Ultraviolet Irradiation of Murine Cytomegalovirus

(Accepted 11 June 1982)

SUMMARY

Ultraviolet irradiation of murine cytomegalovirus (MCMV) caused a rapid dose-related decline in virus infectivity, manifested by virus antigen induction, and in virus production as measured by plaque formation and infectious centre assay. The virus survival curve was multi-component, suggesting host cell-assisted reactivation. Multiplicity reactivation and photoreactivation of MCMV were not observed in these experiments. Productive infection was more sensitive to u.v. irradiation than was virus antigen production, indicating differential inactivation of virus functions. The effects of u.v. irradiation were similar in most respects to those reported for human cytomegalovirus.

Ultraviolet irradiation is known to alter the viability and replication of a number of bacteria and viruses by damaging microbial nucleic acids (Dulbecco, 1978; Setlow & Carrier, 1962). The effects of u.v. light have been characterized for a number of herpesviruses, including pseudorabies virus, herpes simplex virus (HSV) types 1 and 2, Epstein–Barr virus, and human cytomegalovirus (HCMV) (Albrecht et al., 1974; Hirai et al., 1977; Lytle, 1971; Pfefferkorn et al., 1965; Ross et al., 1972; Sairenji & Hinuma, 1975). For several of these herpesviruses it has been demonstrated that while the capacity for initiating productive infections is rapidly diminished by u.v. irradiation, certain other virus-initiated events are much less sensitive to suppression. For example, u.v.-irradiated HSV and HCMV, although no longer able to infect productively certain susceptible cell lines, are nonetheless able to induce transformation of the cells (Albrecht & Rapp, 1973; Davis & Kingsbury, 1976; Duff & Rapp, 1971). The relative insensitivity of early viral protein synthesis to inhibition by u.v. irradiation has also been noted for HCMV (Boldogh et al., 1978; Hirai et al., 1977).

Murine cytomegalovirus (MCMV) has proven a useful model to study both the molecular and pathogenic events of cytomegalovirus infection (Jordan et al., 1977; Lussier, 1975; Mannini & Medearis, 1961). Although u.v. irradiation has been utilized to inactivate MCMV for a variety of experimental purposes (Sethi & Brandis, 1979; Moon et al., 1976; Hudson et al., 1976), the effects of u.v. irradiation on MCMV function and replication have not been characterized.

This study examined the effects of u.v. irradiation on both the ability of the virus to infect cells and on the production of progeny by MCMV. Ultraviolet irradiation caused a dose-related decline in the capacity of virus to infect cells and produce virus progeny. However, it also caused a differential inactivation of virus functions, suggesting certain virus-induced events were more resistant to u.v. irradiation than others. Under the conditions of these experiments, photoreactivation and multiplicity reactivation of u.v.-damaged MCMV were not observed.

The Smith strain of MCMV, originally provided as a 10% (w/v) salivary gland homogenate, was plaque-purified three times in mouse embryo cell (MEC) monolayers. Virus stock was prepared from tissue culture supernatant fluids and portions stored at −70 °C. The titre of the virus stock used for these experiments was 2.4 × 10^6 p.f.u./ml. Plaque assays were performed in secondary MEC monolayers under a 1% tragacanth overlay (Selgrade & Osborne, 1974). An infectious centre assay using MEC monolayers under 1% tragacanth was used to determine the fraction of cells producing MCMV following virus inoculation. Quantification of cells exhibiting virus antigens was evaluated by an indirect immunofluorescence assay (IFA) (Shanley et al., 1979).

For u.v. irradiation, 1.5 ml portions of virus stock, undiluted or diluted 1:50 in MEM/10% newborn calf serum and antibiotics and placed in 60 mm plastic Petri dishes, were exposed, with continuous agitation, to a 15 W General Electric G15T8 short wavelength u.v. light. The energy
Fig. 1. Dose-response curves illustrating the effects of u.v. irradiation on the ability of MCMV to infect MEC (●) measured by plaque assay or to induce MCMV-specific antigens (○) in MEC measured by IFA. The values were calculated from unirradiated virus controls and show the means and standard errors of at least three separate determinations.

