

X-ray and UV mutagenesis in two L5178Y cell strains differing in tumorigenicity, radiosensitivity, and DNA repair

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Summary L5178Y-R (LY-R) and L5178Y-S (LY-S) cells are inversely cross sensitive to X-rays and UV (254 nm) radiation ($D_0^x=0.94$ and 0.69 Gy, $D_q^x=0.32$ and 0.010 Gy; $D_0^{uv}=0.07$ and 5.5 Jm⁻², $D_q^{uv}=3.8$ and 1.6 Jm⁻², for LY-R and LY-S cells, respectively). Mutagenicity of the two cell strains at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus was examined using 6-thioguanine resistance as a marker. LY-S cells showed remarkably low mutability; the spontaneous mutation frequency of LY-S cells was at least two orders of magnitude lower than that for LY-R cells (≤ 0.005 vs. ~ 2.5 mutants per 10^5 survivors). The induced mutation rates for X-rays were 0.95 ± 0.14 and 0.35 ± 0.09 mutants per Gy per 10^5 survivors, and for UV radiation, 21.5 ± 8.2 and 0.04 ± 0.02 mutants per Jm⁻² per 10^5 survivors, for LY-R and LY-S cells, respectively. The results suggest that LY-S cells are efficient in the repair of UV-induced damage but not of lesions produced by ionizing radiation. The reverse appears to be true for LY-R cells. In addition, the low mutability of LY-S cells suggests that the processing of pre-mutational lesions may lead to lethality in this strain.

The two closely related strains of mouse leukaemic lymphoblasts L5178Y-R and L5178Y-S (abbreviated LY-R and LY-S) show inverse cross-sensitivity to two sets of damaging agents (Table I) (for review, see Beer *et al.*, 1983). LY-R and LY-S cells respond in diametrically different manners to dose fractionation (Table II) (Beer *et al.*, 1973). These variations observed at the cellular level are accompanied by differences in post-exposure DNA repair or synthesis. After exposure to 100 Gy of X-rays repair replication was more efficient, and DNA single strand breaks were rejoined more efficiently in LY-R than in LY-S cells (Körner *et al.*, 1977); however, no differences between strand break rejoining rates in the two strains were found after exposure to <10 Gy of γ -rays (Johanson *et al.*, 1982). Lehman (1972) has reported no UV-induced excision repair in his LY cell variant. Marked differences in post-UV DNA synthesis were found between LY-R and LY-S cells (Walicka & Beer, 1979; Walicka *et al.*, 1978, 1980); different patterns of post-exposure [³H]-thymidine incorporation were found for the two cell strains; progeny DNA strands of LY-S cells were smaller following UV exposure; the size of progeny DNA strands of LY-R cells was unchanged. Finally, only LY-R cells were tumorigenic in immunologically non-suppressed animals (Beer *et al.*, in press).

Results of our studies on lethal and mutagenic effects of high and low dose-rate X-irradiations and the detailed protocol for the assay for HGPRT deficiency will be published elsewhere. In the

present paper we suggest possible relationships between sensitivity of L5178Y cells to lethal and mutagenic effects of radiations and the DNA repair abilities of these cells.

Materials and methods

Cell growth and plating

Suspension cultures of LY-R and LY-S cells were grown at 37°C and diluted at appropriate intervals in Fischer's medium supplemented with 10% donor horse serum, 1 g l⁻¹ Pluronic F68 (a gift from BASF, Wyandotte, Michigan), and 0.1 g l⁻¹ sodium pyruvate. Platings were made in the same medium supplemented with 0.32% agar and 50 I.U. penicillin and 50 μ g streptomycin ml⁻¹. The volume of the plating medium was 30 or 100 ml per 100 or 150 mm plastic petri dish, respectively. Macroscopic colonies were counted after 8-12 days of incubation at 37°C.

Irradiation

Cell samples were exposed to X-rays from a General Electric Maximar generator operated at 250 kVp and 15 mA. The dose rate of radiation filtered through 0.5 mm Cu was 0.88 Gy min⁻¹. The cultures were X-irradiated in medium. UV irradiations were performed according to Jacobsen *et al.* (1978). Briefly, a General Electric G15T8

Table I Inverse cross-sensitivity of related mouse leukaemic lymphoblasts to two sets of damaging agents.

