Comparison of the Physical Maps of the DNAs of Two Cytomegalovirus Strains

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SUMMARY

The physical maps of the DNAs of two cytomegalovirus isolates, AD169 and SG, were compared by cross-blot hybridization and by hybridization of nitrocellulose-bound SG fragments with cloned $^{32}$P-labelled AD169 fragments. From this comparison it can be concluded that both physical maps are co-linear to a large extent. Most variability existed at the termini of the long and short component (at the repeats). Other differences were the presence or absence of restriction endonuclease cleavage sites.

INTRODUCTION

Human cytomegalovirus (HCMV), a member of the herpesvirus group, is a ubiquitous pathogen showing a large variety of clinical symptoms (Wright, 1973). The DNA of HCMV has a mol. wt. of $147 \times 10^6$ (Geelen et al., 1978a, b). P. Sheldrick et al. (personal communication) proposed a model for the HCMV DNA (strain AD169) in which the genome consists of two unique sequences, $L$ and $S$, bounded by two sets of redundant sequences. This model was confirmed by Kilpatrick & Huang (1977) using denaturation mapping. The terminal sequences ($TR_1$ and $TR_2$) are repeated in this arrangement in an inverted form ($IR_1$ and $IR_2$). This model was also confirmed by restriction endonuclease analysis by Weststrate et al. (1980), Oram et al. (1982) and Spector et al. (1982). By analogy with the structural organization of HSV-1 (Wilkie, 1976), HSV-2 (Cortini & Wilkie, 1978) and bovine mammillitis virus (Buchman & Roizman, 1978) DNA, it could be derived from the molarity of the terminal fragments of AD169 DNA that the DNA molecules exist in four equimolar subpopulations which differ only in the relative orientation of the unique sequences. The same structural organization was demonstrated in the DNA of the Towne (R. LaFemina & G. S. Hayward, personal communication) and Davis (DeMarchi, 1981) strains, which consist also of four equimolar subpopulations.

The DNAs of different CMV strains share a homology of at least 80% (Huang et al., 1976; Pritchett, 1980) based on reassociation kinetics. The only exception to the high level of homology between the DNAs of the human isolates described up to now is the Colburn strain, which is apparently a simian-CMV-related virus (Huang et al., 1978; R. LaFemina & G. S. Hayward, personal communication).

As we are interested in strain variation, we performed cross-blot hybridization experiments to analyse differences in the genome of the AD169 and the more cell-associated strain SG. The physical maps are compared as an introduction to identify specific gene functions in both genomes.

METHODS

Cells and virus. Three times plaque-purified CMV (strain AD169 and SG) was propagated in diploid human embryonic lung cells grown in roller bottles (surface area approx. 720 cm$^2$), using Eagle's BME medium supplemented with Hanks's salts and 10% newborn calf serum for growth and Eagle's BME medium supplemented with Earle's salts and 5% newborn calf serum for maintenance. The media contained 100 units penicillin/ml and 100 μg streptomycin/ml. The cultures were infected at confluency at a multiplicity of infection of 0.1. The virus was...
harvested from the medium when the cells showed an advanced cytopathic effect and virus DNA was isolated as described by Geelen et al. (1978a, b).

Endonuclease digestion, electrophoresis, estimation of mol. wt. and molarities, blotting, labelling, hybridization and cross-blot hybridization were performed as described previously (Weststrate et al., 1980).

RESULTS

After cleavage of the DNAs of different CMV isolates, electrophoresis patterns are obtained which are unique for a specific isolate, although a large number of co-migrating fragments are found (Geelen & Weststrate, 1980; Huang et al., 1976; Kilpatrick et al., 1976). Cleavage of the DNA with BgIII, HindIII and XbaI resulted in 32, 28 and 21 fragments respectively for AD169 DNA and 36, 32 and 24 fragments respectively for SG DNA.

The molecular weights and molarities of the SG fragments are presented in Table 1. The molecular weights of the AD169 fragments have been published previously (Weststrate et al., 1980). In the electrophoresis patterns of the SG DNA four 0.5 M and four 0.25 M fragments have been identified, indicating the presence of four equimolar subpopulations of the DNA molecules. The electrophoresis patterns of both isolates are presented in Fig. 1, in which it can be seen that a large number of fragments co-migrated in the two isolates. The co-migration became more evident when longer electrophoresis times were used (e.g. 42 h at 30 V).

Although co-migration of restriction endonuclease fragments does not prove their identity, it seems likely that similar cleavage patterns indicate a high degree of sequence homology.

