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Human Cytomegalovirus Elevates Levels of the Cellular Protein p53 in Infected Fibroblasts

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Human cytomegalovirus (HCMV), like other DNA tumor viruses, induces morphological transformation of cells in vitro and stimulates host cell macromolecular synthesis in infected cells. Since other DNA tumor viruses, such as simian virus 40 and adenovirus, have previously been shown to interact with cellular protein p53, we investigated whether infection of cells by HCMV would modulate cellular p53 levels. Our results indicate that HCMV elevates cellular p53 levels on the order of 10- to 20-fold in infected fibroblasts. The induction of elevated p53 levels was dependent upon the presence of active virus and was prevented by neutralizing antibody. The induction of elevated p53 levels was determined not to be due to virus-receptor interactions or HCMV late events. The induction of elevated p53 levels commenced at immediate-early times of the HCMV multiplication cycle (6 h postinfection) and reached maximal levels by 24 h postinfection, before most of the HCMV DNA synthesis was initiated. HCMV immediate-early proteins were clearly shown to be responsible for elevating p53 levels in infected fibroblasts; expression of HCMV immediate-early region 1 and 2 proteins resulted in elevation of p53 levels in transfected human fibroblasts. This is the first report of increased p53 levels caused by HCMV in infected fibroblasts.

Cellular protein p53 is classified as a tumor suppressor gene product (15, 19, 25, 27). The precise biochemical activities and functions of p53 are not clear, and it might well be that p53 has multifunctional regulatory properties (25, 34, 39, 44, 51, 54). Wild-type p53 regulates normal cell proliferation by arresting the cell cycle in the G_1 phase (32, 36). It also acts as a "molecular policeman" by accumulating in response to DNA damage and inhibiting proliferation while repair takes place (21, 22, 24). If repair fails, p53 continues to accumulate until apoptosis occurs (10, 30). Levels of p53 in normal cells are thus kept quite low, and the half-life of the protein is short (6 to 30 min) (42, 43). Information concerning factors that alter cellular p53 levels is thus of scientific and practical importance.

Infection and transformation of cells by certain viruses have been shown to alter cellular p53 levels. Levels of p53 are elevated in cells infected by simian virus 40 (SV40) (11, 28) and adenovirus (45). Studies have shown that the interaction of p53 with SV40 large T antigen in infected and transformed cells and with the adenovirus E1b 55-kDa protein in transformed cells results in a significant increase in the half-life of p53 (26). The interaction of p53 with human papillomavirus type 16 and 18 E6 appears to lead to degradation of p53 (46). It is believed that both mechanisms lead to inactivation of the p53 function. Since p53 is important in cellular functioning, an investigation of the extent of its involvement in the transformation and infection process of other DNA tumor viruses, such as herpesviruses, is necessary. No studies have been reported concerning any interactions between human cytomegalovirus (HCMV) and p53 in permissive infected cells.

HCMV, a herpesvirus, is an important human pathogen, causing widespread reactivable infections in humans (18, 47, 52) and possessing proven oncogenic potential (3, 6, 16, 20, 40). HCMV, like other DNA tumor viruses, morphologically transforms cells in vitro and stimulates host cell macromolecular synthesis in infected cells (2, 20, 40). In this study, we investigated whether infection of human fibroblasts by HCMV affects the level of the cellular p53 protein. We found that p53 protein levels were 10- to 20-fold higher in HCMV-infected cells than in uninfected control cells. Induction of the elevated p53 levels observed in infected cells was dependent on the presence of active HCMV and was prevented by the presence of HCMV neutralizing antibody in the medium. Induction of elevated p53 levels commenced at immediate-early (IE) times of the HCMV multiplication cycle. The involvement of HCMV IE proteins in the induction of elevated p53 levels was shown by transfection of human embryonic lung (HEL) fibroblasts with a plasmid DNA construct expressing HCMV IE region 1 and 2 proteins.

MATERIALS AND METHODS

Cells and virus. HEL, WI38, and MRC5 fibroblasts (American Type Culture Collection, Rockville, Md.) at passages 14 to 24 were used in these experiments. Cell lines were passaged in Eagle's minimal essential medium supplemented with 2 mM glutamine, 10% fetal bovine serum (FBS; Gibco), and penicillin-streptomycin. Cells were infected with HCMV strain Towne as previously described (38) and as indicated in the figure legends, by using medium containing 4% heat-inactivated FBS. Mock-infected control cells were treated with 4% heat-inactivated FBS-containing medium without virus.

Antibodies. Mouse anti-p53 monoclonal antibodies DO-1 and PAb1801 were obtained from Oncogene Science, Inc., San Diego, Calif. The mouse p53 polyclonal antibody and mouse anti-HCMV IE antibody (MAB810; specific for both region 1 and region 2 proteins) were purchased from Chemicon International, Temecula, Calif. Anti-HCMV IE antibody MAB810 very readily detects the major IE region 1 72K protein (which is in abundance), as well as the IE region 2 84K protein; with increased sensitivity, the 50-55K IE region 2 protein is also detected (33). Mouse HCMV neutralizing antibody 14-4b, directed against gp86, and control mouse nonneutralizing mouse antibody 28-19, directed against HCMV pp65, were a

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generous gift of William Britt, University of Alabama at Birmingham. The secondary antibody, a horseradish peroxidase-conjugated goat anti-mouse antibody, was purchased from Kirkegaard & Perry Laboratories, Inc.

Plasmids. HCMV IE gene region 1 and 2-expressing plasmid pHD101SV1 and control plasmid pSVOCMV1 were obtained from Eng-Shang Huang, University of North Carolina at Chapel Hill. Plasmid pHD101SV1 has previously been described (12). Plasmid pHD101SV1 contains the *Eco*RI fragment of the HCMV genome from map units 0.72 to 0.76 inserted into the *Eco*RI site of plasmid pSVOH; expression of IE region 1 and 2 proteins from this fragment is under control of the HCMV IE promoter (12). Plasmid pSVOCMV1 lacks the coding region for HCMV IE region 1 and 2 proteins but contains the HCMV IE promoter.

