Enhanced Capacity of DNA Repair in Human Cytomegalovirus-Infected Cells

YUKIHIRO NISHIYAMA† AND FRED RAPP*

Department of Microbiology and Specialized Cancer Research Center, The Milton S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

Received 6 October 1980/Accepted 30 December 1980

Plaque formation in Vero cells by UV-irradiated herpes simplex virus was enhanced by infection with human cytomegalovirus (HCMV), UV irradiation, or treatment with methylmethanesulfonate. Preinfection of Vero cells with HCMV enhanced reactivation of UV-irradiated herpes simplex virus more significantly than did treatment with UV or methylmethanesulfonate alone. A similar enhancement by HCMV was observed in human embryonic fibroblasts, but not in xeroderma pigmentosum (XP12BE) cells. It was also found that HCMV infection enhanced hydroxyurea-resistant DNA synthesis induced by UV light or methylmethanesulfonate. Alkaline sucrose gradient sedimentation analysis revealed an enhanced rate of synthesis of all size classes of DNA in UV-irradiated HCMV-infected Vero cells. However, HCMV infection did not induce repairable lesions in cellular DNA and did not significantly inhibit host cell DNA synthesis, unlike UV or methylmethanesulfonate. These results indicate that HCMV enhanced DNA repair capacity in the host cells without producing detectable lesions in cellular DNA and without inhibiting DNA synthesis. This repair appeared to be error proof for UV-damaged herpes simplex virus DNA when tested with herpes simplex virus thymidine kinase-negative mutants.

Human cytomegalovirus (HCMV) can stimulate or induce DNA synthesis (8, 25), RNA synthesis (26), protein synthesis (24), thymidine kinase (31), DNA polymerase (11), ornithine decarboxylase (13), and plasminogen activator (30) in permissive and nonpermissive cells. Most of these properties are commonly associated with oncogenic DNA viruses; in fact, persistent infection of virus-permissive human embryo lung fibroblasts (9, 18) and exposure of nonpermissive hamster cells (2) to HCMV results in occasional transformation of these cells.

We previously reported (17) enhanced survival of UV-irradiated herpes simplex virus (HSV) in HCMV-infected, but nonpermissive, primary rabbit kidney cells. The observation suggested that a functional HCMV genome and a caffeine-sensitive process are necessary to express this enhancement. Recent studies have shown that mutagens, such as UV and X irradiation and chemical carcinogens, can enhance survival of UV-irradiated DNA viruses in treated cells (6, 15, 20), and it has been postulated that carcinogens might induce error-prone repair pathways in mammalian cells. Therefore, further studies were undertaken to characterize

this phenomenon in HCMV-infected cells by comparing it with repair induced by mutagenic agents. We report that HCMV enhanced DNA repair in infected cells without producing detectable lesions in cellular DNA, and that this repair may be error proof for UV-damaged HSV DNA.

MATERIALS AND METHODS

Cells and viruses. Vero cells, a stable line of African green monkey cells, Flow 5000 cells (Flow Laboratories, Rockville, Md.), and xeroderma pigmentosum cells (XP12BE; American Type Culture Collection, Rockville, Md.) were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum, 100 U of penicillin, and 100 µg of streptomycin per ml. A mouse cell line (N c1a C110-TK⁻) lacking thymidine kinase (obtained from R. Goldberg, National Institutes of Health, Bethesda, Md.) was propagated in growth medium containing 20 µg of 5-bromodeoxyuridine (BUdR) per ml. HSV type 2 (HSV-2) strain 186 clone 3 isolated from a single plaque and HCMV strain AD169 were used throughout this study. Viruses were propagated in Flow 5000 cells by infecting at low multiplicities (0.01 to 0.1 PFU/cell). Infected cells were harvested when most cells exhibited cytopathic effects. After freezing and thawing three times and eliminating cell debris by centrifugation at 3,000 rpm for 10 min, viruses were stored at -80°C. Clarified lysates of uninfected Flow 5000 cells were used for mock infection.

† Present address: Nagoya University School of Medicine, Showa-Ku, Nagoya, Japan.

Plaque assays. The plaque assay procedures for HSV and HCMV have been described previously (19, 29); however, 0.5% agarose was used as overlay medium in both assays.

Chemicals. [*methyl*- ^3H]thymidine (20.0 Ci/mmol) and [$2\text{-}^{14}\text{C}$]thymidine (51.6 mCi/mmol) were purchased from New England Nuclear Corp., Boston, Mass. Methylmethanesulfonate (MMS) and hydroxyurea were purchased from Aldrich Chemical Company, Inc., Milwaukee, Wis., and Sigma Chemical Company, St. Louis, Mo., respectively, and prepared immediately before use.

