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Human Cytomegalovirus Infection Inhibits Cell Cycle Progression at Multiple Points, Including the Transition from G_1 to S

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Human cytomegalovirus inhibits the growth of human foreskin fibroblast cells by 12 h after infection. Analysis of the cellular DNA content of infected cells by flow cytometry demonstrated that cytomegalovirus does not arrest cell cycle progression at a single point. At least two blockages occur, one of which is in the G_1 phase of the cell cycle. The G_1 arrest introduced by cytomegalovirus infection blocks S-phase entry after serum stimulation.

Human cytomegalovirus (HCMV), a member of the betaherpesvirus family, is a ubiquitous pathogen. HCMV infection is generally asymptomatic in healthy children and adults (reviewed in references 7 and 19). However, HCMV can cause severe disease in unborn children and immunocompromised individuals. HCMV is the leading viral cause of birth defects, a life-threatening opportunistic infection in AIDS, and a common complication in allograft recipients (reviewed in references 7 and 19). During productive infection in cultured human diploid fibroblasts, the entire HCMV replication cycle requires approximately 72 h (reviewed in references 27 and 28). The initial events occurring after viral entry, including virus-induced effects on host cell growth, are presumed to be crucial for the establishment of virus replication and viral pathogenesis.

HCMV has been reported to induce S-phase entry in quiescent cells early after virus infection (reviewed in reference 3). In 1974, St. Jeor et al. (35) concluded that the AD169 strain of HCMV induces cellular DNA synthesis in serum-starved confluent human embryonic lung cells, and their experiments indicated that the induced cellular DNA synthesis represented chromosomal replication instead of repair synthesis. Since then, induced cellular DNA synthesis following HCMV infection has been reported for a variety of different cell lines (4, 6, 8, 13). Cellular DNA synthesis was also observed after infection of cells that are nonpermissive for HCMV replication, leading to the suggestion that viral DNA replication and late gene expression are not required for the effect (reviewed in reference 3). Consistent with the induction of cellular DNA synthesis, increased synthesis or activity of a number of cellular components involved in DNA replication, including thymidine kinase, α-type DNA polymerase, and ornithine decarboxylase, has been reported (11, 17, 20, 39).

Although HCMV has been reported to induce cellular DNA synthesis in cells that have been synchronized in the G_0/G_1 stage of the cell cycle by serum starvation or/and contact inhibition, recent findings (1, 21, 23) have suggested that HCMV infection does not concomitantly result in cell division. Instead, HCMV infection inhibits or delays cellular proliferation by inhibiting host cell cycle progression. AbuBakar et al. (1) observed that HCMV infection prevents proliferation of human lung fibroblast cells following subpassage of contact-inhibited monolayers; a substantially reduced mitotic index was observed in the infected cell culture compared with mock-infected cells. Similarly, Jault et al. (21) observed that HCMV infection prevents serum-starved human foreskin fibroblast (HF) cells from proliferating in response to serum stimulation, and they concluded that HCMV infection induces an arrest in the G_2/M compartment of the cell cycle.

So far, most of the studies on the impact on cellular proliferation following HCMV infection have been carried out on cells whose growth has been synchronized by serum starvation or/and contact inhibition, whereas little is known about the impact of HCMV on cell cycle control of growing cells. In this report, we explore the impact of HCMV infection on cell cycle progression when proliferating cells are infected. We first demonstrate that HCMV infection inhibits the growth of HF cells and that this growth inhibition occurs rapidly following virus infection. Analysis of cellular DNA content by flow cytometry demonstrates that HCMV does not introduce a block at a single point in the host cell cycle. Rather, infection results in arrest in both the G_1 and G_2/M phases of the cell cycle. G_1 arrest induced by HCMV infection is demonstrated to block S-phase entry after serum stimulation.

MATERIALS AND METHODS

Cell culture and virus infection. HF cells were propagated in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum. HF cells used in these studies were between passages 8 and 15. Unless otherwise indicated, HF cells were plated at 3×10^6 to 6×10^6 cells per cm^2. Assays using preconfluent cultures were performed the day after plating at the above-specified densities. Assays requiring confluent cultures used cultures that were plated at the densities specified above and allowed to grow to confluence.

HCMV (strain AD169 [31]) was used to infect cells at a multiplicity of 5 PFU per cell, and phenotypically wild-type adenovirus type 5 (Ad5 *dl*309) was used to infect cells at a multiplicity of 100 PFU per cell; mock-infected control cultures were exposed to an equal volume of medium containing the same serum concentration as was present in the virus stocks. Virus adsorptions were carried out for 1 h at 37°C, and 0 h postinfection (hpi) is defined as the time immediately following the 1-h adsorption period. Virus neutralizations were carried out as described previously (25).

To assay cell growth, HF cells were plated as described above in six-well dishes and mock infected or infected with HCMV. At different times after infection, cells were collected by trypsinization and counted in a hemacytometer. Three wells were assayed for each time point.

