[CANCER RESEARCH 62, 656–660, February 1, 2002]

Advances in Brief

3-Methyladenine DNA Glycosylase-deficient *Aag* Null Mice Display Unexpected Bone Marrow Alkylation Resistance[1]

Richard B. Roth and Leona D. Samson[2]

Division of Bioengineering and Environmental Health, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Abstract

Most cells deficient in 3-methyladenine (3MeA) DNA glycosylase become sensitive to the lethal and clastogenic effects of alkylating agents. Surprisingly, myeloid progenitor bone marrow (BM) cells derived from *Aag* −/− mice were more resistant than those from wild-type mice to the cytotoxic effects of several alkylating agents. Moreover, an alkylation-resistant phenotype was observed *in vivo* using the BM micronucleus assay as a measure of chromosome damage. Flow cytometry indicated that *in vivo* alkylation resistance in *Aag* null BM cells may be restricted to cells of the myeloid lineage. These results show that in particular cell types, the initiation of base excision repair is more lethal to the cell than leaving the damaged bases unrepaired by Aag.

Introduction

All organisms are constantly exposed to alkylating agents, not only from our exogenous environment but also from normal cellular metabolites (1). The many nucleophilic sites of DNA are readily modified by electrophilic alkylating agents, generating alkylated DNA lesions in the process. Among these alkylated lesions, N^7 alkylguanines are usually considered to be innocuous, whereas N^3-alkyladenines and O^6-alkylguanines are known to be both cytotoxic and mutagenic. Alkylated DNA bases are primarily recognized and repaired via the actions of two kinds of repair proteins, O^6-methylguanine DNA MTases[3] and 3MeA DNA glycosylases. MTases directly remove alkyl groups from O^6-alkylguanines (and to a lesser extent from O^4-alkylthymines), toxic lesions generated by most chemotherapeutic alkylating agents. Numerous studies show that MTase-deficient *Escherichia coli*, *Saccharomyces cerevisiae*, and mammalian cells are very sensitive to the mutagenic and cytotoxic effects of chemical alkylating agents (1, 2). 3MeA DNA glycosylases, encoded by *AAG/MPG/ANPG* in humans and *Aag*/*Mpg*/*Anpg* in mice, initiate the BER pathway (3–5). In addition to excising 3MeA, these glycosylases excise DNA bases with methyl or certain other alkyl groups at the N7 and N3 positions of both adenine and guanine; they also excise ϵA) and deaminated adenine (hypoxanthine) DNA bases (1, 6). Aag-initiated BER begins with cleavage of the glycosylic bond connecting the modified base to the deoxyribose sugar; the resulting abasic site can then be recognized by AP endonucleases that cleave the phosphodiester bond 5′ to the abasic site forming a strand break with a 3′ OH and a 5′ deoxyribosephosphate terminus. DNA polymerase β can extend from the 3′ OH terminus. In addition, polymerase β can remove the 5′ deoxyribose phosphate residue via an enzymatic activity residing in its NH_2-terminal domain. BER is completed by either DNA ligase I or a DNA ligase III/XRCCI complex that joins the two resulting ends, restoring the original, unmodified DNA structure (1). It is important to note that each of the intermediates generated during the BER process represents some form of DNA damage and that it is not until DNA ligation is completed that the genome is free from damage. An appreciation of this fact is important for understanding why some surprising and unexpected phenotypes are produced when BER enzymes are inappropriately expressed. Here we show that decreased expression of the *Aag* 3MeA DNA glycosylase confers unexpected alkylation resistance on some mouse tissues, rather than the expected alkylation sensitivity.

Materials and Methods

Generation of Crude Protein Extracts from Mouse Tissues. C57Bl/6J 8-week-old mice were euthanized by CO_2 asphyxiation, and several tissues were immediately harvested, frozen in liquid nitrogen and then processed to generate crude protein extracts (7). In addition, DNA content was determined for each crude sonicate (8).

***In Vitro* Oligonucleotide DNA Glycosylase Assay.** A double-stranded 25mer oligonucleotide containing a single centrally located ϵA residue [5′-GGATCATCGTTTTT(ϵA)GCTACATCGC-3′] was used as template DNA. Glycosylase assays were performed according to Engelward *et al.* (7). A phosphorimager was used both to visualize and to quantitate Aag DNA glycosylase activity.

