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Enhanced mutagenesis parallels enhanced reactivation of herpes virus in a human cell line

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U.v. irradiation of human NB-E cells results in enhanced mutagenesis and enhanced reactivation of u.v.-irradiated H-1 virus grown in those cells (Cornelis *et al.*, 1982). This paper reports a similar study using herpes simplex virus (HSV) in NB-E cells. The mutation frequency of HSV (resistance of virus plaque formation to 40 μg/ml iododeoxycytidine) increased approximately linearly with exposure of the virus to u.v. radiation. HSV grown in unirradiated cells gave a slope of 1.8 x 10^{-5} m^2/J, with 3.2 x 10^{-5} m^2/J for HSV grown in cells irradiated (3 J/m^2) 24 h before infection. There was no evidence for mutagenesis of unirradiated virus by irradiated cells, as seen with H-1 virus. Enhanced reactivation of irradiated HSV in parallel cultures increased virus survival, manifested as a change in slope of the final component of the two-component survival curve from a D_0 of 27 J/m^2 in unirradiated cells to 45 J/m^2 in irradiated cells. Thus, enhanced mutagenesis and enhanced reactivation occurred for irradiated HSV in NB-E cells. The difference in the enhanced mutagenesis of HSV (dependent on damaged DNA sites) and of H-1 virus (primarily independent of damaged DNA sites) is discussed in terms of differences in DNA polymerases.

Key words: mutagenesis/reactivation/herpes virus/human cell

Introduction

Viruses are useful probes for investigating mutagenic repair processes in prokaryotic and eukaryotic cells. The evidence for enhanced mutagenesis of irradiated virus in irradiated monkey kidney cells is conflicting, with some reporting positive findings (DasGupta and Summers, 1978; Sarasin and Benoit, 1980) and others negative results (Lytle *et al.*, 1980; Cornelis *et al.*, 1981a). However, in this last instance there was a significantly enhanced mutation frequency when intact virus was grown on irradiated cells. Cornelis *et al.* (1982) have recently obtained evidence for inducible enhanced mutagenesis in u.v.-irradiated human cells using u.v.-irradiated H-1 virus, a parvovirus containing single-stranded DNA. The experiments reported here employed the same cell line but with a double-stranded DNA-containing viral probe, herpes simplex virus (HSV). The results obtained with the two viruses agree: enhanced viral mutagenesis paralleled the occurrence of enhanced viral reactivation in this human cell line. However, whereas the enhanced mutagenesis was largely independent of damaged DNA with H-1, the mutagenesis apparently required damaged DNA with HSV.

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Results

Enhanced reactivation of u.v.-irradiated HSV

Survival curves of irradiated virus on irradiated and unirradiated cell monolayers gave evidence of enhanced reactivation (Figure 1) for infection 24 h after cell irradiation, in agreement with previous work (Lytle *et al.*, 1976; Coppey and Menezes, 1981). The survival curves displayed the two-component nature typical for this virus and the D_0 of the final component in the unirradiated cells was similar to those found for other, normal human cells (Lytle *et al.*, 1982). The results show that the fraction of surviving virus was higher in irradiated cells, and that the D_0 of the final component increased from 27 J/m^2 in unirradiated cells to 45 J/m^2 in irradiated cells (no change in the initial component). The increase in D_0 is a reliable indicator of enhanced reactivation (Lytle, 1977; Lytle and Hellman, 1981). The reactivation factor is also shown for comparison with results previously published (Lytle *et al.*, 1976). Thus, enhanced reactivation occurred in u.v.-irradiated NB-E cells, as also shown by Cornelis *et al.* (1982) using H-1 virus.

