Increased sensitivity to cell killing and mutagenesis by chemical mutagens in thymidine-kinase-deficient subclones of a Friend murine leukaemia cell line*

By P. G. McKENNA and A. A. YASSEEN

Department of Nursing Studies, the New University of Ulster, Coleraine BT52 1SA, Northern Ireland

SUMMARY

Wild-type Friend murine leukaemia (clone 707) cells and two thymidine-kinase-deficient subclones, 707BUE and 707BUF, were compared for sensitivity to killing and mutagenesis by the chemical mutagens, ethyl methane sulphonate (EMS), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), mitomycin C (MMC), and methyl methane sulphonate (MMS). The two thymidine-kinase-deficient subclones were significantly more sensitive to killing by each of the four chemical mutagens than were wild-type cells. The increased sensitivity to killing by the four mutagens was also reflected in increased mutagenesis (per unit dose of mutagen) to 6-thioguanine resistance. In the light of these results, the significance of thymidine kinase in DNA repair and mutagenesis is discussed.

1. INTRODUCTION

Mutagen-sensitive mutants have proved to be of significant value in elucidating the mechanisms of DNA repair and mutagenesis in prokaryotes. Much of the current knowledge of DNA repair in mammalian cells has come from studies on cell cultures derived from patients suffering from the ultra-violet (UV) irradiation-sensitive hereditary disease, xeroderma pigmentosum. Mutagen-sensitive mutants are increasingly being isolated from a variety of mammalian cultured cell lines (Shiomi & Sato, 1979; Thompson *et al.* 1980; Meuth, 1981). In the majority of cases, however, the exact nature of the mutation conferring mutagen sensitivity has not been apparent.

Friend murine leukaemia cells (clone 707) have a high spontaneous mutation rate at the thymidine kinase locus (McKenna & Hickey, 1981*a*). McKenna & Hickey (1981*b*) have also reported that deficiency of cytosol thymidine kinase (EC. 2 . 7 . 1 . 2 . 1) in Friend leukaemia cells results in increased sensitivity to killing and mutagenesis by UV irradiation. The present communication describes the results of experiments to determine whether thymidine kinase deficiency in Friend cells is also associated with increased sensitivity to chemical mutagens.

2. MATERIALS AND METHODS

(i) *Cells.* Clone 707 of the Friend cell line was originally described by Scher, Holland & Friend (1971). The isolation of subclones 707BUE and 707BUF has been described by McKenna & Hickey (1981*a*).

---

* Request reprints from Dr P. G. McKenna.

(ii) *Culture and medium*. Hams SF12 medium (Flow Laboratories) supplemented with MEM essential amino acids and 16 % horse serum was used. Cells were cloned in this medium plus 0·25 % Difco Noble agar (Paul & Hickey, 1974). Friend cells have a cloning efficiency of approximately 50 %.

(iii) *Determination of survival*. Cells which had been growing in exponential phase were diluted to 2×10^5/ml in non-selective medium. Mutagens were added at the required concentration to cultures for 16 h, after which the cells were washed three times in Hanks BSS, counted and cloned.

(iv) *Mutagenesis*. Chemical mutagens were added at the desired concentration to exponentially growing cultures (2×10^5 cells/ml) for 16 h. The cells were then washed three times in Hanks BSS before resuspending in non-selective medium. After six days, when maximum numbers of 6-thioguanine-resistant mutants are obtained (McKenna, unpublished observation), cells were cloned in soft agar medium containing 6-thioguanine (5·0 μg/ml). Survivals were determined immediately after removal of mutagen as described above.

3. RESULTS

(i) *Survival after mutagen treatment*

Clone 707 and two bromo-deoxyuridine-resistant subclones 707BUE and 707BUF were compared for sensitivity to killing by four chemical mutagens, namely ethyl methane sulphonate (EMS), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), mitomycin C (MMC) and methyl methane sulphonate (MMS). Clone 707BUF has zero thymidine kinase activity while clone 707BUE retains 1·1 % of the level of TK of wild-type cells (McKenna & Hickey, 1981*a*).

(*a*) *Ethyl methane sulphonate*. The survivals of clones 707, 707BUE and 707BUF after EMS treatment are illustrated in Fig. 1*a*. It can be seen that the two thymidine-kinase-deficient subclones have considerably reduced levels of survival, relative to wild-type cells.