BM Cell Clonogenic Survival Assay. BM cells were harvested from the femurs of *Aag* +/+ and *Aag* −/− mice and then treated with MMS, MeLex, TMZ, MMC, BCNU, and thiotepa. Cells were then washed and resuspended in complete medium, mixed with methylcellulose (Stem Cell Technologies, Vancouver, British Columbia, Canada), and plated in duplicate. After 10–14 days, colonies of >50 cells were scored, and percentage survival was determined by comparing treated and untreated duplicate cultures processed similarly.

Micronucleus Assay. Wild-type and *Aag* null mice received i.p. injections of PBS, MMS, MMC, TMZ, and BCNU. Twenty-four h later, BM cells were harvested and processed for analysis (9). PCEs, MN PCEs, and NCEs were then scored using a Nikon Eclipse E800 fluorescent microscope with a ×60 objective. PCEs (2000/mouse) were scored for the presence of micronuclei. In all of the experiments performed, the ratio of PCEs:NCEs was between 40 and 60%, which indicated that the dose of the alkylating agent used was subtoxic.

Flow Cytometry to Determine MMS Sensitivity of Lineage-specific BM Cells. Mice received i.p. injections of PBS or MMS, and 24 h later, BM cells were harvested, washed, and resuspended in HBSS to 1×10^7 cells/ml. For each sample, six 100-μl aliquots were treated with either phycoerythrin-antimouse CD3 (T-cell specific), phycoerythrin-antimouse CD116 (Mac1, macrophage and granulocyte-specific), FITC-antimouse Ly-6G (Gr1, granulocyte-specific), FITC-antimouse CD45R/B220 (B-cell-specific), or IgG isotype nonspecific control antibody (BD PharMingen). A Coulter Elite flow

Received 10/18/01; accepted 12/12/01.

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 by a Leukemia and Lymphoma Society of America Translational Research grant, by NIH Research Grant RO1 CA75576, and by the Kresge NIEHS Center for Environmental Health Research ES 00002 for FACS analyses. R. R. was supported by NIH Radiation Biology Training Grant 5T32CA09078. L. S. is the Ellison American Cancer Society Research Professor. Most of the experiments reported here were carried out in the Department of Cancer Cell Biology, Harvard School of Public Health.

[2] To whom requests for reprints should be addressed, at Division of Bioengineering and Environmental Health, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139. Phone: (617) 258-7813; Fax: (617) 253-8099; E-mail: lsamson@mit.edu.

[3] The abbreviations used are: MTase, methyltransferase; 3 MeA, 3-methyladenine; BER, base excision repair; ϵA, 1-N^6-ethenoadenine; BM, bone marrow; MMS, methyl methanesulfonate; MeLex, methyl lexitropsin; TMZ, temozolomide; MMC, mitomycin C; BCNU, bis-chloronitrosourea; PCE, polychromatic erythrocyte; MN PCE, micronucleated PCE; NCE, normochromatic erythrocyte; ES, embryonic stem; NER, nucleotide excision repair; AP, apurinic/apyrimidinic.

cytometer was used to determine the fraction of each BM cell lineage present before and after alkylating agent treatment.

30-Day Survival Studies. *Aag* +/+ and *Aag* −/− mice received i.p. injections of MMS and MMC. A dose schedule was followed as recommended by Deichmann and LeBlanc (10); one mouse/dose received an injection and six Aag +/+ and *Aag* −/− mice/drug/experiment received an injection with each dose being 50% greater than the preceding dose. The lowest dose at which a mouse dies within 30 days has been shown empirically to be a good estimate of the LD_{50} (10).