UV irradiation. Virus suspensions were diluted 1:10 in Tris-buffered saline, and 1-ml volumes in 60-mm plastic culture plates were exposed to a General Electric G8T5 UV bulb for appropriate time intervals at a dose of 20 ergs per mm^2 per s, as measured by a Black-Ray UV dosimeter model J-225. Dishes were swirled constantly during irradiation. To irradiate cells, the medium was removed and cells were washed with Tris-buffered saline and then irradiated for appropriate time intervals at a dose of 20 ergs per mm^2 per s.

Reactivation assay. Confluent monolayers of HCMV-infected cells or mock-infected Vero cells were irradiated with various doses of UV light or were treated with various concentrations of MMS for 90 min and then incubated for 48 h with maintenance medium containing 2% fetal calf serum. The cells were then infected with appropriate dilutions of UV-irradiated or non-irradiated HSV-2. After a 1-h adsorption period, the cells were overlaid with 0.5% agarose in growth medium, fixed 5 days postinfection (p.i.), and stained with 0.7% crystal violet. Plaques were counted by using a dissecting microscope at $\times 10$ magnification. Virus was UV irradiated for 5 min at 20 ergs per mm^2 per s (6.0×10^3 ergs per mm^2), and the reactivation factor was determined by calculating the ratio of the surviving fraction (ratio of titer of UV-irradiated virus to that of non-irradiated virus) in treated cells to that in mock-infected and untreated cells (17).

Assay of hydroxyurea-resistant DNA synthesis. Confluent monolayers of Vero cells were infected with HCMV (3 PFU/cell) and incubated with maintenance medium for 48 h at 37°C . HCMV-infected or mock-infected cells were irradiated with UV light or treated with MMS after exposure to 20 mM hydroxyurea for 30 min. After UV irradiation, cells were labeled with 10 μCi of [^3H]thymidine per ml in growth medium containing 20 mM hydroxyurea for 2 h. For MMS treatment, cells were incubated with growth medium containing 10 μCi of [^3H]thymidine per ml, 20 mM hydroxyurea, and various concentrations of MMS. The cells were then examined for acid-precipitable material, and radioactivity was measured in a liquid scintillation counter.

DNA analysis by CsCl density gradient equilibrium centrifugation. Confluent monolayers of HCMV-infected Vero cells were labeled with 30 μCi of [^3H]thymidine per ml in growth medium containing 10 μg of BUdR per ml for 2 h, washed, scraped into a small volume of Tris-buffered saline, and pelleted by low-speed centrifugation. When treated with UV light or hydroxyurea, cells were incubated in growth medium containing 10 μg of BUdR per ml for 1 h before treatment. Harvested cells were digested for a mini-

mum of 6 h at 37°C in a mixture of 0.25% Sarkosyl, 0.05 M EDTA, and 0.5% heat-inactivated pronase. Pronase and Sarkosyl were prepared in $0.1\times$ SSC (0.015 M NaCl and 0.0015 M sodium citrate, pH 7.3). After digestion, ^3H -labeled material was centrifuged in a CsCl solution in $0.1\times$ SSC (density, 1.701 g/ml) at 33,000 rpm for 60 h in a Beckman 50.3 fixed-angle rotor. ^{14}C -labeled cellular DNA without BUdR was used as a marker. After centrifugation, fractions of ~ 0.09 ml were collected from the bottoms of the tubes onto Whatman filter paper disks by displacing the CsCl solution with oil. The disks were washed twice in 5% trichloroacetic acid, twice in 95% ethanol, and once in acetone and then air dried. Radioactivity was measured in a liquid scintillation counter.

Alkaline sucrose gradient sedimentation analysis. Vero cells ($\sim 3 \times 10^6$ cells) were plated in 60-mm plastic culture dishes containing growth medium. Parental DNA was prelabeled with 0.2 μCi of [^{14}C]thymidine per ml for 72 h at 37°C . After prelabeling, cells were washed and incubated with nonradioactive growth medium for 2 h. ^{14}C -labeled cells were then infected with HCMV at a multiplicity of 3 PFU/cell. After a 1-h adsorption period, the cells were washed, incubated with maintenance medium containing 2% fetal calf serum for 48 h at 37°C , UV irradiated at a dose of 200 ergs per mm^2 , and labeled with 20 μCi of [^3H]thymidine per ml in prewarmed growth medium for 30 min. The cells were washed twice in warmed medium, chased for 2 h in growth medium containing unlabeled thymidine (4 $\mu\text{g}/\text{ml}$), and trypsinized. A sample (<0.1 ml) containing 10^5 cells was placed directly on 1 ml of lysis solution (0.5 N NaOH, 0.1 M EDTA, 0.2% sodium lauryl sulfate) on top of 31 ml of a preformed 5 to 20% alkaline sucrose gradient (0.25 N NaOH, 0.01 M EDTA, 0.2 M NaCl). After 4 h at room temperature, gradients were centrifuged at 20°C for 15 h at 10,500 rpm in an SW27 rotor (21). Fractions (1 ml) were collected by bottom puncture, 1 mg of calf thymus DNA was added to each fraction, and the DNA was precipitated by the addition of 8 to 10 ml of ice-cold 5% trichloroacetic acid. Each fraction was then filtered through GF/C filters (Whatman) pre-soaked with 5% trichloroacetic acid. The filters were washed sequentially with ice-cold 5% trichloroacetic acid, 70% ethanol, and 95% ethanol and then air dried. The dried filters were then assayed for radioactivity.

