Interaction of Encephalomyocarditis Virus with Ultraviolet-irradiated Host Cells

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Ultraviolet light (UV) impaired the capacity of L cells to support growth of encephalomyocarditis virus. The loss of capacity was partially restored by high multiplicity of infection (MOI). This phenomenon was not due to an increased probability of an infectious virus particle reaching a site of replication undamaged by UV, since UV-inactivated virus at high MOI induced restoration of the capacity to support multiplication of nonirradiated virus adsorbed at low MOI. Multiplicity reactivation of UV-irradiated virus did not play a role in this phenomenon since restoration of capacity took place without multiplication of the UV-irradiated restoring virus. The evidence indicates that restoration of capacity was not due to viral interactions involving genetic exchange. The ability to restore capacity was a property more radioresistant than infectivity, suggesting that the former is a function only of part of the viral genome.

Ultraviolet (UV) radiation affects the capacity of mammalian cells, infected after irradiation, to support viral multiplication. The inactivation of capacity is a function of UV dose, but infection of irradiated cells at high multiplicities partially restores the capacity of the cells to produce virus (9, 11). Some investigators think that the phenomenon of restoration of capacity by a high multiplicity of infection (MOI) in systems involving bacteria and temperate phages is related to viral genetic interactions comparable to multiplicity reactivation (MR; 7). With certain animal viruses and mammalian cells, a similar mechanism might play a role in restoration of capacity since MR does occur in these host-virus systems (1). This investigation was carried out to test this hypothesis with the L cell and encephalomyocarditis (EMC) virus system, in which restoration of capacity does occur (P. Balduzzi, *Bacteriol. Proc.*, p. 135, 1966) and in which markers are available for genetic studies (10). The results indicate that restoration of capacity in this case was not due to mechanisms involving viral genetic exchange, but was probably the result of functional cell-virus interactions.

MATERIALS AND METHODS

Cells. Earl's strain L mouse cells, clone 929, obtained from the American Type Culture Collection, were used in all experiments.

Virus. EMC virus was originally obtained from K.

K. Takemoto and was grown in L cells. The large (r) and small (r⁺) plaque variants (10) were isolated and plaque-purified through three passages.

Media and solutions. L cells were grown and maintained with Eagle's Minimal Essential Medium (MEM) with the addition of 10% inactivated horse serum in a humidified CO₂ incubator at 36 C. Virus and cell dilutions were carried out in tris(hydroxymethyl)aminomethane (Tris)-saline at pH 7.2.

Irradiation of cells and of virus suspensions. Cell monolayers in plastic petri dishes (Falcon Plastics, Los Angeles, Calif.), 15 by 60 mm, were washed with Tris-saline before irradiation. A Westinghouse germicidal lamp at a distance of 27 cm from the open petri dishes in the absence of fluid overlay was used throughout these experiments. By several determinations, the output of the lamp was measured as 30 μ w per cm² per sec at 1 m. The cell monolayers were washed once with Tris-saline after irradiation and before infection, which was carried out as described below.

Virus suspensions in volumes of 2 to 3 ml were similarly irradiated in open petri dishes with frequent agitation. Samples from irradiated virus suspensions were diluted to the proper concentrations in Tris-saline and were assayed for infectivity.

Assays of virus infectivity and infectious centers. Assays of virus infectivity were done on L cells by the plaque method as already described (10). In repeated infectivity assays of the same virus stock, the titers varied over a range of about 0.4 log.

For the infectious center assays, the adsorption of the virus was carried out by exposing cell monolayers in petri dishes to viral suspensions in Tris-saline or medium, by use of volumes of 0.2 or 0.4 ml depending

on the experiment. After 1 hr of adsorption at 36 C with frequent rocking of the plates, the cells were washed three times with 5 ml of Tris-saline and once with 5 ml of MEM without serum. Adsorption of the virus, measured by disappearance of the virus from the inoculum, was 60 to 80% throughout this investigation. The cells were dispersed with trypsin and suspended in MEM plus serum; they were then counted, diluted to the proper concentration in Tris-saline, and further diluted to the desired final concentration in agar medium consisting of Earle's balanced salt solution, 0.1% yeast extract (Difco), 0.1% bovine serum albumin (Armour Pharmaceutical Co., Kankakee, Ill.), 5% inactivated horse serum, and 0.9% Difco Special Agar (Noble). Samples (1-ml) of the cell suspension in agar medium were distributed on monolayers of L cells in petri dishes. When the agar had solidified, the plates were overlaid with 4 ml of the same medium. Agar medium containing neutral red (1:20,000) was added 24 or 48 hr later in volumes of 5 ml, and the infectious centers were counted the following day. The MOI in these experiments was calculated as the ratio of input virus, measured as plaque-forming units (PFU), to the number of cells, counted in a hemacytometer. MOI of UV-irradiated virus refers to the titer of the virus prior to irradiation.