Results

BM Cells Express Low Levels of 3MeA DNA Glycosylase. BM cells, particularly of the myeloid lineage, are acutely sensitive to alkylating agent cytotoxicity. A plausible explanation for such sensitivity is that, relative to other cell types, these cells are deficient in activities that recognize and repair DNA alkylation damage. Indeed, Gerson *et al.* (11) found that both mouse and human BM cells express very low levels of the Mgmt O^6-methylguanine DNA MTase relative to other tissues. Here, we set out to determine whether Aag 3MeA DNA glycosylase levels are similarly low by comparing Aag DNA glycosylase activities in mouse BM, spleen, liver, lung, kidney, brain, and gastrointestinal tract tissues. The activity of this glycosylase was determined by an *in vitro* assay that measures the production of alkali labile abasic sites on release of ϵA bases from an oligonucleotide substrate (see "Materials and Methods"). As depicted in Fig. 1, BM cells expressed the lowest Aag activity among the tissues analyzed, with the exception of the gastrointestinal tract. Liver and brain showed the highest levels of glycosylase activity followed by (in descending order) the kidney, lung, and spleen. [It should be noted that we previously determined that all of the detectable ϵA DNA glycosylase activity in mouse tissue extracts is attributable to the presence of Aag (7)]. These data suggest that BM cells may be limited in their ability to excise alkylated DNA bases and, more importantly, that this limitation may contribute to their extreme sensitivity to alkylation-induced cytotoxicity and clastogenicity. However, it is important to point out that for the analogous Mgmt MTase deficiency in BM cells, the residual very low level of MTase provides surprisingly extensive alkylation protection, as evidenced by the finding that *Mgmt* −/− BM cells of the myeloid lineage are much more sensitive than wild-type BM cells to a number of alkylating agents (12). It seemed to us that this could also hold true for the Aag DNA glycosylase, *i.e.*, that even very low Aag levels might play an important role in protecting BM cells against alkylation toxicity. We, therefore, set out to compare the alkylation sensitivity of BM cells derived from *Aag* −/− and *Aag* +/+ mice.

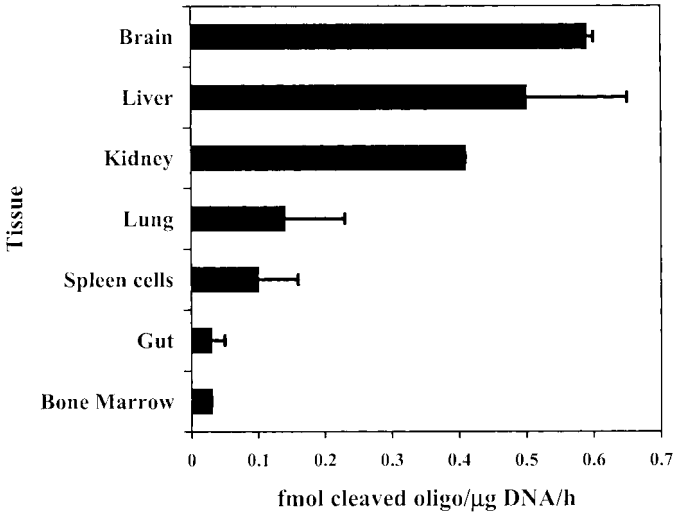

Fig. 1. Aag activities in seven different mouse tissues. The ϵA oligonucleotide glycosylase activity assay was used to determine the level of Aag in cell free extracts of brain, liver, kidney, lung, spleen, gut, and BM mouse tissues. Determinations were performed in triplicate; *error bar*, SE.

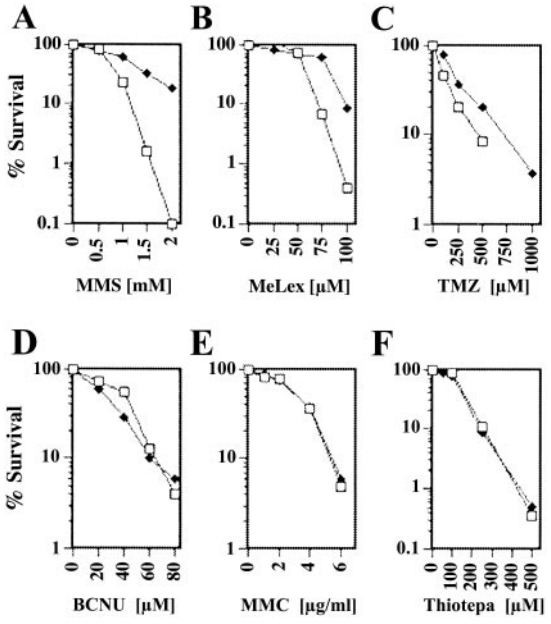

Fig. 2. *Ex vivo* alkylation sensitivity of BM cells from *Aag* null and wild-type mice. Hematopoietic progenitor survival assays were performed to determine the sensitivity of BM cells derived from *Aag* +/+ (□) and *Aag* −/− (◆) mice to: (*A*) methylmethane sulfonate (MMS), (*B*) methyl-lexitropsin (MeLex); (*C*) TMZ; (*D*) BCNU; (*E*) MMC; and (*F*) thiotepa. Experiments were done a minimum of three times each; representative results are shown.

