Demonstration of the Colinearity of Human Cytomegalovirus Genomes and Construction of Restriction Maps of Unknown Isolates Using Cloned Subgenomic Fragments

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SUMMARY

In this study, we have established the colinearity of human cytomegalovirus (HCMV) genomes using stringent conditions of DNA-DNA filter hybridization of HCMV HindIII fragments and cosmid-cloned AD169 strain HCMV DNA fragments. Large cosmid-cloned fragments of AD169 DNA were used for the preparation of radioactive probes by nick translation. These probes were hybridized to HindIII digests of DNA from three fresh isolates of HCMV and to that of the Davis strain. Using published HindIII restriction maps for the AD169 strain as a reference, the results obtained by hybridization allowed us to construct HindIII restriction maps for the genomes of the three fresh isolates. Confirmation of our methodology was found in the correspondence between the HindIII map we constructed for the Davis strain and that published previously. Furthermore, it is shown that variation in the restriction profiles of the unique regions of the genome are due to the absence or gain of restriction sites, and not to a rearrangement of fragments. This technique allows rapid construction of physical maps of the DNA of any fresh isolate for a given restriction enzyme provided the corresponding restriction map of a strain to be used as reference is available.

INTRODUCTION

Human cytomegalovirus (HCMV) is endemic in human populations throughout the world. It has a genome of \( M, 155 \times 10^6 \) with terminal and internal repeat sequences (Kilpatrick & Huang, 1977; Weststrate et al., 1980; Fleckenstein et al., 1982) which could theoretically encode as many as 80 proteins. It shares the property with other herpesviruses of establishing persistence and latency following primary infection and of reactivating upon immunomodulation of its host. Too little is known about strain variation to say whether certain HCMVs show a greater propensity for latency (or persistence) than others. We do not know the roles which viral and/or cellular genes play in the establishment of latency.

HCMV attacks a wide range of tissues, and causes a variety of patterns of disease in various groups. For instance, HCMV causes interstitial pneumonia in perinatally infected children and bone marrow transplant patients but not in congenitally infected infants or renal transplant patients. We do not know whether different virus strains, host factors, or a combination of both play a role in determining HCMV pathology at the individual level.

A possible oncogenic capacity of HCMV has been described in fundamental studies (Gelman et al., 1983; Spector & Vacquier, 1983; Nelson et al., 1982, 1984) as well as in clinical studies (Giraldo et al., 1972; Geder et al., 1976; Huang & Roche, 1978; Melnick et al., 1978; Hashiro et al., 1979). It would be interesting to know whether HCMV strains isolated from tumours differ from non-tumour-associated strains.

To date, comparison of restriction maps of various strains of HCMV has revealed many similarities but no two profiles are identical (Kilpatrick et al., 1976). It is not possible, based on
co-migration alone, to decide whether sequences are homologous or whether co-migrating fragments are located in the same map positions. This is also true for bands that do not co-migrate. It has been suggested that some digestion fragments have the same size and map position in the U₁ and U₅ regions of the genome. LaFemina & Hayward (1980) and later Weststrate et al. (1983) suggested that HCMV genomes might be colinear. There appear to be size differences in the repeat regions (Geelen & Weststrate, 1982; Tamashiro et al., 1984). Although HCMV genomes display overall homology of about 80% (Minson & Darby, 1982), it is not known whether there are regions which show much less homology.

In order eventually to answer questions concerning HCMV strain variation, it is necessary to have restriction maps of unidentified strains for several enzymes, as a basis for studying the molecular epidemiology of HCMV. Therefore, we decided to study simultaneously the sequence homology and map positions of variable (non-co-migrating) bands by DNA-DNA hybridization using cosmid-cloned fragments of the AD169 genome (Fleckenstein et al., 1982). In the present study, we establish a theoretical basis for the rapid construction of cleavage maps of three randomly selected fresh clinical isolates and of the Davis strain using this method and published AD169 HindIII maps as a reference (Weststrate et al., 1980; Greenaway et al., 1982; Oram et al., 1982; Spector et al., 1982; Fleckenstein et al., 1982). We confirm the validity of this approach by constructing a HindIII map for the Davis strain of HCMV and comparing it with that published by De Marchi (1981). Our results show that variation in restriction profiles is due to the loss or gain of restriction sites but not to a genome rearrangement.

**METHODS**

**Virus and cells.** Reference strains AD169 (ATCC) and Davis (ATCC), as well as fresh isolates (C14, C31, C27) from three viraemic patients (generously provided by Dr Y. Perol, St Louis Hospital, Paris, France) were used in this study.

