Comparison of Restriction Site Polymorphisms among Clinical Isolates and Laboratory Strains of Human Cytomegalovirus

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SUMMARY

We have compared HindIII and EcoRI restriction sites in the long and short unique regions of the human cytomegalovirus (HCMV) genome among 20 low passage clinical isolates and four high passage laboratory strains (AD169, Davis, Towne, UW-1). This was done by hybridizing digested DNA on Southern blots with a series of subgenomic cloned fragments of AD169. Fourteen HindIII sites were conserved and three fragments (O, V, W) co-migrated among all strains. Nine HindIII sites found in AD169 were absent in one or more other strains. Eight additional HindIII sites were identified and three more hypothesized by the appearance of slightly smaller fragments. Sixteen EcoRI sites were conserved and six fragments (c, Y, S, W, B, R) co-migrated among all strains. Twelve EcoRI sites were absent or in altered locations and at least seven additional sites were identified in one or more strains. Although no two of these strains were identical throughout the genome, identical patterns of variation in a given region frequently occurred in multiple strains. Polymorphisms occurred throughout the entire genome, including the region specifying immediate early functions. All strains studied showed an identical fragment which hybridized to the transforming fragment of AD169. These restriction site polymorphisms may in the future serve as convenient markers for identification of functional variation among HCMV strains.

INTRODUCTION

Infection with human cytomegalovirus (HCMV) is found worldwide and most frequently is of no obvious clinical consequence. It can, however, be associated with a wide spectrum of disease, particularly when infection occurs in a developing foetus or in an immunocompromised individual. Congenital infection can be inapparent, or can vary clinically from a mild self-limited syndrome to a frequently fatal disease. In some cases neurological sequelae are seen. A proportion of normal adults experiencing primary infection will develop mononucleosis or, rarely, a form of hepatitis or encephalitis (Ho, 1982). HCMV has oncogenic potential (Huang et al., 1984; Nelson et al., 1984) and has been found in association with various human tumours although a cause and effect relationship has not been established (McDougall et al., 1984; Rapp & Robbins, 1984). The factors responsible for this spectrum of manifestations of HCMV infection are largely unknown. Host immunological function and other host factors, route of infection, and size of viral inoculum are undoubtedly of great importance. Variation in the strain of infecting virus is a potential additional factor which may influence the outcome.

Considerable strain variation has been observed among HCMV isolates. Although antigens and polypeptides common to many strains have been demonstrated, antigenic heterogeneity has also been found (Zablotney et al., 1978; Sweet et al., 1979; Huang et al., 1976). Major common nucleic acid sequences among various HCMV strains have been demonstrated by hybridization (Huang et al., 1976). However, electrophoretic analysis of sizes of fragments generated by restriction enzyme digestion of viral DNA have shown that, although some bands co-migrate among many isolates, no wild-type isolates have identical patterns unless they are

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epidemiologically related (Kilpatrick et al., 1976). Published maps of AD169, Towne and a limited number of other HCMV strains document this restriction site variation (LaFemina & Hayward, 1980; Weststrate et al., 1980; Fleckenstein et al., 1982; Spector et al., 1982; Colimon et al., 1985).

The extent to which the diversity found among HCMV strains is reflected in biological and functional differences is not known. The potential for HCMV strain differences influencing disease, perhaps by variation in efficiency of replication, or differences in cell tropism or ability to establish persistent or latent infection, has not been explored. Although antigenic heterogeneity has not been associated with pathogenicity, anatomical site of isolation or geographical origin (Zablotney et al., 1978; Kilpatrick et al., 1976), it has potential significance in developing a vaccine effective against HCMV. Whether all or few strains have the 558 base pair sequence associated with transforming ability in vitro (Nelson et al., 1984) and thus with oncogenic potential is not known. Several high passage laboratory strains have been used in most functional studies of HCMV. To what extent these are representative of strains found in the population is not known.