Inhibition of viral DNA replication. To prevent viral DNA replication, 50 μM ganciclovir (Syntex) was added to the culture medium after virus adsorption. Slot blot assays were performed to confirm the inhibition of HCMV viral DNA replication. Cells were lysed, and total DNA was extracted by overnight proteinase K digestion of cell lysates at 55°C (50 mM Tris [pH 8], 50 mM EDTA, 1% sodium dodecyl sulfate, 100 μg of proteinase K per ml) followed by phenol-chloroform extraction, ethanol precipitation, and RNase treatment (100 μg of RNase A per ml for 1 h at 37°C). Successive fivefold dilutions of the DNA samples were immobilized on a GeneScreen DNA hybridization membrane (New England Nuclear); DNA samples were hybridized with a ^{32}P-labeled DNA fragment obtained from pCGN-IE1 (40) that contains a portion of the HCMV

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IE1 coding region. Signal intensity was quantified with a PhosphorImager (Molecular Dynamics).

Fluorescence-activated cell sorting (FACS) analysis. Cells were labeled with 10 μM bromodeoxyuridine (BrdU; Sigma) for 30 min before harvest. A total of 5×10^5 cells were collected with trypsinization, washed with phosphate-buffered saline (PBS), and fixed with 70% ethanol at −20°C for at least 30 min. Fixed cells were treated with 2 N HCl to remove histones and denature double-stranded DNA. Following neutralization with 0.1 M $Na_2B_4O_7$ (pH 8.5), cells were stained with fluorescein-conjugated anti-BrdU antibody (Becton Dickinson), washed with PBS, and resuspended in PBS containing 10 μg of propidium iodide (Boehringer Mannheim) per ml to measure the DNA content per cell. Propidium iodide- and BrdU-labeled cells were subjected to analysis on an EPICS profile analyzer (Coulter). When required to block cells in the G_2/M phase of the cell cycle, 50 ng of nocodazole (Sigma) per ml was added to the culture medium 12 h before cell harvest.

Assay for stimulation of S-phase entry. Cellular DNA synthesis was monitored by BrdU incorporation (22). Confluent HF cells, which had been directly plated on glass coverslips, were blocked in the G_0/G_1 phase of the cell cycle by incubation in medium supplemented with 0.2% fetal calf serum for 48 h. Prior to mock infection or infection with HCMV or Ad5, the culture medium was removed and stored temporarily. Following the 1-h adsorption, cells were then refed with the original culture medium, and fresh BrdU (10 μM) was added to the medium at 24-h intervals. At different times after infection, cells were fixed with 4% paraformaldehyde in PBS for 10 min at room temperature, rinsed briefly with PBST (PBS with 1% Triton X-100 and 0.5% Tween 20), and reacted with a monoclonal antibody specific for the HCMV IE1 and IE2 proteins (MAB810; Chemicon) or for the Ad5 E1A protein (M73 [16]) in PBST with 0.1% bovine serum albumin (BSA) at room temperature for 1 h. The coverslips were then washed three times with PBST and stained with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (Amersham) in PBST with 0.1% BSA at room temperature for 30 min. After three washes with PBST, coverslips were fixed again in 4% paraformaldehyde, treated with 2 N HCl at 37°C, and subsequently stained with a biotin-conjugated anti-BrdU monoclonal antibody (Zymed) in PBST with 0.1% BSA at room temperature for 1 h. Texas red-conjugated streptavidin (Amersham) was used to visualize BrdU staining. Samples were visualized with a confocal microscope (Bio-Rad model MRC600). At least 500 cells were counted for each sample.

Assay of [^{3}H]thymidine incorporation. Preconfluent HF cells were incubated in medium supplemented with 0.2% fetal calf serum for 48 h. After mock infection or infection with HCMV, cells were overlaid with the original medium. At 12 hpi, medium with 10% fetal calf serum was added to stimulate cell growth. Cells were then incubated for 24-h periods in the presence of 10 μCi of [*methyl*-^{3}H]thymidine ([^{3}H]TdR; specific activity of 71 Ci/mmol; ICN) per ml. At the end of each 24-h labeling period, cells were washed with ice-cold PBS and scraped from the plates, and total DNA was prepared as described above.

To separate viral DNA from cellular DNA, [^{3}H]TdR-labeled DNA was subjected to centrifugation in CsCl solution (1.744 g/ml) at 250,000 × *g* for 14 h. Gradient fractions (100 μl per fraction) were collected by bottom puncture. Solution density was determined from the refractive index ($\rho = 10.8601\eta - 13.4974$ [ρ is density and η is refractive index]), and incorporated radioactivity was determined by scintillation spectroscopy. To determine the total amount of DNA per fraction, a 50-μl aliquot was diluted with 100 μl of H_2O, precipitated with 105 μl of isopropanol, resuspended in 100 μl of TE buffer (10 mM Tris [pH 8], 1 mM EDTA), and subsequently reprecipitated with ethanol precipitation, and the amount of DNA was determined by measurement of A_{260}. Purified viral DNA was analyzed in a separate gradient to serve as a control for the density of viral DNA.