The expected number of infected cells was calculated from the MOI according to the Poisson distribution. The recovery of infectious centers is defined as the ratio of PFU observed to the expected number of infected cells. With nonirradiated cells, this recovery varied between 20 and 60% of the expected value. By lengthening the adsorption period from 30 min to 1 hr, a higher number of infectious centers was usually obtained. Further increases in adsorption period did not appreciably enhance the number of infectious centers.

Owing to the variability in the recovery of infectious centers in different experiments, all data on the recovery of infectious centers (survival of capacity) of UV-irradiated cells are expressed as percentages of the values obtained with nonirradiated cells in the same experiment.

RESULTS

Survival curve of free virus suspensions. Exposure of free virus suspensions to UV light resulted in exponential inactivation of infectivity (Fig. 1). The rate of inactivation varied, however, being higher for virus suspensions diluted in Tris-saline than for serum-containing, undiluted virus preparations. The inactivation curves of two EMC variants, r^+ and r , were exactly the same.

Effect of UV irradiation on cell capacity. A number of cell monolayers were irradiated at different doses and then infected; the cells were counted and assayed by the infectious centers technique. UV light impaired the capacity of L cells to support viral multiplication. The inactivation curve of capacity is multi-hit. The rate of inactivation of capacity varied in different experiments; in all cases, however, it was much lower than the rate of inactivation of virus infectivity.

FIG. 1. Survival of EMC virus UV-irradiated at various doses. A suspension of EMC virus was exposed to UV irradiation. At various times, samples were taken and titrated for infectivity by the plaque method. Virus survival is expressed relative to the titer of nonirradiated virus.

When UV-irradiated cells were infected at low and high MOI, the capacity of the cells infected at high MOI was more UV-resistant than that of cells infected at MOI < 1 (Fig. 2).

This increased resistance is referred to as restoration of capacity by high MOI. A possible explanation for restoration of capacity is that an increase in the MOI increases the probability that an infectious virus particle reaches an undamaged site in the cell and replicates successfully. To test this hypothesis, the following experiments were carried out.

Plates of nonirradiated and UV-irradiated cells were infected at a MOI < 1 with nonirradiated virus and at an increasing MOI with UV-inactivated virus. Similar plates were infected with increasing MOI of the nonirradiated virus alone. The cells were then trypsinized, and the infectious centers were assayed as above. The results of one such experiment are presented in Fig. 3. The recovery of infectious centers in nonirradiated, control cells was approximately the same over the range of MOI used in these experiments. Therefore, the average value of recovery in control cells was taken as 100%, and the recovery in UV-irradiated cells was calculated on that basis. The recovery of infectious centers in UV-irradiated cells increased with increasing MOI,

FIG. 2. Survival of capacity for EMC virus of L cells infected at low and high MOI. A series of L cell monolayers was exposed to UV irradiation for various periods of time and then was infected at MOI = 0.4 (○) or at MOI = 8 (●). After adsorption, the cells were dispersed with trypsin and were plated on monolayers of nonirradiated cells for the infectious center assay. Recovery of infectious centers (see text) at various UV doses is expressed as a percentage of the recovery obtained in nonirradiated cells at the appropriate MOI.

FIG. 3. Restoration of capacity by various MOI of nonirradiated and UV-irradiated EMC virus. Monolayers of nonirradiated control cells and of cells UV-irradiated for 4 min were infected with nonirradiated virus at increasing MOI (left) or with nonirradiated virus at MOI = 0.5 plus UV-irradiated virus at increasing MOI (right). Recovery of infectious centers in UV-irradiated cells is expressed as a percentage of the average recovery obtained in nonirradiated control cells.

indicating restoration of capacity. Also with UV-irradiated virus, restoration of capacity was consistently observed, although to a lesser degree than with nonirradiated virus. With nonirradiated virus, maximal restoration was obtained at a MOI of about 2.5, whereas, with UV-irradiated virus, a plateau was not reached in the range of

MOI used here. The ability of UV-irradiated virus to restore capacity seems to be highly radioresistant as compared to virus survival, since virus irradiated for 3 min (survivors $< 10^{-4}$) was still able to induce a certain degree of restoration of capacity.

The high radioresistance of the ability to restore capacity is shown also by experiments in which plates of UV-irradiated cells were infected at a MOI < 1 with nonirradiated virus, and at high MOI with virus irradiated at increasing doses of UV. The ability of irradiated virus to restore capacity is defined here as the ratio of the number of infectious centers obtained by use of irradiated virus to the number obtained with nonirradiated virus. In Table 1, the results of such an experiment in which the restoring virus is added at a MOI of 15 to cells irradiated for 4 min are presented. Clearly the rate of inactivation of the ability to restore capacity is much more radioresistant than the ability to multiply.

From the experiments described above, it is clear that restoration of capacity is not a consequence of an intact virus particle reaching an undamaged replication site, since UV-irradiated virus still has the ability to bring about this restoration.