As an initial step in studying the significance of HCMV strain variation we have mapped and compared the EcoRI and HindIII restriction sites in the long and short unique regions of the genome among a series of 20 low passage HCMV isolates and four widely used high passage laboratory strains. Mapping was done by hybridizing HCMV restriction fragments with a series of subgenomic cloned fragments of HCMV strain AD169. In this way we have documented where in the HCMV genome variation has occurred and compared patterns of variation among strains in specific regions. We have identified restriction sites conserved among all strains studied, and sites and genomic regions which vary among few or many strains. We have studied the region specifying immediate early functions in greater detail by also comparing XbaI and BamHI sites. In addition, we have tested all strains for their ability to hybridize with the transforming region identified in HCMV strain AD169.

**METHODS**

**Viral strains.** Clinical isolates of HCMV were obtained from cervical secretions and urine of pregnant women receiving routine prenatal care and from cervical secretions, urine, semen and throat secretions of persons attending a sexually transmitted disease (STD) clinic. The details of these isolations have been previously reported (Handsfield et al., 1985; Chandler et al., 1985). Restriction enzyme patterns of 71 clinical isolates from eight pregnant women and 16 STD clinic patients were compared as part of a continuing epidemiological study reported elsewhere (Chandler et al., 1986). From these, 20 isolates showing unique restriction patterns were selected for this study and are described in Table 1. Stocks maintained in our laboratory of high passage prototype strains AD169, Davis, Towne and UW-1 were also used.

**Viral culture.** Isolations from clinical material were carried out in human foetal tonsil fibroblasts or human foreskin fibroblasts (HFF) which had been grown in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum, and antibiotics. Viral isolates were passaged two to five times and frozen at -70 °C for future use. Isolates were re-inoculated into HFF and passaged for a total of 10 or fewer passages for analysis.

**DNA isolation.** Initially, viral cultures were passaged in HFF cells until one 850 cm² roller bottle with a 100% infected monolayer was obtained. Monolayers were harvested by trypsinization. Cells from one roller bottle were suspended in TE buffer (10 mm-Tris, 1 mm-EDTA, pH 8.0) and treated with Pronase (2 mg/ml final concentration) and 0.6% SDS in a final volume of 10 ml at 37 °C for 16 h. Viral DNA was separated from cellular DNA by centrifugation at 55000 r.p.m. in a Beckman L8-80 ultracentrifuge (Ti80 rotor) for 48 h in sodium iodide gradients (0-93 g/ml in TE buffer containing 150 µg/ml ethidium bromide) (Walboomers & ter Schegget, 1976). The viral DNA was dialysed against TE buffer to remove sodium iodide, extracted twice with phenol, twice with chloroform-isooamyl alcohol (24:1), precipitated overnight at -20 °C in 0.2 M-sodium acetate and 2 vol. 95% ethanol, and resuspended in TE buffer.

Subsequently, DNA was prepared without separating viral and cellular DNA. Infected cells from two 75 cm² flasks were similarly harvested and treated with Pronase and SDS. Proteins were removed from total DNA by two extractions each with equal volumes of phenol and chloroform-isooamyl alcohol (24:1). DNA was precipitated overnight at -20 °C in 0.2 M-sodium acetate and 2 vol. 95% ethanol and resuspended in TE buffer.

**Restriction endonuclease digestion.** Viral DNA samples (1 µg each) were digested with the bacterial endonucleases EcoRI, HindIII or BamHI (Bethesda Research Laboratories) according to the manufacturers' directions. HindIII digests were further digested with XbaI or BamHI for some analyses. Half of each digested
Restriction site polymorphism of HCMV