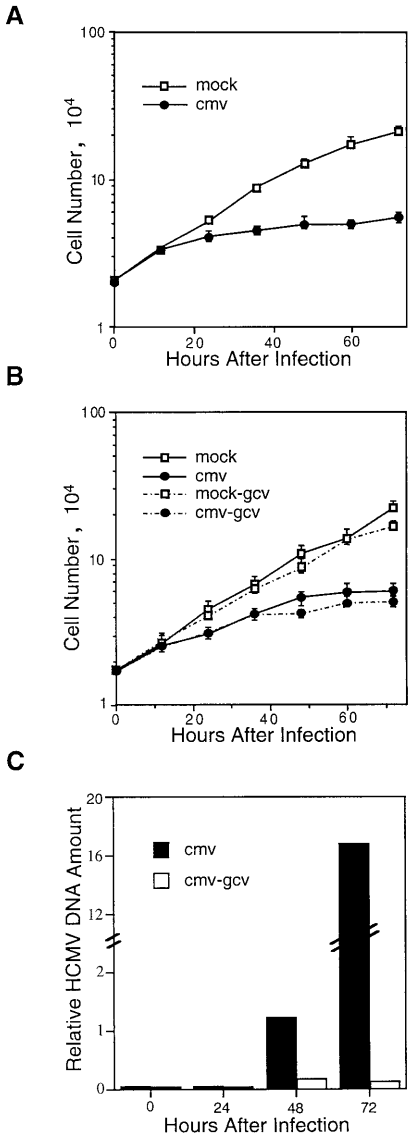

FIG. 1. Growth curve experiments demonstrating the growth-inhibitory effect of HCMV. Cultures of proliferating HF cells were mock infected or infected with HCMV (cmv). (A) At different times after infection, cells were collected by trypsinization and counted. Data are the averages of triplicate determinations. (B) To examine the dependency of the HCMV-induced growth-inhibitory effect on viral replication, ganciclovir (gcv) was added to the culture medium following infection. At different times after infection, cells were collected and counted. Data are the averages of triplicate determinations. (C) To confirm the inhibition of HCMV viral DNA replication by ganciclovir, slot blot assays for viral DNA were performed. Band intensity was quantified with a PhosphorImager.

RESULTS

HF cell growth is rapidly inhibited following HCMV infection. To investigate the impact of HCMV infection on cell growth, cultures of proliferating HF cells were infected, and the number of cells in the cultures was determined at different times after infection (Fig. 1A). The number of the mock-infected HF cells doubled approximately once per 22 h. HCMV-infected HF cells grew at the same rate as the mock-infected cells until 12 hpi, the growth rate of infected cells was slower than that of mock-infected cells between 12 and 24 hpi, and after this time there appeared to be no significant increase in the number of HCMV-infected cells. In other experiments, we have observed an approximately twofold increase in the number of HCMV-infected cells over time following a sharp decline in growth rate at 24 hpi (Fig. 1B). Infections were performed at a multiplicity of 5 PFU per cell, and so >99% of the cells in the culture should be infected. However, at 24 hpi, only about 85% of cells express detectable levels of HCMV immediate-early proteins when assayed by immunofluorescence, and cells that exhibit delayed expression of viral proteins might continue to grow for a period of time. Neutralizing antibody to the gH envelope protein (25) relieved the growth inhibition (data not shown), and so we can conclude that HCMV substantially inhibits the proliferation of HF cells.

Inhibition of HF cell growth by HCMV infection occurs between 12 and 24 hpi, while viral DNA begins to accumulate sometime later than 24 hpi (reviewed in references 27 and 28), suggesting that the growth-inhibitory effect of HCMV does not require viral DNA replication. To confirm that viral DNA replication was not needed to block cell growth, we carried out

a cell growth experiment in the presence of ganciclovir, a specific inhibitor of viral DNA replication (Fig. 1B). When ganciclovir was added to the culture medium following HCMV infection, the level of viral DNA replication was reduced by a factor of about 160 at 72 hpi (Fig. 1C), while the growth-inhibitory effect of HCMV was still observed (Fig. 1B), confirming that the inhibition of cell growth is independent of viral DNA replication.

HCMV blocks progression at multiple points in the cell cycle. The decreased growth rate could result from one of two sources: inhibition of cell proliferation or induction of cell death. No significant cell death was observed by 72 hpi (data not shown), suggesting that HCMV blocks the increase in cell number by halting HF proliferation. Cell proliferation could be blocked by a mechanism that operates independently of the cell's presence in any specific phase of the cell cycle, or it could be blocked by an event specific to a discrete stage of the cell cycle. If HCMV infection induces a specific cell cycle block, then the percentage of infected cells in the cell cycle compartment where the block occurs will be higher than that observed for cells in a mock-infected culture. To determine whether infected cells accumulated in a specific phase of the cell cycle as they cease proliferation, we used FACS to measure the DNA content of cells and distinguish cells in G_1, S, and G_2/M (Fig. 2A). There was no difference in cell cycle distribution between mock-infected and HCMV-infected cells at 12 hpi, and only a small increase in the number of cells in the G_2/M compartment was evident at 24 hpi in infected (27.9%) compared with mock-infected (24.8%) cultures. At 36 hpi, the fraction of HCMV-infected cells with a G_1 or G_2 DNA content was substantially broadened and the number of cells that appeared to be in the S phase increased. Viral DNA replication has begun at this point (Fig. 1C; see also Fig. 5A), and we believe that the change in the FACS profile at this time is substantially due to the accumulation of HCMV DNA, because the increase in DNA content was prevented when ganciclovir was added to the culture medium to inhibit viral DNA synthesis (data not shown). As a result, the 36-hpi data cannot be unambiguously interpreted in terms of the proportion of cells in each cell cycle compartment. Nevertheless, there is little difference in cell cycle distribution between mock-infected and infected cells at 24 hpi, when cell proliferation has substantially decreased (Fig. 1A and B) and viral DNA has not begun to accumulate (Fig. 1C). Since an excess number of