DNA transfections. Plasmid DNA was purified by column chromatography (Qiagen, Inc.). HEL cells at 50 to 80% confluence in 75-cm^2 flasks were transfected with 20 μg of plasmid DNA by using the lipofectamine procedure (Life Technologies, Inc.) in accordance with the manufacturer's instructions. The lipofectamine reagent-DNA complexes were incubated with the cells for 5 h. Extractions were performed 72 h later, and Western blot (immunoblot) analysis was performed as described below.

Immunoblot analysis. Cells were washed in TBS 7.5 (10 mM Tris, [pH 7.5], 150 mM NaCl) and lysed at 4°C by gentle vortexing in lysis buffer (50 mM Tris [pH 7.5], 1 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.5% sodium dodecyl sulfate [SDS], 0.02% NaN_3, 0.0004% NaF, 1 mM phenylmethylsulfonyl fluoride, 2 μg of aprotinin per ml, 0.1 mM leupeptin, 0.1 mg of phosphoramidon per ml). The 16,000 × *g* supernatants were then adjusted to 1 × SDS sample buffer (0.0625 M Tris [pH 6.8], 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.001% bromophenol blue), and equal quantities of each sample were loaded onto an SDS–10% polyacrylamide gel with a 4% stacking gel as described by Laemmli (23). Protein bands were then electroblotted onto polyvinylidene difluoride membranes. The blots were then incubated with TBST containing 5% nonfat powdered milk (10 mM Tris [pH 7.5], 150 mM NaCl, 0.05% Tween 20, 5% nonfat powdered milk) for 1 h at room temperature to block nonspecific sites. The blots were then incubated with the appropriate primary antibody (DO-1 against p53 at 1:100 or MAB810 against HCMV IE antigens at 1:1,000) and processed throughout in accordance with the antibody manufacturer's instructions. The control antibody used was mouse immunoglobulin G (from Sigma Chemical Co.). In most experiments, the secondary antibody used was a horseradish peroxidase-conjugated goat anti-mouse antibody (1:1,000); antigen levels were then detected by a chemiluminescent substrate (Kirkegaard & Perry Laboratories, Inc.). Light emission was measured by using X-ray film. Quantitation was performed by densitometer tracing.

Virus neutralization studies. Equal aliquots of HCMV (~10^7 PFU/ml), enough to infect cells at 1 to 2 PFU per cell, were reacted with hybridoma tissue culture fluid containing control or HCMV neutralizing antibodies for 1 h at 37°C. Most experiments were performed by reacting 50 to 66 μl of a virus suspension with full- or half-strength antibody-containing fluid in a total volume of 1.3 ml. The reacted virus preparations were then used to infect 5 × 10^5 cells in 25-cm^2 tissue culture flasks; adsorption was for 1 h at 37°C. Additional control samples contained cells infected with virus previously incubated with medium at 37 or 25°C, as well as cells mock infected with medium, control antibody, and neutralizing antibody. The virus preparation was removed from the cells after the 1-h adsorption and replaced with medium containing 4% heat-inactivated FBS. Extractions were performed at 24 h postinfection.

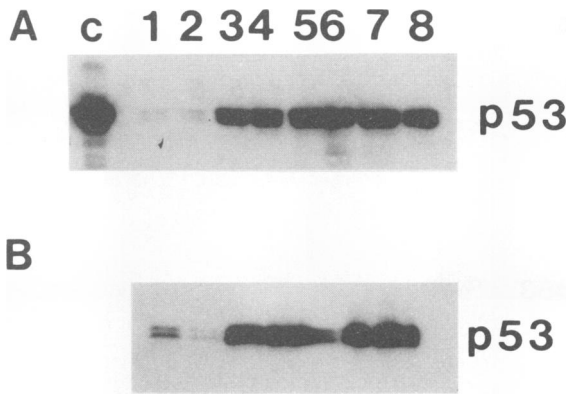

FIG. 1. Immunoblot analysis of p53 protein levels in HCMV-infected (0 to 120 h) HEL cells. HEL cells were infected with HCMV at 2 and 6 PFU per cell, and extractions were performed at various times postinfection. Extracts containing 50 μg of protein were analyzed by immunoblotting (with p53 antibody DO-1) and chemiluminescence detection. (A) Extracts of cells infected with 2 PFU per cell. (B) Extracts of cells infected with 6 PFU per cell. Lanes: 1, mock-infected cells extracted at 0 (A) and 72 (B) h postinfection; 2, HCMV-infected cells extracted at 0 h postinfection; 3 to 8, HCMV-infected cells extracted at 24, 48, 60, 72, 96, and 120 h postinfection, respectively; c, p53 positive control cell line Cos-1.

RESULTS

Immunoblot analysis of p53 protein levels in human fibroblasts after HCMV infection. HEL fibroblasts were infected with HCMV, and extractions were performed at various times postinfection. Figure 1 shows representative results obtained by using chemiluminescence detection and anti-p53 monoclonal antibody DO-1 on cells infected at virus doses of 2 (panel A) and 6 (panel B) PFU per cell. Levels of p53 increased approximately 10- to 20-fold (as determined by densitometer tracing) upon infection of HEL cells with HCMV (lanes 3 to 8) compared with those in mock-infected cells (lane 1) or infected cells extracted at 0 h postinfection (lanes 2). Induction of elevated p53 levels in HCMV-infected cells was maximal by 24 h postinfection (lane 3) and did not increase at later times postinfection (lanes 4 to 8), regardless of whether a viral dose of 2 (panel A) or 6 (panel B) PFU per cell was utilized. The failure to detect a further increase in p53 levels beyond 24 h postinfection was not due to experimental artifacts, since much higher levels of p53 were confirmed in Cos-1 cells (lane C). The same results were obtained with various other p53 antibodies (PAb1801 and a p53 polyclonal antibody from Chemicon) and a colorimetric method of detection. All of the p53 antibodies were demonstrated to be specific for p53, since the control antibody did not react with p53 (data not shown).