Table 1. Sources of clinical isolates of HCMV

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Clinic</th>
<th>Body site or fluid</th>
<th>Sex</th>
<th>Additional isolates with same restriction pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prenatal</td>
<td>Urine</td>
<td>F</td>
<td>3 (urine)</td>
</tr>
<tr>
<td>2</td>
<td>Prenatal</td>
<td>Cervix</td>
<td>F</td>
<td>2 (cervical, 1 urine)</td>
</tr>
<tr>
<td>3</td>
<td>Prenatal</td>
<td>Cervix</td>
<td>F</td>
<td>3 (cervical, 2 urine)</td>
</tr>
<tr>
<td>4</td>
<td>Prenatal</td>
<td>Cervix</td>
<td>F</td>
<td>1 (cervical)</td>
</tr>
<tr>
<td>5</td>
<td>Prenatal</td>
<td>Cervix</td>
<td>F</td>
<td>2 (cervical, 1 urine)</td>
</tr>
<tr>
<td>6</td>
<td>Prenatal</td>
<td>Cervix</td>
<td>F</td>
<td>1 (cervical)</td>
</tr>
<tr>
<td>7</td>
<td>Prenatal</td>
<td>Cervix</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>STD</td>
<td>Urine</td>
<td>F</td>
<td>4 (2 cervical, 1 urine, 1 semen isolate from sexual partner)</td>
</tr>
<tr>
<td>9</td>
<td>STD</td>
<td>Semen</td>
<td>M</td>
<td>2 (1 throat, 1 cervical isolate from sexual partner)</td>
</tr>
<tr>
<td>10</td>
<td>STD</td>
<td>Throat</td>
<td>F</td>
<td>1 (throat)</td>
</tr>
<tr>
<td>11</td>
<td>STD</td>
<td>Cervix</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>STD</td>
<td>Urine</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>STD</td>
<td>Throat</td>
<td>F</td>
<td>9 (5 cervical, 2 urine; 1 throat and 1 urine isolates from baby)</td>
</tr>
<tr>
<td>14</td>
<td>STD</td>
<td>Cervix</td>
<td>F</td>
<td>5 (4 cervical, 1 urine)</td>
</tr>
<tr>
<td>15</td>
<td>STD</td>
<td>Cervix</td>
<td>F</td>
<td>4 (1 cervical, 1 urine; 2 urine isolates from sexual partner)</td>
</tr>
<tr>
<td>16</td>
<td>STD</td>
<td>Cervix</td>
<td>F</td>
<td>3 (1 cervical; 2 urine isolates from sexual partner)</td>
</tr>
<tr>
<td>17</td>
<td>STD</td>
<td>Cervix</td>
<td>F</td>
<td>1 (urine)</td>
</tr>
<tr>
<td>18</td>
<td>STD</td>
<td>Cervix</td>
<td>F</td>
<td></td>
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<tr>
<td>20</td>
<td>STD</td>
<td>Cervix</td>
<td>F</td>
<td></td>
</tr>
</tbody>
</table>

sample was electrophoresed on a 13 × 19 cm horizontal 0.7% agarose gel in E buffer (0.72 M-Tris, 0.1 M-sodium acetate, 0.02 M-EDTA, pH 7.9) for 16 h at 30 V, stained with ethidium bromide, and visualized under u.v. light to ascertain completeness of digestion. The remainder of the digestion mixture was used for hybridization.

**DNA hybridization.** Amounts of digests containing 25 ng DNA were similarly electrophoresed on 13 × 19 cm horizontal 0.7% agarose gels in E buffer, along with *HindIII*-digested lambda phage DNA as a molecular weight marker, for blotting and hybridization. Prior to blotting, gels were depurinated in 0.25 M-HCl, denatured in 1.5 M-NaCl plus 0.5 M-NaOH, and soaked in 1 M-Tris-HCl pH 8.0 plus 1.5 M-NaCl. Transfer of DNA to nitrocellulose filters (Schleicher & Schüll) was carried out in 20 × SSC (1 × SSC is 0.15 M-NaCl, 0.015 M-sodium citrate, pH 8.3) according to the method of Southern (1975). Filters were dried at 80 °C for 2 h in a vacuum oven and prehybridized for a minimum of 2 h at 47 °C in a solution containing 50% formamide, 4 × SSC, 50 mM-sodium phosphate, 1% glycin, 10 mg/ml herring sperm DNA, and 5x Denhardt's reagent (0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinyl pyrrolidone). Hybridization was carried out overnight at 47 °C using 7 × 10^6 c.p.m. of 32P-labelled probe in a mixture of 50% formamide, 3 × SSC, 50 mM-sodium phosphate, 5 mg/ml herring sperm DNA and 10% dextran sulphate. After hybridization, filters were washed twice for 5 min each in 2 × SSC plus 0.1% SDS at room temperature and twice for 1 h each in 0.5% SDS plus 5 mm-Tris, 2.5 mm-EDTA, pH 8.0 at 68 °C. Filters were then exposed to Kodak XAR-5 X-ray film in the presence of intensifying screens at −70 °C for 1 to 5 days.

