Human cytomegalovirus (CMV) causes a variety of clinical manifestations ranging from inapparent to fatal infections. After primary infection, the host harbors the virus as a chronic or latent infection. Recurrent infection often occurs in persons with immunosuppression, allograft reaction, or both, such as in cases of organ transplantation, malignancy, pregnancy, and autoimmune diseases. Restriction endonuclease analysis of viral DNA has been proved to be a powerful tool in epidemiological studies of CMV infections (2, 5–8, 12, 14–16). The limitation to routine application of this analysis is the difficulty of preparing sufficient quantities of viral DNA, because human CMV, especially fresh isolates, replicates very slowly and produces little extracellular virus. To overcome this problem, we prepared DNA from the “Hirt supernatant” (4) of infected cells and subjected it to restriction endonuclease analysis.

Human embryonic lung cells were grown in Eagle minimal essential medium (Nissui Seiyaku Co., Ltd., Tokyo) supplemented with kanamycin (60 μg/ml), 10% fetal bovine serum (Flow Laboratories, Inc., McLean, Va.), and 0.10% NaHCO3. Human CMV (Towne strain), simian CMV (GR2757 strain), and 14 freshly isolated human CMV strains (Table 1) were used. Towne and GR2757 were propagated by inoculating human embryonic lung cells with cell-free virus. The fresh isolates were propagated by mixing infected and uninfected human embryonic lung cells at an infected-to-uninfected cell ratio of approximately 1:10. When more than 75% of cells showed the characteristic cytopathic effect, the cells were washed five times with phosphate-buffered saline (pH 7.0) and lysed gently for 15 min at room temperature with a lysis solution (2 ml per bottle) consisting of 0.01 M Tris (pH 8.0), 0.6% sodium deoxycholate, and 0.01 M EDTA (4). Sodium chloride was then added to a final concentration of 1 M, and the mixture was kept at 4°C overnight. The Hirt supernatant was separated by centrifugation at 17,000 × g for 60 min at 4°C and treated with pronase (1 mg/ml) at 37°C for several hours. After three cycles of extraction with equal volumes of phenol saturated with 0.01 M Tris and 0.001 M EDTA buffer (TE buffer [pH 8.0]), the DNA was precipitated with 2 volumes of isopropanol and 1/10 volume of 3 M sodium acetate at −20°C overnight. The precipitated DNA was washed successively in cold 70, 90, and 100% ethanol and dried. The DNA was dissolved in TE buffer and stored at 4°C until used.

Virions released from infected cells by sonication were freed of cell debris and banded by centrifugation at 25,000 rpm for 2 h in a two-step CsCl gradient. The purified virions were treated as described above to release DNA. CMV DNA (2 μg in 21 μl) was digested twice with 6 U of restriction endonucleases (Takara Shuzo Co., Ltd., Kyoto) in the appropriate reaction mixture at 37°C for 60 min in a final reaction volume of 30 μl. After loading buffer was added to make a final concentration of 10% sucrose and 0.025% bromophenol blue, the mixture was heated at 70°C for 3 min and subjected to electrophoresis in a 0.5% agarose gel for 16 h at 60 mA. DNA bands were stained with ethidium bromide (0.5 μg/ml) and photographed under UV light.

The DNA of one fresh human CMV isolate, Hida strain, was prepared by three different methods and compared quantitatively and qualitatively. The amounts of DNA prepared from the virions, from the infected cells (13), and from the Hirt supernatant were 5.48, and 24 μg per three Roux-type bottles, respectively. Electrophoretic patterns of DNAs prepared by the three different procedures were compared after EcoRI digestion (Fig. 1). The electropherogram of the DNA from the infected whole cells had a heavy background caused by the contaminating cellular DNA. Although there was still some contamination with cellular DNA, the DNA prepared from the Hirt supernatant gave a distinct pattern comparable with that of the DNA from purified virions, except for an additional band originating from the mitochondrial DNA (9).

This technique was applied to identify various isolates of different origin. The DNAs of 14 fresh isolates (Table 1) were prepared from Hirt supernatants, cleaved with restriction enzymes, and subjected to electrophoresis.

The electrophoretic patterns of EcoRI-digested DNAs prepared from various CMV strains were compared (Fig. 2). Among the epidemiologically unrelated strains, there were no identical cleavage patterns. Strains from the same person showed the same cleavage pattern, with one exception. Yama strains isolated at different times and from different sites of the same person showed different cleavage patterns. Ueno et al. (13) recently used infected cell DNA for endonuclease cleavage analysis of herpes simplex virus DNA. In the case of human CMV, however, cleavage pattern analysis by this method was hampered by a thick background of cellular DNA. Usually, fresh human CMV
isolate replicate very slowly in cells, and consequently, the ratio of viral DNA to cellular DNA may be low in the infected cells. Thus, it is necessary to prepare viral DNA-rich fractions with minimal contamination by host cell DNA. Viral DNA with a lower molecular weight than cellular DNA was isolated by the Hirt method (4). This method was extended, and it proved to be useful for the preparation of herpes simplex virus DNA with larger molecular weight (10). We further extended this procedure to human CMV which has a genome one and one-half times larger than that of herpes simplex virus. The DNA prepared from the Hirt supernatant was still contaminated by cellular DNA, especially by mitochondrial DNA. This contamination is inevitable because human CMV stimulates mitochondrial DNA synthesis (3). Mitochondrial DNA is small and closed circular (1), and it separates in the Hirt supernatant. When EcoRI-cleaved mitochondrial DNA was electrophoresed in 0.5% agarose (9), the larger two fragments comigrated and consequently appeared as an additional band as seen in Fig. 1.

However, in practice, the presence of a small amount of host cell DNA in the viral DNA does not interfere with strain identification of CMV isolates. This method requires no isotope and is simple and practical for laboratory analysis of CMV infections.

During the preparation of this manuscript, Rosenthal et al. (11) reported the isolation of human CMV DNA by the Hirt method, and they showed that Hirt-extracted DNA and virion-derived human CMV DNA had similar plaqueing efficiency and shared HindIII restriction cleavage patterns.

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LITERATURE CITED