***Aag* Null BM Cells Are Resistant to Alkylation-induced Cytotoxicity.** Clonogenic survival assays were performed with BM cells harvested from *Aag* +/+ and *Aag* −/− mice. BM cells were treated *ex vivo* with MMS, MeLex, BCNU, TMZ, MMC, or thiotepa and then plated on semisolid media to allow myeloid lineage hematopoietic progenitor (colony-forming unit-granulocyte macrophage, colony-forming unit-GEMM, and BFU-E) colony formation (see "Materials and Methods"). To our surprise, the *Aag* −/− glycosylase-deficient BM cells of the myeloid lineage were more resistant than were wild-type BM cells to several of these alkylating agents, namely MMS, MeLex and TMZ (Fig. 2). This phenomenon is most obvious for the MMS treatment in which *Aag* −/− cells displayed significant resistance over the entire dose range. The fact that *Aag* −/− myeloid BM cells were resistant to killing by MeLex, is of particular importance because this alkylating agent is known to generate almost exclusively 3MeA DNA lesions (13). We may, therefore, conclude that the initiation of BER at 3 MeA DNA lesions in mouse myeloid BM cells turns out to be more toxic for the cell than allowing 3 MeAs to remain unrepaired by the Aag glycosylase. This does not exclude the possibility that Aag-initiated BER at alkylated bases other than 3 MeA (*e.g.*, the 7MeGs induced by MMS and TMZ) might also be more toxic for the cell than leaving those alkylated bases in the genome, unrepaired. *Aag* −/− BM cells were also relatively resistant to the methylating agent TMZ, which indicated that TMZ induces DNA lesions that serve as substrate for the Aag glycosylase and that initiation of BER at these lesions in BM cells leads to cytotoxicity. *Aag* +/+ and *Aag* −/− cells were equally sensitive to BCNU, MMC, and thiotepa. We infer that the DNA base lesions produced by these three agents may not be substrates for the Aag enzyme in these cells, or that these particular base lesions are equally as toxic as their downstream BER intermediates.

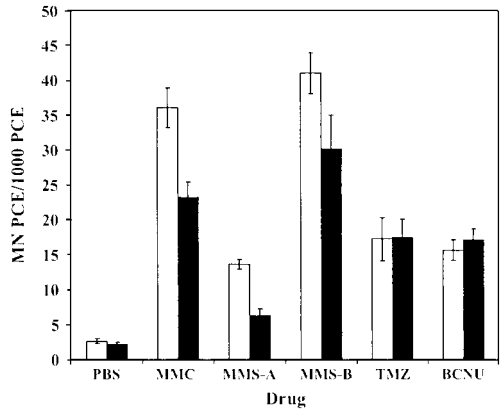

Fig. 3. *In vivo* alkylation sensitivity of BM cells from *Aag* null and wild-type mice. Chromosome damage was measured using the mouse BM micronucleus test (9). Twenty-four h after treatment with each agent, BM cells were harvested from *Aag* +/+ (□) and *Aag* −/− (■) mice, and MN PCEs were monitored as described in "Materials and Methods." At least four mice/group were used; and the doses were as follows: MMC, 2mg/kg; MMS-A, 30 mg/kg; MMS-B, 60 mg/kg; TMZ, 10 mg/kg; BCNU, 7 mg/kg. Data are shown as the mean ± SE.

***Aag* Null BM Cells Display *in Vivo* Alkylation Resistance.** The micronucleus test is commonly used to assess large-scale chromosome damage in mammalian cells after *in vivo* treatment with a DNA damaging agent. In this study, *Aag* +/+ and *Aag* −/− mice received i.p. injections of MMS, MMC, TMZ, BCNU, or a vehicle control, 24 h before harvesting BM cells to monitor the generation of MN PCEs that reflects the extent of clastogenicity. *Aag* null cells were more resistant than wild-type cells to the clastogenic effects of MMS and MMC because both MMS and MMC induced significantly more chromosomal damage in the repair proficient *Aag* +/+ mice *versus* the repair deficient *Aag* −/− mice (Fig. 3). In contrast, *Aag* status had no influence on *in vivo* clastogenicity after BCNU and TMZ exposure. Thus, from both the *ex vivo* hematopoietic progenitor survival assays and the *in vivo* micronucleus assay in PCEs, our results clearly indicate that *Aag* null BM cells are more resistant than wild-type cells to the lethal and clastogenic effects of the alkylating agent MMS. However, for MMC and TMZ the two assays did not concur, in that *Aag* status influenced clastogenicity but not cytotoxicity for MMC, and influenced cytotoxicity but not clastogenicity for TMZ. We believe these complex results reflect the fact that each assay monitors damage sensitivity in different cell types and, moreover, that individual cell types display complex phenotypic differences.