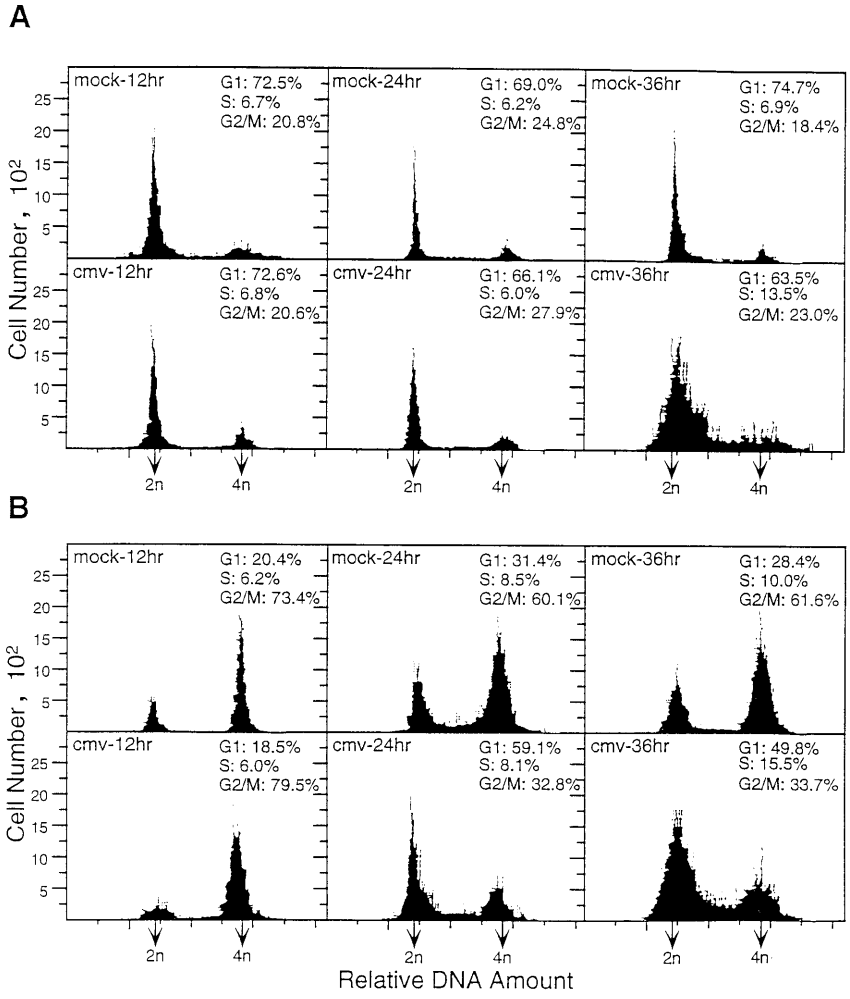

FIG. 2. FACS analysis demonstrating that cell cycle progression is arrested at multiple points following HCMV infection. Cultures of proliferating HF cells were mock infected or infected with HCMV (cmv). (A) At different times after infection, cells were harvested and cellular DNA content was stained with propidium iodide and subjected to analysis on an EPICS profile analyzer. (B) Twelve hours before cell harvest, nocodazole was added to the culture medium. After harvesting, cellular DNA content was analyzed. Data shown in panels A and B are from a single experiment representative of three independent experiments with similar results.

infected cells did not accumulate in an individual cell cycle compartment, blocks must occur at a minimum of two points in the cell cycle, one in G_1 and one in G_2/M.

Jault et al. (21) have recently shown that HCMV infection leads to a cell cycle block in the G_2/M compartment, and so we focused our attention on the apparent block in G_1. Our initial approach was to block proliferating cells in the G_2/M compartments by treatment with nocodazole and then to test whether HCMV infection perturbed the ability of cells to accumulate at the drug-induced block. If HCMV induces a block in the G_1