(*b*) *N-methyl-N'-nitro-N-nitrosoguanidine*. The survivals of clones 707 and the two thymidine-kinase-deficient subclones after MNNG treatment are illustrated in Fig. 1*b*. Again both 707BUE and 707BUF have considerably reduced levels of survival relative to clone 707.

(*c*) *Mitomycin C*. Fig. 1*c* illustrates the survival curves of clones 707, 707BUE and 707BUF to MMC treatment. Once more the two thymidine-kinase-deficient subclones are considerably more sensitive than wild-type cells.

(*d*) *Methyl methane sulphonate*. The survival curve of clones 707, 707BUE and 707BUF after MMS treatment is shown in Fig. 1*d*. As before, there is reduced survival in subclones 707BUE and 707BUF relative to clone 707.

(ii) *Mutagenesis*

The induction of 6-thioguanine-resistant mutants by the four chemical mutagens in wild-type clone 707 and in subclones 707BUE and 707BUF was measured and the results are presented in Table 1.

(*a*) *Ethyl methane sulphonate*. At a dose of 100 μg/ml EMS there was an approximately fourfold increase in mutant frequency in subclone 707BUE and a sevenfold increase in 707BUF, relative to clone 707 (Table 1). A dose of 200 μg/ml EMS induced a sixfold increase in mutant frequency in subclones 707BUE and 707BUF relative to clone 707.

(*b*) *N-methyl-N'-nitro-N-nitrosoguanidine*. A dose of 0·10 μg/ml MNNG induced greater increases in mutant frequency in subclones 707BUE and 707BUF than did a dose of
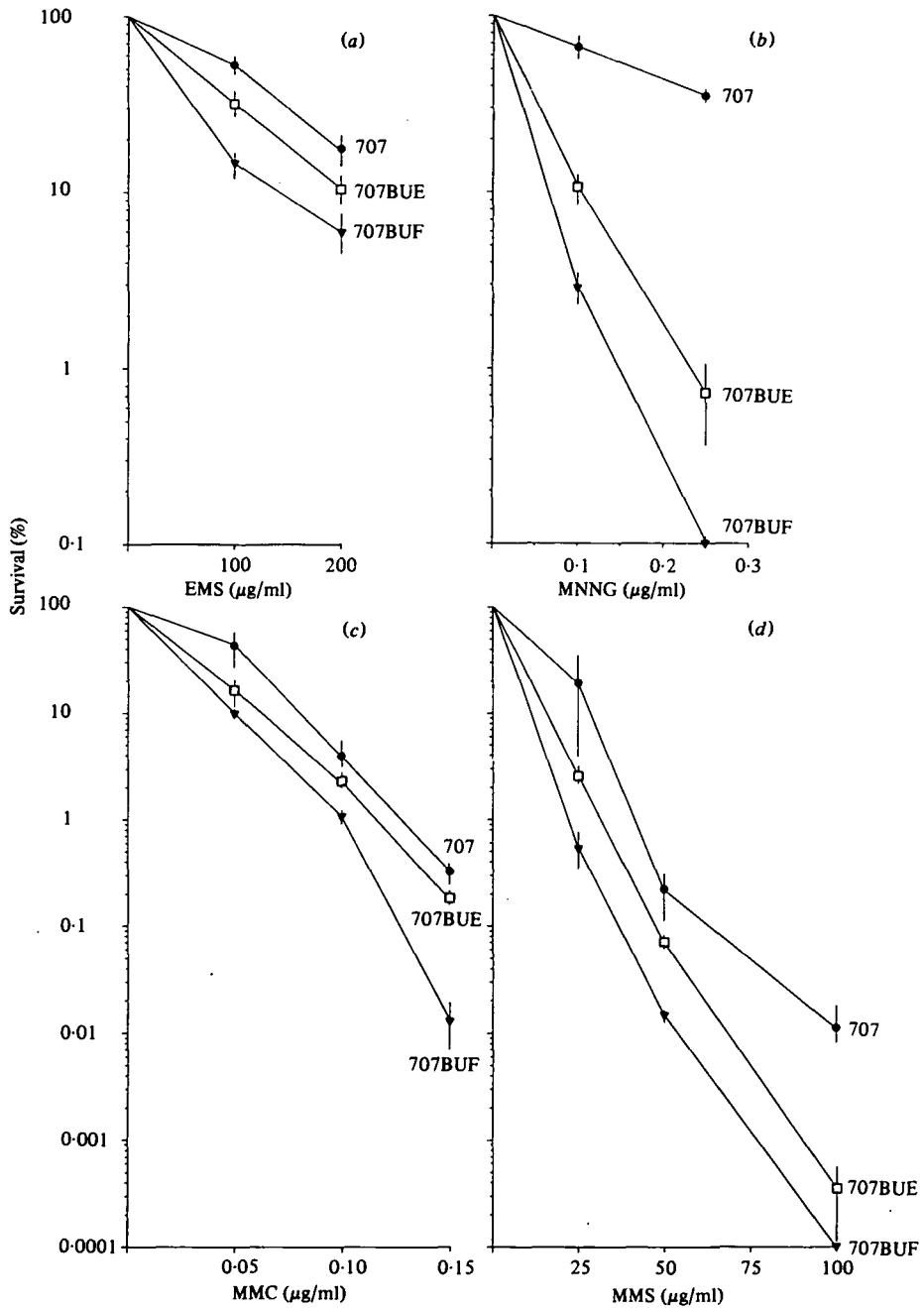
Fig. 1. Survival after mutagen treatment. (*a*) EMS, (*b*) MNNG, (*c*) MMC, (*d*) MMS. Each point represents the mean of duplicate experiments and the bar represents the standard error of the mean.