**Probes.** The probes used for hybridization consisted of a series of seven subgenomic fragments of HCMV strain AD169 cloned in the cosmid vector pHC79 which were provided by Dr B. Fleckenstein (Fleckenstein et al., 1982). These included pCM1015, pCM1049, pCM1029, pCM1058, pCM104 and pCM1052. We used an additional cosmid clone, pCM1039, which was also provided by Dr Fleckenstein. This clone contains the *HindIII* S, P, a, U, b, c, and L fragments. The inserts in these vectors span the entire genome with some overlap.

In addition, we used several individual AD169 restriction fragments which had been cloned in the plasmid pACYC184 (Jahn et al., 1984) or pBR322 (Nelson et al., 1984). Bacteria containing cosmid or plasmid clones were grown in LB medium (1% tryptone, 0.5% yeast extract, Difco) and the clones were amplified during exponential growth by the addition of chloramphenicol. Cosmid or plasmid DNA was prepared by alkaline lysis and further purified by centrifugation through caesium chloride gradients. Probes were 32P-labelled by nick translation to a specific activity of 2 × 10^6 to 4 × 10^6 c.p.m./ug (Rigby et al., 1977). Probes were denatured by heating at 68 °C in 0.1 M-NaOH for 3 min prior to mixing with hybridization solution.

**RESULTS**

Comparison of *HindIII* and *EcoRI* restriction site maps for 20 low passage clinical isolates of HCMV and the four high passage laboratory strains AD169, Davis, Towne and UW-1 are
Restriction site polymorphism of HCMV

shown in Fig. 1 and 2 respectively. In comparing restriction sites, we refer to sites and fragments on the basis of AD169 fragment nomenclature (Fleckenstein et al., 1982). The figures compare, by arbitrarily divided regions of the genome, the variations we observed and the frequency at which each specific group of polymorphisms occurred.

HindIII site variation

The following HindIII sites were conserved in all the strains examined: I-O, O-Y, Y-N, J-Z, Z-M, M-F, F-D, b-U, R-T, T-E, H-V, V-W, W-X and X-Q. The fragments O, V and W co-migrated among all strains. Sites or combinations of sites which were present in AD169 but absent in one or more other strains are shown in Fig. 1, 3(a), 4(a) and 5(a) and include N-J, D-L, D-L-c, c-b, U-a, U-a-P, P-S-R, S-R and E-K.

Additional HindIII sites appeared in one strain or occurred repeatedly in a number of different strains. An additional HindIII site was found in J in 12 isolates, Davis and Towne, generating 12 kb and 1.5 kb fragments which hybridized to pCM1015 (Fig. 3a). An additional site in M in UW-1 generated 6-1 kb and 5-4 kb fragments which hybridized with pCM1049 (Fig. 6a). Two fragments of 9-2 kb and 11-5 kb which hybridized with both pCM1049 and pCM1029 in two isolates indicated an additional site in F (Fig. 6a). In the above cases the locations of the additional sites within the respective fragments were not determined.

A 4-2 kb fragment detected with pCM1058 (Fig. 5a) indicated an additional HindIII site in R in UW-1 and two isolates. UW-1 and one isolate had an 8-6 kb fragment (S-R minus R') and the other isolate a 16-3 kb fragment (P-S-R minus R'). Thus, this site can be located 4-2 kb from the R-T site. Identity of bands containing all or part of the genome corresponding to R of AD169 was confirmed by hybridization with pCM5009, a probe consisting of this fragment alone.

An additional HindIII site in T in one isolate generated 4-1 and 2-2 kb fragments as shown schematically in Fig. 7. The site was located 2-2 kb to the right of the R-T site but left of the XbaI site located in this fragment, determined by hybridizing HindIII- and HindIII-XbaI-digested DNA with pGJ10.