BM Cell Resistance to Alkylating Agents in *Aag* Null Animals Is Lineage-specific. In Fig. 2 and 3, we showed that *Aag* expression renders hematopoietic progenitors of the myeloid lineage, as well as PCEs (also of the myeloid lineage), sensitive to the toxic effects of MMS. The BM compartment is composed of many different cell types and lineages, and we reasoned that each one might express different 3MeA DNA glycosylase levels (as well as different levels of other repair enzymes); as a result each lineage may show differences in their MMS sensitive phenotype. To test this, BM was harvested from *Aag* null and wild-type mice 24 h after i.p. injection with MMS (30 mg/kg) or vehicle and analyzed for the remaining levels of different cell lineages. In vehicle-treated *Aag* null and wild-type mice the distribution of granulocytes, macrophages, T, or B lymphocytes, was not significantly different (data not shown). However, after MMS exposure, BM cells with granulocyte and macrophage lineage markers were significantly depleted in *Aag*-expressing wild-type mice relative to *Aag* null mice. In contrast, cells staining positive for B- or T-lymphocyte markers were equally represented in both lineages of mice (data not shown). These findings indicate that 3MeA DNA glycosylase status modulates MMS sensitivity in a lineage specific fashion, and that the action of the glycosylase on MMS-induced DNA damage produces a toxic effect in the myeloid (granulocyte and macrophage), but not the lymphoid (B and T lymphocyte) lineages.

The *Aag* Phenotype Has No Influence on Alkylating Agent-induced Animal Lethality. To determine whether 3MeA DNA glycosylase expression influences animal lethality after alkylating agent treatment, 30-day survival assays were performed to estimate LD_{50} values for MMS in wild-type and *Aag* null mice. Engelward *et al.* (14) and Allan *et al.* (15) had previously shown that *Aag* null ES cells are sensitive to the cytotoxic and clastogenic effects of MMS. On the basis of these findings one might expect *Aag* null animals to be MMS sensitive. Alternatively, based on the alkylation resistance of *Aag* null BM cells *versus* wild-type BM cells described in our current report, one might expect *Aag* null animals to be MMS resistant. In fact, neither expectation turned out to be correct. The LD_{50} experiments indicated no significant differences in MMS-induced lethality between wild-type and *Aag* null mice, with an approximate LD_{50} value of 150 mg/kg (data not shown). Thus, Aag 3MeA DNA glycosylase status does not appear to modulate lethality to MMS in the whole animal, although for individual cell types, the presence or absence of this DNA repair activity can have a profound effect on MMS-induced toxicity. Whether Aag status modulates alkylation-induced tumorigenesis is under investigation.

Discussion

The repair of potentially cytotoxic O^6-alkylguanine DNA lesions is a one-step direct reversal process that does not involve the cleavage of any covalent bonds in DNA other than that connecting the unwanted alkyl group to the O^6 position of guanine. In contrast, all excision repair processes require multiple steps involving the cleavage of several covalent bonds at sites flanking the DNA damage, followed by new DNA synthesis and DNA ligation. For BER, this means the generation of several types of damaged DNA as intermediates in the repair process. Given that each intermediate, if left unrepaired, can itself be toxic to the cell, it is not surprising that producing an imbalance between BER enzymes might lead to increased mutation or cell death. Indeed, a growing body of evidence, using bacterial and yeast model systems, has recently emerged to support this notion (16, 17). Here we demonstrate that imbalances can produce changes in alkylation sensitivity in an animal, namely the mouse, and more importantly, that the changes can be either positive or negative (or neutral) depending on the specific cell and tissue type.

Initial studies showed that *Aag* −/− mouse ES cells are more sensitive than wild-type cells to the chromosome damage and cell killing that follows alkylation treatment (14, 15). This observation was not surprising because it was analogous to the earlier characterization of 3MeA DNA glycosylase deficient *E. coli* and *S. cerevisiae* model organisms, and because it was consistent with 3MeA lesions blocking DNA replication (1, 18). Elder *et al.* (19) also found that *Aag* −/− mouse PEFs are more sensitive to MMS-induced cytotoxicity, and that *Aag* −/− T lymphocytes are more sensitive to MMS-induced mutation. Taking all these results together, our present observation that certain Aag −/− BM cells are more resistant to alkylating agent-induced cytotoxicity and clastogenicity than their *Aag* +/+ counterparts was quite unexpected. However, some of our more recent studies using *S. cerevisiae*, *Schizosaccharomyces pombe* and *E. coli* as model organisms for studying BER might provide a framework for understanding how the same genotypic change in mouse ES, PEF, and BM cells could produce such different phenotypes. A brief summary of these studies follows.

