 parental line (Fig. 3), but had twice as much DNA. Therefore a limiting amount of acceptor protein (3, 24) just sufficient to remove all O^6MeG from a Mex^+ parent will still leave 50% of the adduct present in the hybrid. Notwithstanding this relation, the Tk −/− line, which removed O^6MeG about as well as do the ROT-5 × L6 hybrids (Fig. 3 and 4) was almost as sensitive to MNNG-induced killing as was the absolute Mex^- L6 line (Fig. 5).

DISCUSSION

The inability of some lymphoblastoid lines to remove the alkylation product O^6MeG probably does not reflect a genetic deficiency of the individuals from whom the lines were derived, but is more likely a consequence of cell specialization or transformation. For example, rat liver hepatocytes can remove O^6MeG, whereas nonparenchymal cells are relatively deficient (17); brain tissue from neonate rats excises little O^6-ethylguanine, but liver cells remove large amounts (10). Most (but not all) simian virus 40-transformed human fibroblast lines have lost their capacity to remove O^6MeG (7); normal fibroblasts which are Mex^+ and transformed lymphoblastoid cells which are Mex^- can be obtained from the same individual (Y. Shiloh, personal communication). One of us (R.S.) has isolated two lymphoblastoid lines, one Mex^+ and the second Mex^-, from the same male individual. This observation does appear to rule out genetic polymorphism or X inactivation as a cause of the Mex^- phenotype, but is compatible with the hypothesis that there is a cellular poly-

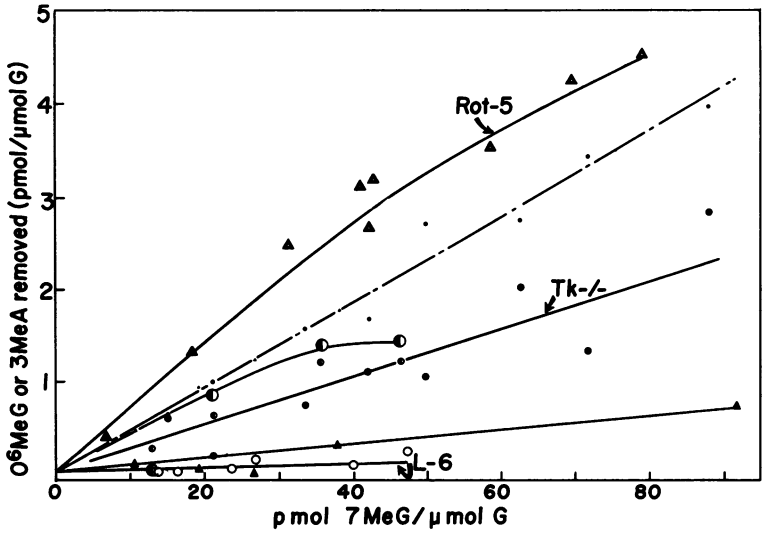


FIG. 4. Removal of O^6MeG and 3-methyladenine by hybrids of ROT-5 × Tk −/− and Tk −/− × TGL2. Removal was determined as described in the text and in the legend to Fig. 3. Solid lines, O^6MeG removal; dashed line and smaller symbols, 3-methyladenine removal. Symbols: ○, strain L6; △, ROT-5 (same data as for Fig. 3); ● and ●, Tk −/−; ◐ and ◐, ROT-5 × Tk −/−; ▲ and ▲, TGL2 × Tk −/− hybrid.

morphism among lymphocytes or that cell transformation (in this case by Epstein-Barr virus) either selects or produces cells which are Mex^-.

Our data show that cells of the ROT-5 × L6 hybrid no. 2 have about 40 to 50% of the ROT-5 parental capacity for the removal of O^6MeG per mole of guanine. Since the cells of the hybrid have approximately twice the parental chromosome number, they contain about twice the DNA, and our finding can be restated by saying that hybrid cells remove as much O^6MeG per cell as does their Mex^+ parent. The introduction of the Mex^- chromosomes has little or no effect on the O^6MeG removal capacity of the hybrid! It is therefore unlikely that a diffusible regulator shuts off the expression of the genes on the Mex^+ chromosome or that such a regulator activates the Mex^- chromosomes. Some control mechanism in which the chromosomes act autonomously is operative. It could be argued that the cellular machinery can only make that amount of O^6MeG acceptor produced in the Mex^+ strains so that it is not possible for the Mex^- genome to be activated in the hybrid. However, the finding that the hybrids remove the same amount of 3-methyladenine per mole of guanine as do the parental lines means that the cellular capacity for removal of this adduct is doubled in the hybrid. Therefore, cellular capacity for 3-methyladenine removal depends on the number of chromosomes which can supply message. There is no obvious reason why cell capacity should be dependent on chromosome number with 3-methyladenine and not with O^6MeG. We therefore conclude that some structural chromosomal difference results in the failure of the Mex^- strains to manufacture acceptor protein. The finding that the Tk −/− strain has a stable, intermediate level of removal must mean that there are alternative chromosomal states.

