Carcinogenesis vol.20 no.11 pp.2089–2094, 1999

Human O^6-alkylguanine-DNA alkyltransferase: protection against alkylating agents and sensitization to dibromoalkanes

Nieves Abril, Francisco L.Luque-Romero, Fred C.Christians[1,2], Lance P.Encell[1], Lawrence A.Loeb[1] and Carmen Pueyo[3]

Departamento de Bioquímica y Biología Molecular, Avda. de Medina Azahara s/n, Universidad de Córdoba, 14071-Córdoba, España and [1]The Joseph Gottstein Memorial Cancer Research Laboratory, Departments of Pathology and Biochemistry, University of Washington, Seattle, WA 98195-7705, USA

[2]Present address: Affymetrix, 3380 Central Expressway, Santa Clara, CA 95051, USA

[3]To whom correspondence should be addressed
Email: bb1pucuc@uco.es

***O*^{6}-alkylguanine-DNA alkyltransferase (AGT) is a suicide protein that corrects DNA damage by alkylating agents and may also serve to activate environmental carcinogens. We expressed human wild-type and two active mutant AGTs in bacteria that lack endogenous AGT and are also defective in nucleotide excision repair, to examine the ability of the AGTs to protect *Escherichia coli* from DNA damage by different types of alkylating agents and, oppositely, to sensitize cells to the genotoxic effects of dibromoalkanes (DBAs). Control bacteria carrying the cloning vector alone were extremely sensitive to mutagenesis by low, noncytotoxic doses of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG). Expression of human wild-type AGT prevented most of this enlarged susceptibility to MNNG mutagenesis. Oppositely, cell killing required much higher MNNG concentrations and prevention by wild-type AGT was much less effective. Mutants V139F and V139F/P140R/L142M protected bacteria against MNNG-induced cytotoxicity more effectively than the wild-type AGT, but protection against the less stringent mutagenesis assay was variable. Subtle differences between wild-type AGT and the two mutant variants were further revealed by assaying protection against mutagenesis by more complex alkylating agents, such as *N*-ethyl-*N*-nitrosourea and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. Unlike wild-type and V139F, the triple mutant variant, V139F/P140R/L142M was unaffected by the AGT inhibitor, *O*^{6}-benzylguanine. Wild-type AGT and V139F potentiated the genotoxic effects of DBAs; however, the triple mutant virtually failed to sensitize the bacteria to these agents. These experiments provide evidence that in addition to the active site cysteine at position 145, the proline at position 140 might be important in defining the capacity by which AGTs modulate genotoxicity by environmentally relevant DBAs. The ability of AGTs to activate dibromoalkanes suggests that this DNA repair enzyme could be altered, and if expressed in tumors might be lethal by enhancing the activation of specific chemotherapeutic prodrugs.**

Abbreviations: AGT, O^6-alkylguanine-DNA alkyltransferase; BG, O^6-benzylguanine; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; DBAs, dibromoalkanes; DBE, 1,2-dibromoethane (or ethylene dibromide); DBM, dibromomethane; ENU, *N*-ethyl-*N*-nitrosourea; GSH, glutathione; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; O^6-alkG, O^6-alkylguanine; O^6-meG, O^6-methylguanine.

Introduction

Alkylating agents that damage DNA and are used extensively in cancer chemotherapy are also mutagenic and carcinogenic. They alkylate DNA at multiple sites on nucleotide bases, sugars and phosphates; yet there is substantial agreement that the major mutagenic and lethal lesions involve the O^6 position of guanine. Repair of O^6-alkylguanine (O^6-alkG) is carried out predominantly by O^6-alkylguanine-DNA alkyltransferase (AGT). This enzyme attenuates the mutagenic and lethal effects of methylating and ethylating agents in virtually all organisms studied (1,2), and the levels of this enzyme in tumors frequently parallel the response of the tumors to chemotherapeutic bifunctional haloethylnitrosoureas [e.g. 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)]. Treatment of patients with alkylating agents is usually limited by myelosuppression due to the cytotoxic effects of these agents on bone marrow. As a result it has been proposed that the enhanced expression of AGT or mutant AGTs could protect bone marrow cells and permit dose escalation of therapeutic alkylating agents.

Previous studies from one of our laboratories (6,7) suggested a new role for AGT in activating dibromoalkanes (DBAs) to enhance their reactivity with DNA, thus increasing toxicity, mutagenesis and carcinogenesis. 1,2-Dibromoethane (DBE), used extensively in industry (3), is mutagenic in microorganisms, yeast and other fungi, plants, insects, mammals and human cells (4). DBE is also carcinogenic in experimental animals, and probably carcinogenic to humans (5). When human or bacterial AGT was expressed in *Escherichia coli*, a significant increase in mutagenesis and cytotoxicity was observed following exposure to both DBE and dibromomethane (DBM) (6,7). This unexpected finding was the first evidence that a DNA repair protein can enhance, rather than prevent, the genotoxicity of environmental chemical carcinogens.

