ORIGINAL ARTICLE

INVESTIGATION OF DNA PREPARED FROM HUMAN CYTOMEGALOVIRUS INFECTED CELLS

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ABSTRACT: The effects of human cytomegalovirus (HCMV) infection on human fibroblast cell genomes were investigated using agarose gel electrophoresis. At selected intervals post-infection (PI), cellular DNA from mock-treated and HCMV-infected cells were prepared in low melting point agarose plugs. Results obtained following electrophoresis of the cellular DNA indicate that HCMV infection did not result in extensive degradation of the cellular DNA, even in samples obtained from cells which showed > 95% cytopathologic effects. High molecular weight DNA (> 23 Kb) comparable to that of the mock-treated samples were noted in all HCMV infected DNA samples. Digestion of the DNA samples with restriction endonucleases, *Eco*R I, *Not* I, *Sfi* I, and *Nru* I, however, resulted in the appearance of smaller DNA fragments (< 23 Kb) in samples obtained on day 3, 4, and 5 PI. Since these DNA bands appeared only in the infected cells, it was likely that these were the HCMV genomic DNA fragments. Findings presented in this study support the notion that the cellular DNA of HCMV-infected cells could remained intact and functional. *(JUMMEC 1996 1(1): 21-24)*

KEY WORDS: Cytomegalovirus, chromosomes, DNA, genomes, restriction enzymes

Introduction

Human cytomegalovirus (HCMV) is a common pathogen which infects and establishes life-long persistent and latent infection. The virus is teratogenic and has been associated with induction of congenital abnormalities (1). A number of studies have shown that infection by the virus results in host cell chromosomal damages and mitotic perturbation (2, 3) which could contribute to induction of congenital abnormalities. In a recent investigation, Boldogh *et al.* (4) demonstrate that the high molecular weight DNA extracted from HCMV infected human cells could transform normal human fibroblasts. This finding suggests that the cellular DNA of HCMV infected cells is intact and functional. The following investigation is initiated to examine the effects of HCMV infection on the host cell genome.

Materials and Methods

Cell culture and virus infection

Human lung fibroblast cells (MRC-5) purchased from the American Type Tissue Culture (ATCC; Rockville, MD, USA) were used in this study. Cells were cultured and maintained in Eagle's minimal essential medium (EMEM) supplemented with 10% fetal calf serum (PAA Laboratories, Linz, Austria). Virus inoculum was prepared as previously described (2). Briefly, confluent MRC-5 cells in T-175 cm^2 tissue culture flasks (Nunc, Roskilde, Denmark) were infected with stock culture of HCMV AD169 purchased from ATCC. The cultures were incubated at room temperature for an hour with gentle shaking at about every 15 minutes. After 1 hour the inoculum was replaced with fresh growth medium and the infected cells were incubated at 37°C until about 80 percent of the infected cells showed significant cytopathologic effects (CPE). Virus titer was determined using a modified method of Wentworth and French (5) as previously described (2).

Cell harvest and preparation of DNA in agarose plugs

In a typical experiment, confluent MRC-5 cells were infected with virus inoculum to give an estimated multiplicity of infection (MOI) of about 5 PFU/cell. At selected intervals PI, the growth medium was removed and cells were rinsed twice with phosphate buffered saline (PBS). After the washings cell monolayers were scraped using disposable plastic cell scraper and transferred into centrifuge tubes. Cells were then centrifuged at 800x g for 7 min. The supernatant was discarded and cells were resuspended in prewarmed

(42°C) L-buffer (0.01M Tris-HCl, pH 7.6; 0.02M NaCl; 0.1M EDTA). Equal volume of molten (42°C) 1.5% (w/v) low melting point (LMP) agarose (InCert agarose, FMC Bioproducts, Rockland ME, USA) was then added to the cell suspension. The cell suspension was immediately transferred into a prechilled agarose plug mold (Bio-Rad Laboratories, Hercules, CA, USA). Samples were refrigerated at 4°C for about 10 min and the agarose plugs were removed into universal bottles.