Fig. 2. The relationship of plaque formation and relative virus concentration for MCMV (5 x 10^4 p.f.u./ml) irradiated with various doses of u.v. light. The ordinate shows the mean number of plaques formed in MEC monolayer at various virus dilutions. The abscissa indicates the relative virus concentration based on the plaque count obtained at the highest virus dilution for each u.v. dose. The u.v. doses were 0 (○), 72 (●), 144 (□) and 432 (■) J/m². Lines fitted to the data points by linear regression analysis all had a slope of 1 or less, indicating that multiplicity reactivation was not occurring. Identical results were obtained for undiluted MCMV stock (2.6 x 10^6 p.f.u./ml).

output, measured by a black ray ultraviolet meter (Ultraviolet Products Inc., San Gabriel, Ca, U.S.A.), was found to be 9.8, 4.4 and 2.4 J/m²/s at 10 cm, 20 cm and 30 cm respectively.

The effects of various doses of u.v. on MCMV were evaluated by several methods. Initially, the inactivation of MCMV was determined by plaque assay following various u.v. exposures (Fig. 1). Exposure of MCMV to u.v. light resulted in a rapid, dose-related decline in virus titre. As with the other herpesviruses studied, the virus survival curve for MCMV infection and virus production was multi-component, indicating resistance to inactivation at higher u.v. doses (Albrecht et al., 1974; Lytle, 1971; Pfefferkorn et al., 1965; Ross et al., 1972; Sairenji & Hinuma, 1975). The inactivation curve for virus filtered through a 0.45 μm filter to remove virus aggregates was also multi-component as was the survival curve observed for MCMV prepared in phosphate-buffered saline rather than MEM/10% newborn calf serum. Unlike the discontinuous survival curve reported for HCMV, the inactivation curve for MCMV was continuous over the energy ranges studied (Albrecht et al., 1974). The energy required for one-hit inactivation of MCMV (approx. 20 J/m²) was similar to that reported for HCMV (23 J/m²) and substantially greater than that for HSV (8 J/m²) (Albrecht et al., 1974). In all experiments, the plaques which formed in MEC culture after virus irradiation were noticeably smaller than control plaques.

The ability of u.v.-irradiated MCMV to infect MEC and induce virus antigens detectable by IFA was also evaluated (Fig. 1). There was marked decline in the percentage of cells expressing MCMV antigens following inoculation with u.v.-irradiated virus. The reduction in number of cells bearing virus antigens, following inoculation with virus treated with various doses of u.v. light, was also a multi-component curve.

For some viruses, the flattening of the u.v. inactivation curve has been attributed to multiplicity reactivation (Abel, 1962; Albrecht et al., 1974; Ross et al., 1972). This process is thought to occur when multiple infection of cells with several damaged virions leads to productive
infection. This phenomenon can be shown experimentally when the number of plaques seen in a plaque assay increases out of proportion to a relative virus concentration (Dulbecco, 1978). For MCMV, a linear relationship between actual plaque formation and the plaque formation expected for the relative virus dilution (relative virus concentration) was found for MCMV at virus concentrations of $5.4 \times 10^4$ and $2.6 \times 10^6$ p.f.u./ml at all u.v. doses studied (Fig. 2). These findings indicate that multiplicity reactivation was not occurring under these conditions and could not account for the resistant portion of the u.v. inactivation curve. In contrast, Albrecht and co-workers reported evidence for multiplicity reactivation for HCMV at u.v. doses greater than $80$ J/m$^2$ occurring at multiplicities of infection as low as 0.003 (Albrecht et al., 1974). These authors felt that multiplicity reactivation accounted for both the u.v. resistance and the discontinuity seen in the HCMV survival curves. They postulated that a large population of defective HCMV particles present prior to u.v. irradiation might permit co-operative infection to compensate for genetic damage. Alternatively, introduction of virus DNA from defective virions might augment cell-assisted virus DNA repair. Although the experiments shown in Fig. 2 employed an m.o.i. of 0.05, a number of experiments employing undiluted stock virus ($2.4 \times 10^6$ p.f.u./ml) at an m.o.i. of 1.0 also failed to demonstrate multiplicity reactivation (data not shown) as an important mechanism for MCMV survival.