The availability of mutant AGTs has facilitated studies on the function of this enzyme in the repair of DNA damage by alkylating agents and now in the activation of DBAs. Random mutagenesis was followed by positive genetic selection to create large libraries of human AGTs. The mutant enzymes were then selected on the basis of complementing AGT-deficient *E.coli* (8). One mutant (V139F) provided the repair-deficient bacteria with greater protection than the wild-type protein against both the cytotoxic and mutagenic effects of the methylating agent, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG). A triple mutant (V139F/P140R/L142M) was efficacious in protecting *E.coli* against MNNG and also rendered the bacteria exceptionally resistant to the AGT inhibitor, O^6-benzylguanine (BG). These mutant AGTs are currently being evaluated for potential use in protecting bone marrow by gene therapy in patients who receive high doses

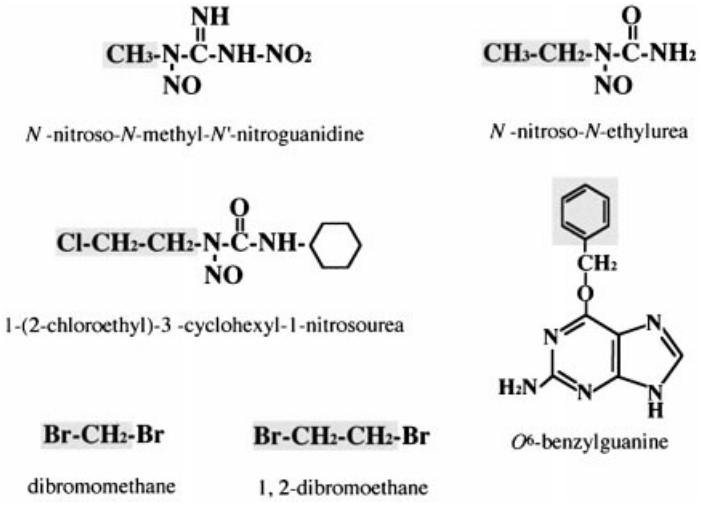

Fig. 1. Structure of the chemicals used in this study.

of alkylating agents, as well as in patients who are to receive BG to overcome the recalcitrant resistance of many tumors to alkylation-based chemotherapeutic regimens (9). Here we compare the ability of these two mutants and wild-type AGT in protecting *E.coli* against the lethal and mutagenic effects of the ethylating agent *N*-ethyl-*N*-nitrosourea (ENU), and the chloroethylating chemotherapeutic agent CCNU. To explore the putative mechanism by which AGT plays a role in the sensitization towards DBA genotoxicity, the cytotoxicity and mutagenesis induced by DBE and DBM were also quantified.

Materials and methods

MNNG, ENU, DBE, DBM and CCNU (Figure 1) were purchased from Sigma (St Louis, Mo). BG was generously provided by Dr R. Moschel (National Cancer Institute, Frederick, MD). MNNG, ENU, DBE, DBM and BG were dissolved in dimethyl sulfoxide (DMSO), and CCNU in *N*,*N*-dimethylformamide. Chemical structures are given in Figure 1. Plasmids used in this work were pUC118 containing the human AGT cDNA or a derivative mutant variant designated V139F and V139F/P140R/L142M, respectively (8,9). The level of the wild-type AGT in bacteria lacking endogenous AGT activity has been reported to be indistinguishable from the level of the V139F variant (8), and it is assumed that a negligible difference exists between the levels of the wild-type AGT and that of V139F/P140R/L142M (9). Plasmids were introduced into *E.coli* K-12 UC978 [*araD81*, *arg56*, *nad113*, Δ (*uvrB-bio*), *ogt1*::Kan^r, *ada10*::Tn*10*] (10). The new bacterial strains were designated UC1292 (vector), UC1291 (wild-type AGT), UC1298 (V139F) and UC1289 (V139F/P140R/L142M). UC1292, carrying the cloning vector pUC118 was used as reference. Culture media were as described (11).

Mutagenesis was assayed by selecting forward mutations to L-arabinose resistance (Ara^r) in a medium containing L-arabinose and a carbon source (glycerol) that fails to repress the *araDAB* operon expression (12). The selective plates were as follows: VB-salts containing Difco-agar (17 g/l), L-arabinose (2 g/l), glycerol (2 g/l), arginine (40 μg/ml), D-biotin (5 μg/ml), thiamine (5 μg/ml), nicotinic acid (5 μg/ml) and carbenicillin (32 μg/ml). For mutagenesis determinations, bacteria were grown at 37°C for 12 h with shaking (90 r.p.m.) in Luria-Bertani (LB) nutrient medium in the presence of carbenicillin (50 μg/ml). Cells were then harvested by centrifugation and resuspended in VB-salts (0.2 M phosphate buffer, pH 7 for CCNU). BG (final concentration, 100 μM) or its solvent, DMSO (final concentration, 3%), was added to 1 ml of bacterial suspension (~10^8 cells), and the cells were incubated at 37°C for 20 min with shaking (90 r.p.m). Then, the mutagen was added and the incubations continued for an additional 20 min (40 min for CCNU). Aliquots of 0.1 ml (~10^7 bacteria) were then combined in 2 ml of molten top agar and poured on selective plates. Incubations with the mutagen solvent were used to establish the spontaneous mutation rate. All bacterial strains exhibited similar background numbers of spontaneous Ara^r mutants (<300 mutants/10^7 bacteria plated). This number was unaffected by the BG treatment. The number of mutants induced per dose of compound (mutants/nmol or μmol) was estimated as the slope of the linear regression line fitted to the increasing portion of the corresponding dose–response relationship. Differences between slopes were done by regression ANOVA analysis. *P*-values for some of the comparisons are indicated in the text. For survival determinations in the presence and absence of BG, ~10^5 bacteria were exposed to each dose of mutagen, as described above. Following mutagen treatment, aliquots of 0.1 ml (~10^4 bacteria) were combined in 2 ml of molten top agar and plated on LB nutrient agar (VB minimal medium plates for ENU and CCNU) with carbenicillin (50 μg/ml). Bacterial colonies were counted automatically (Analytical Measuring System Ltd., UK, model 40-10). All data represent averages from at least two duplicate plates. Each assay was repeated on at least two separate occasions using a wide range of mutagen concentrations.