Virus was isolated from heparinized blood which had been drawn under sterile conditions (10 ml). After a 2 h decantation, leukocytes and plasma were transferred to monolayers of MRC-5 human lung fibroblasts (Jacobs et al., 1970) grown in Eagle’s MEM supplemented with 10% foetal calf serum, ampicillin (100 μg/ml) and kanamycin (200 μg/ml). After 6 h incubation at 37 °C, culture supernatants were replaced with fresh growth medium (Ferchal et al., 1971). When cytopathic effects appeared, cells were trypsinized and transferred at a 1/4 surface ratio to fresh monolayers of MRC-5 cells. Passages were repeated until four flasks (150 cm²) showing 100% cytopathic effects were obtained.

**Extraction of viral DNA.** After discarding culture supernatants, 5 ml lysis buffer (0·1 M-Tris-HCl pH 7-5, 0·01 M-EDTA, 0·5% SDS) was added to the cells of each 150 cm² flask. Chromosomal DNA was removed from lysates by precipitation with NaCl (0·25 vol. 5 M-NaCl) overnight at 4 °C according to Hirt (1967). Following centrifugation at 20000 g for 30 min at 4 °C, supernatants containing viral DNA were collected. Viral DNA was deproteinized by digestion with proteinase K (Merck) (200 μg/ml of supernatant) for 48 h at 37 °C. Proteins were then extracted from DNA with phenol–chloroform (by vol., phenol saturated with 1 M-Tris base and chloroform containing 2% isoamyl alcohol). DNA in the aqueous phase was precipitated overnight at − 30 °C with 2 vol. ethanol in the presence of 0·2 M-sodium acetate and collected by centrifugation at 20000 g for 30 min at 4 °C.

**Purification of viral DNA.** DNA was purified on gradients of sodium iodide (density 1·86 g/ml) in 0·05 M-Tris-HCl pH 7·5 containing 0·01 M-EDTA, 0·5 mg/ml sodium bisulphate, and 30 μg/ml ethidium bromide. Gradients were centrifuged in a 50Ti rotor (Beckman) at 42000 r.p.m. for 72 h at 20 °C. Bands visualized by u.v. light were collected and ethidium bromide was removed by treatment with 2-butanol. DNA was then dialysed against a total buffer consisted of 0·02 M-Tris-HCl pH 7·4, 0·007 M-MgCl₂, 0·06 M-NaCl, and XbaI buffer consisted of 0·006 M-Tris–HCl pH 7·9, 0·005 M-MgCl₂, 0·15 M-NaCl. Digestion was stopped by heating for 5 min at 65 °C. Digested DNA was stored at 4 °C in the presence of 1 mM-EDTA at pH 7·5, after verifying the extent of cleavage by electrophoresis (see below).

**Agarose gel electrophoresis.** Digested viral DNA was separated by electrophoresis in a horizontal gel system. Gels consisted of 0·6% type II agarose (Sigma) in a Tris–acetate buffer (0·04 M-Tris, 0·01 M-EDTA, adjusted to pH 7·7 with glacial acetic acid). A HindIII digest of phage lambda DNA was used as molecular weight markers. Migration was carried out at room temperature at 30 mA for 16 h. Gels were then stained with ethidium bromide and photographed with a Polaroid camera using an orange filter.
Transfer of viral DNA to nitrocellulose. Gels were incubated for 10 min in a 0.25 M-HCl solution. After washing in distilled water, they were incubated for two 15 min periods in a solution of 1.5 M-NaCl and 0.5 M-NaOH, then once for 40 min in 0.5 M-Tris-HCl pH 7.0 containing 3 M-NaCl. DNA was then transferred to nitrocellulose (Schleicher & Schüll, BA85, 0.45 μm) according to Southern (1975). After 24 h of transfer, filters were washed for 20 min in 2 × SSC (0.3 M-NaCl, 0.03 M-sodium citrate) and dried for 15 min at 37 °C. DNA was fixed on the filters by heating for at least 2 h at 80 °C.

Preparation of radioactive probes. Bacteria (Escherichia coli K12) transfected with cosmids containing subgenomic fragments of AD169 HCMV DNA were generously provided by Dr B. Fleckenstein (Universität Erlangen, F.R.G.); cosmids were amplified according to his published procedures (Fleckenstein et al., 1982). Total AD169 DNA was extracted and gradient-purified as described above. DNAs were labelled in vitro by nick translation (Rigby et al. 1977). The reaction was carried out at 15 °C for 75 min in the presence of 2 μCi each of α-32P-labelled dCTP and TTP (800 Ci/mmol) and 4 × 10^{-6} M each of unlabelled dATP and dGTP. Labelled DNA was separated from unincorporated deoxynucleoside triphosphates by centrifugation through a Sephadex G-50 column prepared in 0.1 M-Tris-HCl pH 7.5 buffer containing 0.01 M-EDTA. Columns were prepared by filling 5 ml syringes with Sephadex G-50 equilibrated with the above buffer and centrifuging for 3 min at 1500 g to dry the gel. The reaction mixture was then carefully applied to the column (height 4.5 cm) and centrifuged for 3 min at 1500 g. Free nucleotides were retained on the column and the labelled DNA was collected in a siliconized Eppendorf tube at the bottom of the column. The specific radioactivity of the DNA was then determined by scintillation counting.