In Davis and 15 isolates an additional HindIII site in E generated 14-5 and 7 kb fragments (Fig. 5a). The site was placed 7 kb from the E-K site since the probe pGJ7 (6-8 kb, Fig. 7) detected only the 7 kb fragment.

An additional site in K generated a 1-7 kb fragment detected by pCM1058 in UW-1, Towne, Davis and 19 isolates (Fig. 5a). One isolate lacked the E-K site and showed a 14-5 kb fragment (E) and an 8-7 kb fragment corresponding to the 1-7 kb K' fragment plus the 7-0 kb E' fragment.

A 14-5 kb band hybridized to pCM104 in Towne and four isolates, indicating an additional site in H within the short unique segment of the genome (Fig. 8a).

In some strains certain fragments were slightly smaller than the corresponding AD169 fragments. This probably indicates the presence of additional HindIII sites generating fragments too small to be detected under our conditions since corresponding decreases in the sizes of EcoRI fragments were not seen. In UW-1 and one isolate, Y was smaller by approximately 0-2 kb (Fig. 3a). Fragment Z was smaller in Towne by about 0-4 kb (Fig. 6a). In five isolates, X was slightly smaller than in the other strains (Fig. 8a).

The fragment of Davis corresponding to T of AD169 was slightly larger compared to the other strains (Fig. 5a). This increase was localized to a BamHI-HindIII fragment as shown in Fig. 7.

Fig. 1. Patterns of HindIII restriction site variation in 20 clinical isolates of HCMV, Towne, Davis and UW-1 in comparison to AD169. Patterns of variation are shown by groupings of several fragments which are identified by all or part of a particular probe. The heights of the pattern groupings are proportional to the total number of strains showing the pattern; names and numbers to the left of the groups indicate the specific laboratory strains and numbers of clinical isolates showing that pattern. Asterisks on the AD169 map indicate HindIII sites conserved among all strains studied. Arrows indicate sites present in AD169 but absent in one or more other strains. Sites present in one or more strains but not present in AD169 are indicated by dashed lines. Whether or not a particular site has been positioned unambiguously within a fragment is discussed in the text. Question marks indicate probable sites in strains in which a fragment is smaller than the corresponding AD169 fragment by <0-5 kb.
Expansion of Restriction Site Polymorphism of HCMV (a) kb
23.1 - 9.42 6.42 4.36 2.32 2.02
(b) Fragments AD 169
3 4 5 1 2 3 4
N K
2185 Fragments
7 8 Q, I i q I i
Number of isolates 6 1 12 1
Lab strains AD 169 Davis Towne UW 1
I pCM1015; iL 7 6 1 1 1
ADI69 UW-I Davis Towne
~ ??? ~ ~
12.1 kb ? 9.9 kb
10.8 kb 17 kb t
1.5 kb
9 kb 7 kb?
AD169
1 2 3 4 5
Fragments
(b) AD169
1 2 3 4 5 6 7 8
G, K
Q Q' Q'
S Y, Q' Y Y'
c
? 
12.1 kb 9.9 kb
17 kb 10.8 kb
11.5 kb
10 kb
9 kb
7 kb
Fig. 3. Autoradiograms of Southern blots of DNA from representative HCMV strains digested with HindIII (a) and EcoRI (b) and hybridized with pCM1015. In both, lane 1 shows the AD169 fragments. The letters on the right identify the positions of identical and variant fragments in the remaining lanes. The number of clinical isolates and the specific laboratory strains showing the pattern in a given lane are listed at the bottom in this and subsequent figures. In (b) and in several subsequent figures not all patterns observed are shown. Symbols used in the maps are described in the legend to Fig. 1.

EcoRI Site Variation

The following EcoRI sites were conserved in all strains examined: K–c, c–Y, Y–S, S–Q, Q–b, b–Z, E–M, P–V, I–A, D–W, W–B, B–R, R–d, c–J, J–a and C–L. The fragments c, Y, S, W, B and R co-migrated among all strains. The absence of seven sites or combinations of sites which were present in AD169 generated fragments having sizes expected from fusion of the AD169 fragments as shown in Fig. 2, 4(b), 5(b), 6(b) and 8(b). These sites included Z–E, M–P, A–D, d–e, N–X, X–T and X–T–C.