Assay for mutation frequency. Confluent monolayers of Vero cells were infected with HCMV at a multiplicity of 3 PFU/cell. After incubation for 48 h at 37°C , the cells were infected with UV-irradiated or non-irradiated HSV-2 at a multiplicity of 1 PFU/cell. After adsorption for 1 h, cells were washed twice with Tris-buffered saline, incubated with growth medium, and then harvested 24 h p.i. Infectivity was assayed in Vero and N cla cl10-TK $^-$ cells. For assay of HSV-TK $^-$ mutants, confluent monolayers of N cla cl10-TK $^-$ cells were infected with and allowed to adsorb to various dilutions of virus for 2 h and then incubated with growth medium containing 20 μg of BUdR per ml and anti-HSV antiserum (rabbit-immune serum). Cells were fixed and stained 72 h p.i., and plaques were counted by using a dissecting microscope at $\times 10$ magnification. The number of plaques from at least four dishes was averaged for each assay point, and the

mutation frequency was then determined by calculating the ratio of virus in N cIa cl10-TK⁻ cells to virus in Vero cells.

RESULTS

Reactivation of UV-irradiated HSV in HCMV-infected cells. Figure 1 shows reactivation of UV-irradiated HSV in HCMV-infected or mock-infected Vero cells treated with UV light or MMS. Confluent monolayers of Vero cells were infected with HCMV at a multiplicity of 3 PFU/cell. After incubation for 24 h, cells were irradiated with UV light or treated with MMS for 90 min, and incubated for 48 h at 37°C. The cells were then infected with UV-irradiated HSV (<0.005 PFU/cell) and overlaid with 0.5% agarose in growth medium, and the plaques were counted 5 days p.i. Enhancement induced by UV or MMS was dose dependent; maximal reactivation factors were approximately 12 and 5 in UV-treated and MMS-treated mock-infected cells, respectively, a range at which treated cells could be maintained during HSV plaque formation without extensive damage. As reported previously (17), enhanced reactivation was also observed in HCMV-infected cells. UV or MMS treatment of HCMV-infected cells markedly enhanced reactivation of UV-irradiated HSV. The

maximal reactivation factor was approximately 20 to 25 in HCMV-preinfected Vero cells treated with UV or MMS.

The effect of HCMV infection on reactivation of UV-irradiated HSV was examined in human embryonic fibroblasts (Flow 5000 cells) and xeroderma pigmentosum fibroblasts (XP12BE cells) (Table 1). XP12BE cells are known to have decreased host cell reactivation due to defective excision repair mechanisms (1, 10). Because of HCMV cytopathology, virus yields instead of plaque numbers were determined. When Flow 5000 cells were infected with UV-irradiated HSV, virus yields were always higher in HCMV-infected cultures than in mock-infected cultures. This enhancement was most prominent in cultures infected with HSV irradiated for 15 min, but was not observed in XP12BE cells. Growth of non-irradiated HSV was inhibited slightly by HCMV infection in both cell lines.

Repair replication in HCMV-infected cells. These experiments were performed to investigate whether HCMV infection affected repair replication of host cell DNA. In the first set of experiments, neutral CsCl density gradient analysis was carried out by using BUdR density and [³H]thymidine radioisotope labeling to determine whether HCMV caused DNA lesions which effected repair replication. At 48 h after HCMV infection, the cells were incubated for 2 h in growth medium containing 10 µg of BUdR and 30 µCi of [³H]thymidine per ml. Repair replication was undetectable at 48 h p.i. in HCMV-infected Vero cells (Fig. 2a). Even when hydroxyurea was used to suppress normal replication, peaks corresponding to the position of ¹⁴C-labeled cell DNA were not observed (Fig. 2b). In contrast, repair replication was easily detected in UV-irradiated cultures (Fig. 2c). We also examined this mechanism 24 p.i. in HCMV-infected Vero cells and 24, 48, and 96 h p.i. in HCMV-infected Flow 5000 cells; however, we did not detect peaks corresponding to repair replication (data not shown).