Results

Protection from alkylating agents by AGT and variants

V139F and V139F/P140R/L142M are mutant AGTs that were previously selected from random libraries in bacteria lacking AGT for their ability to confer resistance to the methylating agent, MNNG. The triple mutant, V139F/P140R/L142M was identified by screening the library for resistance to MNNG + BG (8). Larger alkyl groups that are substrates for AGT are also subject to removal by nucleotide excision repair. In these studies, we utilized bacteria that lack both AGT and nucleotide excision repair pathways so that the role of AGT in protecting against compounds (Figure 1) that generate larger adducts could be examined.

Survival and mutagenesis were quantitated following treatment of repair-defective bacteria expressing either wild-type AGT, V139F or V139F/P140R/L142M with varying concentrations of MNNG ± 100 μM BG (Figure 2). Cell death occurred at higher concentrations of MNNG than that required for mutagenesis, which is in agreement with previous results (8). As expected, MNNG was the most toxic to bacteria expressing the vector alone, while the mutants, V139F and V139F/P140R/L142M offered the cells enhanced protection over wild-type AGT. As was the case for survival, MNNG was highly mutagenic to bacteria carrying the cloning vector alone. However, this mutagenic effect was extensively reduced by each of the AGTs; a >50-fold reduction in mutation rate was observed in bacteria expressing wild-type AGT. This difference was observed at doses of MNNG that were 10-fold lower than those previously used with AGT-deficient but nucleotide excision repair-proficient bacteria when measuring the induction of rifampicin-resistance mutations (8).

The data in Figure 2 confirm previous findings on protection from MNNG cytotoxicity demonstrating that while 100 μM BG did not affect the protective ability of V139F/P140R/L142M, it provided a maximum inhibitory effect on the survival of cells expressing the wild-type AGT (9). New valuable information in Figure 2 is that: (i) V139F protected *E.coli* from both lethality and mutagenesis by MNNG better than V139F/P140R/L142M; (ii) protection by V139F/P140R/L142M from the mutagenic effects of MNNG was not greater than that offered by wild-type ($P = 0.33$); and (iii) 100 μM BG did not inhibit all AGT present in the cells, since, at all doses of MNNG, bacteria harboring the wild-type or the V139F protein showed lower mutagenesis than bacteria carrying the cloning vector.

We examined the ability of wild-type AGT and the two mutants to protect *E.coli* from mutagenesis induced by the ethylating agent, ENU and the chloroethylating agent, CCNU (Figure 3; Table I). The data indicate that in the absence of BG, both wild-type and mutant AGTs were less effective at protecting against ENU and CCNU mutagenesis than against MNNG mutagenesis. This is in agreement with evidence showing that the rate of repair of O^6-alkG decreases as the

