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Resticted Growth of Human Cytomegalovirus in UV-Irradiated WI-38 Human Fibroblasts[1] (38726)

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Introduction. The replication of human cytomegalovirus (CMV), a herpes group virus is apparently intimately related to host cell functions. Synthesis of both cellular DNA (1) and RNA (2) is stimulated in infected cells, and inhibition of CMV by rifampin may be mediated through its effect on cellular RNA polymerase (3). In addition pretreatment of cells with 2-iodo-2-deoxyuridine (IUdR) results in enhanced replication of CMV (4, 5).

Ultraviolet (uv) light exposure damages cellular DNA. We here report that preirradiation of human fibroblasts insufficient to affect the growth of Herpes simplex virus (HSV), markedly inhibited CMV replication.

Materials and Methods. Viruses. Towne, a strain of human CMV isolated in our laboratory from an infant and serially passaged in WI-38 human fibroblasts (6), was used throughout the experiments.

HSV type 2 was obtained from Dr. A. Nahmias (Emory University) and propagated in WI-38 cells.

Cells. WI-38 human diploid cells were obtained from Leonard Hayflick (Stanford University) and used between the 20th and 35th passage level.

Medium. Eagle's minimal essential medium (MEM) supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), gentamicin (20 μg/ml), and fetal calf serum at 10% for cell growth or 2% for maintenance was used.

Infectivity assays. Viral infectivity titers were obtained by assay of CPE in tube cultures of WI-38 cells or by plaque assay (7). The 50% end points were determined the 14th day after infection and calculated by the method of Reed and Muench.

Incorporation of ^{3}H-uridine and ^{3}H-thymidine. The cells were pulsed for 2 hr in a medium containing 10 μCi of ^{3}H-thymidine (dT) or 10 μCi ^{3}H-uridine (U). Incorporation was stopped by decanting the pulsing medium, washing the monolayer twice with cold phosphate-buffered saline (PBS) and dissolving the cells in 1 ml of 0.1 *N* sodium hydroxide for the analysis of DNA or 1% sodium dodecylsulfate (SDS) for the analysis of RNA. In both cases 10% trichloroacetic acid (TCA) was added to precipitate nucleic acid and the precipitate was examined for radioactive counts by scintillation spectrophotometry.

Ultraviolet irradiation. Confluent monolayers of cells (in 3-cm diameter, Falcon, plastic petri dishes) were irradiated at room temperature at a distance of 22 cm from the germicidal uv tube. This gave an incident-dose rate of 1861 ergs/mm^2/min.

Results. Effect of uv-irradiation on viral growth. Confluent monolayers were exposed to uv-light for 0, 2, 3, or 8 sec immediately prior to virus infection. CMV was then adsorbed to the monolayer for 1 hr at 37°. Infectivity was assayed 14 days postinfection (p.i.). The early cell rounding and early antigen formation (6) that we have described for CMV was also seen in uv-irradiated cells. However, the yield of infectious virus was decreased more than 4 log 10 in cells irradiated for 3 sec. On the other hand, HSV replicated as well in 3-sec-irradiated cells as in the control cells (Table I), although at 8 sec HSV replication was reduced. HSV replication was also not affected in cells irradiated for 3 sec 3 days previous to virus inoculation (data not shown).

To determine at what point the viability of uv-irradiated cells was compromised, the cells were trypsinized after uv-exposure. The

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3-sec-irradiated cells grew at the same rate as nonirradiated cells, as determined by cell counts at confluence, but 8-sec-irradiated cells grew more slowly.

Effect of uv irradiation of viral growth cycle. Confluent monolayer cell cultures (in 3-cm diameter petri dishes) were infected at a multiplicity of 10. Virus was allowed to adsorb for 1 hr. At indicated intervals, infected cultures were exposed to uv light for 3 sec. Infectivity of cell-associated virus was assayed at 96 hr p.i. The yield of virus was markedly decreased in cultures exposed to uv light at 0, 24, or 48 hr p.i., but not at 72 hr p.i. (Table II).

TABLE I. CMV REPLICATION IN UV-IRRADIATED WI-38 CELLS.