	LY-R	LY-S		LY-R	LY-S
X Rays	Resistant	Sensitive	Lidocaine	No Difference	
³ H Compounds			KCN		
Nitrogen Mustard			UV Radiation	Sensitive	Resistant
Dimethylmyleran			Heat (43°C)		
Neutrons	cis-PAD				
Misonidazole	No Difference		Ledakrin		
Anti-mycin A			Ouabain		

Table II Effects of X-ray and UV-radiation dose fractionation on survival of LY-R and LY-S cells (based on Beer *et al.*, 1973).

Radiation	Change in survival caused by dose fractionation	
	LY-R	LY-S
X-rays	Increase	Decrease (!)
UV	Decrease (!)	Increase

germicidal ultraviolet lamp was used with an aperture giving a fluence rate of 0.12 W m^{-2} at 65 cm from the lamp. The samples were irradiated in suspension ($1.5 \times 10^6 \text{ cells ml}^{-1}$) in phosphate buffered saline. The thickness of the cell suspension was < 1 mm.

Survival

For determination of survival, the irradiated samples and sham-irradiated controls were appropriately diluted and plated immediately after exposure. Macroscopic colonies were counted after 8–12 days of incubation, and surviving fractions were computed.

Mutation frequency

To allow expression of 6-thioguanine (TG)

resistance caused by a deficiency in hypoxanthine-guanine phosphoribosyl transferase (HGPRT), the irradiated samples and appropriate controls were cultured for either 6–10 days (LY-R) or 8–10 days (LY-S). In the X-ray experiments, sample size was maintained at $\geq 10^7$ cells during expression. In UV experiments the sample size was maintained at $\geq 6 \times 10^6$ and $\geq 1.2 \times 10^6$ for LY-S and LY-R cells, respectively. Following expression, appropriately diluted parallel samples of the cultures were plated in agar medium, supplemented with either $5 \mu\text{g ml}^{-1}$ of TG (for growth of mutant colonies) or no TG, for measurement of clonogenicity. To compute mutation frequency, the number of TG-resistant (TG^r) colonies was divided by the number of colonies formed in TG-free medium, with appropriate corrections for sample size.

Results

Lethality and mutagenicity data for LY-R and LY-S cells exposed to X-rays or UV radiation are plotted in Figure 1. The linear scale used in this figure to show induced mutability enables us to show only the data point for the lowest UV-exposure for LY-R cells. All mutation data are additionally presented in Figure 2 using a logarithmic plot. This figure also includes information on the spontaneous mutation frequencies in the two cell lines. The data in Figure 2 are plotted against surviving fraction to adjust for differences in lethality.

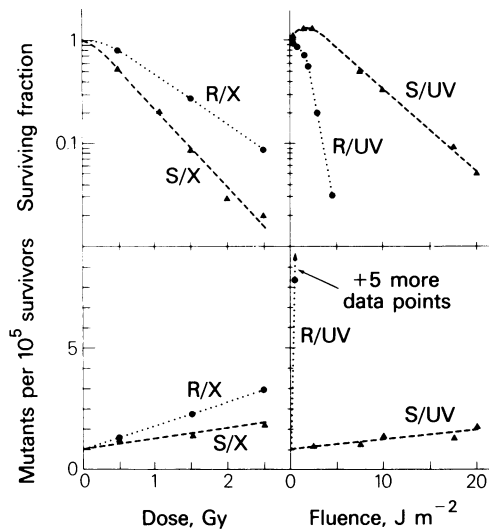


Figure 1 Relationships between dose of X-rays or fluence of UV-radiation and killing or induction of TG^f mutants in populations of LY-R and LY-S cells. ●---●:LY-R cells, ▲---▲:LY-S cells. Data for survival confirm previously published radio- and photobiological parameters for LY-R and LY-S cells. Mutation frequency points represent the means of at least 5 experiments for X-rays and an average of 2 experiments for UV radiation. Additional data points for UV-irradiated LY-R cells were obtained at 1.0, 1.6, 2.0, 3.0, and 4.6 J m⁻² (not plotted here, see Figure 2). Mutation data were plotted after subtraction of mutation frequencies determined in unirradiated controls. A small increase of survival of LY-S cells at low UV fluences results from a stimulating effect of such fluences (Beer *et al.*, 1981).