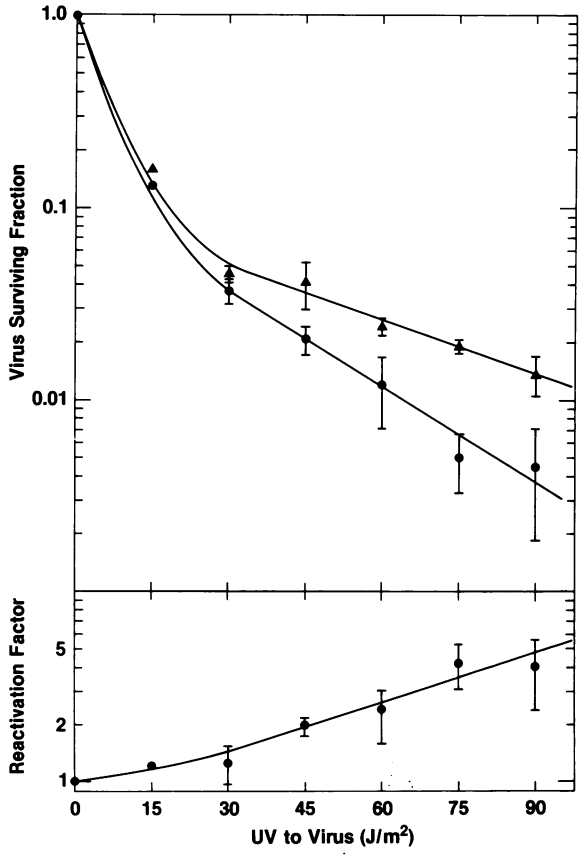

Fig. 1. Enhanced reactivation of u.v.-irradiated HSV in u.v.-irradiated NB-E cells. **Upper panel**: survival curves of HSV on irradiated (3 J/m^2) (▲) and unirradiated (●) NB-E cells, infection 24 h after cell irradiation. **Lower panel**: reactivation factor calculated from above data, ratio of virus surviving fraction in irradiated cells to that in unirradiated cells.

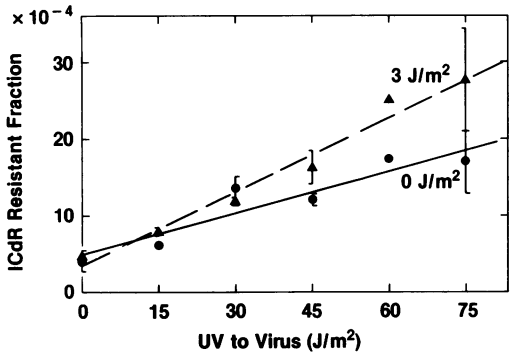

Fig. 2. Mutagenesis of u.v.-irradiated HSV grown in u.v.-irradiated (3 J/m² 24 h pre-infection) (▲) and unirradiated (●) NB-E cells.

Enhanced mutagenesis of u.v.-irradiated HSV

The mutation frequency of irradiated virus was determined for virus grown in irradiated and unirradiated cells (Figure 2). There was no enhancement of mutagenesis of unirradiated virus in irradiated cells. This finding contrasts with previous results with HSV in CV-1 cells (DasGupta and Summers, 1978; Lytle *et al.*, 1980); a small change may have been masked by the relatively high spontaneous mutation frequency. The lack of enhanced mutagenesis of intact HSV also contrasts with the 2-fold increase found with intact H-1 virus in these and other cells (Cornelis *et al.*, 1981b, 1982).