A number of u.v.-damaged bacteria and viruses have been observed to regain viability when exposed to light of longer wavelength (Jagger, 1958). Photoreactivation has been observed for both pseudorabies virus and HSV (Pfefferkorn & Coady, 1968; Pfefferkorn et al., 1965; Ross et al., 1972; Zavadova & Zavada, 1968). This phenomenon is thought to result from light-assisted enzymic elimination of the thymine dimers induced by u.v. irradiation (Jagger, 1958; Setlow & Carrier, 1962, 1964). For herpesviruses, the process appears to be highly dependent on the cell type supporting virus replication, occurring more efficiently in avian than in mammalian cells (Pfefferkorn & Coady, 1968). To determine whether MEC are able to assist in photoreactivation of virus, virus endpoint plaque titrations of control and u.v.-irradiated virus pools were performed. Following virus adsorption, infected monolayers were exposed to white light for 2 h. All stages of the virus titration, except for white light exposure, were performed in a dark room. The virus titre observed in the white light-treated monolayers was $2.3 \times 10^3$ p.f.u./ml compared to $2.5 \times 10^3$ p.f.u./ml for infected monolayers not exposed to light. These findings suggest that photoreactivation of MCMV in MEC cells did not occur and, therefore, did not play a role in the resistant phase of the u.v. irradiation curve.

Studies of u.v. irradiation of HCMV have reported a dose-dependent sequential inactivation of HCMV genomic function (Boldogh et al., 1978; Hirai et al., 1977). Virus treated with less than 2600 J/m$^2$ u.v. light was able to infect cells and copies of virus genome were demonstrable, by nucleic acid hybridization techniques, in the cell nuclei. In addition, u.v.-inactivated HCMV was able to induce a number of virus-associated functions, including stimulation of host cell DNA synthesis and induction of virus antigens. To determine whether MCMV is similar, the ability of u.v.-irradiated virus to replicate in MEC was compared to the induction of virus antigens following infection.

Ultraviolet irradiated (792 J/m$^2$) or unirradiated virus was used to infect MEC monolayers. Following inoculation, the percentage of cells producing virus was determined by infectious centre assay. The overall infection of cells by u.v.-irradiated and control virus was determined by the percentage of cells expressing virus antigens detectable by IFA 18 h after inoculation.

Following infection with non-irradiated MCMV, the fractions of MEC producing virus and expressing virus antigens were $17.5 \pm 2.6\%$ and $15.6 \pm 2.5\%$ respectively. In contrast, u.v. irradiation of virus reduced the percentage of cells producing virus to $0.048 \pm 0.01\%$ while $3.1 \pm 0.7\%$ of cells exhibited virus antigens. This observation suggests that at certain doses, u.v. irradiation of MCMV may cause a differential inactivation of the virus genome, blocking productive infection but not infection and partial genomic expression. Other virus functions, such as cell transformation or virus interactions with cellular metabolism, were not investigated.

The effects of u.v. irradiation of MCMV are similar in many respects to those seen with HCMV, although a number of interesting differences were noted. Ultraviolet irradiation should prove a useful tool in the study of the biochemical and pathogenic behaviour of MCMV.
I am indebted to Jo Lovetinsky and Jean Morningstar for technical assistance in preparing this manuscript and to E. L. Pesanti for his support. These studies were supported by a U.S. Public Health Service Grant (No. AI05612) and a grant from the American Heart Association (No. 80-994).

Department of Internal Medicine
University of Iowa Hospitals and Clinics
Iowa City, Iowa 52242, U.S.A.

† Present address: Department of Medicine, University of Connecticut Health Center and Newington Veterans Medical Center, Newington, Connecticut 06111, U.S.A.

REFERENCES


(Received 1 March 1982)