Human Cytomegalovirus

II. Lack of Relatedness to DNA of Herpes Simplex I and II, Epstein-Barr Virus, and Nonhuman Strains of Cytomegalovirus

ENG-SHANG HUANG AND JOSEPH S. PAGANO

Department of Medicine and Department of Bacteriology and Immunology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514

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The purified DNA of human cytomegalovirus, radiolabeled *in vitro*, was examined for homology to Epstein-Barr virus, herpes simplex type I and II, and simian and murine cytomegalovirus DNA by DNA-DNA reassociation kinetics analyses with the S1 enzyme differential digestion technique. Cross-matching of the DNAs showed no relatedness or less than 5% detectable homology.

Viruses within the herpes group have similarities structurally and biologically. They appear identical under the electron microscope, all containing a nucleocapsid structure and outer envelope (7, 17). The molecular weight of the DNA of herpes simplex (HSV) type I, type II, cytomegalovirus (CMV), and Epstein-Barr virus (EBV) are similar, in the range of 10^8 daltons (9, 11, 14). These viruses exhibit striking tendencies toward latent infection of the human host: in leukocytes, in the male and female genital tract, and in other sites (4, 5, 7, 8, 12). HSV and CMV characteristically produce polykaryocytes as a feature of their cytopathic effect; mouse and simian CMV also induce such a tissue reaction. Immunological studies of the relatedness between HSV and varicella-zoster viruses indicate that these viruses share common antigens (16).

However, there is no clear evidence of immunologic relatedness between human CMV and CMV isolated from other animal species, nor between CMV and HSV or EBV. As part of a study of the genetic relatedness among viruses in the herpes group we show here the first evidence of the lack of detectable nucleic acid homology, determined by DNA-DNA renaturation kinetics analyses, between a strain of human CMV and other members of the herpes group.

MATERIALS AND METHODS

Viruses. We used the AD-169 strain of human cytomegalovirus (15), the KOS strain of HSV I and the 196-2 strain of HSV II (courtesy of E. R. Alexander), EBV from HR1K cells (14), the C57-1a strain of murine CMV (courtesy of K. S. Kim), and the simian strain of CMV, GR2757 (6, 13).

The human and simian strains of CMV were

cultivated in WI38 cells, HSV I and II were grown in Hep 2 or WI38 cells, and the mouse CMV was grown in mouse embryo fibroblasts. The virus was prepared by infection of roller-bottle cultures at a multiplicity of infection of 1 to 2 PFU/ml and harvested from the extracellular fluid essentially as described before (9).

Purification of virus and extraction of viral DNA. The viruses were purified and the viral DNA was extracted from the purified virions as described before (9).

Radioisotopic labeling of the viral DNA *in vitro*. This was done with Kornberg's enzyme as described before in detail for both CMV and EBV (9, 14). The specific activities of the viral DNAs were as follows: AD-169, 1.7×10^6 counts per min per μg ; EBV DNA, 2.7×10^6 counts per min per μg .

Renaturation kinetics analyses and preparation of S1 nuclease (*Aspergillus oryzae*). The purified viral DNAs were reduced by ultrasonic vibrations to fragments of $\sim 5S$ (9). The mixtures of DNA were denatured at 100 C for 15 min in 0.01 M Tris buffer (pH 7.4) and EDTA (0.0025 M). The concentration of salt was adjusted to 1.2 N NaCl, and portions were placed in 100- μl pipettes and sealed; hybridization was conducted at 66 C (9, 14). The S1 enzyme was prepared by the method of Ando (2) with additional chromatographic steps, both through DEAE-cellulose and Sephadex G75. After intervals of hybridization the DNA in one of the capillary tubes was diluted to 0.6 ml with water, and a portion was used for determination of total acid-insoluble radioactivity. The other portion was mixed with 50 μl of S1 enzyme (activity, 14,000 U/ml) and 20 μg of sonicated double-stranded calf-thymus DNA to protect the reassociated [^3H]DNA from double-stranded DNase activity. The reaction was carried out in 0.035 M acetate buffer containing 0.0004 M zinc chloride and 0.1 M NaCl, pH 4.5 (2), in a volume of 1 ml. After 1 h at 37 C the precipitable radioactivity was counted on membrane filters.

Testing of the S1 enzyme with two lots of *in vitro* labeled CMV DNA and one of EBV DNA indicated

that 93 to 96% of sonicated denatured DNA, and 4 to 7% of native DNA, were sensitive to the enzyme.

RESULTS

For these determinations it was important to establish first the freedom of the labeled index CMV DNA from detectable host cell DNA contamination, and this has been done with the same preparation of strain AD-169 DNA (9). The experimental results plotted in Fig. 1 show no detectable homology between the human CMV strain, AD-169, and either HSV I, HSV II, or EBV. The addition of unlabeled AD-169 DNA to the renaturation mixture gave a Cot_{50} value of around 3×10^{-4} mol s/liter, as expected from the increase in concentration of the AD-169 DNA to 4 μ g/ml, compared with 6×10^{-2} mol s/liter for 0.02 μ g of 3 H-labeled AD-169 indicator DNA. The mixture with the additional CMV DNA was the 100% control and demonstrated at least 95% homology, in contrast to the heterologous DNAs which did not accelerate the renaturation of the index radiolabeled AD-169 DNA. This result indicated less

than 5% homology between the CMV DNA and the three heterologous viral DNAs of human origin.