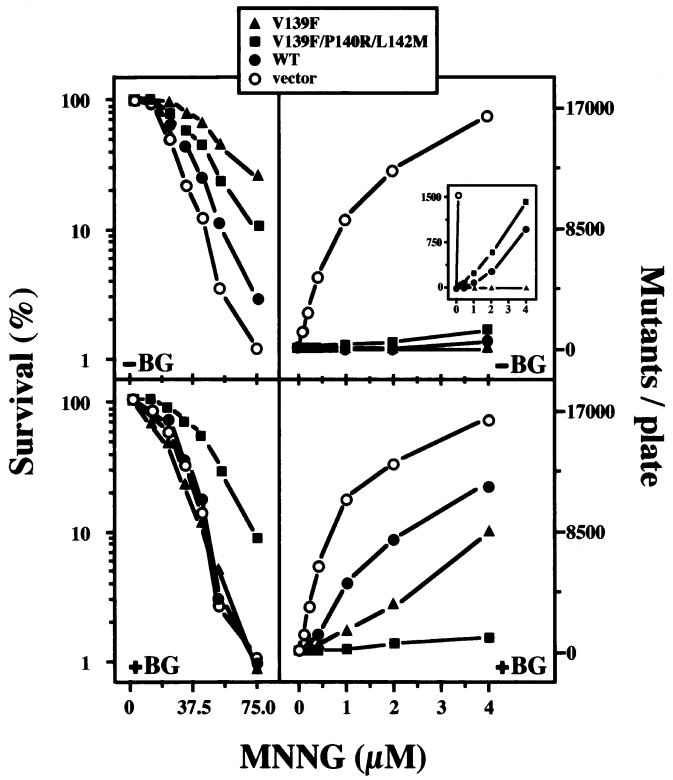

Fig. 2. MNNG-induced cytotoxicity and mutagenesis. Bacteria containing the cloning vector (UC1292, open circles) or plasmids expressing either the human wild-type AGT (UC1291, closed circles) or the V139F (UC1298, closed triangles) or V139F/P140R/L142M (UC1289, closed squares) mutant version were treated with increasing amounts of MNNG in the presence (+BG) or the absence of (–BG) 100 μM BG (Materials and methods). The percentage survival and the numbers of Ara^r mutants induced per selective plate (total – spontaneous counts) were plotted as a function of the tested dose of mutagen. Values from a representative experiment are shown. (Inset) Data corresponding to the lowest scale range of Ara^r induced mutants.

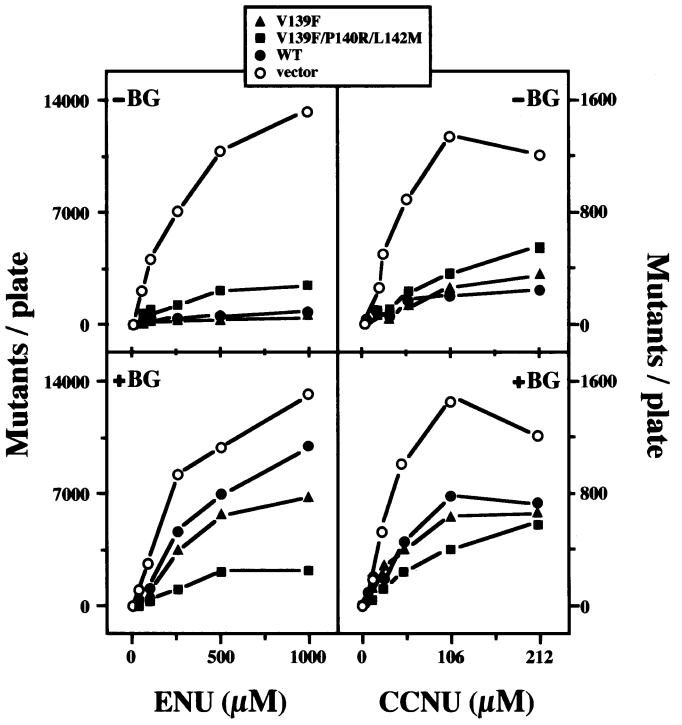

Fig. 3. ENU- and CCNU-induced mutagenesis. Bacteria containing the cloning vector (UC1292, open circles) or plasmids expressing either the human wild-type AGT (UC1291, closed circles) or the V139F (UC1298, closed triangles) or V139F/P140R/L142M (UC1289, closed squares) mutant version were treated with increasing amounts of ENU or CCNU in the presence (+BG) or the absence of (–BG) 100 μM BG (Materials and methods). The numbers of Ara^r mutants induced per selective plate (total – spontaneous counts) were plotted as a function of the tested dose of mutagen. Values from a representative experiment are shown.

size of the alkyl group increases. V139F offered better protection than V139F/P140R/L142M against ENU and CCNU mutagenesis ($P < 0.01$), but in contrast to the MNNG results, this protection was similar to that exhibited by wild-type AGT ($P \geqslant 0.21$). This order of effectiveness was maintained in protection against killing by either ENU or CCNU, where wild-type AGT $\approx$ V139F $>$ V139F/P140R/L142M (Figure 4). As was the case with MNNG, the protective ability of V139F/P140R/L142M against ENU and CCNU mutagenesis was unaffected by BG ($P \geqslant 0.41$), while both V139F and wild-type AGT were highly sensitive to this inhibitor (Figure 3; Table I).

Sensitization to DBAs

In marked contrast to the results obtained with the alkylating agents, bacteria carrying the wild-type AGT or the V139F mutant version were substantially more sensitive than control cells to both cytotoxicity and mutagenesis by DBE (Figure 5) and DBM (Figure 6). Interestingly, V139F/P140R/L142M showed much lower efficiency than the other two AGTs in enhancing cytotoxicity and mutagenesis caused by DBAs. Inactivation of AGT by BG virtually abolished the sensitization promoted by the wild-type and V139F proteins; however, the presence of BG had no effect on the bacteria harboring V139F/P140R/L142M.

Discussion

Human AGT mutants have been engineered by either site-directed or random sequence mutagenesis, and their putative utilities for gene therapy have been initially inferred on the basis of the mutants being able to prevent an AGT-defective strain of *E.coli* from killing by high (and in some cases multiple) doses of MNNG (~70–270 μM) (8,13,14). The induction of L-arabinose resistance provides a sensitive forward mutation assay for detection and quantification of the mutagenic potency of chemical carcinogens (15,16). We utilized this sensitive mutagenesis assay to evaluate the ability of human wild-type AGT and two mutant variants of AGT to protect *E.coli* from DNA damage by different types of alkylating agents and, oppositely, to sensitize cells to the genotoxic effects of DBAs. Bacteria that lacked endogenous AGT activity were also defective in nucleotide excision repair (*ada ogt uvr* triple mutant), because we have previously shown that differences in sensitivity to mutagenesis by both long-chain alkylating agents (10) and DBAs (6) between AGT-proficient and -deficient bacteria are vastly increased in a Uvr defective background.