To investigate whether the elevation of p53 in cells infected with HCMV was not a characteristic unique to HEL cells, other permissive cells were infected with HCMV. Both WI38 and MRC5 human fibroblasts infected with HCMV at 2 PFU per cell had elevated p53 levels comparable to those observed in HEL cells (data not shown). This indicated that infection with HCMV causes elevation of p53 levels in various permissive human fibroblastic cells.

Elevation of p53 protein levels in HCMV-infected cells is dependent on the presence of active virus. To investigate whether HCMV was indeed responsible for the induction of

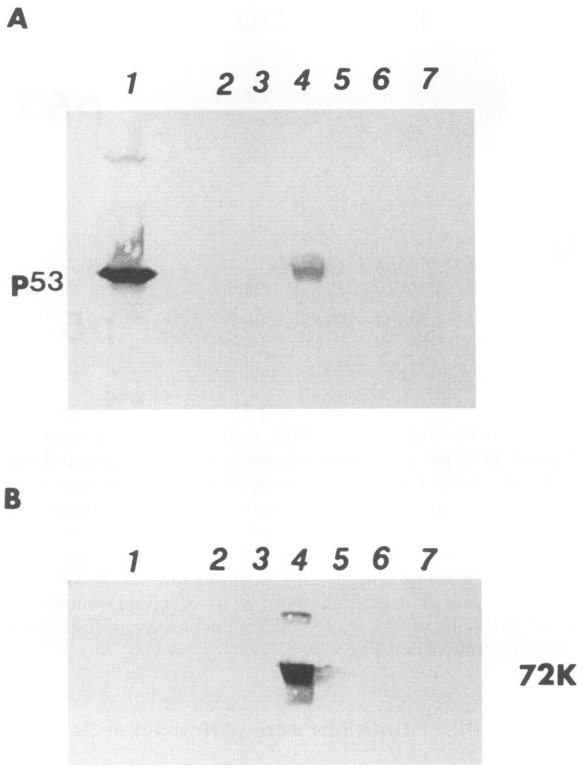

FIG. 2. Effects of virus inactivation and removal on elevation of p53 levels in HCMV-infected cells. Equal aliquots of HCMV enough to infect cells at 1 to 2 PFU per cell were either heat inactivated, left untreated, or passed through a 0.1-μm-pore-size Millipore filter (Ultrafree-CL low-protein-binding Durapore membrane filter used to retain the virus). The aliquots were then used to infect HEL cells. Extractions were performed at 24 h postinfection, and immunoblot analysis (of 100 μg of protein per lane) was performed with alkaline phosphatase-conjugated goat anti-mouse antibody (0.2 μg/ml; Oncogene Science, Inc.) as the secondary antibody and a color reaction. (A) p53 protein levels detected by p53 antibody DO-1. (B) HCMV IE antigen levels detected by HCMV antibody MAB810. Lanes: 1, Cos-1, a p53 positive control cell line; 2 and 3, mock-infected HEL cells maintained in 10 and 4% FBS-containing media, respectively; 4, HEL cells infected with active HCMV (no heat inactivation); 5, HEL cells infected with heat-inactivated HCMV (56°C, 30 min); 6, HEL cells infected with a 0.1-μm HCMV filtrate (no heat inactivation); 7, HEL cells infected with a heat-inactivated (56°C, 30 min) 0.1-μm HCMV filtrate.

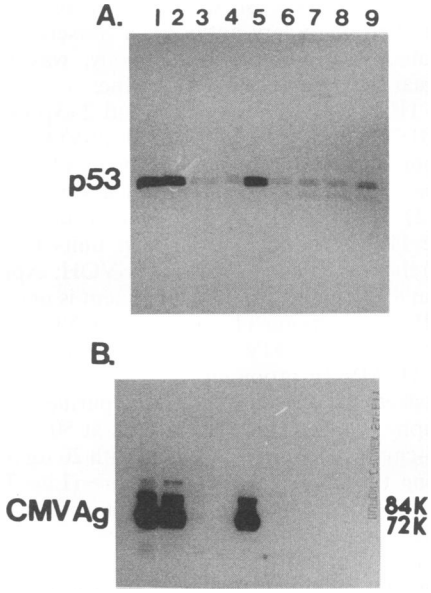

FIG. 3. Effect of neutralizing the infectivity of HCMV on the elevation of p53 levels in infected cells. Equal aliquots of HCMV (10^7 PFU/ml and enough to infect cells at 1 to 2 PFU per cell) were treated as described in Materials and Methods and then used to infect HEL cells. Extractions were performed at 24 h postinfection, and immunoblot analysis was performed as described in Materials and Methods. (A) Levels of p53 protein (detected by p53 antibody DO-1) in 25 μg of extracts. (B) Levels of HCMV IE antigen (detected by antibody MAB810) in 10 μg of extracts. Lanes: 1, cells infected with an HCMV aliquot which had been kept at 25°C for 1 h; 2, cells infected with an HCMV aliquot which had been kept at 37°C for 1 h; 3 and 4, cells infected with an HCMV aliquot which had been reacted at 37°C for 1 h with HCMV neutralizing monoclonal antibody 14-4b (directed against HCMV gp86) at full strength and half strength, respectively; 5, cells infected with an HCMV aliquot which had been reacted at 37°C for 1 h with control HCMV nonneutralizing monoclonal antibody 28-19 (directed against pp65) at full strength; 6, cells mock infected with medium which had been kept at 37°C for 1 h; 7 and 8, cells mock infected with media containing a neutralizing antibody at full strength and at half strength, respectively; 9, cells mock infected with a control nonneutralizing antibody at full strength. CMVAg, cytomegalovirus antigen.