Additional EcoRI sites, compared to AD169, were documented. In one isolate, 2.9 and 3.5 kb fragments hybridized with pCM1015, replacing the 6.4 kb Q fragment (Fig. 3b). No 15.2 kb band (E) was seen hybridizing to pCM1029 or pCM1049 in Davis and five isolates (Fig. 6b). Hybridization with a cloned E fragment (pCM5002) identified a single band of 7.6 kb in Davis and four isolates indicating an additional EcoRI site approximately in the centre of the fragment. In the fifth isolate, bands of 11.1 kb (Z–E') and 7.6 kb (E') were seen. Davis and 12

Fig. 2. Patterns of EcoRI restriction site variation in 20 clinical isolates of HCMV, Towne, Davis and UW-1 in comparison to AD169. See Fig. 1 for explanation of symbols used. Italicized numbers associated with the G and K fragments indicate sizes in kilobases of observed bands which probably correspond to these fragments.
Fig. 4. Autoradiograms of Southern blots of DNA from representative HCMV strains digested with HindIII (a) and EcoRI (b) and hybridized with pCM1039. Lanes 10 (a) and 4 (b) show AD169 fragments. The letters on the left identify the positions of identical and variant fragments in the remaining lanes. Symbols used in the maps are described in the legend to Fig. 1; size markers are as in Fig. 3.

Fig. 5. Autoradiograms of Southern blots of DNA from representative HCMV strains digested with HindIII (a) and EcoRI (b) and hybridized with pCM1058. Lane 11 in each shows the AD169 fragments. The letters on the left identify the positions of identical and variant fragments in the remaining lanes. Symbols used in the maps are described in the legend to Fig. 1; size markers are as in Fig. 3.
isolates had 5.5 and 1 kb bands which hybridized with pCM1029 and pCM1049 instead of a 6.5 kb band, indicating an additional EcoRI site in P (Fig. 6b). Towne and four isolates which also lacked the 6.5 kb band had 5.5 kb (P') and 8.2 kb (M + P') bands, thus locating the site closest to the M fragment.

The A-D fusion fragment which hybridized with pCM1039 was smaller in UW-1 and three isolates. These strains also showed a 7.5 kb fragment (Fig. 4b). This fragment was cloned from one of the isolates. It hybridized to the HindIII D fragment which placed the additional EcoRI site 7.5 kb from the I-A site. Eight isolates had an additional EcoRI site in J as indicated by hybridization of a 9.6 kb band instead of a 10.6 kb band with pCM1058 (Fig. 5b). This was placed nearest the e-J site because hybridization with pGJ6 detected both fragments of J (shown schematically in Fig. 7). A band in Towne which hybridized to pCM1015 was approximately 0.2 kb smaller than Q of AD169 suggesting an additional EcoRI site in Q (Fig. 3b). One isolate, when hybridized with pCM1058, had a 1.5 kb band instead of the 1.7 kb e fragment, suggesting an additional site in e (Fig. 5b).

Several other variations were seen. Six isolates had no 3.3 kb band (a) but instead a 4 kb band after hybridization with pCM1058 (Fig. 5b). This may represent an a-O EcoRI site further into the O fragment region compared to AD169. One isolate, when hybridized with pCM1029, showed a difference in location of an EcoRI site in the V and I fragments (not shown). Bands of 6.5 and 9.1 kb instead of 11.4 kb (I) and 4.2 kb (V) were seen. Two isolates had slightly larger fragments replacing b after hybridization with pCM1049 (Fig. 6b) with no corresponding decrease in size of the adjacent fragments Q or Z.