When the logarithm of the induced mutation frequency was plotted versus the logarithm of u.v. exposure to the virus (not shown), the slopes were 1.1 and 1.3 for unirradiated and irradiated cells, respectively, indicating that the relationship between mutagenesis and u.v. exposure is approximately linear. This was also true for herpes virus grown in normal skin fibroblasts from a clinically normal individual, from a xeroderma pigmentosum (group A) patient, and from an individual whose cells had somewhat reduced post-replication repair *in vitro* (Lytle *et al.*, 1982; Hitchins *et al.*, 1982). The only known exception for HSV is for virus grown in a xeroderma pigmentosum variant cell line where the viral mutation frequency increased with the square of the u.v. exposure (Lytle *et al.*, 1982). The data were therefore plotted linearly as shown in Figure 2. The mutation frequency of irradiated virus grown in unirradiated cells increased with u.v. exposure to the virus with a slope of 1.8×10^{-5} m²/J (correlation coefficient 0.77). This value is the same as that found earlier in normal human skin fibroblasts (Lytle *et al.*, 1982). These data support the earlier statement that integrated SV40, as in the present transformed NB-E line, does not affect u.v. mutagenesis of HSV. The mutation frequency for virus grown in irradiated cells was higher, with a slope of 3.2×10^{-5} m²/J (correlation coefficient 0.87). The slopes for the two cases are statistically different at the $p = 0.02$ level. The increase in slope indicates that the enhanced mutagenesis required damaged sites in the HSV DNA.

Discussion

Enhanced mutagenesis of u.v.-irradiated HSV occurred in u.v.-irradiated NB-E cells, in parallel with u.v.-enhanced reactivation of the irradiated virus. This result agrees with the enhanced mutagenesis of irradiated H-1 virus in this cell line reported by Cornelis *et al.* (1982). These data, therefore, show that two rather dissimilar viruses can be used as probes for enhanced viral mutagenesis in mammalian cells.

Previously (Lytle *et al.*, 1982), we concluded that mutagenesis of irradiated HSV in unirradiated human cells occurred on unexcised lesions by an, as yet, unknown process. DNA replication was mentioned as a likely process for mutation fixation; in fact, the HSV DNA polymerase has recently been shown to be involved in viral mutagenesis (Hall and Almy, 1982). The background mutagenesis of intact HSV, perhaps *via* the HSV polymerase, may have been maximized in these transformed NB-E cells, since there was no increase when the cells were irradiated. The enhanced mutagenesis of irradiated virus may have resulted from the HSV DNA polymerase becoming less accurate when bypassing DNA lesions as a result of modification by the irradiated cell, as is probably the case for a cellular DNA polymerase in these irradiated NB-E cells (Cornelis *et al.*, 1982).

While enhanced mutagenesis has been found for both HSV and H-1 virus in u.v.-irradiated NB-E cells, major quantitative differences were found. The mutagenesis of HSV was dependent on damage to the virus and was not apparent for intact virus. Mutagenesis of H-1 virus occurred for intact virus and showed only slight additional increases for irradiated virus. The polymerases used for initial DNA replication have different sources for the two viruses: HSV codes for its own DNA polymerase (Purifoy and Powell, 1977), and H-1 virus employs a host cell DNA polymerase (Rhode, 1974). Presumably the utilization of different DNA polymerases by the two viruses, together with the different strandedness of their respective DNAs, gave rise to the quantitative differences in enhanced mutagenesis.

NB-E cells were derived from normal human tissue, have normal levels of host cell reactivation and normal dose response for mutagenesis of irradiated virus. However, they were transformed by SV40 and have been maintained in culture for many passages (Shein and Enders, 1962). Therefore, while evidence for enhanced mutagenesis has been found in these human cells, one must be mindful that evidence is still lacking for normal human cells. One finding which may indicate abnormal behavior of NB-E cells is the high level of spontaneous mutagenesis of intact HSV. This finding is consistent with evidence that growth of intact HSV in certain human tumor cells gives higher spontaneous mutagenesis than growth in normal human cells (Bockstahler *et al.*, unpublished data). Thus, in this respect, the transformed NB-E cells gave results more similar to tumor cells than to normal cells.

Enhanced reactivation was found under the same conditions under which enhanced mutagenesis occurred. Quantitatively the increases were similar: the reactivation as indicated by D_0 or by dose reduction factor increased by 60–70%, the mutagenesis as indicated by linear slope increased 60–90%. Cause and effect is not apparent from this evidence, however.