The nature of this control mechanism is made difficult to define by the complex relationship between the O^6MeG lesion and cytotoxicity. We found (Fig. 5) that Mex^+ × Mex^- hybrids had a resistance to MNNG-induced killing intermediate to that of the parents. It can also be argued that the effectiveness of MNNG in inducing killing is related to the amount of O^6MeG induced. The parent strain of L6 (L33-6-1) is inactivated by methyl methanesulfonate more readily than is the parental strain of ROT-5 (Raji). At 37% survival the methyl methanesulfonate dose reduction factor comparing Raji with L33-6-1 is 4 (12). The dose reduction factor for MNNG killing of ROT-5 compared with L6 at 37% survival is 3/0.04 (Fig. 5) = 75. MNNG is 75/4 = 18.8 times more effective in the differential killing of L6 than is methyl methanesulfonate. But MNNG is about 21 times more effective in producing O^6MeG than is methyl methanesulfonate (16). This calculation and a similar one made previously (30) along with the findings (30, 32; hybrid data above, Fig. 5) that sensitivity to MNNG and inability to remove O^6MeG are characteristics that tend to occur together, suggest that MNNG-induced lethality and failure to remove O^6MeG are in some way related. However, the similar sensitivity of L6

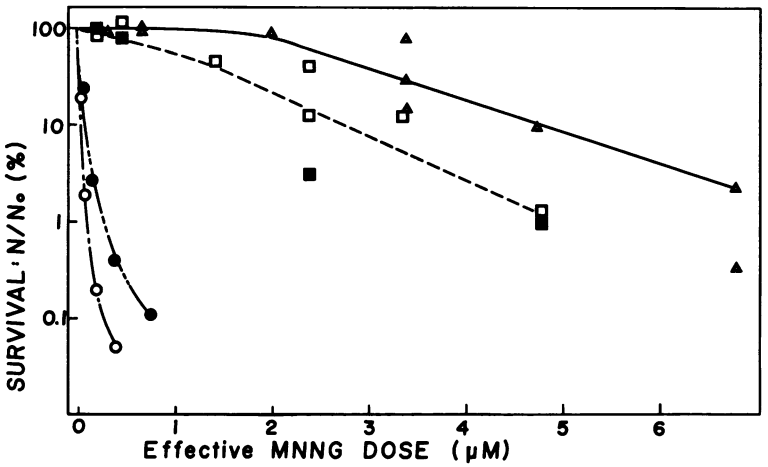


FIG. 5. Survival of lymphoblastoid strains and their hybrids after treatment with MNNG. Cells were treated with MNNG as described in the text. The cells were then diluted and plated in microwells, and the number of colonies was determined after 3 weeks of incubation at 37°C. Survival was calculated as described by Kraemer et al. (15). The effective MNNG concentration is calculated by correcting for differing reactivity as shown in Fig. 2 so that the dose shown is that which would be observed if all cells had the reactivity of ROT-5. Symbols: △, ROT-5; ○, L6; □, hybrid clone no. 2; ■, hybrid clone no. 1.1; ●, Tk −/−. The values from several different experiments are recorded.

and Tk −/− to MNNG, notwithstanding their different O^6MeG removal capability (Fig. 4), argues that some additional factor is involved in lethality. Data in the literature on close derivatives of the strains we use support this conclusion. In the study of Slapikoff et al. (34), WI-L2 (= Tk −/−) had about half the MNNG-induced mutability of MIT-2 (= L6), as would be expected if the ability to remove O^6MeG determined mutagenic response. However, both were almost identically sensitive to the killing action of MNNG as compared with a lymphoblastoid line relatively resistant to both killing and mutagenesis. We therefore suppose that strain Tk −/− and strain L6 are deficient in a reaction which repairs alkylation damage and which would otherwise result in lethality but not mutagenicity. This hypothetical alkylation damage would have to be produced in proportion to the amount of O^6MeG made to account for the relative effects of methyl methanesulfonate and MNNG discussed above. For example, it might be that the removal of O^6MeG is only the first step in a repair series that prevents lethality and that it is the later step(s) which is deficient in Tk −/−.