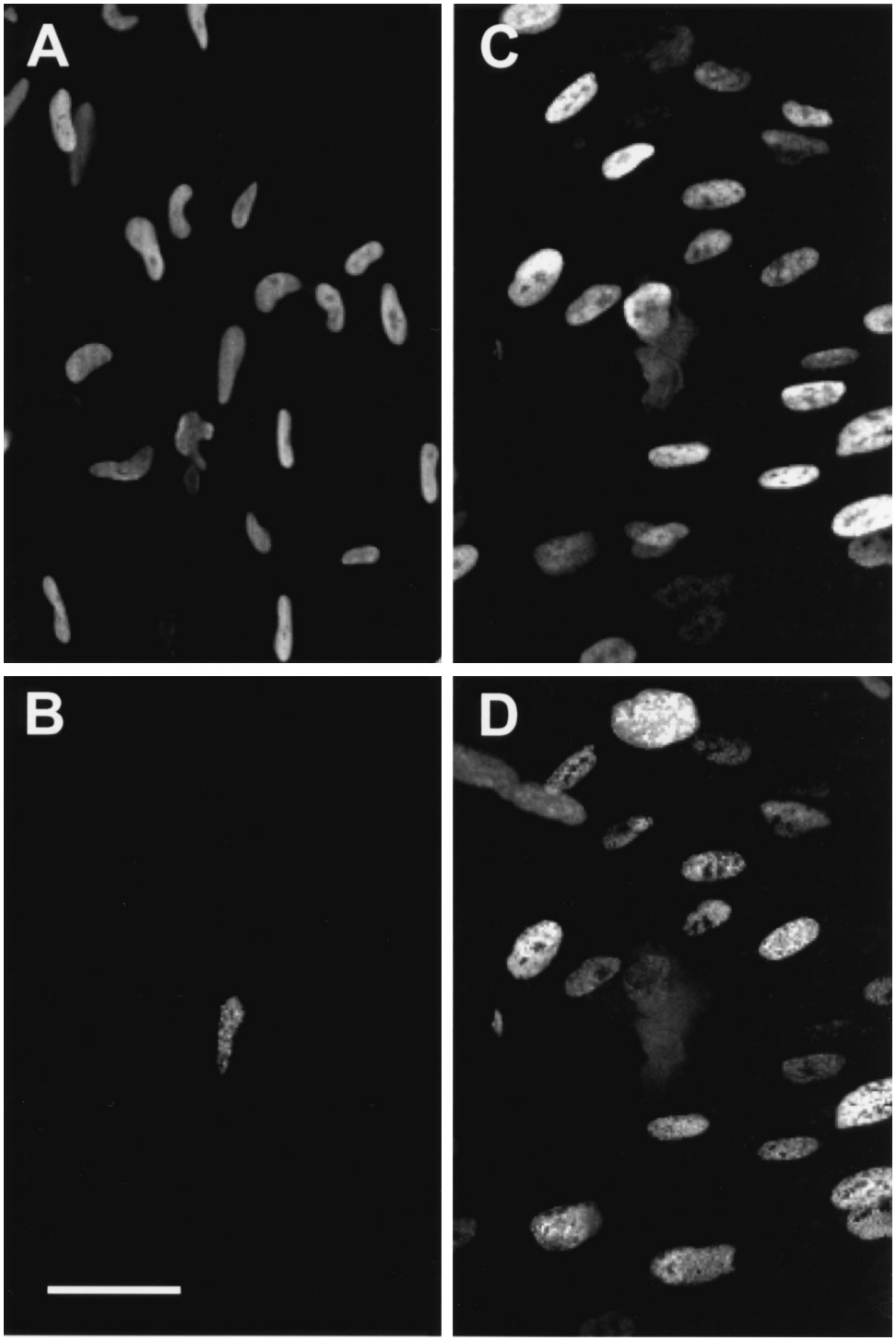

FIG. 3. Confocal immunofluorescence assays demonstrating that HCMV infection does not significantly induce S-phase entry. After synchronization in the G_0/G_1 phase by serum starvation, confluent HF cells were infected with HCMV (A and B) or Ad5 (C and D). BrdU was added immediately after virus adsorption. At 24 hpi, cells were fixed and assayed by double immunofluorescence for expression of viral antigens (HCMV IE1 and IE2 [A] or Ad5 E1A [C]) and for DNA synthesis (B and D). The bar represents 50 μm.