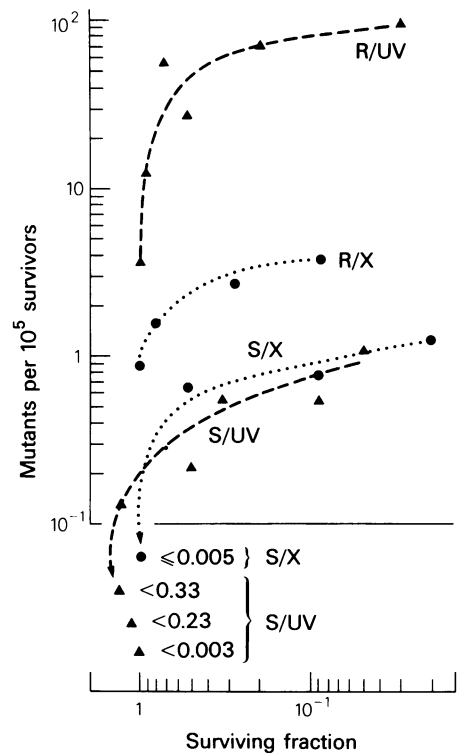


Figure 2 Relationships between survival of LY-R and LY-S cells exposed to X-rays or UV radiation and induction of TG^f mutants. (...) LY-R; (---) LY-S cells; ●: X-rays; ▲: UV-radiation. Spontaneous mutation frequencies, i.e., those found in unirradiated controls, are included. Mutation data are plotted without subtracting the spontaneous mutation frequencies. The spontaneous mutation frequencies were determined in 5 and 7 independent X-ray experiments and in 6 and 10 UV experiments, for LY-R and LY-S cells, respectively. For number of determinations of induced mutation frequencies and for explanation of >1 surviving fraction values see legend to Figure 1.

The exposure-survival data illustrate the differences in sensitivity of the two cell strains and their inverse cross-sensitivity to X-rays and UV radiation. The sensitivity difference was particularly pronounced for UV radiation. Radiobiological parameters were: D₀=0.94 and 0.69 Gy, D_q=0.32 and 0.010 Gy, and photobiological parameters were: D₀=0.7 and 5.5 J m⁻², D_q=3.8 and 1.6 J m⁻², for LY-R and LY-S cells, respectively.

The frequency of TG^f mutants in unirradiated control LY-S populations was very low (Figure 2, ≤ 5 × 10⁻⁸ mutants per surviving cell). In 23/31 experiments no TG^f mutant colonies were found after plating up to 8 × 10⁷ LY-S cells. Spontaneous mutation frequencies in LY-R cells were at least two orders of magnitude higher than those for LY-S-cells (Figure 2). The X-ray and UV-induced mutation rates for LY-R and LY-S cells are given in Table III. Relatively few mutants of LY-S cells were induced by either ionizing or UV radiation.

For LY-R cells, UV was found to be considerably more mutagenic than X-rays after equitoxic doses.

Table III Induction rates for TG^f mutants in X-ray or UV treated LY-R and LY-S cells

Cell strain	Number of mutants per 10 ⁵ survivors	
	Per Gy (X-rays)	Per J m ⁻² (UV)
LY-R	0.95 ± 0.14	21.5 ± 8.2
LY-S	0.35 ± 0.09	0.04 ± 0.02

Discussion

We have found that mutability of LY-R and LY-S cells does not parallel the inverse cross sensitivity of the two strains to the lethal effects of ionizing and UV radiations. LY-S cells were *more* sensitive to the lethal effects of ionizing radiation but *less* mutable by this agent than were LY-R cells. However, LY-S cells were *less* sensitive to both the lethal and mutagenic effects of UV radiation than were LY-R cells.

Since LY-R cells have been found to convert to LY-S cells during prolonged growth in culture (Beer *et al.*, 1983), it is possible that the differences between the two cell strains are caused by a mutation(s) in one or more genes. Alternatively, a chromosomal rearrangement could occur resulting in a change in expression of one or several genes.

As compared to LY-R cells, LY-S cells show a very low frequency of TG^r cells in untreated populations, as well as in cultures exposed to ionizing or UV radiation. This property of LY-S cells could be a result of amplification of the HGPRT gene; however, two findings rule against this possibility. First, only a 3-fold difference in mutability between the two strains was observed following X-irradiation; a larger difference would be expected if LY-S cells possessed two or more active HGPRT genes. Second, results of preliminary experiments, not reported here, indicate that LY-S cells are also less mutable than LY-R cells at the Na⁺/K⁺ ATPase locus as assayed by ouabain resistance (H.H. Evans, unpublished results).