Enhanced reactivation was manifested primarily in the final component of the survival curve. The "repair" reflected in this component has been proposed to be irrelevant to mutagenesis of irradiated virus (Lytle *et al.*, 1982). The shape of the mutagenesis dose–response curve could yield information on the possible relevance of enhanced reactivation to mutagenesis. A strictly linear mutagenesis curve is evidence against enhanced reactivation being important for mutagenesis, because a linear response will not occur when only 10% or less of the infected cells possess mutagenic repair

(the slope of the mutagenesis curve would change at the same dose at which the survival curve changed). On the other hand, a biphasic mutagenesis response curve could be evidence of a causal relationship. The uncertainty in our mutagenesis data, however, precludes a definite conclusion as to whether the mutagenesis response curve is linear. Therefore, the relationship of enhanced reactivation to enhanced mutagenesis for HSV remains unknown. Now that there is evidence for enhanced mutagenesis in transformed human cells, it is desirable to investigate further the relationship between enhanced reactivation and enhanced mutagenesis in normal human cells.

Materials and methods

Virus and cells

The macro-plaque strain of HSV type 1 was used. The methods of virus stock preparation and plaque assay conditions have been described (Lytle, 1971). Virus stocks were grown in CV-1 (TC7) monkey kidney cells at 34°C.

Human embryonic kidney cells transformed by SV40 (NB-E) cells were the same cell line used to study enhanced mutagenesis with H-1 virus (Cornelis *et al.*, 1982). R.Martin (National Cancer Institute, Bethesda, MD) has found that SV40 infection *per se* does not cause cellular mutations (Martin *et al.*, 1981). Stock cultures were grown in Eagle's Minimal Essential Medium supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY) and 2 mM L-glutamine. Medium for experimental cultures also contained 50 I.U. penicillin/ml and 50 mg streptomycin/ml. African green monkey kidney cells (CV-1, TC7) were used to assay the mutated virus stocks. Culture medium for these cells has been described (Lytle *et al.*, 1980).

Irradiation

Virus suspensions in Dulbecco's phosphate buffered saline (DPBS) were irradiated by a germicidal lamp (General Electric G8T5, emission primarily at 254 nm) at 0.8 W/m^2. Virus irradiation procedures and dosimetric procedures have been described (Lytle, 1971). Freshly confluent monolayers of NB-E cells were rinsed with DPBS and irradiated at 0.5 W/m^2.

Virus mutant assay

Assay of mutant virus consisted of determining the fraction of the virus population capable of producing plaques in CV-1 monkey kidney cells in the presence of 40 μg/ml iododeoxycytidine (ICdR). The mutation frequency is expressed as the ICdR resistant fraction (ICdR^r), i.e., the ratio of the viral titer assayed in the presence of ICdR to the titer assayed without ICdR (Lytle *et al.*, 1980).

Experimental protocol

The basic protocol for determining enhanced mutagenesis consisted of three steps: (1) irradiating freshly confluent monolayers of NB-E cells in 150 mm plastic Petri dishes (Costar, Cambridge, MA) and incubating them for 24 h; (2) infecting these cells with u.v.-irradiated herpes virus (multiplicity of infection = 0.2; based on pre-irradiation titer) to produce progeny of the irradiated virus; and (3) assaying the progeny virus stocks on the CV-1 monkey kidney cells in the presence or absence of ICdR to determine the mutation frequencies to ICdR^r of the different virus stocks. Details of the mutagenesis aspects of this study were the same as in previous studies (Lytle *et al.*, 1980, 1982).

Enhanced reactivation was determined by irradiating cell monolayers in 60 mm plastic Petri dishes, incubating for 24 h to allow induction of the enhanced reactivation, and infecting with irradiated virus to measure viral survival. Comparison of the viral survival in irradiated cells with that in unirradiated cells was used to determine the occurrence of enhanced reactivation of the irradiated virus.

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