DNA preparation

Proteinase K lysis solution consisting of 1% N-lauryl-sarcosine, 0.2% sodium deoxycholate, and Proteinase K (1 mg/ml) in L-Buffer was added into the universal bottles containing the agarose plugs. Samples were incubated in the lysis solution at 50°C for 48 hr in a shaking waterbath. After 48 hours, lysis solution was removed and the agarose plugs consisting of the DNA were rinsed three times with TE (10 mM Tris, pH7.4; 0.1 mM EDTA) buffer. At every washing steps the agarose plugs were incubated in TE at 50°C for 15 min with constant shaking. After the final washing TE buffer was removed and the agarose plugs were transferred into clean sterile microcentrifuge tubes. The plugs were stored in 0.45 M EDTA until needed

Restriction enzyme digestion of DNA

Agarose plugs containing the DNA were sliced into thin sections (8 x 1.5 mm) and rinsed twice with distilled water. Restriction enzyme (RE) solutions prepared as recommended by the manufacturer were then added to the samples and the agarose plugs were incubated on ice for an hour. Samples were incubated at 37°C for about 16 hr (overnight). Following which the digestion solution was replaced with a freshly prepared RE solution and samples were incubated at 37°C for an additional 5 hr. All digestions using *Sfi* I restriction enzyme, however, was done at 50°C. After digestion, the DNA samples were rinsed with TE to remove the restriction enzymes.

Electrophoresis of the DNA fragments

Agarose plugs containing the DNA were sliced into thin sections on clean glass coverslips and loaded into a 1% agarose gel prepared in 0.5X TBE (0.045M Tris-borate; 0.001M EDTA) buffer. The wells were sealed with molten agarose (1% agarose) and DNA was electrophoresed at 40 - 45V for 16 hr at 4°C in 0.5X TBE buffer. Following electrophoresis, gels were stained with ethidium bromide and photographed under ultraviolet light illumination at 302 nm wavelength.

Enzymes and reagents

Reagents, chemicals, and detergents used in preparation of DNA samples were purchased from Sigma Chemical Company, St. Lois MO, USA. All reagents for tissue culture and agarose gel electrophoresis were purchased from Life Technologies (Grand Island, NY, USA). Restriction enzymes were purchased from New England Biolabs, Beverly, MA, USA.

Results and Discussion

Agarose gel electrophoresis of DNA samples obtained from mock-treated and HCMV-infected human fibroblasts, MRC-5, indicated that there was no substantial DNA degradation, in the form of extensive DNA smearing in these samples (Figure 1). Most of the DNA was noted to migrate lower than the 23 Kb marker along side the yeast marker which contained DNA fragments of about 3.5 to 5.7 Mb. Even though equal volume of samples from an approximately equal number of cells were loaded into the agarose gel wells, it was apparent that substantial amount of DNA was present in samples in lane 4, 5, and 6 which were obtained from HCMV-infected cells on day 3, 4, and 5 PI respectively. Despite the substantial amount of DNA present in these samples, the expected DNA smearing was not evident. It was unlikely that the lack of DNA smearing was due to an overwhelming presence of uninfected cells in the HCMV-infected samples, since immunofluorescent staining of cells similarly treated with HCMV inoculum indicated that more than 95% of the cells showed the presence of HCMV antigens, suggesting that almost all cells were infected (data not shown). Increased amount of DNA in the HCMV-infected samples beginning on day 3 PI onwards could indicate the newly synthesized viral DNA. It has been reported that initiation of HCMV DNA replication could take place as early as at 24 hr PI and production of viral progeny could be detected by 48 hr PI (6). Using the conventional agarose gel electrophoresis, however, it was not possible to differentiate between the viral and cellular DNA.

DNA samples of mock-treated and HCMV-infected cells were examined further by using restriction endonucleases. The DNA was digested with *Eco*R I and the rare cutting restriction enzymes *Not* I, *Nru* I, and *Sfi* I. It was noted that *Eco*R I digestion of the samples obtained on day 4 and 5 PI yielded distinctive DNA fragments varying in size from about 1.5 Kb to more than 23 Kb (Figure 2). Comparable DNA fragments, however, were not noted in mock-infected samples and samples obtained after 6, 24, and 48 hr PI. The majority of the DNA has a molecular weight of about 16 Kb which was significantly lower than that of the undigested samples (Figure 1). Higher molecular weight DNA fragments (> 23 Kb) were noted distinctively in samples obtained on day 4 and 5 PI. Digestion of the samples with *Not* I, on the other hand, resulted in at least 15 distinguishable DNA fragments (Figure 3). Eight of the fragments have molecular weights ranging in size