0·25 μg/ml in clone 707 (Table 1). Similarly a dose of 0·20 μg/ml MNNG induced a greater increase in mutant frequency in 707BUE and 707BUF than was observed in clone 707 with a dose of 0·50 μg/ml.

(c) *Mitomycin C*. A dose of 0·025 μg/ml MMC induced a small but significant increase in mutant frequency in subclone 707BUE relative to wild-type cells (Table 1). A similar dose induced a much greater increase in mutant frequency in subclone 707BUF. A dose

Table 1. *Results of mutagenesis experiments**

Mutagen	Cells	Dose (μg/ml)	Survival (%)	Increase in 6-TG^r frequency ($\times 10^{-6}$)
EMS	707	100	48·78	59
		200	22·42	110
	707BUE	100	37·32	233
		200	8·09	665
	707BUF	100	12·70	424
		200	3·21	725
MNNG	707	0·25	44·06	93
		0·50	38·75	188
	707BUE	0·10	9·97	107
		0·20	1·45	244
	707BUF	0·10	3·65	178
		0·20	0·225	255
MMC	707	0·025	98·5	30
		0·05	40·18	57
	707BUE	0·025	54·1	35
		0·05	16·42	82
	707BUF	0·025	52·2	102
		0·05	12·69	149
MMS	707	15	76·89	53
		25	20·32	90
	707BUE	15	22·21	81
		25	3·27	121
	707BUF	15	4·71	113
		25	0·67	139

* Each value is the mean result of at least two independent experiments.

of 0·05 μg/ml MMC also induced significantly greater mutagenesis in subclones 707BUE and 707BUF than in wild-type (clone 707) cells.

(d) *Methyl methane sulphonate*. The results of mutagenesis experiments with MMS to 6-thioguanine resistance are also shown in Table 1. A dose of 15 μg/ml MMS induced a $1\frac{1}{2}$-fold increase in mutant frequency in subclone 707BUE and a greater than twofold increase in subclone 707BUF, relative to wild-type cells. A dose of 25 μg/ml MMS induced an increase in mutant frequency in subclones 707BUE and 707BUF of approximately $1\frac{1}{2}$-fold, relative to clone 707.

DISCUSSION

It is clear from the results described that the two thymidine-kinase-deficient Friend erythroleukaemia subclones, 707BUE and 707BUF, are significantly more sensitive than

wild-type (clone 707) cells to killing by the four chemical mutagens tested, EMS, MNNG, MMC and MMS. The increased killing in the thymidine-kinase-deficient subclones relative to wild-type cells is also reflected in increased mutagenesis per unit dose of mutagen to 6-thioguanine resistance. These and the similar results reported earlier for UV sensitivity in thymidine-kinase-deficient Friend cells (McKenna & Hickey, 1981*b*) raise questions as to the significance of thymidine kinase in DNA repair and mutagenesis.

It is noteworthy that in all experiments and with all mutagens, subclone 707BUF was more sensitive to killing and mutagenesis than was subclone 707BUE. This may be due to the fact that 707BUE retains 1·1 % of the level of thymidine kinase of clone 707 whereas no activity is detectable in 707BUF.