Hydroxyurea-resistant DNA synthesis induced by UV light or MMS was measured in HCMV-infected or uninfected Vero cells since excision repair replication in mammalian cells can be assessed by measuring the rate of [³H]thymidine incorporation after adding hydroxyurea to inhibit normal semiconservative DNA synthesis (4). At a concentration of 20 mM of hydroxyurea, more than 95% of semiconservative DNA synthesis was inhibited. Confluent monolayers of HCMV-infected or mock-infected cells were exposed to 20 mM of hydroxyurea for 30 min 48 h p.i., irradiated with various doses of UV light, and then incubated with 10 µCi of

FIG. 1. Reactivation of UV-irradiated HSV after treatment of HCMV-infected (●) or mock-infected (○) Vero cells with UV light (a) or MMS (b). Confluent monolayers of Vero cells were infected with HCMV and then treated with various doses of UV light or MMS. The reactivation factor was determined by calculating the ratio of the surviving fraction (ratio of titer of UV-irradiated virus to that of non-irradiated virus) in treated cells to that in mock-infected and untreated cells. Each point represents the average of four to six separate experiments. Vertical bars show the standard deviation of the mean.

TABLE 1. *Effect of HCMV infection on growth of UV-irradiated HSV in human embryonic fibroblasts and xeroderma pigmentosum fibroblasts*

Cells	HCMV infection ^a	Virus yield (PFU/ml) ^b			
		Non-irradiated ^c	UV irradiated ^d		
			6 min	10 min	15 min
Human embryonic fibroblasts	+	8.0×10^5	7.3×10^5	1.0×10^4	5.8×10^3
	-	1.9×10^5 (<1) ^e	2.5×10^5 (2.9)	4.0×10^3 (2.5)	<10 ($>10^3$)
Xeroderma pigmentosum fibroblasts	+	2.4×10^5	1.0×10^3	2.6×10^2	<10
	-	3.7×10^5 (<1)	8.0×10^3 (<1)	4.7×10^2 (<1)	<10

^a Confluent monolayers of cells were infected with HCMV at a multiplicity of 0.1 PFU/cell. After a 1-h adsorption period, cells were infected with UV-irradiated or non-irradiated HSV.

^b Virus was harvested 48 h after HSV infection.

^c Cells were infected with non-irradiated HSV at a low multiplicity (100 PFU/culture).

^d Virus was UV irradiated for appropriate times at a dose of 20 ergs per mm² per s. Cells were inoculated at a multiplicity equivalent to 0.3 PFU/cell.

^e Numbers within parentheses express the ratio of HSV titers in HCMV-infected cultures to those in uninfected cultures.

FIG. 2. *CsCl* density gradient DNA analysis of HCMV-infected Vero cells labeled with BUdR and [³H]thymidine. Confluent monolayers of Vero cells were infected with HCMV at a multiplicity of 3 PFU/cell; 48 h later the cells were labeled with BUdR and [³H]thymidine for 2 h in the absence (a) or presence (b) of 20 mM of hydroxyurea or irradiated with UV light (c) at a dose of 200 ergs per mm² before labeling; ○, ¹⁴C-labeled cellular DNA of normal density used as a marker; ●, DNA from HCMV-infected cells; \, buoyant density.

[³H]thymidine per ml in the presence of hydroxyurea. When treated with MMS, cells were incubated with growth medium containing [³H]thymidine, hydroxyurea and various concentrations of MMS for 2 h. Hydroxyurea-resistant DNA synthesis was dependent on the dose of UV or MMS and was enhanced in HCMV-infected cultures (Fig. 3). The extent of enhancement increased with higher UV and MMS doses.

As in bacteria, newly synthesized cell DNA in UV-irradiated mammalian cells is smaller than that in non-irradiated cells, and these small DNAs join to form larger DNAs that are similar in size to those in non-irradiated cells. The size distribution of newly synthesized cell DNA from HCMV-infected or mock-infected Vero cells exposed to UV light was studied by alkaline sucrose gradient sedimentation. ¹⁴C-labeled Vero cells were infected with HCMV, irradiated with

FIG. 3. Hydroxyurea-resistant DNA synthesis of HCMV-infected or mock-infected Vero cells after treatment with UV light (a) or MMS (b). The UV-irradiated or MMS-treated cells were labeled with 10 μ Ci of [³H]thymidine per ml for 2 h at 48 h p.i. in the presence of 20 mM of hydroxyurea; ●, HCMV-infected cells; ○, mock-infected cells. The results show counts per minute per 2×10^5 cells. Each point represents the average of duplicate cultures.