elevated p53 protein levels in HCMV-infected cells, the experiment whose results are shown in Fig. 2A and B was performed. Similar aliquots of HCMV Towne strain (needed to infect HEL cells at 2 PFU per cell) were subjected to heat inactivation at 56°C for 30 min (lane 5), filtration through a 0.1-μm-pore-size Millipore filter (Ultrafree-CL low-protein-binding Durapore membrane filter; catalog no. UFC4 OVV 25) (lane 6), or filtration through a 0.1-μm-pore-size filter followed by heat inactivation at 56°C for 30 min (lane 7) or left untreated (lane 4). The aliquots were then used to infect HEL cells. HEL cells infected with heat-inactivated virus (Fig. 2B, lanes 5 and 7) or the 0.1-μm virus filtrate (Fig. 2B, lane 6) failed to show a cytopathic effect (data not shown) or detectable HCMV IE antigens; this implied that the virus in these preparations was either inactivated by heat or filtered out of the aliquots. These preparations lacking active virus failed to induce elevated levels of p53 upon infection of HEL cells (Fig. 2A, lanes 5 to 7), while untreated control aliquots containing active virus (Fig. 2B, lane 4) were able to (Fig. 2A, lane 4). Mock-infected cells cultured in 10% fetal bovine serum-containing medium (Fig. 2A, lane 2) failed to show an increase in p53 levels compared with mock-infected cells cultured in 4% fetal bovine serum-containing medium (Fig. 2A, lane 3). This observation, along with the fact that the 0.1-μm filtrate failed to elevate p53 levels, indicates that growth factors are not responsible for elevating p53 levels in infected cells under these experimental conditions. Together, all of these observations suggest that the induction of elevated p53 levels in HCMV-infected cells is dependent on the presence of active virus.

Since both heat inactivation and filtration were not specific for removal of HCMV, the role of virus in elevating p53 levels was further investigated by performing virus neutralization studies (Fig. 3A). Infection of HEL cells for 24 h with a virus dose of 1 to 2 PFU per cell resulted in the usual elevation of p53 levels (compared with the mock-infected cells shown in lane 6), regardless of whether the virus aliquot had previously

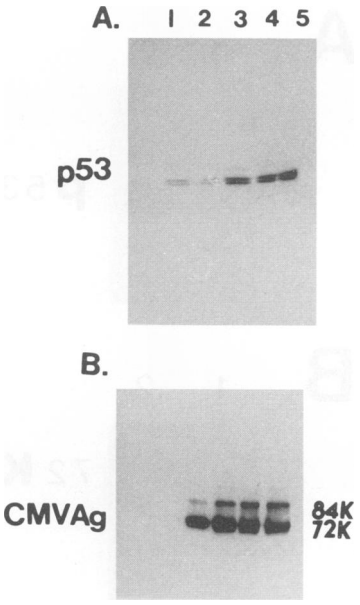

FIG. 4. Effect of virus dose on p53 protein levels in HCMV-infected cells. HEL cells were infected with various doses of HCMV, and extractions were performed at 8.5 h postinfection. Immunoblot analysis was performed as described in Materials and Methods. (A) Levels of p53 (detected by the DO-1 antibody) in 25 μg of extract protein. (B) Levels of HCMV IE antigens (detected by the MAB810 antibody) in 10 μg of extract protein. Lanes: 1, mock-infected cells; 2 to 5, cells infected with HCMV at virus doses of 0.25, 0.5, 1, and 2 PFU per cell, respectively. CMVAg, cytomegalovirus antigen.

been kept for 1 h at 25°C (lane 1) or at 37°C (lane 2). However, infection of HEL cells with a similar HCMV aliquot which had been reacted at 37°C with HCMV neutralizing monoclonal antibody 14-4b (against gp86) for 1 h at full strength (lane 3) or at half strength (lane 4) resulted in no elevation of p53 levels. A similar HCMV aliquot reacted with control nonneutralizing HCMV antibody 28-19, directed against the pp65 protein (lane 5), was still able to elevate p53 levels (lane 6). The observed effects were not artifacts of experimental procedures, for both the neutralizing and nonneutralizing antibodies had no effects on p53 levels in mock-infected cells (lanes 7 to 9). HCMV IE antigens were detected only in cells infected by nonneutralized virus (see Fig. 5B, lanes 1, 2, and 5), indicating that the neutralization was effective. These results thus show that HCMV is directly responsible for elevating p53 levels in infected cells.

Effect of virus dose on elevation of p53 protein levels in HCMV-infected cells. Since induction of elevated p53 levels in HCMV-infected HEL cells was maximal at 2 PFU per cell (Fig. 1), the effect of the virus dose at a low multiplicity of infection was investigated. HEL cells were infected with HCMV Towne strain at various multiplicities of infection (0.25, 0.5, 1, and 2 PFU per cell), and extractions were performed at 8.5 h postinfection (a time point right after elevation of p53 commences). Figure 4A shows that elevated p53 levels were detected between virus doses of 0.5 and 2 PFU per cell and not at 0.25 PFU per cell. As the virus dose increased from 0.5 to 2 PFU per cell, only a very small increase in p53 levels was detected at 8.5 h postinfection. Experiments performed at 24 and 48 h postinfection showed the same results. These observations thus suggest that induction of elevated p53 levels in HCMV-infected cells increases with increasing viral doses but a cooperativity effect occurs. Under these experimental conditions, levels of HCMV IE antigens also did not significantly increase between 0.5 and 2 PFU per cell (Fig. 4B), although the immunoblot assay was linear with increasing antigen concentrations within this range (data not shown). In line with these observations, DeMarchi and Kaplan (13) have previously reported that virus antigens in nonarrested confluent HEL cells were detected in 25 to 50% of the cells, even though the multiplicity was varied from 1 to 10 PFU per cell. Together, all of these observations suggest that the induction of elevated p53 levels in HCMV-infected cells occurs once a certain threshold of inducing viral factors has been obtained within the infected cells.