McKenna & Hickey (1981*b*) interpreted the UV sensitivity of thymidine-kinase-deficient Friend cells as probably being due to misincorporation of nucleotides into DNA as a result of imbalance in deoxyribonucleoside triphosphate pools. Thymidine triphosphate (dTTP) has an important regulatory role to play in the reduction of ribonucleotides to deoxyribonucleotides by ribonucleoside diphosphate reductase. High levels of dTTP inhibit cytosine diphosphate reduction. Conversely dTTP is required as an allosteric effector for the reduction of purine ribonucleotides (Moore & Hulbert, 1966). Thymidine kinase provides a shunt which allows a rapid increase in the concentration of dTTP to a level which permits the correction of any imbalance in the deoxyribonucleoside triphosphate pools. This process might be impeded by a deficiency in thymidine kinase, particularly when conditions demand a rapid induction of DNA synthesis as, for example, in DNA excision repair.

The concept the alterations in deoxyribonucleoside triphosphate pool levels may lead to mutations has also been put forward as an explanation for enhanced mutation frequencies in Chinese hamster cells after thymidine supplementation of culture medium (Bradley & Sharkey, 1978; Peterson *et al.* 1978). Further support has come from the observations of Meuth (1981) that thy⁻ mutants of Chinese hamster ovary (CHO) cells have increased sensitivity to killing by UV and monofunctional alkylating agents and increased mutagenesis by EMS at low doses. Thy⁻ mutants are dependent on an exogenous source of thymidine and have a consequent imbalance in their deoxycytidine triphosphate (dCTP)/dTTP ratio.

It therefore seems likely that a function of thymidine kinase is to ensure balanced deoxyribonucleoside triphosphate pools for DNA synthesis. It is worth noting that both wild-type Friend cells and thymidine-kinase-deficient subclones have similar growth rates, and spontaneous mutation frequencies (McKenna & Yasseen, unpublished observation). This implies that thymidine kinase is of more significance in DNA repair than in normal DNA synthesis.

REFERENCES

BRADLEY, M. D. & SHARKEY, N. (1978). Mutagenicity of thymidine to cultured Chinese hamster cells. *Nature* **274**, 607–608.

McKENNA, P. G. & HICKEY, I. (1981*a*). The thymidine kinase locus of Friend erythroleukaemic cells. 1. Mutation rates and properties of mutants. *Mutation Research* **80**, 187–199.

McKENNA, P. G. & HICKEY, I. (1981*b*). UV sensitivity in thymidine kinase deficient mouse erythroleukaemia cells. *Cell Biology International Reports* **5**, 555–561.

MEUTH, M. (1981). Role of deoxynucleoside triphosphate pools in the cytotoxic and mutagenic effects of DNA alkylating agents. *Somatic Cell Genetics* **7**, 89–102.

Moore, E. C. & Hulbert, R. B. (1966). Enzymatic synthesis of deoxyribonucleotides. X. Reduction of purine ribonucleotides; allosteric behaviour and substrate specificity of the enzyme system from *Escherichia coli*. *Journal of Biological Chemistry* **241**, 2540–2549.

Paul, J. & Hickey, I. (1974). Haemoglobin synthesis in inducible, uninducible and hybrid Friend cell clones. *Experimental Cell Research* **87**, 20–30.

Peterson, A. R., Landolph, J. R., Peterson, H. & Heidelberger, C. (1978). Mutagenesis of Chinese hamster cells is facilitated by thymidine and deoxycytidine. *Nature* **276**, 508–510.

Reichard, P., Canellakis, Z. N. & Canellakis, E. S. (1969). Studies on a possible regulatory mechanism for the biosynthesis of deoxyribonucleic acid. *Journal of Biological Chemistry* **236**, 2414–2519.

Scher, W., Holland, J. G. & Friend, C. (1971). Hemoglobin synthesis in murine virus-induced leukemic cells *in vitro*. *Blood* **37**, 428–437.

Shiomi, T. & Sato, K. (1979). Isolation of UV-sensitive variants of human FL cells by a viral suicide method. *Somatic Cell Genetics* **5**, 193–201.

Thompson, L. H., Rubin, J. S., Cleaver, J. E., Whitmore, G. F. & Brookman, K. (1980). A screening method for isolating DNA repair-deficient mutants of CHO cells. *Somatic Cell Genetics* **6**, 391–405.