UV light at a dose of 200 ergs per mm² 48 h later, labeled with [³H]thymidine (20 μ Ci/ml) for 30 min, and chased for 2 h. Trypsinized cells (10^5) were placed directly in lysis solution on top of a preformed 5 to 20% alkaline sucrose gradient. UV irradiation inhibited large-size DNA synthesis, but stimulated [³H]thymidine incorporation in small-size DNA fractions (fractions 20 to 28) in both HCMV-infected and mock-infected cells (Fig. 4). In UV-irradiated HCMV-infected cells, the overall rate of DNA synthesis was enhanced compared with that in UV-irradiated mock-infected cells; however, the size distribution profiles (expressed as percent incorporation of [³H]thymidine) were similar.

HCMV-infected cell DNA synthesis and growth. Mutagenic agents or inducing agents of the "SOS" function, a type of inducible DNA repair in bacteria, are known to inhibit host cell DNA synthesis (28). Reactivation of UV-irradiated simian virus 40 in mammalian cells was enhanced by treatment with cycloheximide, an inhibitor of DNA synthesis that does not produce detectable lesions in DNA (20). Therefore, we examined the effect of HCMV on Vero cell DNA synthesis and growth and compared it with that of UV-treated or MMS-treated cells. Confluent monolayers of Vero cells were infected with HCMV at a multiplicity of 3 PFU/cell, UV irradiated (50 ergs per mm²), or treated with 0.5 mM MMS for 90 min. These treatments in-

creased reactivation of UV-irradiated HSV by approximately three- to fivefold. DNA synthesis of UV-treated or MMS-treated cells was markedly suppressed after treatment, whereas that of HCMV-infected cells was suppressed by approximately 20% 24 h p.i., but was enhanced thereafter (Fig. 5). Similarly, cell growth was greatly inhibited by UV irradiation or MMS treatment. However, HCMV infection of Vero cells did not suppress cell growth (Fig. 6).

Mutation frequency of HSV in HCMV-infected cells. A recent study (6) showed that UV irradiation of mammalian cells before infection increased mutagenesis of UV-irradiated and non-irradiated HSV in a manner similar to Weigle reactivation (27) of UV-irradiated phage λ in *Escherichia coli*. However, attempts to demonstrate enhanced mutagenesis of UV-irradiated adenovirus in UV-irradiated human fibroblasts have not been successful (7, 10). Lytle et al. (16) demonstrated that in monkey kidney cells UV reactivation alone is not mutagenic for HSV, although UV reactivation and multiplicity reactivation together are somewhat mutagenic.

Experiments were performed to determine whether induced or enhanced repair pathways in HCMV-infected cells were error proof or error prone for UV-damaged HSV DNA. Confluent monolayers of Vero cells were infected with HCMV (3 PFU/cell). After incubation for 48 h, cells were infected with UV-irradiated or non-irradiated HSV (1 PFU/cell). Virus was harvested 24 h p.i. and assayed in Vero or N cla cl10-TK⁻ cells (Table 2). The mutation frequency increased after UV irradiation of the virus; however, when irradiated HSV was grown in HCMV-infected cells, the mutation frequency decreased.

DISCUSSION

The present study demonstrates that HCMV infection of Vero cells increased DNA repair of UV-damaged HSV DNA and UV- and MMS-damaged cellular DNA. These data suggest that this enhanced DNA repair may not require inhibition of host cell DNA synthesis or production of repairable lesions in cellular DNA, and that the enhanced repair activities appear to be error proof for UV-damaged HSV DNA.

Survival of UV-irradiated DNA viruses in mammalian cells may be related to several types of reactivation mechanisms including host cell reactivation, UV and X-ray reactivation, multiplicity reactivation, and photoreactivation. Because enhanced reactivation in HCMV-infected Vero cells can be observed in cells infected with UV-irradiated HSV at multiplicities less than 0.005 PFU/cell and maintained in darkness, multiplicity reactivation and photoreactivation

FIG. 4. Alkaline sucrose gradient profiles of newly synthesized DNA from HCMV-infected or mock-infected Vero cells irradiated with UV light. HCMV-infected or mock-infected cells were irradiated with UV light at a dose of 200 ergs per mm^2 and pulse-labeled for 30 min with [^3H]thymidine, chased for 2 h, and centrifuged at 10,500 rpm for 15 h at 20°C ; ●, non-irradiated HCMV-infected cells; ○, non-irradiated mock-infected cells; ■, UV-irradiated HCMV-infected cells; □, UV-irradiated mock-infected cells. Parental DNA (×) labeled with [^{14}C]thymidine from mock-infected cells irradiated with UV light.