In *S. cerevisiae*, overexpression of the Mag1 3MeA DNA gly-

cosylase increases spontaneous mutation and sensitizes cells to MMS-induced cytotoxicity (12, 18). This phenotype was largely suppressed by the cooverexpression of an AP endonuclease, which indicated that it is a glycosylase/AP endonuclease imbalance that produces the mutator and alkylation sensitive phenotypes, presumably by leading to the accumulation of mutagenic and cytotoxic abasic sites in the yeast genome (12). Posnick and Samson (17) showed that overexpression of 3MeA DNA glycosylases in *E. coli* similarly increased spontaneous mutation and MMS-sensitivity. It is therefore clear that improper balance, and thus improper coordination, between consecutive BER steps can be detrimental to the cell. Indeed, biochemical evidence now supports the notion that the individual steps of BER are coordinated via specific protein-protein and protein-DNA interactions (20, 21). In addition, we recently demonstrated unexpected cross-talk between BER and other DNA repair pathways. Memisoglu and Samson (22) knocked out the major 3MeA DNA glycosylase in three *S. pombe* strains with different repair capacities, and this resulted in three different phenotypes. In wild-type cells, glycosylase deficiency had virtually no effect on MMS-sensitivity, indicating that BER normally plays a minor role in 3MeA repair in this organism; other experiments indicated that NER and homologous recombination repair play much more predominant roles in preventing the toxicity induced by 3MeA. In NER-deficient cells, the elimination of 3 MeA DNA glycosylase rendered cells MMS-sensitive, which indicated that BER can play a back-up role when NER is absent. However, elimination of the same glycosylase in recombination-deficient cells rendered them MMS resistant. We infer from this surprising result that one or more of the intermediate steps in BER is limiting in *S. pombe* and that the homologous recombination repair pathway normally processes the strand breaks that would accumulate under these conditions. Thus, in the absence of recombination the cell actually survives better if BER is not initiated at 3MeA lesions, because 3MeAs are presumably less toxic to the cell than unrepaired strand breaks. The replication blocking 3MeA lesions may be relatively less toxic than DNA strand breaks for one or both of the following reasons: (*a*) NER can complete the repair process at 3MeAs; (*b*) 3MeAs can be bypassed by translesion DNA polymerases (23). These results using microbial cells may help provide hypotheses as to why mouse ES, PEF, and BM cells show different alkylation-sensitive phenotypes when the Aag 3MeA DNA glycosylase is eliminated.

Aag-elimination renders ES and PEF cells MMS sensitive (14, 15), indicating that Aag activity protects against alkylation-induced cell death. Presumably, in these cells, BER, initiated by wild-type levels of Aag, can be properly completed to a ligated DNA product, and such completion may be achieved via the downstream BER enzymes or via homologous recombination at BER intermediates. However, in the myeloid BM cells it is possible that Aag-initiated BER does not always go to completion, either by BER or by recombination, resulting in longer-lived BER intermediates, and thus longer-lived DNA strand breaks. Because unrepaired DNA strand breaks are known to be cytotoxic, and are likely to be more toxic than unrepaired alkylated bases, Aag-elimination would render such cells MMS-resistant. Indeed, this may explain why the BM tissue has evolved to express such low levels of the Aag 3MeA DNA glycosylase. One prediction from this model that is currently being tested, is that the introduction of extra 3MeA DNA glycosylase activity via gene transfer into BM stem cells would render BM more alkylation sensitive than wild-type BM. This possibility has significant implications for the design of gene therapy vectors for conferring alkylation resistance to the BM of patients undergoing cancer chemotherapy.