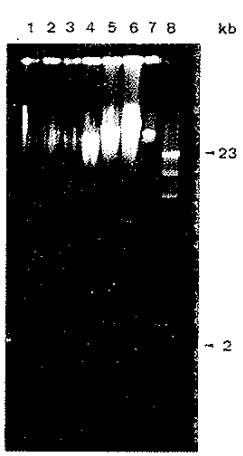

Figure 1. *Agarose gel electrophoresis of DNA prepared from mock- and HCMV- infected human lung fibroblast cells (MRC-5). DNA samples from mock-infected (lane 1) and HCMV-infected at 24 (lane 2), 48 (lane 3), 72 (lane 4), 96 (lane 5), and 120 (lane 6) hr post-infection (PI) were prepared in agarose plugs as described in the Materials and Methods. Samples were electrophoresed using 1% agarose (w/v) at 40V for 16 hr at 4°C in 0.5X TBE buffer. Estimation of the molecular weights of the DNA samples were made using the yeast marker (lane 7) and the* Hind III*-digested lambda DNA (lane 8).*

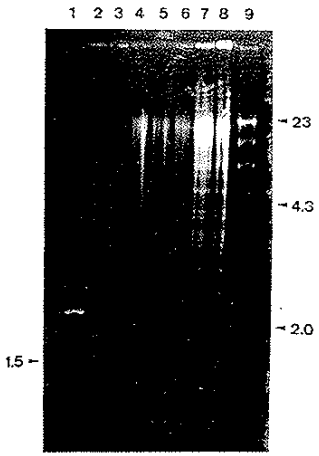

Figure 2. *EcoR I digested DNA samples from mock-treated (lane 2) and HCMV-infected MRC 5 cells harvested at 6 (lane 3), 24 (lane 4), 48 (lane 5), 72 (lane 6), 96 (lane 7), and 120 (lane 8) hr PI. The molecular weight markers were the 100 bp marker (lane 1), and the* Hind III*-digested lambda DNA (lane 9). DNA samples and electrophoresis conditions were as described in the legend of Figure 1. The DNA markers were labelled in kilobases.*

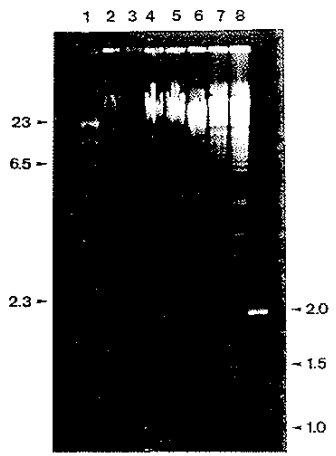

Figure 3. Not I *digested DNA samples from mock-treated (lane 2) and HCMV-infected MRC 5 cells harvested at 6 (lane 3), 24 (lane 4), 48 (lane 5), 72 (lane 6), 96 (lane 7), and 120 (lane 8) hr PI. The molecular weight markers were the* Hind III*-digested lambda DNA (lane 1). DNA samples and electrophoresis conditions were as described in the legend of Figure 1. The DNA markers were labelled in kilobases.*

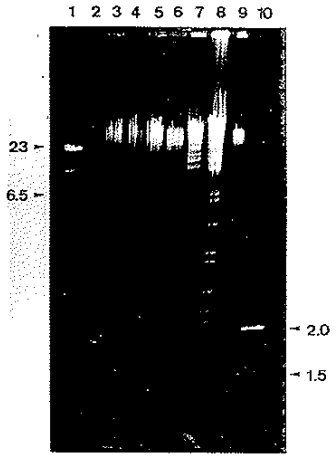

Figure 4. Sfi I *digested DNA samples from mock-treated (lane 2) and HCMV-infected MRC 5 cells harvested at 6 (lane 3), 24 (lane 4), 48 (lane 5), 72 (lane 6), 96 (lane 7), and 120 (lane 8) hr PI. The molecular weight markers were the* Hind III*-digested lambda DNA (lane 1), low molecular weight yeast marker (lane 9), and 100 bp marker (lane 10). DNA samples and electrophoresis conditions were as described in the legend of Figure 1. The DNA markers were labelled in kilobases.*