may not be involved in this phenomenon. Moreover, it seems unlikely that recombination between HCMV and HSV DNAs plays a role in enhanced survival of UV-irradiated HSV in HCMV-infected cells because: (i) HSV-2 DNA does not share detectable base sequence homology with HCMV DNA (12); (ii) enhanced reactivation of UV-irradiated HSV by HCMV was observed in human embryonic fibroblasts, but not in xeroderma pigmentosum fibroblasts (XP12BE cells), which are known to be defective in an early step of excision repair (Table 1); (iii) enhanced reactivation was observed in the second component of the survival curves of UV-irradiated HSV; (iv) reactivation was maximal in cells infected with HCMV at a multiplicity of 1 PFU/cell; (v) maximal enhancement was attained even in cells infected with UV-irradiated HCMV in which infectivity was reduced by more than 95%; and (vi) enhancement was sensitive to caffeine (17). These observations also suggest that DNA repair mechanisms may be involved in enhanced reactivation of UV-irradiated HSV in HCMV-infected cells, but that recombination and complementation are not involved. It is

possible that HCMV may enhance constitutive repair activities or induce new repair pathways that are repressed under normal conditions.

In the bacteria system, it has been well established that treatment with UV light or chemical mutagens enhances the survival of UV-irradiated phage due to the induction of the SOS repair function (28). In mammalian cells, the existence of an inducible SOS response has been suggested to explain the enhanced reactivation of UV-irradiated DNA viruses in UV-treated or chemical carcinogen-treated cells (6, 15, 20); however, little information is available on the biological characteristics and enzymology of inducible repair pathways. Although we do not know the types of repair pathways responsible for enhanced reactivation in HCMV-infected cells, enhanced repair activities appear to be error proof for UV-damaged HSV DNA because the mutation frequency of UV-irradiated HSV decreased after growth in HCMV-infected cells.

SOS repair activity in *E. coli* is induced in response to various agents that damage host cell DNA or interrupt its replication. Survival of UV-irradiated DNA viruses in mammalian cells

is enhanced by pretreatment of cells with DNA-damaging agents and inhibitors of cellular DNA synthesis. However, we could not detect DNA lesions in HCMV-infected cells that induced repair replication. In addition, HCMV did not significantly suppress host cell DNA synthesis or cell growth. On the contrary, infection with

HCMV stimulated host cell DNA synthesis after 48 h p.i. Recent studies have shown that stimulation by phytohemagglutinin or concanavalin A increases the capacity of lymphocytes to initiate repair synthesis induced by chemical carcinogens severalfold; this increase is induced concomitantly with DNA synthesis (3, 22, 23). Our experiments showed that the enhanced reactivation in HCMV-preinfected Vero cells treated with UV irradiation or MMS 24 h p.i. exceeded the maximum in mock-infected treated cells,

FIG. 5. Effect of HCMV infection, UV irradiation, or MMS treatment on cellular DNA synthesis. Confluent monolayers of Vero cells were infected with HCMV at a multiplicity of 3 PFU/cell (●), irradiated with UV light at a dose of 50 ergs per mm^2 (□), or treated with MMS for 90 min at a concentration of 0.5 mM (▲) and maintained at 37°C. At various intervals after treatment, the cells were incubated with fresh growth medium containing 10 μCi of [^3H]thymidine per ml for 60 min. The cells were processed for acid-precipitable material, and incorporated radioactivity was measured in a liquid scintillation counter. Each point represents the average of duplicate cultures.

FIG. 6. Effect of HCMV infection (a), UV irradiation (b), or MMS treatment (c) on cell growth. Confluent monolayers of Vero cells were infected with HCMV at a multiplicity of 3 PFU/cell, irradiated with UV light, or treated with MMS for 90 min. After this treatment, cells were washed with Tris-buffered saline, trypsinized, and seeded into 60-mm plastic culture plates at a concentration of 10^5 cells/ml. Cells were then incubated with growth medium at 37°C. N_t/N_0 , Cell number at given time divided by cell number at time 0. Uninfected control cells (○); HCMV-infected cells (●); cells irradiated with UV at a dose of 50 ergs per mm^2 (■) and 100 ergs per mm^2 (◆); cells treated with MMS at a concentration of 0.5 mM (▲) or 1 mM (▼).

TABLE 2. Frequency of HSV-TK⁻ mutants after growth in HCMV-infected Vero cells

Expt. no.	Treatment		Virus titer ^a		Mutation frequency
	Cell ^b	Virus ^c	Vero cells	N cIA cl10-TK ⁻ cells	
1	None	UV irradiated	3.4×10^7	2.6×10^3	7.6×10^{-5}
	HCMV infected	UV irradiated	7.9×10^7	1.5×10^3	1.9×10^{-5}
	None	None	1.4×10^8	1.2×10^3	8.5×10^{-6}
	HCMV infected	None	8.5×10^7	8.0×10^2	9.4×10^{-6}
2	None	UV irradiated	2.8×10^7	2.4×10^3	8.6×10^{-5}
	HCMV infected	UV irradiated	5.8×10^7	1.6×10^3	2.8×10^{-5}

^a Virus was harvested 24 h p.i. and assayed in Vero and N cIA cl10-TK⁻ cells. Titers represent averages of duplicate plates.