One in three people in the developed world will be diagnosed with cancer at some point during their lifetime, a significant fraction of whom will undergo alkylating agent chemotherapy. It is, therefore, inevitable that large numbers of individuals in our society will be deliberately exposed to alkylating agents despite the inevitable myelosuppressive side-effects of such agents (24, 25). For this reason, we seek to understand exactly how BM cells can be protected from alkylation-induced toxicity. In this regard, it is notable that, on their characterization, two DNA alkylation repair-deficient mouse models have each produced surprising results. Initially, it seemed logical that low Mgmt and Aag levels in BM were primarily responsible for the alkylation sensitivity of this tissue. However, it turns out that the low level of Mgmt plays an unexpectedly profound role in protecting BM against alkylating agents (12, 26), and that the low level of Aag in BM cells plays an unexpected negative role in protecting against alkylating agents. We surmise that before we can understand exactly how cells are protected against alkylation damage, it is important to understand how these repair activities integrate with other repair activities being expressed in the cell, and further, to understand how DNA repair overall integrates with other determinants of alkylation-induced toxicity.

Acknowledgments

We thank Amy Imrich and Yaoyu Ning of the Kresge National Institute of Environmental Health Sciences Center for Environmental Health Research ES 00002 for FACS analyses. We thank current and former members of the Samson laboratory for insightful comments and Brian Glassner for initial DNA glycosylase assays.

References

1. Friedberg, E. C., Walker, G. C., and Siede, W. DNA Repair and Mutagenesis. Washington, DC: American Society for Microbiology, 1995.
2. Samson, L. The suicidal DNA repair methyltransferases of microbes. Mol. Microbiol., *6:* 825–831, 1992.
3. Chakravarti, D., Ibeanu, G. C., Tano, K., and Mitra, S. Cloning and expression in *Escherichia coli* of a human cDNA encoding the DNA repair protein *N*-methylpurine-DNA glycosylase. J. Biol. Chem., *266:* 15710–15715, 1991.
4. Engelward, B. P., Boosalis, M. S., Chen, B. J., Deng, Z., Siciliano, M. J., and Samson, L. D. Cloning and characterization of a mouse 3-methyladenine/7-methyl-guanine/3-methylguanine DNA glycosylase cDNA whose gene maps to chromosome 11. Carcinogenesis (Lond.), *14:* 175–181, 1993.
5. Samson, L., Derfler, B., Boosalis, M., and Call, K. Cloning and characterization of a 3-methyladenine DNA glycosylase cDNA from human cells whose gene maps to chromosome 16. Proc. Natl. Acad. Sci. USA, *88:* 9127–9131, 1991.
6. Wyatt, M. D., Allan, J. M., Lau, A. Y., Ellenberger, T. E., and Samson, L. D. 3-methyladenine DNA glycosylases: structure, function, and biological importance. Bioessays, *21:* 668–676, 1999.
7. Engelward, B. P., Weeda, G., Wyatt, M. D., Broekhof, J. L., de Wit, J., Donker, I., Allan, J. M., Gold, B., Hoeijmakers, J. H., and Samson, L. D. Base excision repair deficient mice lacking the *Aag* alkyladenine DNA glycosylase. Proc. Natl. Acad. Sci. USA, *94:* 13087–13092, 1997.
8. Teare, J. M., Islam, R., Flanagan, R., Gallagher, S., Davies, M. G., and Grabau, C. Measurement of nucleic acid concentrations using the DyNA Quant and the GeneQuant. Biotechniques, *22:* 1170–1174, 1997.
9. Tinwell, H., and Ashby, J. Comparison of Acridine Orange and Giemsa stains in several mouse bone marrow micronucleus assays, including a triple dose study. Mutagenesis, *4:* 476–481, 1989.
10. Deichmann, W. B., and LeBlanc, T. J. Determination of approximate lethal dose with about six animals. J. Ind. Hyg. Toxicol., *25:* 415–417, 1943.
11. Gerson, S. L., Trey, J. E., Miller, K., and Berger, N. A. Comparison of O^6-alkylguanine-DNA alkyltransferase activity based on cellular DNA content in human, rat, and mouse tissues. Carcinogenesis (Lond.), *7:* 745–749, 1986.
12. Glassner, B. J., Weeda, G., Allan, J. M., Broekhof, J. L. M., Carls, N. H. E., Donker, I., Engelward, B. P., Hampson, R. J., Hersmus, R., Hickman, M. J., Roth, R. B., Warren, H. B., Wu, M. M., Hoeijmakers, J. H. J., and Samson, L. D. DNA repair methyltransferase (Mgmt) knockout mice are sensitive to the lethal effects of chemotherapeutic alkylating agents. Mutagenesis, *14:* 339–347, 1999.
13. Zhang, Y., Chen, F. X., Mehta, P., and Gold, B. Groove- and sequence-selective alkylation of DNA by sulfonate esters tethered to lexitropsins. Biochemistry, *32:* 7954–7965, 1993.
14. Engelward, B. P., Dreslin, A., Christensen, J., Huszar, D., Kurahara, C., and Samson, L. Repair-deficient 3-methyladenine DNA glycosylase homozygous mutant mouse cells have increased sensitivity to alkylation-induced chromosome damage and cell killing. EMBO J., *15:* 945–952, 1996.