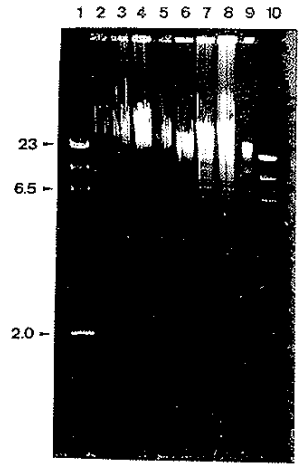

Figure 5. Nru I *digested DNA samples from mock-treated (lane 2) and HCMV-infected MRC 5 cells harvested at 6 (lane 3), 24 (lane 4), 48 (lane 5), 72 (lane 6), 96 (lane 7), and 120 (lane 8) hr PI. The molecular weight markers were the 100 bp marker mixed with the* Hind III*-digested lambda DNA (lane 1), low molecular weight yeast marker (lane 9) and* HindIII*-digested lambda DNA (lane 10). DNA samples and electrophoresis conditions were as described in the legend of Figure 1. The DNA markers were labelled in kilobases.*

between 1.4 to 6.5 Kb. The rest of the distinguishable fragments clustered between 6.5 to 23 Kb in a ladder-like arrangement differing in size by about 2.6 Kb. These fragments were noted to appear beginning on day 2 PI. Nevertheless, similar to the mock-infected DNA samples, most of the DNA from HCMV-infected samples remained larger than 23 Kb.

Sfi I digestion of the DNA samples obtained from HCMV-infected cells on day 4 and 5 gave at least 13 fragments smaller than 23 Kb (Figure 4). A cluster of DNA fragments was noted between 6.5 to 23 Kb and a well separated group of at least 9 DNA fragments ranging in size from 1.7 to 6.5 Kb. Evidence of *Sfi* I digested DNA fragments can be observed in HCMV-infected DNA samples obtained on day 2 PI onwards. On the other hand, digestion of the DNA samples with *Nru* I restriction enzyme gave rise to at least 20 DNA fragments of smaller then 23 Kb. Similarly with *Not* I, digestion with either *Sfi* I or *Nru* I resulted in high molecular weight DNA (> 23 Kb) which migrated in a comparable manner to that of the undigested samples (Figure 1). These results which differ significantly from that of the *Eco*R I digested DNA samples were not surprising, since *Eco*R I digestion of human genome on average gave DNA fragments of about 5 Kb. In contrast, *Not* I and *Sfi* I digestions normally gave fragments of about 100 and 30 Kb respectively. The high molecular weight DNA (> 23 Kb) noted in *Not* I, *Sfi* I, and *Nru* I digestions of the DNA samples probably represent several large cellular genomic DNA fragments which could not be resolved using conventional agarose gel electrophoresis. These findings did not exclude the possibility that HCMV infection results in induction of chromosome breaks but indicates that chromosome pulverization or degradation did not take place in HCMV infected cells. Our observations support an earlier report (2) which suggests that HCMV infection induces premature chromosome condensation (PCC) which appeared microscopically as pulverized chromosomes. Thus, it is possible that DNA fragments of the HCMV infected cells may contain intact genes that could transform normal fibroblast cells as reported by Boldogh *et al.* (4).

The smaller (< 23 Kb) DNA fragments noted in restriction enzyme digested samples beginning on day 3 PI could represent the newly synthesized viral DNA. The appearance of these fragments coincided with the reported appearance of viral DNA in HCMV-infected cells (7). Furthermore, digestion of the DNA samples from HCMV-infected cells with *Eco*R I gave rise to smaller fragments (< 23 Kb) comparable to that obtained using viral DNA purified by isopycnic centrifugation in cesium chloride (8).

In the present study we also showed that HCMV infected cellular DNA embedded in low melting point agarose could be used for restriction endonuclease digestion. Using this procedure it was not necessary to purify the DNA. Thus, the restriction enzyme digestion patterns of the HCMV DNA useful for epidemiological investigation could be obtained rapidly. Results from this investigation suggest also that the rare cutting restriction enzymes *Not* I and *Sfi* I which gave distinctive DNA fragments could be useful in epidemiological investigation of HCMV infection in addition to the commonly used endonucleases such as *Eco*R I, *Bam*H I, *Bgl* II, *Hind* III, and *Xba* I (9, 10, 11, 12).

Acknowledgements

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