An alternative way of looking at the data is to say that in ROT-5, L6, and their hybrids the repair processes controlling O^6MeG removal (mutagenicity) (34) and toxicity are turned on together, whereas in Tk −/− these reactions are separable. A similar situation occurs in bacteria where mutation can alter the inducibility of the O^6MeG removal reaction without altering the inducibility of the reaction which protects against cytotoxicity (13). How a gene control mechanism could act at a chromosomal level and could, in some (but not all) cases control separable O^6MeG removal and MNNG-induced cytotoxicity repair reactions remains to be determined. The separation, in human cells, of mutagenic and inactivating alkylating lesions has been observed in HeLa variants that become resistant to the inactivating effects of monofunctional alkylating agents while retaining high mutagenic sensitivity (2).

ACKNOWLEDGMENTS

This work was supported by Public Health Service contract NO1-CP-85669 from the National Cancer Institute, by Public Health Service grants CA 14599 and GM 07816 from the National Institutes of Health, and by a grant from the Department of Energy. R.S. and K.L. were trainees on a Public Health Service training grant in environmental biology sponsored by the National Cancer Institute; V.L. was a trainee on Public Health Service genetics program GM 07197 supported by the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Ayres, K.** 1982. High cloning efficiency of human lymphoid cells in agarose without feeder layers. J. Natl. Cancer Inst. **68**:919–924.
2. **Baker, R., W. Van Voorhis, and L. Spencer.** 1979. HeLa cell variants that differ in sensitivity to monofunctional alkylating agents with independence of cytotoxic and mutagenic responses. Proc. Natl. Acad. Sci. U.S.A. **76**:5249–5253.
3. **Bogden, J., A. Eastman, and E. Bresnick.** 1981. A system in mouse liver for the repair of O^6-methylguanine lesions in methylated DNA. Nucleic Acids Res. **9**:3089–3103.
4. **Cairns, J.** 1980. Efficiency of the adaptive response of *Escherichia coli* to alkylating agents. Nature (London) **286**:176–178.
5. **Corsaro, C., and B. Migeon.** 1978. Gene expression in euploid human hybrid cells: ouabain resistance is codominant. Somat. Cell Genet. **4**:531–540.
6. **Davidson, R., and P. Gerald.** 1977. Induction of mammalian cell hybridization by polyethylene glycol. Methods Cell Physiol. **15**:325–338.
7. **Day, R., and C. Ziolkowski.** 1979. Human brain tumor cell strains with deficient host cell reactivation of N-methyl-N′-nitro-N-nitrosoguanidine-damaged adenovirus 5. Nature (London) **279**:797–799.
8. **Day, R., C. Ziolkowski, D. Scudiero, S. Meyer, A. Lubiniecki, A. Girardi, S. Galloway, and G. Bynum.** 1980. Defective repair of alkylated DNA by human tumour and SV40-transformed human cell strains. Nature (London) **288**:724–727.
9. **Farber, R., and R. Davidson.** 1977. Differences in the order of termination of DNA replication in human chromosomes in peripheral blood lymphocytes and skin fibroblasts from the same individual. Cytogenet. Cell Genet. **18**:349–363.
10. **Goth, R., and M. Rajewsky.** 1974. Persistence of O^6-ethylguanine in rat brain DNA: correlation with nervous system-specific carcinogenesis by ethylnitrosourea. Proc. Natl. Acad. Sci. U.S.A. **71**:639–643.
11. **Hansen, D., and J. Stadler.** 1977. Increased polyethylene glycol-mediated fusion competence in mitotic cells of a mouse lymphoid cell line. Somat. Cell Genet. **3**:473–482.
12. **Higgins, N. P., and B. Strauss.** 1979. Differences in the ability of human lymphoblastoid lines to exclude bromodeoxyuridine and in their sensitivity to methyl methanesulfonate and to incorporated [^{3}H]thymidine. Cancer Res. **39**:312–320.
13. **Jeggo, P.** 1979. Isolation and characterization of *Escherichia coli* K-12 mutants unable to induce the adaptive response to simple alkylating agents. J. Bacteriol. **139**:783–791.
14. **Kennett, R.** 1979. Cell fusion. Methods Enzymol. **58**:345–367.
15. **Kraemer, K., H. Waters, and J. Buchanan.** 1980. Survival of human lymphoblastoid cells after DNA damage measured by growth in microtiter wells. Mutat. Res. **72**:285–294.
16. **Lawley, P., and C. Thatcher.** 1970. Methylation of deoxyribonucleic acid in cultured mammalian cells by N-methyl-N′-nitro-N-nitrosoguanidine: the influence of cellular thiol concentrations on the extent of methylation and the 6-oxygen atom of guanine as a site of methylation. Biochem. J. **116**:693–707.
17. **Lewis, J., and J. Swenberg.** 1980. Differential repair of O^6-methylguanine in DNA of rat hepatocytes and nonparenchymal cells. Nature (London) **288**:185–187.
18. **Lindahl, T.** 1979. DNA glycosylases, endonucleases for apurinic/apyrimidinic sites and base excision repair. Prog. Nucleic Acids Res. Mol. Biol. **22**:135–192.
19. **Littlefield, J.** 1964. Selection of hybrids from matings of fibroblasts *in vitro* and their presumed recombinants. Science **145**:709–710.
20. **Loveless, A.** 1969. Possible relevance of O-6 alkylation of deoxyguanosine to the mutagenicity and carcinogenicity of nitrosamines and nitrosamides. Nature (London) **223**:206–207.
21. **Mehta, J., D. Ludlum, A. Renard, and W. Verly.** 1981. Repair of O^6-ethylguanine in DNA by a chromatin fraction from rat liver: transfer of the ethyl group to an acceptor protein. Proc. Natl. Acad. Sci. U.S.A. **78**:6766–6770.