Escherichia coli defective strain carrying the cloning vector as control (UC1292) was extremely sensitive to mutagenesis by low, non-cytotoxic doses of MNNG. Expression of human wild-type AGT in these cells prevented most (98%) of the mutagenesis induced by MNNG. Cell killing required higher MNNG concentrations, and prevention by wild-type AGT was much less effective. These results are explained by differences in the contribution of O^6-methylguanine (O^6-meG) to the

Table I. Comparative effects of human AGTs on mutagenic potencies of alkylating agents and DBAs[a]

Mutagen	UC1292 (vector)		UC1291 (WT)		UC1298 (V139F)		UC1289 (V139F/P140R/L142M)	
	−BG	+BG	−BG	+BG	−BG	+BG	−BG	+BG
MNNG (μM)	12 487	14 625	234	5087	0	2147	331	258
ENU (μM)	23	23	0.8	15	0.4	12	4.0	4.6
CCNU (μM)	31	28	2.4	8.0	1.9	6.7	3.1	3.5
DBE (mM)	114	120	5760	108	6515	113	157	162
DBM (mM)	0	0	1174	0	836	32	44	51

[a]Mutagenic potencies, expressed as Ara^r mutants induced per dose of mutagen, were calculated from the corresponding dose–response curves as described in Materials and methods. Data are averages from independent experiments. SD values did not exceed 15% of the mean. All bacterial strains were assayed in parallel.

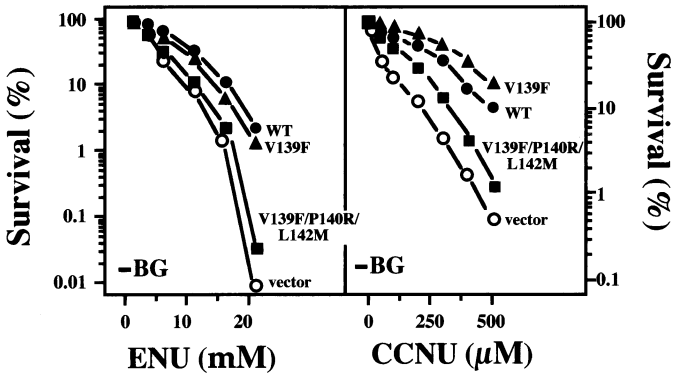

Fig. 4. ENU- and CCNU-induced cytotoxicity. Bacteria containing the cloning vector (UC1292, open circles) or plasmids expressing either the human wild-type AGT (UC1291, closed circles) or the V139F (UC1298, closed triangles) or V139F/P140R/L142M (UC1289, closed squares) mutant version were treated with increasing amounts of ENU or CCNU in the absence of BG (Materials and methods). The percentage survival was plotted as a function of the tested dose of mutagen. Values from a representative experiment are shown.

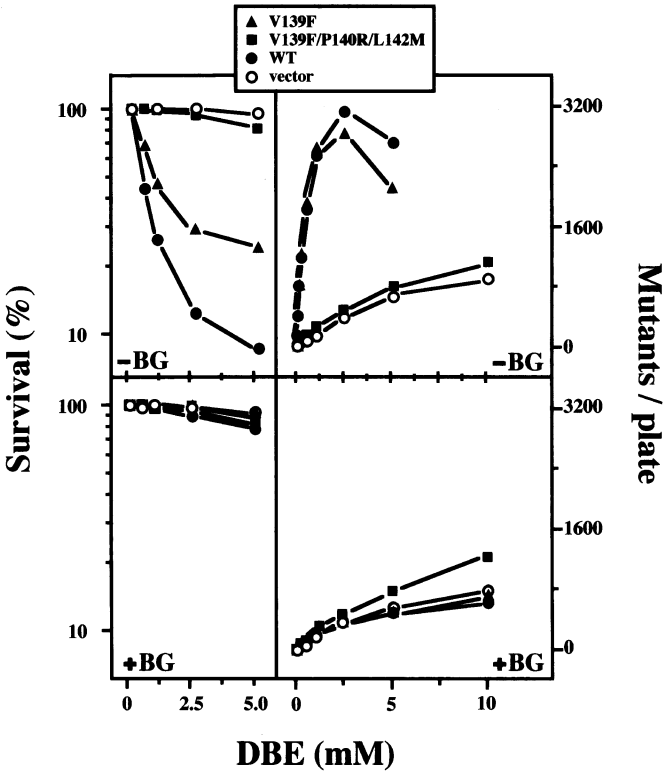

Fig. 5. DBE-induced cytotoxicity and mutagenesis. Bacteria containing the cloning vector (UC1292, open circles) or plasmids expressing either the human wild-type AGT (UC1291, closed circles) or the V139F (UC1298, closed triangles) or V139F/P140R/L142M (UC1289, closed squares) mutant version were treated with increasing amounts of DBE in the presence (+BG) or the absence of (–BG) 100 μM BG (Materials and methods). The percentage survival and the numbers of Ara^r mutants induced per selective plate (total – spontaneous counts) were plotted as a function of the tested dose of mutagen. Values from a representative experiment are shown.