A possible objection to this conclusion is that in these experiments the UV-irradiated virus produced viable virus by MR phenomena. Although MR occurs with EMC virus (Fig. 4), it does not seem to play a role in restoration of capacity. Evidence on this point derives from experiments in which two plaque variants of EMC virus were used to investigate the role of MR phenomena in restoration of capacity. In the experiment presented in Table 2, UV-irradiated cells were infected at a MOI of 0.8 with the r^+ variant. UV-irradiated r virus was added at MOI

TABLE 1. Survival of EMC virus infectivity and of the ability to restore capacity

UV dose (min)	Virus survival (%)	Ability to restore capacity (%) ^a
0	100	100
1	2	44
2	0.06	26
3	0.00077	20
4	0.000045	7.4

^a Survival of the ability of irradiated virus to restore capacity is expressed as a percentage of the ability to restore capacity of nonirradiated virus at the same high MOI. In each case, the ability to restore capacity is corrected for the recovery of infectious centers obtained with low MOI of nonirradiated virus. See text for further details.

FIG. 4. Multiplicity reactivation of EMC virus inactivated by UV radiation. A suspension of EMC virus was exposed to UV irradiation. At various times, samples were used to infect nonirradiated cells at MOI = 1 (○) or at MOI = 12 (●). The MOI was calculated on the basis of nonirradiated virus. Recovery of infectious centers at various UV doses is expressed relative to the recovery obtained with nonirradiated virus at the appropriate MOI.

= 0.36 and 3.6. Restoration of the capacity to support multiplication of nonirradiated r^+ variant occurred without any multiplication of the UV-irradiated r variant or rescue of the r marker. This evidence also indicates that restoration of capacity takes place independently of viral genetic interactions involving recombination.

DISCUSSION

During this investigation, several facts already known about the effect of UV irradiation on infection of mammalian cells with picornaviruses were confirmed. The inactivation curve of EMC virus was found to be exponential, as could be anticipated from the results of other authors (1, 4, 5) with poliovirus. MR phenomena occur with EMC virus, although to a limited extent as compared with T even phages (2). Drake (1) observed a similar low degree of MR with poliovirus. The capacity of the cells was more resistant to UV than was the free virus, as already shown for poliovirus (4, 5). The capacity curve of L cells for EMC virus is a multi-hit curve with a low extrapolation value varying with the MOI; the meaning of the extrapolation value is not clear. Dulbecco (3), on the basis of studies on the inactivation of infective centers, suggests that one or a few centers for the synthesis of poliovirus are present in the cell. Evidence of another nature for the existence of such centers is still lacking. In our hands, contrary to the results obtained by others (4, 6), the capacity curve did not level off at a residual capacity of about 10%, but decreased constantly for the whole range of UV doses employed. It has been suggested that the variation in the resistance of capacity to radiation of a cell population is a reflection of variation in cellular size, the larger cells being more resistant (8). Although no direct measurement of cell volumes has been done in this study, the cell size appeared constant through-

TABLE 2. Restoration of capacity for EMC virus, r^+ variant, induced by UV-irradiated r variant

Cells	Virus	PFU/cells	Percentage of control	RI ^a	r plaques
Control	r^{+b}	$224/8 \times 10^2$	100	—	0
UV-irradiated	r^+	$152/9 \times 10^4$	0.6	—	0
UV-irradiated	$r^+ + r^c$	$340/9 \times 10^4$	1.35	2.2	0
UV-irradiated	$r^+ + r^d$	$528/9 \times 10^4$	2.1	3.5	1
UV-irradiated	r^d	$24/2.16 \times 10^6$	0.0036	—	24

^a Restoration index = PFU per cell infected with r^+ and r virus divided by PFU per cell infected with r^+ virus alone.

^b Nonirradiated r^+ variant; MOI = 0.8.

^c UV-irradiated r variant (survival 2.3×10^{-4}); MOI = 0.36.

^d UV-irradiated r variant (survival 2.3×10^{-4}); MOI = 3.6.

out the investigation. It is possible that the lack of a tail in our capacity curves reflects the uniformity in cell size.

As in other systems (9), the capacity of cells multiply infected is more resistant than that of cells infected by $\text{MOI} < 1$. The experiments with UV-irradiated virus argue against the possibility that such increased resistance reflects an increased probability of a virus particle coming in contact with an undamaged site in the UV-irradiated cell.

Some investigators think that the loss of capacity of *Escherichia coli* K12S for λ phage is due to an inactivation of infectivity of the phage within the irradiated cell, and that the restoration of capacity by multiple infection is caused by a process similar to MR (7). By using two plaque size variants in the experiments of Table 2, restoration occurred without any multiplication of the UV-irradiated restoring virus or rescue of the plaque size marker. These results indicate that genetic exchange between viruses does not play a significant role in restoration of capacity.

The nature of the viral function responsible for this phenomenon is not known. If it is a function of the viral genome, the inactivation rate of the ability to restore capacity, as compared to virus survival, would indicate that a minor fraction of the viral genome can carry on this function.

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