Considerable variation was seen in the G-i-g-K region which hybridized with pCM1015 (Fig. 3b). Three isolates were identical to AD169. Among ten strains with 9-9 kb bands similar to K of AD169 and fragments corresponding to i and g, seven isolates and UW-1 had 11.5 kb bands, one isolate a 10 kb band, and Davis a 17 kb band, all probably corresponding in part to G.
Fig. 7. Patterns of HindIII, EcoRI, BamHI and XbaI restriction site variation in the region of the HCMV genome specifying immediate early functions. Two HindIII sites, two EcoRI sites and one BamHI site not found in AD169 but found in other HCMV strains are indicated by dashed lines. The arrow indicates an EcoRI site which is absent in Davis and two clinical isolates. The BamHI–HindIII fragment which is larger in Davis compared to other strains is bracketed. The immediate early RNAs are those described by Jahn et al. (1984).

Fig. 8. Autoradiograms of Southern blots of DNA from representative HCMV strains digested with HindIII (a) and EcoRI (b) and hybridized with pCM104. Lanes 5 (a) and 4 (b) show the AD169 fragments. The letters on the left identify the positions of identical and variant fragments in the remaining lanes. Symbols used in the maps are described in the legend to Fig. 1; size markers are as in Fig. 3.
(12.5 kb) of AD169. Since we did not see smaller bands we could identify as part of G we hypothesized additional sites located beyond the region identified by pCM1015. The co-migration of the HindIII O fragment in all strains rules out the possibility of insertions or deletions in the corresponding portion of EcoRI G; however, this is an alternative explanation of the variation in the region where we have suggested additional sites. Seven isolates and Towne had no bands the sizes of i or g but instead a 1.1 kb band, and a 10.8 kb band probably corresponding to K. Differently sized bands and possible map configurations can be seen in Fig. 2 and 3(b).

Immediate early region

Several immediate early mRNAs have been mapped to the HindIII fragments T and E of AD169 (Jahn et al., 1984). Fig. 7 shows the HindIII, EcoRI, BamHI and XbaI sites of AD169 and the strains in which we have studied this region. Some variation was seen. Davis, UW-1 and 16 isolates had a HindIII site 6.5 kb from the E-K site which may be within the 2.3 kb transcript. Eight isolates had an EcoRI site 10 kb from the e-J site in the J fragment. One isolate had an additional EcoRI site within the EcoRI e fragment, and Davis and two isolates did not have the EcoRI site between the d and e fragments. These EcoRI sites are all within the 7 kb transcript and two are within the 4 kb transcript as well. The BamHI–HindIII fragment cloned as pGJ10 and within the 5 kb transcript was larger in Davis compared to the other strains. Four isolates had an additional BamHI site identified by probing with pGJ7. This site was located 1.8 kb from the BamHI site on the left of pGJ7, and within the 1.9 kb transcript. No variation in XbaI sites was seen.

Transforming region

HindIII + XbaI-digested DNA was hybridized with pCM4127, a clone containing the 558 base pair transforming sequence of HCMV strain AD169. Hybridization to a 2.85 kb HindIII–XbaI fragment was seen in all isolates tested and in the strains Towne, Davis and UW-1, examples of which are shown in Fig. 9.

DISCUSSION

In this study, we have compared patterns of EcoRI and HindIII restriction site polymorphism among 20 clinical isolates of HCMV and four common laboratory strains by hybridizing digested DNA with a series of cloned fragments of the AD169 genome. Although others have mapped HCMV strains in this manner (Colimon et al., 1985) and used this technique to make epidemiological comparisons (Spector et al., 1984), comparisons of the frequencies of specific restriction site differences in large numbers of strains have not previously been made.

We have observed that 16 of the 29 EcoRI sites and 14 of the 23 HindIII sites found in the unique regions of AD169 were conserved among all 24 strains. Six EcoRI and three HindIII fragments co-migrated among all strains. We have shown, as have others (Chou et al., 1984; Spector et al., 1984; Colimon et al., 1985), that hybridization occurs with AD169 in all strains under stringent conditions throughout the genome. Thus, over the entire length of the genome a high degree of homology exists among all strains. The variable restriction sites that we observed occurred throughout the entire length of the unique portions of the genome. Thus, it also appears that no extensive length of DNA lacks some degree of variation. Clearly, we have only defined a small fraction of the potential spectrum of variation of the HCMV genome. Undoubtedly, examination of more isolates would identify additional variable EcoRI and HindIII sites. Among the three isolates studied by Colimon et al. (1985), they describe an additional HindIII site within the J fragment and loss of the R–T site, which we did not observe. In addition, cleavage by other restriction enzymes would identify more extensive variation and enable definition of its scope more fully.