The cross-blot hybridization technique was used to analyse the homology between co-migrating fragments of AD169 and SG DNA. To determine whether cross-blot hybridizations can be used to identify homologous regions, a homologous cross-blot hybridization was first performed as shown in Fig. 2. As it is difficult to identify both small and large fragments in one electrophoresis run, short (Fig. 2a) and long (Fig. 2b) electrophoresis times were used (18 h and 42 h at 1.5 V/cm respectively). As can be seen from this figure, a diagonal of hybridization spots and additional hybridization spots were found caused by fragments containing a repeat (0.5 M and 0.25 M fragments). From this figure it can also be seen that both large (e.g. fragment A) and small (e.g. fragment i and j) fragments are transferred and hybridize well by this technique. In addition, homologous termini (e.g. fragment a) hybridize to themselves, although these are found in low amounts. Even if the terminal fragments are found in a broad band due to size heterogeneity, hybridization can be detected (e.g. fragment A). In our hands the cross-blot hybridization technique gave reproducible quantitative results. Additional evidence that transfer of CMV DNA fragments to nitrocellulose occurred quantitatively is based on the following experiment: nitrocellulose-bound CMV fragments were hybridized with 32P-labelled CMV DNA and after hybridization the electrophoresis patterns were scanned. These scans are comparable to the scans of the EtBr-stained electrophoresis patterns (data not shown).

The results of the heterologous cross-blot hybridizations are presented in Table 2. Fig. 3 shows the cross-blot hybridization between the HindIII fragments of the AD169 and SG DNA. From these data it can be concluded that most co-migrating fragments hybridize with each other, indicating a high degree of sequence homology between these DNAs. No hybridization or only a weak hybridization was observed between most of the submolar fragments of AD169 and SG DNA, indicating a low level of homology in these regions. As this low level of hybridization observed could result from low prevalence of fragments containing a repeat or from the sometimes rather high background in the high mol. wt. area, additional hybridization experiments were performed. Nitrocellulose-bound BgIII, HindIII and XbaI fragments of SG DNA were hybridized with cloned AD169 fragments (kindly supplied by B. Fleckenstein, Erlangen, F.R.G.).

AD169 HindIII GIV (Fig. 4) hybridized with SG HindIII O, Z, W and G, with SG BgII D, J, K and Q and with SG XbaI V, S, N, O and I but not with SG HindIII b and SG XbaI X. As SG HindIII b and SG XbaI X are 0.5 M terminal fragments containing the repeat delimiting the short unique sequence, it can be concluded that these repeats of the AD169 and SG DNA do not hybridize with each other. This was confirmed by other hybridization experiments, as AD169 HindIII GU, JI and JU did not hybridize with SG HindIII b and SG XbaI X (data not shown).
Cytomegalovirus DNA maps

Fig. 1. Restriction endonuclease profiles of HCMV DNA, strain AD169 and SG. CMV DNA (1 μg) was cleaved by (a) BglII, (b) HindIII and (c) XbaI as described previously (Weststrate et al., 1980) and electrophoresed on 0.6% agarose gels. Lambda DNA was cleaved by HindIII and co-electrophoresed. The restriction endonuclease fragments are designated by capital letters in order of decreasing size according to a convention adopted at the Herpesvirus Workshop in Bologna (1981).

<table>
<thead>
<tr>
<th>BglII fragment</th>
<th>Mol. wt. (x 10^-6)</th>
<th>Molar ratio</th>
<th>HindIII fragment</th>
<th>Mol. wt. (x 10^-6)</th>
<th>Molar ratio</th>
<th>XbaI fragment</th>
<th>Mol. wt. (x 10^-6)</th>
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<td>H</td>
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Fig. 2. Cross-blot hybridization of $^{32}$P-labelled $Bgl$II fragments with unlabelled $Bgl$II fragments of SG DNA (homologous cross-blot hybridization). $Bgl$II fragments were electrophoresed for (a) 18 h at 30 V or (b) 42 h at 30 V. Asterisks indicate submolar fragments.
AD169 *Hind*III JUXWV hybridized with SG *Hind*III e, X, W, U and Z and with SG *Bgl*II L, i, E and Q. As SG *Bgl*II a and AD169 *Hind*III J are located at corresponding termini of SG and AD169 DNA respectively, but do not hybridize with each other, it can be concluded that the repeats bordering the long unique sequence of the SG and AD169 genome do not hybridize with each other. From the hybridization of SG *Hind*III Z with AD169 *Hind*III GIV and JUXWV, it can be concluded that the Z fragment, which is a near terminal unique fragment from the right end of SG UL, is homologous to the repeats bordering the AD169 UL region.

SG *Hind*III Y and SG *Bgl*II g and U are 1 m fragments which do not hybridize with the corresponding AD169 fragments, or with other parts of the AD169 DNA using cloned AD169 *Hind*III fragments, indicating that these fragments are unique for the SG DNA. These data were confirmed by hybridization of total $^{32}$P-labelled AD169 DNA to nitrocellulose-bound SG *Hind*III and *Bgl*II fragments, in which experiments SG *Hind*III Y and SG *Bgl*II g and U were not detected (data not shown).