Figure 2 shows the results of the experiment in which labeled EBV DNA was used in comparison with either unlabeled herpes simplex type I, type II, or AD-169 DNA, as well as unlabeled EBV DNA for the 100% homologous control DNA. Here again there is no evidence of detectable homology between the EBV DNA and the other human viral DNAs.

In Fig. 3 we show the lack of relatedness between human cytomegalovirus DNA and cytomegaloviruses of simian and murine origin. Once again there is no evidence of homology within the same tolerances of sensitivity.

DISCUSSION

Among other reasons, we made these determinations because antisera to purified preparations of EBV are not available, and there has been a description of a CF antigen common to some herpes group viruses (16). More important, it is essential to establish the nonrelated-

FIG. 1. Reassociation kinetics analysis of tritiated human cytomegalovirus DNA with the DNA of herpes simplex virus I, herpes simplex virus II, and Epstein-Barr virus. Sonically disrupted 3 H-labeled CMV DNA (strain AD-169), 0.02 μ g (3.4×10^4 counts/min), 20 μ g of calf thymus DNA, and 4 μ g of unlabeled viral DNA were mixed and denatured in presence of 0.01 M Tris-hydrochloride (pH 7.4) and 0.0025 M EDTA. The salt concentration was then adjusted to 1.2 N NaCl. The hybridization was carried out at 66 C, and the fraction of reassociated 3 H-DNA was analyzed by S1 enzyme differential digestion as described in Materials and Methods. Calf thymus control DNA, 4 μ g (O); HSV I DNA (Δ); HSV II DNA ($\blacktriangle$); EBV DNA ($\bullet$); and AD-169 DNA ($\times$).

FIG. 2. Reassociation kinetics analysis of tritiated Epstein-Barr viral DNA with the DNA of herpes simplex virus I, herpes simplex virus II, and cytomegalovirus DNA. Sonically disrupted ^3H -labeled EBV DNA, $0.02\text{ }\mu\text{g}$ (5.4×10^4 counts/min), $20\text{ }\mu\text{g}$ of calf thymus DNA, and $3\text{ }\mu\text{g}$ of unlabeled viral DNA were mixed. The experiment was carried out as described in Fig. 1. Calf thymus control DNA (○); HSV I DNA (△); HSV II DNA (●); AD-169 DNA (□); and EBV DNA (×).

FIG. 3. Reassociation kinetics analysis of tritiated human cytomegalovirus DNA with the DNA of simian cytomegalovirus and murine cytomegalovirus. Sonically disrupted ^3H -labeled CMV AD-169 DNA, $0.02\text{ }\mu\text{g}$ (3.4×10^4 counts/min), $20\text{ }\mu\text{g}$ of calf thymus DNA, and $4\text{ }\mu\text{g}$ of unlabeled viral DNA were mixed, and the experiment was carried out as in Fig. 1. Calf thymus control DNA (○); simian (GR 2757) CMV DNA (●); murine (C57-1a) CMV DNA (□); and human CMV (AD-169) DNA (×).

ness of these viral DNAs because of the increasing use of nucleic acid probes for the detection of EBV, HSV, and CMV DNA in cells and tumor tissues that might harbor only a portion of the genome in a latent state. This possibility takes on added relevance because human leukocytes may contain all three viruses, and the cervix and human semen may harbor at least two of them (4, 5, 12).

The analysis of the renaturation kinetics of mixtures of DNA is a more sensitive method for the determination of homology than DNA-DNA hybridization on membrane filters which lacks the kinetic element of the analysis. DNA renaturation kinetics analyses are, however, probably not as sensitive as heteroduplex mapping (3) which can show homology between sequences of as few as 40 base pairs.

We also looked for immunologic indications of relatedness of the simian strain of cytomegalovirus, GR 2757, with the human strain, AD-169, but found no cross-reactivity either by complement fixation or immunofluorescence tests (10). Dreesman and Benyesh-Melnick et al. failed to detect relatedness between these monkey and human strains (6, 13). Kim et al. showed no relation between the mouse and the human strain (K. S. Kim, personal communication). Anderson et al. (1) also presented evidence that the complement-fixing antigen of human CMV possesses a high degree of specificity when compared with HSV, varicella-zoster, and EBV.

In terms of density the DNA of the human and the mouse CMV strains are identical (1.716 g/cm³), EBV DNA has a density of 1.718 g/cm³ (14), and the simian CMV strain has a density of 1.710 g/cm³ (9); all are in contrast to HSV I and HSV II DNA with densities of 1.726 and 1.728 g/cm³, respectively (11).

The next step is interstrain analysis of human CMV isolates to determine whether there are variants that could account for the wide spectrum of biological behavior of CMV in the human host.

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