mutagenesis and cytotoxicity caused by MNNG. O^6-meG is the major cause of mutagenesis by alkylating agents in *E.coli*, particularly in the absence of both AGT and nucleotide excision repair (17,18). With respect to its role in cytotoxicity, unrepaired O^6-meG lesions may trigger bacterial death through repeated, futile DNA mismatch repair (19). Alternatively, cytotoxicity by methylating agents in *E.coli* is largely ascribed to *N*-alkylpurines which are substrates for repair by DNA glycosylases (20).

Christians and Loeb (8) reported that V139F is more active than wild-type AGT in protecting AGT-deficient *E.coli* from MNNG-induced cell killing. We have now extended these results using bacteria that are also deficient in nucleotide excision repair. A dose of 4 μM MNNG increased the background level of Ara^r mutants 4.5 times in bacteria expressing the wild-type protein, while those expressing V139F remain insensitive to the mutagenic action of this methylating compound. Christians *et al*. (9) reported that V139F/P140R/L142M (like V139F) is more effective than wild-type AGT in the protection of *E.coli* from MNNG-induced cell killing. Though we were able to repeat this finding using bacteria lacking AGT and nucleotide excision repair, we did not detect significant differences in the abilities of these two AGTs to protect bacteria from the mutagenic effects of low doses of MNNG. It should therefore be noted that conclusions based on MNNG survival at relatively high concentrations do not necessarily apply to the much less stringent mutagenesis assay.

Subtle differences between wild-type AGT and the two mutant variants were further revealed by assaying protection against mutagenesis by ENU and CCNU. V139F protected bacteria against these alkylating agents with similar efficiency to wild-type AGT, in contrast to the higher levels of protection provided by this mutant against MNNG. V139F/P140R/L142M protected *E.coli* from ENU mutagenesis with lower efficiency (up to 5-fold) than the wild-type. Hence, conclusions based on protection against MNNG mutagenesis do not necessarily apply to other alkylating agents such as ENU and CCNU.

BG, a competitive inhibitor and thus a potent inactivator of human wild-type AGT, is in clinical trials for sensitizing

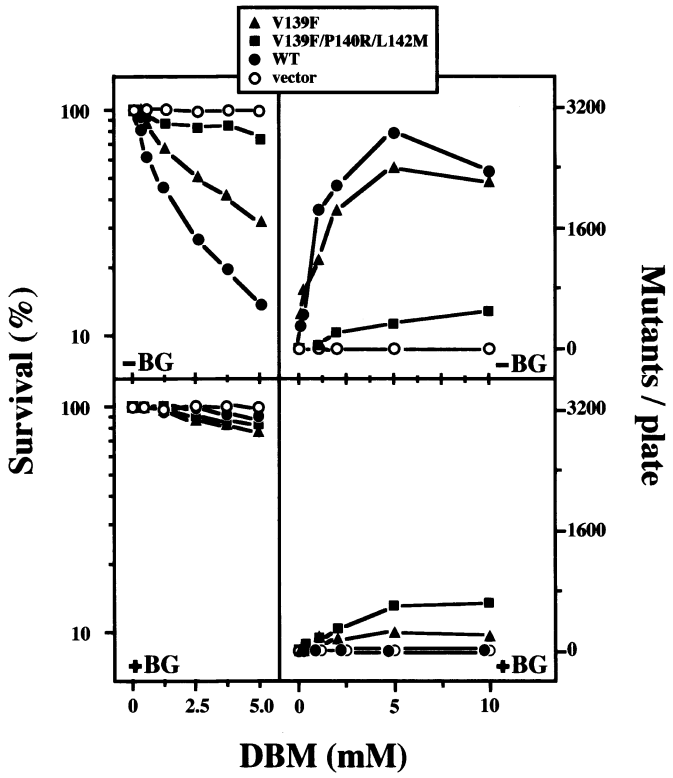

Fig. 6. DBM-induced cytotoxicity and mutagenesis. Bacteria containing the cloning vector (UC1292, open circles) or plasmids expressing either the human wild-type AGT (UC1291, closed circles) or the V139F (UC1298, closed triangles) or V139F/P140R/L142M (UC1289, closed squares) mutant version were treated with increasing amounts of DBM in the presence (+BG) or the absence of (–BG) 100 μM BG (Materials and methods). The percentage survival and the numbers of Ara^r mutants induced per selective plate (total – spontaneous counts) were plotted as a function of the tested dose of mutagen. Values from a representative experiment are shown.

tumors to alkylating agents. The results reported here confirm and extend previous studies on the resistance of V139F/P140R/L142M to concentrations of BG as high as 100 μM (9). It has been proposed that BG-resistant AGT mutants have a more sterically hindered active site, preventing the relatively large benzyl group from entering (9,21). This may also explain why V139F/P140R/L142M is less effective at preventing mutagenesis by larger size alkylating agents.