15. Allan, J. M., Engelward, B. P., Dreslin, A. J., Wyatt, M. D., Tomasz, M., and Samson, L. D. Mammalian 3-methyladenine DNA glycosylase protects against the toxicity and clastogenicity of certain chemotherapeutic DNA cross-linking agents. Cancer Res., *58:* 3965–3973, 1998.
16. Glassner, B. J., Rasmussen, L. J., Najarian, M. T., Posnick, L. M., and Samson, L. D. Generation of a strong mutator phenotype in yeast by imbalanced base excision repair. Proc. Natl. Acad. Sci. USA, *95:* 9997–10002, 1998.
17. Posnick, L. M., and Samson, L. D. Imbalanced base excision repair increases spontaneous mutation and alkylation sensitivity in *Escherichia coli*. J. Bacteriol., *181:* 6763–6771, 1999.
18. Xiao, W., and Samson, L. *In vivo* evidence for endogenous DNA alkylation damage as a source of spontaneous mutation in eukaryotic cells. Proc. Natl. Acad. Sci. USA, *90:* 2117–2121, 1993.
19. Elder, R. H., Jansen, J. G., Weeks, R. J., Willington, M. A., Deans, B., Watson, A. J., Mynett, J. J., Bailey, J. A., Cooper, D. P., Rafferty, J. A., Heeran, M. C., Wijnhoven, S. W. P., van Zeeland, A. A., and Margison, G. P. Alkylpurine-DNA-*N*-glycosylase knockout mice show increased susceptibility to induction of mutations by methyl methanesulfonate. Mol. Cell. Biol., *18:* 5828–5837, 1998.
20. Parikh, S. S., Putnam, C. D., and Tainer, J. A. Lessons learned from structural results on uracil-DNA glycosylase. Mutat. Res., *460:* 183–199, 2000.
21. Wilson, S. H., and Kunkel, T. A. Passing the baton in base excision repair. Nat. Struct. Biol., *7:* 176–178, 2000.
22. Memisoglu, A., and Samson, L. Contribution of base excision repair, nucleotide excision repair, and DNA recombination to alkylation resistance of the fission yeast *Schizosaccharomyces pombe*. J. Bacteriol., *182:* 2104–2112, 2000.
23. Eckert, K. A., and Opresko, P. L. DNA polymerase mutagenic bypass and proof-reading of endogenous DNA lesions. Mutat. Res., *424:* 221–236, 1999.
24. Pedersen-Bjergaard, J., Osterlind, K., Hansen, M., Philip, P., Pedersen, A. G., and Hansen, H. H. Acute nonlymphocytic leukemia, preleukemia, and solid tumours following intensive chemotherapy of small cell carcinoma of the lung. Blood, *66:* 1393–1397, 1985.
25. Pedersen-Bjergaard, J., Ersboll, J., Sorensen, J. M., Keiding, N., Larsen, S. O., Philip, P., Larsen, M. S., Schultz, H., and Nissen, N. I. Risk of acute nonlymphocytic leukemia and preleukemia in patients treated with cyclophosphamide for non-Hodgkin's lymphomas. Comparison with results obtained in patients treated for Hodgkin's disease and ovarian carcinoma with other alkylating agents. Ann. Intern. Med., *103:* 195–200, 1985.
26. Tsuzuki, T., Sakumi, K., Shiraishi, A., Kawate, H., Igarashi, H., Iwakuma, T., Tominaga, Y., Zhang, S., Shimizu, S., Ishikawa, T., Nakamura, K., Nakao, K., Katsuki, M., and Sekiguchi, M. Targeted disruption of the *DNA repair methyltransferase* gene renders mice hypersensitive to alkylating agent. Carcinogenesis (Lond.), *17:* 1215–1220, 1996.

3-Methyladenine DNA Glycosylase-deficient *Aag* Null Mice Display Unexpected Bone Marrow Alkylation Resistance

Richard B. Roth and Leona D. Samson

Cancer Res 2002;62:656-660.