DNA–DNA hybridization. Hybridization conditions used were calculated based on a 56% G + C content such that an average of 90% homology was required for detection (Minson & Darby, 1982). Filters were prehybridized by incubation for 2 h at 68 °C in a mixture containing 10 × Denhardt’s solution (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin), 6 × SSC, and 0.15 M-sodium phosphate buffer pH 7 (final concentrations). Radioactive DNA probes were denatured by heating at 100 °C for 5 min, followed by rapid cooling. Hybridization was carried out in 50 to 80 ml of prehybridization solution containing a total of 5 × 10^6 c.p.m. for 24 h at 68 °C. Filters were then washed as follows: three times for 20 min in 500 ml 2 × SSC with 0.1% SDS, three times for 20 min in 500 ml 1 × SSC with 0.1% SDS, four times for 15 min in 1 litre of 0.1 × SSC with 0.1% SDS.

After drying, filters were exposed to Kodak X-Omat AR film with an intensifying screen at room temperature for 24 to 72 h. To ensure detection of any weak hybridizations, filters were routinely re-exposed for 1 week.

RESULTS

Restriction profiles

Restriction profiles for strains AD169, Davis and the three fresh isolates of HCMV generated by digestion with the enzyme HindIII, as well as hybridization patterns with total AD169 DNA, are presented in Fig. 1. Our profiles for AD169 and Davis corresponded to those described by others (Spector et al., 1982; De Marchi, 1981). Examination of the HindIII profiles revealed many fragments which co-migrated. Bands that did so most clearly are indicated by arrows (Fig. 1). For convenience, homologous fragments of all strains are referred to using the designations of AD169 fragments (Fleckenstein et al., 1982).

Hybridization in the repeat regions with HindIII digests of viral DNA

When pCM4000 comprising the extreme right end of the AD169 HindIII E fragment (Fig. 2) was hybridized with the other four virus DNAs and compared to its hybridization with that of AD169, it was seen that the cosmid hybridized to all five, with fragment E of AD169 (14.3 × 10^6 Mr), and bands of 9 × 10^6 Mr from Davis and the other three strains. This shows that the sequences of AD169 in pCM4000 are located in a lower Mr fragment having the same size in the other four strains and suggests that these fragments which co-migrate in gels carry the same sequences.

Cosmid pCM1050 was used to examine the repeat and flanking unique sequences, within which the insert of pCM4000 is included. As expected, pCM1050 hybridized to homologous fragments of AD169 DNA digested by HindIII (Fig. 3). It recognized the junction G fragment (13.2 × 10^6 Mr) which, in conformation IV, is composed of fragments K and Q (8.55 × 10^6 Mr and 4.6 × 10^6 Mr, respectively). It also included the one-molar E fragment (14.3 × 10^6 Mr). Due to the inverted repeats, pCM1050 also hybridized with the half-molar fragments H and I (11.6 × 10^6 and 11.53 × 10^6 Mr, respectively), and the quarter-molar fragments A, B and C (23.8 × 10^6, 20.2 × 10^6 and 16.3 × 10^6 Mr, respectively).
Fig. 1. (a) HindIII restriction profiles of strains AD169 (lane 1), Davis (lane 2), and of three fresh isolates C14 (lane 3), C31 (lane 4) and C27 (lane 5). A HindIII digest of lambda phage DNA, used as molecular weight marker, was run in the same gel and Mr are indicated (×10⁶) at the left. Band nomenclature is that of strain AD169 used as reference. Fragments that clearly co-migrate are indicated by arrowheads. (b) Hybridization of HindIII digests of AD169 (lane 1), Davis (lane 2), and three fresh isolates C14 (lane 3), C31 (lane 4) and C27 (lane 5) with a total AD169 DNA probe. Dotted lines running between (a) and (b) indicate bands common to all five strains. The filter was purposely overexposed so as to detect and illustrate the hybridization pattern of AD169 DNA with that of the isolate in lane 5.

When DNAs of the Davis strain and isolates C14, C31 and C27 digested with HindIII were hybridized to pCM1050, all the fragments that hybridized in AD169 were seen except for the one corresponding to the E fragment of AD169. Three 'new' bands were seen with Mr of 9 × 10⁶, 4.4 × 10⁶ and 1.1 × 10⁶. The sum of these Mr values corresponded almost exactly to the Mr of fragment E of AD169. These results, and those obtained with pCM4000, show that Davis and the fresh isolates have two new HindIII sites in the region represented by E in AD169. The position of one of these within HindIII E generated the 9 × 10⁶ and 4.4 × 10⁶ M, fragments, but it cannot be deduced whether the 1.1 × 10⁶ M, fragment is part of HindIII E or K, although our unpublished results suggest that it is a part of the E fragment.