In vitro and *in vivo* DNA adduct formation by DBE is dependent on metabolic conversion via conjugation with glutathione (GSH). The half-mustard formed from DBE rearranges to form a reactive episulfonium ion, which is thought to be the ultimate metabolite that can react with DNA (3). A similar mechanism has been proposed for dihalomethanes (22). The role of GSH in the metabolic activation of these chemical carcinogens is rather unusual, considering it is generally thought of as a detoxifying agent. In contrast to the familiar role that AGT plays in protecting cells from alkylating agents, we have recently reported that bacterial or mammalian DNA AGTs can sensitize *E.coli* to both lethality and mutagenesis by DBAs (6,7). Such studies are consistent with the following two hypotheses. (i) DBA reacts first with the active site cysteine of AGT. Such a reaction would activate the compound, as postulated for the GSH-dependent activation pathway. (ii) A GSH–DBA conjugate reacts first with guanine in DNA. Subsequently, the AGT might become tightly bound to DNA at this lesion, generating a complex of increased cytotoxic/mutagenic potential. Mutant AGTs provide us with the opportunity to examine the relative ability of wild-type and the two mutant AGTs to influence DBA cytotoxicity and mutagenesis, both in the presence or the absence of the AGT inhibitor, BG. As reported previously (7), expression in *E.coli* of wild-type AGT caused a remarkable increase in mutagenesis and lethality upon exposure to DBE and DBM. Here we further demonstrate that the AGT-mediated sensitization to DBAs can be reversed by depleting AGT with BG, indicating an absolute requirement for active AGT in this response. Moreover, we show that although the single substitution V139F has little effect on the protein's ability to promote DBA genotoxicity, the triple substitution V139F/P140R/L142M rendered this variant virtually unable to potentiate toxicity or mutagenesis by DBAs.

As it has already been mentioned, the resistance of V139F/P140R/L142M to depletion by BG has been attributed to steric hindrance at the active site, provoked primarily by the substitution of the proline residue at position 140 (9,14). The diminished ability of V139F/P140R/L142M to sensitize *E.coli* to the lethal and mutagenic effects of DBE and DBM may also be due to such a steric effect. In the case of DBE, one can additionally speculate that insertion of arginine possessing a positively charged side chain at position 140 might prevent the attack by the reactive episulfonium ion. We have reported previously that the *E.coli* Ada AGT is unable to promote DBA mutagenicity. The other bacterial AGT, Ogt does enhance DBA mutagenicity, but with an efficiency ~50-fold lower than that of wild-type human AGT (6,7). Ada contains an alanine at the equivalent of position 140, rendering the protein resistant to BG. In the Ogt, which shows some sensitivity to BG but is much less suceptible than the human AGT, the proline is replaced by a serine yet there is another proline located two residues earlier in the sequence (2,21). All these observations indicate that the proline residue at position 140 is important for the ability of AGTs to promote DBA genotoxicity.

Whilst the mechanism by which the human AGT promotes DBA genotoxicity has yet to be established in detail, the recent findings that human AGT sensitizes human fibroblasts to both the lethal and mutagenic effects of DBE (N.Abril and G.P.Margison, personal communication) supports the idea that high levels of human AGT expression might be an increased risk factor in both the toxic and mutagenic effects of environmentally relevant DBAs. The results also indicate that different mutant AGTs might be used in cancer gene therapy either to protect normal tissues or to ablate tumor cells. The observation that the V139F/P140R/L142M variant is incapable of promoting the deleterious actions of DBAs increases the interest of creating new mutant AGTs for protecting host tissue, as AGTs have been proposed to be used in gene therapy for the protection of susceptible cell populations, particularly bone marrow. As a result it may be feasible to limit the major toxicity of alkylating agents. The finding that AGTs can activate DNA damaging agents presents the possibility that mutant AGTs created for the ability to activate specific prodrugs might be used directly for ablation of tumors. The introduction of genes expressing these mutant enzymes into tumors may render them specifically susceptible to particular chemotherapeutic agents as exemplified by the DBAs.

Acknowledgements

This work was supported by grant P95-0557-CO2-01 (DGES), and by Junta de Andalucía (group CVI 0187). L.A.L was supported by a grant from the

U.S. NIH, CA78885. L.P.E. was supported by a postdoctoral training grant from NIEHS (T32 ES07032).