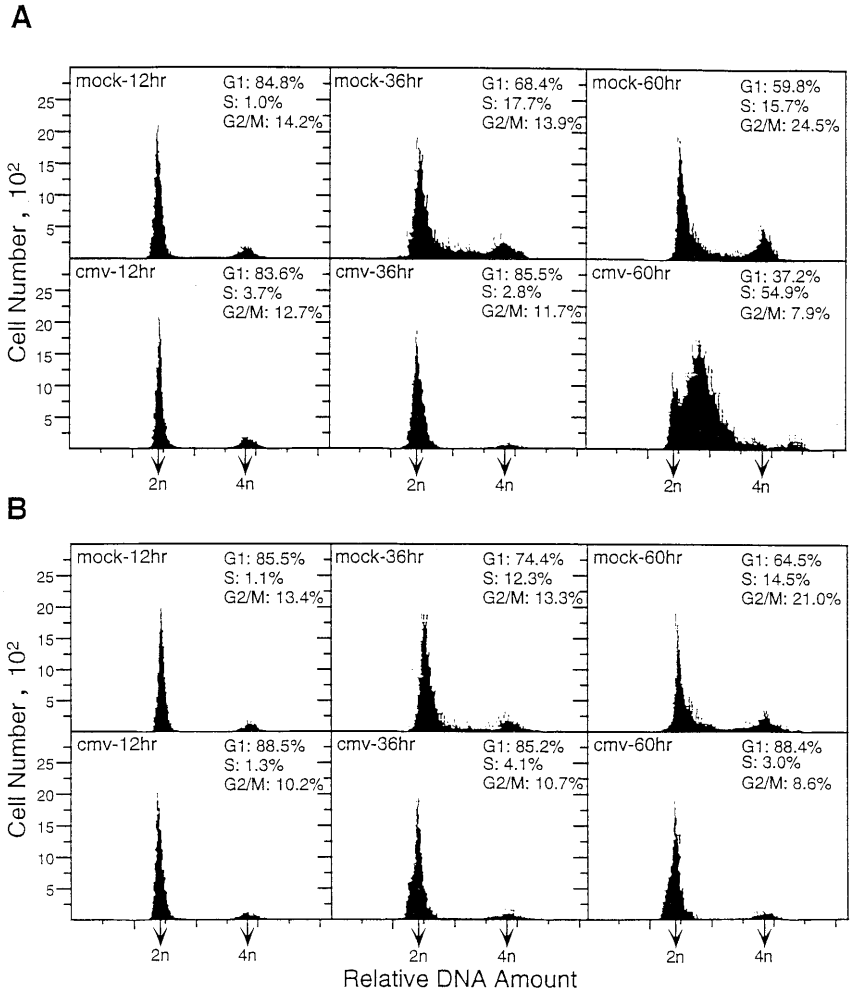

FIG. 4. FACS analysis demonstrating the suppression of S-phase entry following HCMV infection. After synchronization in the G_0/G_1 phase by serum starvation, preconfluent HF cells were mock infected or infected with HCMV (cmv). At 12 hpi, medium with 10% fetal calf serum was added to stimulate cell growth. (A) At different times after infection, cells were harvested and cellular DNA content was analyzed. (B) To prevent viral DNA replication, ganciclovir was added to the culture medium following infection. Cells were harvested at different times after infection, and cellular DNA content was analyzed. Data shown in panels A and B are from a single experiment representative of three independent experiments with similar results.

compartment, then fewer cells should accumulate in G_2/M in the presence of the drug. Accordingly, HCMV-infected and mock-infected HF cells were treated with 50 ng of nocodazole per ml for 12 h before harvesting at different times after infection. As would be expected, in the presence of nocodazole (Fig. 2B), a much higher proportion of cells accumulate in the G_2/M compartment than was observed in the absence of the drug (Fig. 2A). At 12 h, both mock-infected and HCMV-infected cells accumulated to high levels (>75% of the cell population) in the G_2/M compartment (Fig. 2B). Similar fractions of the two cell populations resided in the G_1 and G_2/M compartments, suggesting that no block in G_1 exists at this time after infection. However, at 24 h, the cell cycle distribution of mock-infected and infected cultures is quite different (Fig. 2B). The G_1 population consisted of a much higher proportion of cells in the infected (59.1%) than in mock-infected (31.4%) cultures. A similar increase in G_1 cells was observed at 36 h (Fig. 2B), although the increase in the G_1 compartment is probably underestimated as a result of HCMV DNA replication, which increases cell DNA content and moves a portion of G_1 cells into the S and G_2/M regions of the FACS profile. The increase in the number of infected compared with mock-infected cells in the G_1 compartment of nocodazole-treated cultures is consistent with an HCMV-induced block in the G_1 phase of the cell cycle.

HCMV infection does not significantly induce S-phase entry. Our FACS analyses demonstrating a block in the G_1 compartment were carried out on asynchronized cells. To further examine the G_1 arrest following HCMV infection, we determined whether HF cells, synchronized in the G_0/G_1 phase by maintenance in medium containing 0.2% fetal calf serum, can enter the S phase of the cell cycle following HCMV infection and continued maintenance in low serum. We used a BrdU incorporation assay to examine whether cells infected in the G_0 phase of the cell cycle moved into the S phase and synthesized DNA. At 24 hpi, 85% of HCMV-infected cells stained positive for the expression of viral immediate-early proteins, IE1 and IE2 (Fig. 3A), but only 3% of these cells stained positive for BrdU incorporation (Fig. 3B), which is similar to the percentage of mock-infected G_0/G_1 cells that stained positive for BrdU incorporation (data not shown). Adenovirus, which is known to induce cells to enter the S phase subsequent to infection (32, 37, 38), was also tested in a control experiment. When HF cells synchronized in the G_0/G_1 phase by mainte-