Involvement of viral IE antigens in induction of elevated p53 levels in HCMV-infected cells. Elevation of p53 in HCMV-infected cells was not blocked by inhibitors of HCMV DNA synthesis (such as phosphonoformic acid at 200 μg/ml; data not shown) and is thus due to events in the multiplication cycle of HCMV prior to HCMV DNA synthesis. Events in the HCMV multiplication cycle that might be responsible for elevating p53 levels thus included virus-receptor interactions and the action of HCMV IE and/or early proteins. The role of virus-receptor interactions was tested and ruled out by using UV light inactivation of HCMV (unpublished observations) in accordance with the procedure of AbuBakar et al. (1). Since the induction of elevated p53 levels in HCMV-infected HEL cells was maximal by 24 h postinfection (Fig. 1), the effect of the virus on cellular p53 levels at earlier times (0 to 24 h) postinfection was investigated. Figure 5A indicates that induction of elevated p53 levels was observed as early as 6 h postinfection (lane 5). Levels of p53 continued to rise slowly up to 12 h postinfection, reaching maximal levels at 24 h postinfection. Figure 5A shows the results obtained with 0.5 PFU per cell; however, an infection dose of 2 PFU per cell gave similar p53 kinetics (data not shown and Fig. 4). An investigation of how the kinetics of p53 induction correlate with HCMV IE antigen levels in these cells gave the results shown in Fig. 5B. At a virus dose of 0.5 PFU per cell, HCMV IE antigens (the 72K IE region 1 protein is visible) were detectable as early as 4 h postinfection (lane 4), reached maximal levels by 10 h postinfection (lane 7), and then declined very slightly. Under these experimental conditions, the immunoblotting assay was linear with increasing p53 and HCMV antigen quantities (data not shown). Since induction of elevated p53 levels in infected cells was observed right after synthesis of HCMV IE proteins was detected, these results favored the possible involvement of HCMV IE antigens, rather than early antigens, in the induction of elevated p53 levels in HCMV-infected cells.

To directly test the role of HCMV IE antigens in the induction of elevated p53 levels, a control plasmid (pSVO CMV1) and a DNA construct (pHD101SV1) encoding HCMV IE region 1 and 2 proteins were separately transfected into HEL cells (Fig. 6). The results show that transfection of plasmid pHD101SV1 (which expresses the HCMV IE proteins) resulted in elevation of p53 levels (Fig. 6A, lane 2) over those detected in HEL cells transfected with control plasmid pSVOCMV1 (lacking HCMV IE regions 1 and 2) (Fig. 6A, lane 1). HCMV IE antibody MAB810 was able to detect the expression of the most abundant 72K IE region 1 protein in HEL cells transfected with pHD101SV1 (Fig. 6B, lane 2) but not in cells transfected with pSVOCMV1 (Fig. 6B, lane 1). These results thus show that HCMV IE antigens are directly involved in the induction of elevated p53 levels in HCMV-infected cells.

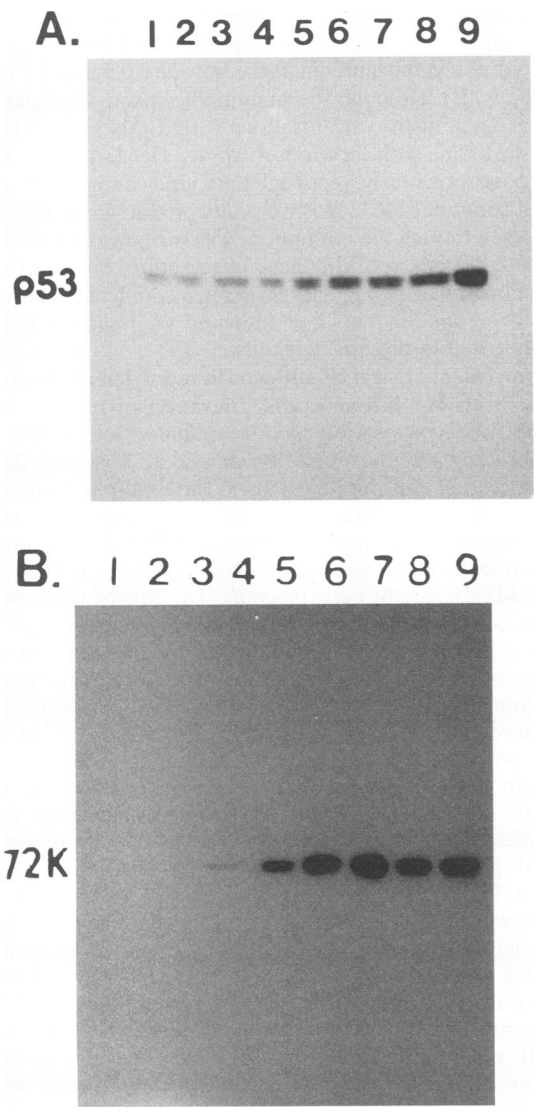

FIG. 5. Analysis of p53 and HCMV IE antigen levels in HCMV-infected HEL cells at early times postinfection. HEL cells were infected with an HCMV dose of 0.5 PFU per cell. Extractions were then performed at various times (0 to 24 h) postinfection, and samples were analyzed by immunoblotting and chemiluminescence detection. (A) p53 protein levels (detected by the DO-1 antibody) in 25 μg of protein. (B) HCMV IE protein levels (detected by the MAB810 antibody) in 10 μg of protein. Lanes: 1, mock-infected cells; 2 to 9, HCMV-infected cells collected at 0, 2, 4, 6, 8, 10, 12, and 24 h postinfection, respectively.

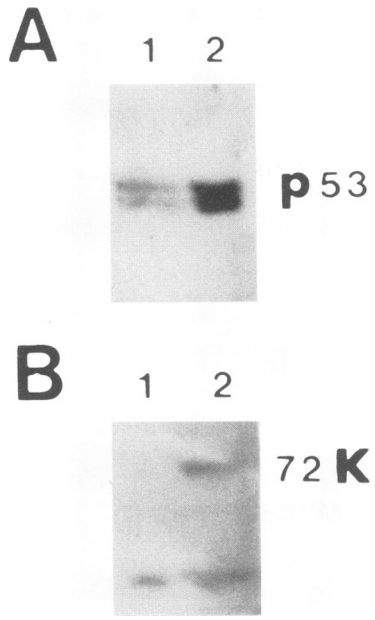

FIG. 6. Induction of elevated p53 levels in HEL cells by transfection of a DNA construct expressing HCMV IE proteins. HEL cells (10^6) at 50% confluence in 75-cm^2 flasks were transfected by using the lipofectamine procedure (see Materials and Methods), with either 20 μg of pHD101SV1 (an HCMV IE region 1 and 2 protein-expressing construct) or 20 μg of pSVOCMV1 (a control plasmid lacking the HCMV IE protein-coding region). Extractions were performed 72 h later, and Western blot analysis was performed on 100 μg of transfected cell extract protein as described in Materials and Methods. (A) p53 protein levels detected by the DO-1 antibody. (B) HCMV IE antigens detected by the MAB810 antibody. Lanes: 1, cells transfected with control plasmid pSVOCMV1; 2, cells transfected with the HCMV IE proteins expressing construct pHD101SV1.