SG *Bgl*II g and U (Fig. 2) did hybridize in the homologous cross-blot hybridization; thus, the lack of hybridization in the heterologous cross-blots was not due to the technique itself. In the filter hybridization, fragments which are smaller than SG *Bgl*II a and U and *Hind*III Y were still detectable (Fig. 4). Thus, our filter hybridization technique is sensitive enough to detect hybridization of $^{32}$P-labelled AD169 *Hind*III fragments with for example SG *Bgl*II a. As SG *Bgl*II a and U and SG *Hind*III Y were not detected it can be concluded that these fragments are unique for the SG DNA.
Table 2. Results of the heterologous cross-blot hybridizations of the BglII, HindIII and XbaI fragments of AD169 and SG DNA

<table>
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<tr>
<th>BglII fragment</th>
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<th>XbaI fragment</th>
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<td>AD169 SG</td>
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</tr>
<tr>
<td>C</td>
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* ND, Not detected.
† AD169 BglII H hybridizes with SG BglII F.

**DISCUSSION**

The results of the hybridization experiments are summarized in Fig. 5, in which the physical maps of the AD169 and SG DNA are compared. Based on restriction endonuclease analysis published earlier (Weststrate et al., 1980), analysis of cloned fragments in plasmids (D. Spector, personal communication) and overlapping cloned fragments (B. Fleckenstein & J. Collins, personal communication) and additional re-cleavage analysis by cross-blot hybridization (Weststrate, 1981), slightly modified physical maps for the AD169 DNA were obtained (Weststrate, 1981). These maps are largely consistent with the maps published recently by Oram et al. (1982) and Spector et al. (1982). Physical maps of the SG DNA were constructed using cross-blot hybridization experiments, identification of terminal fragments and double-digestion of fragments (Weststrate, 1981). The SG DNA consists of a long segment of 125 × 10^6 and a short segment of 25 × 10^6 mol. wt. The repeats bordering the long unique sequence and the short unique sequence have a maximal tool. wt. of 0.5 × 10^6 and 1.8 × 10^6 respectively, as BglII a (0.5 M; 1.8 × 10^6) and XbaI (0.5 M; 1.5 × 10^6) are located at the termini of the long and short unique sequence respectively. These values have been confirmed by P. Sheldrick (personal communication) by electron microscopy.

From a comparison of the physical maps of the AD169 and SG DNA it can be concluded that both DNAs are largely co-linear. The greatest variability between the DNAs was detected in the redundant sequences at the termini of the L and S component. Comparison of the structural organization of several HCMV DNAs shows large differences in the molecular weights of the repeats delimiting the long unique sequence, while the range in molecular weights of the repeats delimiting the small unique sequence is much smaller. TRs and IRs of AD169 (P. Sheldrick, personal communication), SG (Weststrate, 1981), Davis (DeMarchi, 1981) and Towne (R.
LaFemina & G. S. Hayward, personal communication) have a mol. wt. of $1.6 \times 10^6$, $1.5 \times 10^6$, $1.3 \times 10^6$ and $1.8 \times 10^6$ respectively. TR$_1$ and IR$_1$ have a mol. wt. of $5.0 \times 10^6$, $1.8 \times 10^6$, $2.7 \times 10^6$ and $6.6 \times 10^6$ respectively.

Other differences between DNAs of AD169 and SG involve the presence or absence of restriction endonuclease cleavage sites.

One striking difference between DNAs from the SG and AD196 isolates is the presence of regions of the genomes which have little or no detectable cross-hybridization. The regions which fail to cross-hybridize fall into two categories: DNA sequences located in the repeated regions
which border the S and L segments, and DNA sequences from the left end of the SG U_L region (e.g. BglII g and U) which did not hybridize to AD169 DNA. Failure to detect cross-hybridization was not due to experimental protocols since the diagnostic fragments were readily detectable in homologous hybridization experiments using DNA from the same isolate (either SG or AD169). Furthermore, fragments smaller than the diagnostic fragments could easily be detected in heterologous cross-hybridization experiments. Equally striking was the detection of marked hybridization homology between DNA from the extreme right end of the long unique region (UL) of SG (HindIII Z) to the repeat sequences bordering the UL region of AD169. These strain differences close to the ends of the S and L segments presumably reflect deletions and substitutions arising during strain evolution or laboratory isolation. At present, our understanding of CMV replication is not adequate enough to suggest how or when such diversity occurred.

REFERENCES


*(Received 2 February 1982)*