22. **Montesano, R., H. Bresil, G. Planche-Martel, G. Margeson, and A. Pegg.** 1980. Effect of chronic treatment of rats with dimethylnitrosamine on the removal of O^6-methylguanine from DNA. Cancer Res. **40**:452–458.
23. **Nicoll, J., P. Swann, and A. Pegg.** 1975. Effect of dimethylnitrosamine on persistence of methylated guanines in rat liver and kidney DNA. Nature (London) **254**:261–262.
24. **Olsson, M., and T. Lindahl.** 1980. Repair of alkylated DNA in *Escherichia coli*. Methyl group transfer from O^6-methylguanine to a protein cysteine residue. J. Biol. Chem. **255**:10569–10571.
25. **Pegg, A.** 1977. Formation and metabolism of alkylated nucleosides: possible role in carcinogenesis by nitro compounds and alkylating agents. Adv. Cancer Res. **25**:195–209.
26. **Pulvertaft, R.** 1965. A study of malignant tumors in Nigeria by short-term tissue culture. J. Clin. Pathol. **18**:261–273.
27. **Samson, L., and J. Schwartz.** 1980. Evidence for an adaptive DNA repair pathway in CHO and human skin fibroblast cell lines. Nature (London) **287**:861–863.
28. **Sato, K., R. Slesinski, and J. Littlefield.** 1972. Chemical mutagenesis at the phosphoribosyltransferase locus in cultured human lymphoblasts. Proc. Natl. Acad. Sci. U.S.A. **69**:1244–1248.
29. **Schendel, P.** 1981. Inducible repair systems and their implications for toxicology. CRC Crit. Rev. Toxicol. **8**:311–362.
30. **Shiloh, Y., and Y. Becker.** 1981. Kinetics of O^6-methylguanine repair in human normal and ataxia telangiectasia cell lines and correlation of repair capacity with cellular sensitivity to methylating agents. Cancer Res. **41**:5114–5120.
31. **Sklar, R., K. Brady, and B. Strauss.** 1981. Limited capacity for the removal of O^6-methylguanine and its regeneration in a human lymphoma line. Carcinogenesis **2**:1293–1298.
32. **Sklar, R., and B. Strauss.** 1981. Removal of O^6-methylguanine from DNA of normal and xeroderma pigmentosum-derived lymphoblastoid cell lines. Nature (London) **289**:417–420.
33. **Skopek, T., H. Liber, B. Penman, and W. Thilly.** 1978. Isolation of a human lymphoblastoid line heterozygous at the thymidine kinase locus: possibility for a rapid human cell mutation assay. Biochem. Biophys. Res. Commun. **84**:411–416.
34. **Slapikoff, S., B. Andon, and W. Thilly.** 1980. Comparison of toxicity and mutagenicity of methylnitrosourea, methylnitronitrosoguanidine and ICR-191 among human lymphoblast lines. Mutat. Res. **70**:365–371.