DISCUSSION

In this report, we have demonstrated for the first time that infection of human fibroblasts with HCMV induces elevated levels of cellular protein p53. This elevation was observed in all of the permissive cell lines tested. The involvement of HCMV in this process was shown by virus neutralization studies, virus heat inactivation studies, and studies involving physical removal of the virus from infecting fluids. Previous reports have demonstrated the elevation of p53 protein levels in cells infected and transformed by SV40 and adenovirus (11, 28, 45). A very recent report by Speir et al. (48) has shown that infection of nonpermissive human smooth muscle cells in vitro by HCMV elevated p53 levels. Not all DNA tumor viruses, however, have been found to elevate p53 protein levels; human papillomavirus E6 promotes the degradation of p53 in cells containing human papillomavirus types 16 and 18. Therefore, the modulation of cellular p53 levels in infected cells will have to be determined for each individual type of virus, and the HCMV system provides an additional system in which the multifunctional nature of p53 (25, 34, 39, 44, 51, 54), an important tumor suppressor gene product, can be studied.

HCMV gene expression, which occurs after virus attachment, penetration, and uncoating, is coordinated into IE, early, and late phases; the late phase begins with the onset of HCMV DNA synthesis (49). Our experiments determined that late events within the HCMV multiplication cycle are not involved in the induction of elevated p53 levels, since p53 levels in HCMV-infected cells were not affected by inhibitors of HCMV DNA synthesis (data not shown). Studies utilizing UV light-inactivated HCMV (which could still penetrate a cell but had different degrees of viral DNA template damage [1]) determined that HCMV gene expression, rather than virus-receptor interactions, is required for virus-induced elevation of p53 levels in infected fibroblasts (data not shown). Subsequently, HCMV IE gene products were clearly implicated in the induction of elevated p53 levels in infected HEL cells; expression of IE antigens within transfected HEL cells resulted in induction of elevated p53 levels. Furthermore, induction of elevated p53 levels in infected cells occurred right after the synthesis of IE antigens was detected. Speir et al. (48) have

recently shown that elevation of p53 levels in HCMV-infected human smooth muscle cells correlated temporally with the expression of the HCMV IE 84K protein; those researchers, however, did not perform transfection studies to prove this point. Our experiments do not specify which of the IE proteins (the 72K IE 1 or the 84K IE 2 protein) is involved in elevating p53 levels in infected fibroblasts; this, along with other details of the mechanism by which elevated p53 levels are induced within HCMV-infected cells is under investigation.

It is interesting that levels of the p53 protein in normal cells markedly increase upon stimulation of quiescent-cell growth by serum or mitogens (35, 37, 41, 51) and upon treatment of cells by DNA-damaging agents (17, 21, 31, 55). Albrecht and coworkers have previously shown that HCMV infection causes DNA damage (1), and infection of quiescent cells with HCMV activates cellular biochemical and physiological responses which in some aspects resemble growth factor-induced cell activation (2, 4, 5). We have noted that the magnitude and induction kinetics of p53 elevation in HCMV-infected human fibroblasts are within a range similar to that of those obtained upon growth stimulation of cells (41) or induction of DNA damage (17). It is also interesting that Albrecht and coworkers (7) have determined that the DNA damage caused by HCMV correlates with the expression of IE gene products in infected cells. All of these factors, as they relate to HCMV-induced elevation of p53 levels in infected cells, are under investigation.

Pulse-chase studies indicate that HCMV-infected cells have a higher than normal rate of p53 protein synthesis, as well as increased p53 stability; no stable-complex formation between p53 and any viral or cellular proteins was observed under conditions in which p53-SV40 large T antigen complexes were detected in Cos-1 cells (37a). Thus, complex formation, if it occurs, is not very stable. In contrast to these findings, Speir et al. (48) have observed complex formation between the HCMV IE 84K protein and p53, as assayed in a doubly infected baculovirus expression system. Apart from the use of a different system, those researchers did not specify the conditions of their assay (which could explain the discrepancy) and reported that less than 10% of the proteins were in a complex. It is important to note that elevated p53 levels in infected cells can occur in the absence of complex formation and that complex formation does not always account for the stability of p53. The HCMV-infected cell system may possess similarities to the adenovirus-infected cell system, in which no stable complex formation was observed, despite elevated p53 levels caused by increased synthesis (8, 45). Stabilization of the p53 protein by the adenovirus 5 E1A protein and not by E1B protein, which complexes with p53 in transformed cells, has also been reported (29). On the other end, several reports (14, 50) point out that the increased metabolic stability of p53 in SV40-transformed cells may actually be mediated through an effect of SV40 on cellular properties rather than, or in addition to, complex formation per se. Thus, the role of complex formation in the stability of p53 in the best-studied SV40 system is still undefined.

Cellular protein p53 has a multifunctional nature, and its precise biochemical functions are not clear (25, 34, 39, 44, 51, 54). There are indications that p53 is involved in the infection processes of DNA tumor viruses, such as herpes simplex virus, Epstein-Barr virus, and SV40 (9, 53, 56). We do not know the significance of the elevation of p53 levels by HCMV infection. Albrecht et al. (2) have reported that infection of nonarrested confluent cells with HCMV resulted in cell activation accompanied by inhibition of cellular DNA synthesis, which was apparent within the first 25 h postexposure; it is possible that this was mediated by p53. On the other end, Speir et al. (48) have reported that HCMV IE 84K protein-mediated inhibition of p53 may contribute to the development of coronary restenosis. Thus, dissection of the HCMV-infected cell system with regard to p53 will further our understanding of virus-cellular interactions, as well as the regulation and function of p53 during lytic infection and in cell growth control. This is currently under investigation.