We have not detected any major deletions or insertions of DNA when comparing these strains. However, since we have not attempted to resolve all the ambiguities in our maps it is possible that smaller changes of this nature exist. We did identify fragments which were smaller by <0.5 kb in a few isolates compared to the others, e.g. HindIII Y and X and EcoRI e. The fragments generated by the opposite enzyme in the corresponding segment of DNA did not
Isolates

Fig. 9. Autoradiogram of Southern blot of DNA from AD169, Towne, Davis and three clinical isolates digested with HindIII and XbaI and probed with pCM4127, the transforming region of AD169. The 2.85 kb band was identical in all HCMV strains studied.

show the same decrease in size. Thus, we hypothesized additional restriction sites which generated fragments too small to be detected in our gels. Towne showed similar decreases in size of both HindIII Z and EcoRI Q compared to AD169. This could represent a small deletion or additional closely spaced sites in both fragments.

We had anticipated that the region of the genome specifying immediate early functions would be highly conserved but in fact some differences were observed. Some of these occurred within mapped transcripts. It is interesting that all strains studied contained sequences which hybridized with the AD 169 transforming region under stringent conditions. However, whether minor differences exist in any isolate such that the fragment does not transform cells in vitro can only be determined by functional studies.

With a given HCMV isolate, as with herpes simplex virus (HSV), the genotypic pattern of restriction sites is stable through many tissue culture passages, upon repeated isolations from a human host, and upon transmission to a new host (Huang et al., 1980). This suggests a low mutation rate and that the variations observed have probably been accumulating in the population over a long period of time. It can be seen in Fig. 1 and 2 that most of the polymorphisms we observed occurred in multiple isolates. Since all our clinical isolates were obtained locally some of the patterns we observed may represent geographical clustering of particular variants. In some regions of the genome AD169, Davis and Towne, which were recovered elsewhere, show unique patterns. One of our local isolates from a recent immigrant from Taiwan was unique in two portions of the genome: the size of the fragments in the EcoRI G–i–g–K region (Fig. 2, pattern seen fourth from the bottom with pCM1015) and the loss of the HindIII E–K site (Fig. 1, pattern seen in bottom panel with pCM1058). However, in other regions this isolate grouped with other Seattle isolates. Likewise some of the polymorphisms observed in the three isolates from Paris mapped by Colimon et al. (1985) were different from
ours, as previously discussed. As has been seen with HSV, evidence of geographical clustering of HCMV variants will likely require study of large numbers of isolates from widely separated and relatively isolated population groups. Chaney et al. (1983) found no groupings with a geographical basis in a study of 29 HSV-1 and 38 HSV-2 isolates from two regions of Canada. However, Sakaoka et al. (1986) grouped 93 HSV-1 isolates from Japan and 32 from Kenya on the basis of restriction site polymorphisms and found significant differences between isolates from the two countries as well as similarities among isolates from within each country.

With so few genes mapped on the HCMV genome it is not possible at this time to consider any functional differences among these strains on the basis of restriction site differences. Clearly, since we isolated virus strains with identical EcoRI and HindIII sites from multiple body sites from 14 of 20 individuals we have not identified any difference associated with tropism for oral or genitourinary sites. A genomic difference associated with a tropism for lymphocytes may eventually be found, since differences in the ability of clinical HCMV isolates to infect lymphocytes in vitro may relate to strain differences and/or degree of passage in tissue culture (Rice et al., 1984). No specific difference which can be related to length of time in tissue culture passage has been found between the laboratory strains and the clinical isolates in our study. Other restriction enzymes might detect a difference or perhaps any change associated with tissue culture occurs immediately or at least prior to the five to 10 passages to which we subjected our isolates. We have also not studied the terminal repeat regions of the genome in this regard. In general, correlation of functional differences with genomic variation among HCMV strains will have to be done as individual genes are mapped and sequenced. As differences are found these and other restriction site polymorphisms may serve as convenient markers for identification of functional variation.

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