References

1. Samson,L. (1992) The suicidal DNA repair methyltransferases of microbes. *Mol. Microbiol.*, **6**, 825–831.
2. Pegg,A.E. and Byers,T.L. (1992) Repair of DNA containing O^6-alkylguanine. *FASEB J.*, **6**, 2302–2310.
3. Guengerich,F.P., Min,K.-S., Persmark,M., Kim,M.-S., Humphreys,W.G., Cmarick,J.M. and Thier,R. (1994) Dihaloalkanes and polyhaloalkanes. In Hemminki,K., Dipple,A., Shuker,D.E.G., Kadlubar,F.F., Segerbäck,D. and Bartsch,H. (eds) *DNA Adducts: Identification and Biological Significance.* IARC Scientific Publications No. 125, IARC, Lyon, pp. 57–72.
4. Rannug,U. (1980) Genotoxic effects of 1,2-dibromoethane and 1,2-dichloroethane. *Mutat. Res.*, **76**, 269–295.
5. IARC (1987) *Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. IARC Scientific Publications No. 7 (suppl.), IARC, Lyon.
6. Abril,N., Luque-Romero,F.L., Prieto-Alamo,M.-J., Margison,G.P. and Pueyo,C. (1995) *ogt* alkyltransferase enhances dibromoalkane mutagenicity in excision repair-deficient *Escherichia coli* K-12. *Mol. Carcinog.*, **12**, 110–117.
7. Abril,N., Luque-Romero,F.L., Prieto-Alamo,M.-J., Rafferty,J.A., Margison,G.P. and Pueyo,C. (1997) Bacterial and mammalian DNA alkyltransferases sensitize *Escherichia coli* to the lethal and mutagenic effects of dibromoalkanes. *Carcinogenesis*, **18**, 1883–1888.
8. Christians,F.C. and Loeb,L.A. (1996) Novel human DNA alkyltransferases obtained by random substitution and genetic selection in bacteria. *Proc. Natl Acad. Sci. USA*, **93**, 6124–6128.
9. Christians,F.C., Dawson,B.J., Coates,M.M. and Loeb,L.A. (1997) Creation of human alkyltransferases resistant to O^6-benzylguanine. *Cancer Res.*, **57**, 2007–2012.
10. Abril,N., Ferrezuelo,F., Prieto-Alamo,M.-J., Rafferty,J.A., Margison,G.P. and Pueyo,C. (1996) Contribution of *ogt*-encoded alkyltransferase to resistance to chloroethylnitrosoureas in nucleotide excision repair-deficient *Escherichia coli*. *Carcinogenesis*, **17**, 1609–1614.
11. Gerhardt,P., Murray,R.G.E., Wood,W.A. and Krieg,N.R. (1994) *Methods for General and Molecular Bacteriology.* American Society for Microbiology, Washington, DC.
12. Pueyo,C. and Ruiz-Rubio,M. (1984) The L-arabinose resistance test with *Salmonella typhimurium*. In de Serres,F.J. (ed.) *Chemical Mutagens: Principles and Methods for their Detection.* Plenum Press, New York, NY, Vol. 9, pp. 89–109.
13. Encell,L.P., Coates,M.M. and Loeb,L.A. (1998) Engineering human DNA alkyltransferases for gene therapy using random sequence mutagenesis. *Cancer Res.*, **58**, 1013–1020.
14. Xu-Welliver,M., Kanugula,S. and Pegg,A.E. (1998) Isolation of human O^6-alkylguanine-DNA alkyltransferase mutants highly resistant to inactivation by O^6-benzylguanine. *Cancer Res.*, **58**, 1936–1945.
15. Dorado,G. and Pueyo,C. (1988) L-arabinose resistance test with *Salmonella typhimurium* as a primary tool for carcinogen screening. *Cancer Res.*, **48**, 907–912.
16. Roldán-Arjona,T., Luque-Romero,F.L., Ruiz-Rubio,M. and Pueyo,C. (1990) Quantitative relationship between mutagenic potency in the Ara test of *Salmonella typhimurium* and carcinogenic potency in rodents. A study of 11 direct-acting monofuctional alkylating agents. *Carcinogenesis*, **11**, 975–980.
17. Horsfall,M.J., Gordon,A.J.E., Burns,P.A., Zielenska,M., van der Vliet, G.M.E. and Glickman,B.W. (1990) Mutational specificity of alkylating agents and the influence of DNA repair. *Environ. Mol. Mutagen.*, **15**, 107–122.
18. Abril,N., Roldán-Arjona,T., Prieto-Alamo,M.-J., van Zeeland,A.A. and Pueyo,C. (1992) Mutagenesis and DNA repair for alkylation damages in *Escherichia coli* K-12. *Environ. Mol. Mutagen.*, **19**, 288–296.
19. Rasmussen,L.J. and Samson,L. (1996) The *Escherichia coli* MutS DNA mismatch binding protein specifically binds O^6-methylguanine DNA lesions. *Carcinogenesis*, **17**, 2085–2088.
20. Evensen,G. and Seeberg,E. (1982) Adaptation to alkylation resistance involves the induction of a DNA glycosylase. *Nature*, **296**, 773–775.
21. Pegg,A.E., Boosalis,M., Samson,L., Moschel,R.C., Byers,T.L., Swenn,K. and Dolan,M.E. (1993) Mechanism of inactivation of human O^6-alkylguanine-DNA alkyltransferase by O^6-benzylguanine. *Biochemistry*, **3**, 11998–12006.
22. Thier,R., Taylor,J.B., Pemble,S.E., Humphreys,W.G., Persmark,M., Ketterer,B. and Guengerich,F.P. (1993) Expression of mammalian glutathione *S*-transferase 5-5 in *Salmonella typhimurium* TA1535 leads to base-pair mutations upon exposure to dihalomethanes. *Proc. Natl Acad. Sci. USA*, **90**, 8576–8580.

Received May 4, 1999; revised June 30, 1999; accepted July 3, 1999