^b Confluent monolayers of Vero cells were infected with HCMV at a multiplicity of 3 PFU/cell or mock infected 48 h before HSV infection.

^c Virus was irradiated for 5 min at a dose of 20 ergs per mm^2 per s.

although DNA synthesis in treated HCMV-infected cells was suppressed compared with that of untreated cells. These observations suggest that neither inhibition nor stimulation of host cell DNA was directly responsible for enhanced reactivation in HCMV-infected cells. Although the mechanisms remain unclear, early HCMV functions may play a role in this phenomenon (17).

UV-induced or MMS-induced hydroxyurea-resistant DNA synthesis was enhanced by preinfection of cells with HCMV. This enhancement was also shown by CsCl gradient analysis using the combined BUdR density and [^3H]thymidine radioisotope labeling (data not shown). The extent of enhancement was dependent on the UV or MMS dose. Because HCMV did not produce repairable DNA lesions at detectable levels and the total DNA content of HCMV-infected Vero cells was the same as that of uninfected cells at 48 h p.i., as determined by the method of Burton (5) with diphenylamine (unpublished observation), it is reasonable to assume that the enhancement in HCMV-infected cells resulted from an enhanced rate of excision repair. Besides excision repair, damaged mammalian cells also have a repair process termed postreplication repair whereby small segments of newly synthesized DNAs are joined to form DNA of parental size without repairing lesions before DNA replication (14). Because the overall rate of DNA synthesis was enhanced in UV-irradiated HCMV-infected cells (Fig. 3), HCMV stimulates normal DNA replication (Fig. 3) (25), and postreplication repair is closely associated with DNA replication (10, 14), we propose that UV-irradiated HCMV-infected cells have an enhanced rate of postreplication repair compared with mock-infected UV-irradiated cells.

Based on the repair system in bacteria, it has been hypothesized that excision repair is an error-proof process, whereas postreplication repair is an error-prone process leading to production of somatic mutations and cancer (10, 14). In DNA-damaged cells, there may be competition between prereplication and postreplication repair, and DNA replication before DNA repair may lead to cell death, mutation, or cell transformation. Although enhanced repair activities in HCMV-infected cells appeared to be error proof for UV-damaged HSV DNA, the possibility that HCMV might induce error-prone repair pathways for host cell DNA remains undetermined. An appropriate system to determine whether HCMV-infected nonpermissive cells show higher mutation frequencies than uninfected cells when treated with mutagenic agents may provide some insight into virus and chemical cocarcinogenesis.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grants P01 CA 27503 and CA 18450 from the National Cancer Institute.