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REFERENCES

1. **AbuBakar, S., W. W. Au, M. S. Legator, and T. Albrecht.** 1988. Induction of chromosome aberrations and mitotic arrest by cytomegalovirus in human cells. Environ. Mol. Mutagen. **12**:409–420.
2. **Albrecht, T., I. Boldogh, M. Fons, C. H. Lee, S. AbuBakar, J. M. Russell, and W. V. Au.** 1989. Cell activation responses to cytomegalovirus infection: relationship to the phasing of CMV replication and to the induction of cellular damage. Subcell. Biochem. **15**:157–202.
3. **Albrecht, T., and F. Rapp.** 1973. Malignant transformation of hamster embryo fibroblasts following exposure to ultraviolet-irradiated human cytomegalovirus. Virology **55**:53–61.
4. **Boldogh, I., S. AbuBakar, C. Z. Deng, and T. Albrecht.** 1991. Transcriptional activation of cellular oncogenes *fos*, *jun*, and *myc* by human cytomegalovirus. J. Virol. **65**:1568–1571.
5. **Boldogh, I., S. AbuBakar, D. Millinoff, C. Z. Deng, and T. Albrecht.** 1991. Cellular oncogene activation by human cytomegalovirus: lack of correlation with virus infectivity and immediate early gene expression. Arch. Virol. **118**:163–177.
6. **Boldogh, I., E. Gonczol, and L. Vaczi.** 1978. Transformation of hamster embryonic fibroblast cells by UV-irradiated human cytomegalovirus. Acta Microbiol. Acad. Sci. Hung. **25**:269–275.
7. **Boldogh, I., E.-S. Huang, J. Baskar, L. Gergely, and T. Albrecht.** 1992. Oncogenic transformation by cellular DNA isolated from human cytomegalovirus-infected cells. Intervirology **34**:62–73.
8. **Braithwaite, A., C. Nelson, A. Skulimowski, J. McGovern, D. Pigott, and J. Jenkins.** 1990. Transactivation of the p53 oncogene by E1A gene products. Virology **177**:595–605.
9. **Braithwaite, A. W., H.-W. Sturzecher, C. Addison, K. Palmer, K. Rudge, and J. R. Jenkins.** 1987. Mouse p53 inhibits SV40 origin dependent DNA replication. Nature (London) **329**:458–460.
10. **Clarke, A. R., C. A. Pudie, D. J. Harrison, R. G. Morris, C. C. Bird, M. L. Hooper, and A. H. Wyllie.** 1993. Thymocyte apoptosis induced by p53 dependent and independent pathways. Nature (London) **362**:849–852.
11. **Crawford, L.** 1983. The 53,000-dalton cellular protein and its role in transformation. Int. Rev. Exp. Pathol. **25**:1–50.
12. **Davis, M. G., S. C. Kenney, J. Kamine, J. S. Pagano, and E. S. Huang.** 1987. Immediate early gene region of human cytomegalovirus trans-activates the promoter of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **84**:8642–8646.
13. **DeMarchi, J. M., and A. S. Kaplan.** 1977. Physiological state of human embryonic lung cells affects their response to human cytomegalovirus. J. Virol. **23**:126–132.
14. **Deppert, W., and M. Haug.** 1986. Evidence for free and metabolically stable p53 protein in nuclear subfractions of simian virus 40-transformed cells. Mol. Cell. Biol. **6**:2233–2240.
15. **Finlay, C. A., P. W. Hinds, and A. J. Levine.** 1989. The p53 proto-oncogene can act as a suppressor of transformation. Cell **57**:1083–1093.
16. **Geder, L., R. O'Neill, and F. Rapp.** 1976. Oncogenic transformation of human embryo lung cells by human cytomegalovirus. Science **192**:1134–1137.
17. **Hall, P. A., P. H. McKee, H. P. Menage, R. Dover, and D. Lane.** 1993. High levels of p53 protein in UV-irradiated normal