A number of other mechanisms could explain the low mutation frequencies in LY-S cells and/or the differences between induced mutation rates in LY-R and LY-S cells. They include (1) low viability or a slow growth rate of TG^r (and ouabain-

resistant) mutants of LY-S cells, (2) defects in genes necessary for mutational changes in LY-S cells; and (3) differences in the efficiency and accuracy of post-exposure DNA replication or repair in LY-R vs. LY-S cells. No information is available on viability of LY-S mutants or on defects in the genes necessary for mutational changes. Therefore, for the purpose of this discussion we will consider only differences in DNA replication and repair.

We suggest that LY-R cells are deficient in the repair of UV-induced lesions and that in these cells replication occurs on a damaged template leading to mutations as well as to lethality. In agreement with this hypothesis, sedimentation studies have shown that the molecular weight of progeny DNA strands was not reduced following exposure of LY-R cells to UV-radiation (Walicka *et al.*, 1978, 1980). From the results described above, it appears that LY-R cells are more efficient in the repair of X-ray induced damage than UV-induced damage. Therefore, less mutations and lethality are produced by ionizing radiation than by UV radiation in LY-R cells. We suggest that LY-S cells, in contrast, can repair UV-induced lesions effectively but are deficient in the repair of damage induced by ionizing radiation. Instead, excessive DNA degradation has been reported to occur in LY-S cells following exposure to ionizing radiation (Ueno & Lett, 1979). Such degradation may be the reason for the X-ray sensitivity of LY-S cells as well as for the low X-ray induced mutability of these cells.

Many facts suggest a relationship between the efficiency and accuracy of DNA repair and radiation-induced lethality and mutagenicity. However, it is possible that phenomena other than DNA repair may be involved in the differences we have observed between the two cell strains. Whether DNA repair is a determining or contributing factor in these differences is a subject for future investigation.

References

- BEER, J.Z., BUDZICKA, E., NIEPOKOJCZYCKA, E., ROSIEK, O., SZUMIEL, I. & WALICKA, M. (1983). Loss of tumorigenicity with simultaneous changes in radio- and photosensitivity during *in vitro* growth of L5178Y murine lymphoma cells. *Cancer Res.*, (in press).
- BEER, J.Z., JACOBSON, E.D. & KRELL, K. (1981). Stimulation of L5178Y mouse lymphoma cells by small fluences of UV radiation. In *Symposium on Biological Effects and Measurements of Light Sources*, HHS Publication FDA 81-8156, Rockville, Maryland, p. 137.
- BEER, J.Z., SZUMIEL, I. & WALICKA, M. (1973). Cross-sensitivities to UV-light and X-rays of two strains of murine lymphoma cells *in vitro*. *Studia Biophys.* (Berlin), **36/37**, 175.
- JACOBSON, E.D., KRELL, K., DEMPSEY, M.J., LUGO, M.H., ELLINGSON, O. & HENCH II, C.W. (1978). Toxicity and mutagenicity of radiation from fluorescent lamps and a sunlamp in L5178Y mouse lymphoma cells. *Mutat. Res.*, **51**, 61.
- JOHANSON, K.-J., WŁODEK, D. & SZUMIEL, I. (1982). DNA repair and replication in radiation-sensitive and -resistant mouse lymphoma cells γ -irradiated under aerobic and hypoxic conditions. *Int. J. Radiat. Biol.*, **41**, 261.
- KÖRNER, I., WALICKA, M., MALZ, W. & BEER, J.Z. (1977). DNA repair in two L5178Y cell lines with different X-ray sensitivities. *Studia Biophys.* (Berlin), **61**, 141.

- LEHMANN, A.R. (1972). Postreplication repair of DNA in ultraviolet-irradiated mammalian cells. *J. Mol. Biol.*, **66**, 319.
- UENO, A.M. & LETT, J.T. (1979). Deficient repair and degradation of DNA in L5178Y S/S cells. *Radiat. Res.*, **79**, 424.
- WALICKA, M. & BEER, J.Z. (1979). UV-light sensitivity and split-dose effects in two strains of murine leukemic L5178Y cells. *Fortschr. Onkol.*, **4**, 73.
- WALICKA, M., KÖRNER, I., MALZ, W. & BEER, J.Z. (1978). The effect of caffeine on post-replication repair and survival in two L5178Y cell lines with different sensitivities to UV irradiation. *Mutat. Res.*, **52**, 265.
- WALICKA, M., KÖRNER, I., MALZ, W. & BEER, J.Z. (1980). Post-replication repair of DNA in X or UV-irradiated L5178Y-R and L5178Y-S cells. *Nukleonika*, **25**, 759.