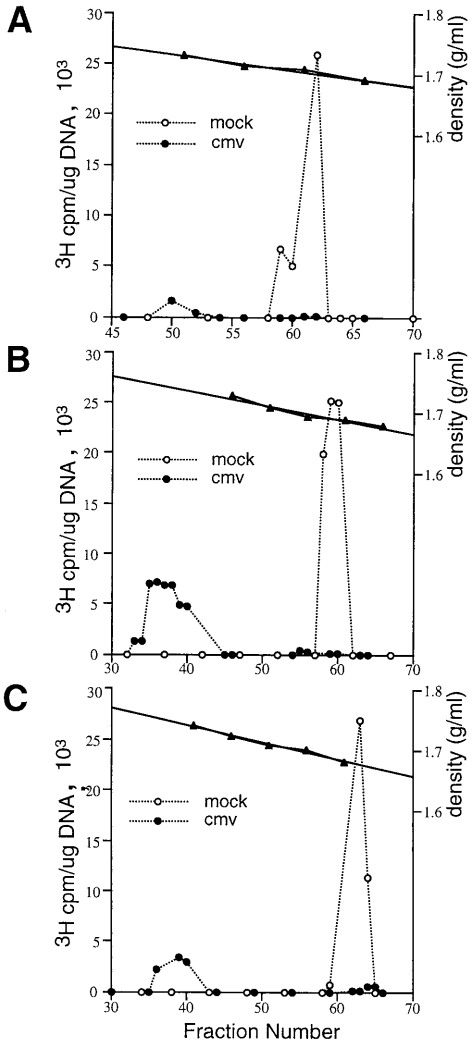

FIG. 5. Equilibrium density centrifugation analysis demonstrating the suppression of S-phase entry following HCMV infection. After synchronization in the G_0/G_1 phase by serum starvation, preconfluent HF cells were mock infected or infected with HCMV (cmv). At 12 hpi, medium with 10% fetal calf serum was added to stimulate cell growth. At 12, 48, and 84 hpi, [^{3}H]TdR was added to the culture medium. After a 24-h incubation, cells were harvested at 36 hpi (A), 72 hpi (B), and 108 hpi (C). DNA was extracted and subjected to CsCl gradient centrifugation. For each collected fraction, density was calculated from its refractive index ($\rho = 10.8601\eta - 13.4974$); [^{3}H]TdR incorporation was determined by scintillation spectroscopy, and total DNA amount was measured by A_{260}. Data shown are from a single experiment representative of three independent experiments with similar results.

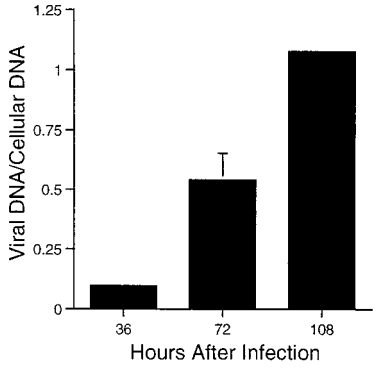

FIG. 6. Relative amounts of viral and cellular DNA in HCMV-infected cells. Preconfluent HF cells were infected and DNA was analyzed as described in the legend to Fig. 5. The ratio of the amount of viral DNA to that of cellular DNA is plotted at different times after infection. Data shown are the average values from two independent experiments.

nance in medium containing 0.2% serum were infected with Ad5, 45% of E1A-expressing cells showed BrdU incorporation at 24 hpi (Fig. 3C and D). The increased BrdU incorporation in the adenovirus-infected cells was not due to viral DNA replication, because Southern blot analysis demonstrated that adenovirus DNA replication had not started by 24 hpi (data not shown). In contrast to adenovirus, HCMV does not significantly induce cells to move from G_0 into the S phase of the cell cycle.

HCMV infection inhibits S-phase entry after stimulation with serum. Since HCMV blocked cell cycle progression in asynchronized HF cell populations maintained in medium containing 10% fetal calf serum, we suspected that HCMV infection might block S-phase entry after serum stimulation of cells residing in the G_0/G_1 compartment. To test this possibility, HF cells were synchronized in the G_0/G_1 phase by maintenance in 0.2% serum and then either mock infected or infected with HCMV. Twelve hours later, cultures were refed with medium containing 10% fetal calf serum to reverse the G_0/G_1 block. Mock-infected cells started to proliferate in the serum-rich medium, while the number of HCMV-infected cells did not increase (data not shown). FACS analysis revealed that by 24 h after serum addition, the number of mock-infected cells in the G_0/G_1 compartment decreased from 85.0 to 68.4% as the mock-infected cells reentered the S phase (Fig. 4A, mock-36hr). In contrast, the entry of cells into the S phase was completely suppressed in the HCMV-infected cells (Fig. 4A, cmv-36hr). With additional time, a high proportion of cells exhibited a cellular DNA content greater than 2*n* (Fig. 4A, cmv-60hr). However, the increased DNA content is not due to the increased synthesis of cellular DNA but rather is due to the replication of HCMV viral DNA, because the increase in DNA content was prevented when ganciclovir was added to the culture medium to inhibit viral DNA synthesis (Fig. 4B, cmv-60hr).

S-phase entry after serum stimulation was also monitored by measuring the amount of [^{3}H]thymidine incorporated into newly synthesized DNA. HF cells synchronized in the G_0/G_1 compartment by maintenance in medium containing 0.2% serum were either mock infected or infected with HCMV. Cultures were refed with medium containing 10% fetal calf serum, and cells were labeled with [^{3}H]thymidine for the final 24 h before DNA was extracted at 36, 72, and 108 h. Samples were subjected to density gradient centrifugation to separate viral DNA from cellular DNA. Much more cellular DNA was synthesized in mock-infected than infected cells at each time assayed (Fig. 5). In fact, little if any ^{3}H-labeled DNA banding at the density of cellular DNA was produced in HCMV-infected cells, while ^{3}H-labeled DNA banding at the density of HCMV DNA was evident. The amount of viral DNA relative to cellular DNA increased with time, until at 108 hpi, the cultures contained equal amounts of viral and cellular DNA (Fig. 6).

Taken together, the FACS and equilibrium density experiments demonstrate that the G_1 arrest introduced by HCMV infection blocks S-phase entry after serum stimulation.