human skin. Oncogene **8**:203–207.
18. **Ho, M.** 1990. Epidemiology of cytomegalovirus infections. Rev. Infect. Dis. **12**:S701–S710.
19. **Hollstein, M., D. Sidransky, B. Vogelstein, and C. Harris.** 1991. p53 mutations in human cancers. Science **253**:49–53.
20. **Huang, E. S., E. C. Mar, I. Boldogh, and J. Baskar.** 1984. The oncogenicity of human cytomegalovirus. Birth Defects Orig. Artic. Ser. **20**:193–211.
21. **Kastan, M. B., O. Onyekwere, D. Sidransky, B. Vogelstein, and R. W. Craig.** 1991. Participation of p53 protein in the cellular response to DNA damage. Cancer Res. **51**:6304–6311.
22. **Kastan, M. B., Q. Zhan, W. S. El-Deiry, F. Carrier, T. Jacks, W. V. Walsh, B. S. Plunkett, B. Vogelstein, and A. J. Fornace.** 1992. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell **71**:587–597.
23. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
24. **Lane, D. P.** 1992. p53, guardian of the genome. Nature (London) **358**:15–16.
25. **Lane, D. P., and S. Benchimol.** 1990. p53: oncogene or anti-oncogene? Genes Dev. **4**:1–8.
26. **Levine, A. J.** 1990. The p53 protein and its interaction with the oncogenic products of the small DNA tumor viruses. Virology **177**:419–426.
27. **Levine, A. J., J. Momand, and C. A. Finlay.** 1991. The p53 tumor suppressor gene. Nature (London) **351**:453–456.
28. **Linzer, D. I. H., and A. J. Levine.** 1979. Characterization of a 54,000 MW cellular SV40 tumor antigen present in SV40 transformed cells and uninfected embryonal carcinoma cells. Cell **17**:43–52.
29. **Lowe, S. W., and H. E. Ruley.** 1993. Stabilization of the p53 tumor suppressor is induced by adenovirus 5 E1A and accompanies apoptosis. Genes Dev. **7**:735–745.
30. **Lowe, S. W., E. M. Schmidt, S. W. Smith, B. A. Osborne, and T. Jacks.** 1993. p53 is required for radiation induced apoptosis in mouse thymocytes. Nature (London) **362**:847–849.
31. **Maltzman, W., and L. Czyzyk.** 1984. UV irradiation stimulates levels of p53 cellular tumor antigen in nontransformed mouse cells. Mol. Cell. Biol. **4**:1689–1694.
32. **Martinez, J., I. Georgoff, and A. J. Levine.** 1991. Cellular localization and cell cycle regulation by a temperature sensitive p53 protein. Genes Dev. **5**:151–159.
33. **Mazeron, M. C., G. Jahn, and B. Plachter.** 1992. Monoclonal antibody E-13 (M-810) to human cytomegalovirus recognizes an epitope encoded by exon 2 of the major immediate early gene. J. Gen. Virol. **73**:2699–2703.
34. **Mercer, W. E.** 1992. Cell cycle regulation and the p53 tumor suppressor protein. Crit. Rev. Eukaryotic Gene Expression **2**(3): 251–263.
35. **Mercer, W. E., and R. Baserga.** 1985. Expression of the p53 protein during the cell cycle of human peripheral blood lymphocytes. Exp. Cell Res. **160**(1):31–46.
36. **Miller, J. H.** 1991. The role of p53 in normal control of cell proliferation. Curr. Opin. Cell Biol. **34**:282–286.
37. **Montenarh, M.** 1992. Biochemical, immunological and functional aspects of the growth suppressor/oncoprotein p53. Crit. Rev. Oncogenesis **3**:233–256.

37a. **Muganda, P., R. Carrasco, and Q. Qian.** Unpublished data.

38. **Muganda-Ojiaku, P. M., and E. S. Huang.** 1987. Alteration of protein phosphorylation patterns in cell lines morphologically transformed by human cytomegalovirus. Cancer Biochem. Biophys. **9**:179–189.
39. **Quartin, R. S., C. A. Finlay, and A. J. Levine.** 1992. The p53 gene and gene product, p. 87–104. *In* P. Sharp (ed.), Nuclear processes and oncogenes. Academic Press, San Diego, Calif.
40. **Rapp, F., and D. Robins.** 1984. Cytomegalovirus and human cancer. Birth Defects Orig. Artic. Ser. **20**:175–192.
41. **Reich, N. C., and A. J. Levine.** 1984. Growth regulation of a cellular tumor antigen, p53, in non-transformed cells. Nature (London) **308**:199–201.
42. **Reich, N. C., M. Oren, and A. J. Levine.** 1983. Two distinct mechanisms regulate the levels of a cellular tumor antigen, p53. Mol. Cell. Biol. **3**:2143–2150.
43. **Rogel, A., M. Popliker, C. G. Webb, and M. Oren.** 1985. p53 cellular tumor antigen: analysis of mRNA levels in normal adult tissues, embryos, and tumors. Mol. Cell. Biol. **5**:2851–2855.
44. **Ruppert, J. M., and B. Stillman.** 1993. Analysis of a protein-binding domain of p53. Mol. Cell. Biol. **13**:3811–3820.
45. **Sarnow, P., Y. S. Ho, J. Williams, and A. J. Levine.** 1982. Adenovirus E1b-58 kd tumor antigen and SV40 large tumor antigen are physically associated with the same 54 kd cellular protein in transformed cells. Cell **28**:387–394.
46. **Scheffner, M., B. A. Werness, J. M. Huibregtse, A. J. Levine, and P. M. Howley.** 1990. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell **63**:1129–1136.
47. **Schooley, R. T.** 1990. Cytomegalovirus in the setting of infection with human immunodeficiency virus. Rev. Infect. Dis. **12**:S811–S819.
48. **Speir, E., R. Modali, E.-S. Huang, M. B. Leon, F. Shawl, T. Finkel, and S. E. Epstein.** 1994. Potential role of human cytomegalovirus and p53 interaction in coronary restenosis. Science **265**:391–394.
49. **Stinski, M. F.** 1990. Cytomegalovirus and its replication, p. 1959–1980. *In* B. N. Fields, D. M. Knipe, et al. (ed.), Virology, 2nd ed. Raven Press, New York.
50. **Tieman, F., and W. Deppert.** 1994. Stabilization of the tumor suppressor p53 during cellular transformation by simian virus 40: influence of viral and cellular factors and biological consequences. J. Virol. **68**:2869–2878.
51. **Ullrich, S. J., C. W. Anderson, W. E. Mercer, and E. Apella.** 1992. The p53 tumor suppressor protein, a modulator of cell proliferation. J. Biol. Chem. **267**(22):15259–15262.
52. **Weller, T. H.** 1971. The cytomegaloviruses: ubiquitous agents with proven clinical manifestations. N. Engl. J. Med. **285**:203–274.
53. **Wilcock, D., and D. P. Lane.** 1991. Localization of p53, retinoblastoma and host replication proteins at sites of viral replication in herpes-infected cells. Nature (London) **349**:429–431.
54. **Yin, Y., M. A. Tainsky, F. Z. Bischoff, L. C. Strong, and G. M. Wahl.** 1992. Wild type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. Cell **70**:937–948.
55. **Zhan, Q., F. Carrier, and A. J. Fornace, Jr.** 1993. Induction of cellular p53 activity by DNA-damaging agents and growth arrest. Mol. Cell. Biol. **13**:4242–4250.
56. **Zhang, Q., D. Gutsch, and S. Kenney.** 1994. Functional and physical interaction between p53 and BZLF1: implications for Epstein-Barr virus latency. Mol. Cell. Biol. **14**:1929–1938.