LITERATURE CITED

1. Aaronson, S. A., and C. D. Lytle. 1970. Decreased host cell reactivation of irradiated SV40 virus in xeroderma pigmentosum. *Nature* (London) **228**:359-361.
2. Albrecht, T., and F. Rapp. 1973. Malignant transformation of hamster embryo fibroblasts following exposure to ultraviolet-irradiated human cytomegalovirus. *Virology* **55**:53-61.
3. Bertazzoni, U., M. Stefanini, G. P. Noy, E. Giulotto, F. Nuzzo, A. Falaschi, and S. Spadari. 1976. Variations of DNA polymerases- α and - β during prolonged stimulation of human lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* **73**:785-789.
4. Brandt, W. N., W. G. Flamm, and N. J. Bernheim. 1972. The value of hydroxyurea in assessing repair synthesis of DNA in HeLa cells. *Chem. Biol. Interact.* **5**:327-339.
5. Burton, K. 1956. A study of the condition and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62**:315-323.
6. DasGupta, U. B., and W. C. Summers. 1978. Ultraviolet reactivation of herpes simplex virus is mutagenic and inducible in mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* **75**:2378-2381.
7. Day, R. S., III, and C. Ziolkowski. 1978. Studies on UV-induced viral reversion, cockayne's syndrome, and MNNG damage using adenovirus-5, p. 535-539. In P. C. Hanawalt, E. C. Friedberg, and C. F. Fox (ed.), *DNA repair mechanisms*. Academic Press, Inc., New York.
8. Furukawa, T., S. Sakuma, and S. A. Plotkin. 1976. Human cytomegalovirus infection of WI-38 cells stimulates mitochondrial DNA synthesis. *Nature* (London) **262**:414-416.
9. Geder, L., R. Lausch, F. O'Neill, and F. Rapp. 1976. Oncogenic transformation of human embryo lung cells by human cytomegalovirus. *Science* **192**:1134-1137.
10. Hanawalt, P. C., P. K. Cooper, A. K. Ganesan, and C. A. Smith. 1979. DNA repair in bacteria and mammalian cells. *Annu. Rev. Biochem.* **48**:783-836.
11. Hirai, K., and Y. Watanabe. 1976. Induction of α -type DNA polymerases in human cytomegalovirus-infected WI-38 cells. *Biochim. Biophys. Acta* **447**:328-339.
12. Huang, E.-S., and J. S. Pagano. 1974. Human cytomegalovirus. II. Lack of relatedness to DNA of herpes simplex I and II, Epstein-Barr virus, and nonhuman strains of cytomegalovirus. *J. Virol.* **13**:642-645.
13. Isom, H. C. 1979. Stimulation of ornithine decarboxylase by human cytomegalovirus. *J. Gen. Virol.* **42**:265-278.
14. Lehman, A. R. 1974. Postreplication repair of DNA in mammalian cells. *Life Sci.* **15**:2005-2016.
15. Lytle, C. D., J. Coppey, and W. D. Taylor. 1978. Enhanced survival of ultraviolet-irradiated herpes simplex virus in carcinogen-pretreated cells. *Nature* (London) **272**:60-62.
16. Lytle, C. D., J. G. Goddard, and C.-H. Lin. 1980. Repair and mutagenesis of herpes simplex virus in UV-irradiated monkey cells. *Mutat. Res.* **70**:139-149.
17. Nishiyama, Y., and R. Rapp. 1980. Enhanced survival of ultraviolet-irradiated herpes simplex virus in human cytomegalovirus-infected cells. *Virology* **100**:189-193.
18. Rapp, F., L. Geder, D. Murasko, R. Lausch, R. Ladda, E.-S. Huang, and M. M. Webber. 1975. Long-term persistence of cytomegalovirus genome in cultured human cells of prostatic origin. *J. Virol.* **16**:982-990.
19. Rapp, F., and N. Turner. 1978. Biochemical transformation of mouse cells by herpes simplex virus types 1

- and 2: comparison of different methods for inactivation of viruses. *Arch. Virol.* **56**:77-87.
20. **Sarasin, A. R., and P. C. Hanawalt.** 1978. Carcinogens enhance survival of UV-irradiated simian virus 40 in treated monkey kidney cells: induction of a recovery pathway? *Proc. Natl. Acad. Sci. U.S.A.* **75**:346-350.
 21. **Sarasin, A. R., C. A. Smith, and P. C. Hanawalt.** 1977. Repair of DNA in human cells after treatment with activated aflatoxin B. *Cancer Res.* **37**:1786-1793.
 22. **Scudiero, D., A. Norin, P. Karran, and B. Strauss.** 1976. DNA excision-repair deficiency of human peripheral blood lymphocytes treated with chemical carcinogens. *Cancer Res.* **36**:1397-1403.
 23. **Sirover, M. A.** 1979. Induction of the DNA repair enzyme uracil-DNA glycosylase in stimulated human lymphocytes. *Cancer Res.* **39**:2090-2095.
 24. **Stinski, M. F.** 1977. Synthesis of proteins and glycoproteins in cells infected with human cytomegalovirus. *J. Virol.* **23**:751-767.
 25. **St. Jeor, S. C., T. B. Albrecht, F. D. Funk, and F. Rapp.** 1974. Stimulation of cellular DNA synthesis by human cytomegalovirus. *J. Virol.* **13**:353-362.
 26. **Tanaka, S., T. Furukawa, and S. A. Plotkin.** 1975. Human cytomegalovirus stimulates host cell RNA synthesis. *J. Virol.* **15**:297-304.
 27. **Weigle, J. J.** 1953. Induction of mutations in a bacterial virus. *Proc. Natl. Acad. Sci. U.S.A.* **39**:628-636.
 28. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. *Bacteriol. Rev.* **40**:869-907.
 29. **Yamanishi, K., M. Fogel, and F. Rapp.** 1980. Effect of caffeine on the replication of nonirradiated and ultraviolet-irradiated cytomegalovirus. *Intervirology* **10**:241-253.
 30. **Yamanishi, K., and F. Rapp.** 1979. Production of plasminogen activator by human and hamster cells infected with human cytomegalovirus. *J. Virol.* **31**:415-419.
 31. **Závada, V., V. Erban, D. Režacová, and V. Vonka.** 1976. Thymidine-kinase in cytomegalovirus infected cells. *Arch. Virol.* **52**:333-339.