DISCUSSION

St. Jeor et al. (35) reported some years ago that infection of growth-arrested primary human embryonic lung cells with

HCMV led to the induction of cellular DNA synthesis. Viral and cellular DNAs were distinguished by equilibrium density centrifugation, and UV-irradiated virus failed to induce cellular DNA synthesis. Similar reports of HCMV-induced cellular DNA replication by others (4, 6, 13, 36, 39), coupled with the observations that HCMV infection can induce several enzymes that function in DNA metabolism (5, 11, 20) and that viral immediate-early proteins deregulate the S-phase-specific E2F transcriptional activator protein (15, 26, 33), led to the view that HCMV induces infected cells to enter the S phase of the cell cycle and synthesize cellular DNA (reviewed in references 3 and 28).

Our present results argue that HCMV does not induce infected human diploid fibroblasts to enter the S phase or synthesize cellular DNA. HCMV blocks cellular proliferation (Fig. 1) and institutes blocks to cell cycle progression that cause cells to accumulate in the G_1 and G_2/M compartments (Fig. 2). In contrast to adenovirus, HCMV is not able to induce G_0-arrested cells to enter the S phase and synthesize cellular DNA (Fig. 3). In fact, HCMV blocks S-phase entry and cellular DNA replication when cells residing in the G_0 compartment are stimulated with serum (Fig. 4 and 5). D. Dittmer and E. S. Mocarski also have concluded that HCMV infection blocks the progression of HF cells beyond the G_1/S border (10a). Thus, in permissive human diploid fibroblasts, HCMV blocks cellular DNA replication and proliferation as does herpes simplex virus (3, 30) and the more closely related (14) human herpesvirus 6 (12, 18).

The reason for the discrepancy between our results and some of the earlier work discussed above is not entirely clear. It is possible that some of the earlier studies (35, 39) used too low a multiplicity of infection, and the cellular DNA replication that was monitored actually occurred in cells that were not infected. In other cases, cellular DNA replication might have resulted from a high proportion of defective virus in HCMV stocks. Virus stocks that were serially passaged without dilution and stocks that were UV irradiated have been reported to induce cellular DNA replication in a portion of the infected culture, whereas no cellular replication was detected in cultures infected with virus stocks derived from plaque-purified virus that was passaged at low input multiplicities (9, 10). Further, DeMarchi (8) observed cellular DNA replication in nonpermissive rabbit kidney cells that expressed some, but not all, HCMV immediate-early and early viral gene products after infection. Possibly, expression of a subset of viral genes from defective or UV-inactivated genomes or infection of a cell type that limits viral gene expression can lead to the induction of cellular DNA synthesis, but when the entire HCMV gene repertoire is expressed in permissive human cells, cellular DNA synthesis is blocked. This is an intriguing possibility since it predicts that HCMV encodes gene products that can both stimulate and block cellular DNA synthesis. Possibly, subsets of the viral genes are expressed in different cell types or under different physiological conditions in natural infections. Conceivably, this could lead to cell growth in some instances and a block to proliferation in others.

Since HCMV-infected cells which had ceased proliferation did not accumulate in a single cell cycle compartment (Fig. 2A), HCMV must institute blocks at a minimum of two points in the cell cycle. We have documented a block in late G_1, and Jault et al. (21) recently described a block in G_2/M. We cannot be certain whether there are more than two points at which HCMV institutes blocks to cell cycle progression. However, examination of the FACS data for infected and mock-infected cells (Fig. 2A) raises the possibility that a virus-induced block also occurs in the S phase. At 24 h after infection, when cells have substantially ceased proliferation (Fig. 1), the percentages of cells in the S phase of the cell cycle are similar in infected (6.0%) and mock-infected (6.2%) cultures. If infected cells contain blocks to cell cycle progression in only G_1 and G_2, one would predict that the S compartment would empty as cells move from S to G_2 and no new cells move from G_1 to S. Since a portion of the population continues to reside in the S phase (Fig. 2A), it seems likely that there is also a block to cell cycle progression in this compartment. A similar result is evident in nocodazole-blocked cultures (Fig. 2B); the S compartment does not empty in cells that are blocked in both the G_1 and G_2 compartments. It is conceivable that progression is frozen at whatever point in the cell cycle the infected cells happen to reside as the virus-encoded or induced cellular product responsible for the block reaches a critical concentration.

We do not yet know the mechanism by which HCMV induces blocks to cell cycle progression. The cell cycle blocks occur in the absence of HCMV DNA replication, and so they must not depend on the synthesis of late viral proteins. Cell cycle progression could be modulated by immediate-early or early viral gene products or by virion proteins. Alternatively, the interaction of the virus with its receptor could initiate a cascade of events that lead to the cell cycle blocks, although this seems unlikely since UV-inactivated virus has been reported to induce cellular DNA replication (9, 10).

The viral gene products might act through the cellular tumor suppressor, p53, to block cell cycle progression. HCMV infection causes a substantial increase in the level of p53 (29, 34), and high levels of p53 can lead to arrest in both the G_1 and G_2/M compartments of the cell cycle (2, 24). Therefore, elevated p53 levels could account for blocks to cell cycle progression in multiple compartments, as is observed in HCMV-infected cells. However, the activity of p53 might be inhibited in infected cells by the HCMV IE2 protein, which reportedly binds to p53 and inhibits its transcriptional regulatory function (34). Work is in progress to identify the viral proteins that are responsible for instituting cell cycle blocks in HCMV-infected cells.

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