Time of uv (sec)	Early cell rounding due to CMV	CMV infectivity[a]	HSV infectivity[a]
2	++	$10^{6.5}$	$10^{8.0}$
3	++	$10^{3.5}$	$10^{8.5}$
8	++	$10^{1.0}$	$10^{3.0}$
No uv	++	$10^{8.0}$	$10^{7.5}$

[a] Tissue culture infectious doses $(TCID)_{50}$.

TABLE II. EFFECT OF POST INFECTION, 3-SEC UV-IRRADIATION ON CMV REPLICATION CYCLE.

uv Irradiation (hr, p.i.)	Infectivity at 96 hr p.i. (PFU/ml)
No uv	6.0×10^7
0	2.5×10^4
24	3.0×10^4
48	8.0×10^5
72	7.0×10^7

Effect of uv irradiation on macromolecular synthesis of CMV-infected cells. We reported that CMV stimulates host cell RNA synthesis preceding viral DNA synthesis (3). Studies of RNA and DNA synthesis in uv-irradiated CMV-infected cells were carried out to see whether stimulation of host cell RNA occurred.

Cells were irradiated for 3 sec or left unirradiated immediately prior to infection at a multiplicity of 10. At 48 hr p.i., incorporation of ^{3}H-dT or ^{3}H-U was measured. In CMV-infected cultures, there was apparent DNA synthesis above the control values. Although not determined in this experiment, other studies (6) have shown that this is mainly due to viral DNA synthesis. Ultraviolet irradiation sharply decreased the thymidine incorporation. As expected (2) CMV infection increased uridine incorporation by 360% above that in control cells. This increase was abolished by uv irradiation. Thus CMV-induced stimulation of host cell RNA synthesis did not occur in uv-irradiated cultures (Table III).

Discussion. CMV replication was inhibited when the host cells were irradiated by uv immediately prior to infection, evidently through damage to the host cell genome. Ultraviolet is known to damage the cell through the formation of dT dimers and the disruption of cellular DNA. Since the inhibitory effect occurred as late as 48 hr p.i., i.e., during the latent period of CMV replication, and was not apparent after viral DNA synthesis was in full swing, it is probable that the block occurs at a step preceding macromolecular synthesis.

Our results could be interpreted as show-

TABLE III. EFFECT OF CMV INFECTION ON RNA AND DNA SYNTHESIS IN CELLS UV IRRADIATED FOR 3 SEC, COMPARED TO UNIRRADIATED CELLS

	RNA synthesis[b]				DNA synthesis[c]			
	CMV infection		No infection		CMV infection		No infection	
Treatment[a]	cpm	%	cpm	%	cpm	%	cpm	%
Unirradiated	8,302	360	2,302	100	45,656	232	19,611	100
uv Irradiated	2,196	94	1,693	69	27,382	139	18,956	96

[a] Irradiation at 3 sec immediately prior to infection at multiplicity of 10.
[b] Incorporation of ^{3}H-dT 48 hr p.i.
[c] Incorporation of ^{3}H-U 48 hr p.i.

ing that the replication of CMV is more dependent on host cell functions than is the replication of Herpes simplex. These studies do not show which host cell functions are important to CMV replication. Nevertheless, it is interesting to speculate from our earlier results showing that host cell RNA stimulation preceded viral growth, and our present finding that synthesis of both host cell RNA and infectious virus were impaired by uv irradiation, that damage to cellular DNA causes poor transcription of cellular RNA and thus poor synthesis of viral DNA. However, further evidence is necessary to enable us to distinguish between the effect of uv irradiation on cellular DNA and RNA metabolism in relation to CMV replication.

Summary. The growth of human CMV was inhibited by uv irradiation of cells prior to infection or during the 48-hr latent period of virus replication but not after virus synthesis began. The duration of uv exposure sufficient to inhibit CMV replication was insufficient to inhibit replication of Herpes simplex and did not prevent uninfected cells from dividing normally. The effect of uv irradiation on CMV replication may have been mediated through prevention of the stimulating effect of the virus on host cell RNA(s) synthesis.

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