A heterogeneity of bands was observed, being particularly evident at the level of AD169 HindIII fragment Q with Mr ranging from 5.3 × 10⁶ to 4.1 × 10⁶. The bands varied in Mr by multiples of 300000. Similar heterogeneity was also observed for the other four strains, always with a multiple of 300000 between the bands. Although one of these 'Q' fragments in strain Davis appeared to hybridize more intensely, this was due to the presence of the 4.4 × 10⁶ M, fragment homologous to AD169 E which has the same Mr as one of the Q bands.
Restriction maps of HCMV isolates

Fig. 2. Hybridization of plasmid pCM4000 with DNA from strains AD169 (lane 1), Davis (lane 2), and isolates C14 (lane 3), C31 (lane 4) and C27 (lane 5) digested by HindIII. mol. wt. Markers as in Fig. 1. Arrowheads indicate the Mr of hybridizing bands. The map position of pCM4000 is presented under the hybridization pattern.

Hybridization in the long unique region with HindIII fragments of HCMV DNA

Cosmid pCM1007 was used to investigate the long unique region adjacent to, and to the right of, the AD169 E fragment. When pCM1007 was hybridized to Davis, C14, C31 and C27 DNA, hybridization patterns different from that of AD169 were observed in each case (Fig. 4). Thus, the diversity of hybridization patterns reflects a variation in the presence of certain restriction sites, while fragments covered by this cosmid on AD169 are colinear with those of the other strains.

pCM1007 hybridized to Davis DNA at HindIII fragments P, a, U and b. New bands of $8.5 \times 10^6$ M\(_r\) and $23.6 \times 10^6$ M\(_r\) were revealed. The $8.5 \times 10^6$ M\(_r\) band corresponded to HindIII R + S, indicating the loss of a restriction site between these two fragments. The $23.6 \times 10^6$ M\(_r\) fragment corresponded to the sum of the M\(_r\) of AD169 HindIII fragments c, L and D, again indicating the loss of sites between these fragments.

With isolate C14, pCM1007 hybridized to fragments equivalent to AD169, HindIII P, a, U, b and c. There was loss of the HindIII R–S and L–D sites.
Restriction maps of HCMV isolates

On isolate C31, hybridization occurred with bands equivalent to AD169 HindIII fragments P, a and U. Here again, the R–S restriction site was absent. In addition, hybridization occurred to a fragment (9.2 × 10⁶ Mr) corresponding to the combined Mr of fragments HindIII b, c and L, indicating the loss of restriction sites between these fragments.

Finally, pCM1007 hybridized to fragments equivalent to HindIII S, P, and b on isolate C27. Hybridization to a new band of 5.2 × 10⁶ Mr was seen; this corresponds to the sum of fragments a and U. There was also a loss of sites between HindIII fragments c and L, and L and D as reflected by the appearance of a band of 23.6 × 10⁶ Mr.

Hybridization with cosmid pCM1029, which covers AD169 fragments D and F, confirmed the loss of HindIII L–D sites in Davis, C14 and C27. pCM1029 hybridized to high Mr, bands corresponding to L + D (strain C14; results not shown) and to c + L + D (strains Davis and C27) (Fig. 5). AD169 HindIII fragment F was present and had the same Mr in all strains (data not shown).

Hybridization of cosmid pCM1017 to Davis and the three isolates revealed the conservation in these strains of restriction sites found in AD169 (Fig. 6). However, in all cases additional restriction sites appeared within the AD169 J fragment. This was illustrated by a reduction in the Mr of the HindIII J fragment for the four other strains and in the appearance of a small Mr fragment. In the case of C31, there were two new restriction sites within J, resulting in hybridization with a Mr 1.7 × 10⁶ fragment, as well as with the low molecular weight fragment seen in the other three strains.

Hybridization with cosmid pCM1035 showed the presence of the V band and therefore the conservation of the HindIII H–V and V–W restriction sites in the four strains (Fig. 7). Use of cosmid pCM1072 (containing HindIII fragments C, X and W) confirmed the colinearity of bands W and X for all four strains (results not shown).

Hybridization with XbaI digests of HCMV DNA

AD169 HindIII cosmids were hybridized with XbaI digests of viral DNAs, not only to confirm colinearity but also to demonstrate that fragments cloned using one enzyme can serve in the construction of restriction maps using a different enzyme.

pCM1007 hybridized to AD169 XbaI fragments F, G and H (Fig. 8) and to equivalent fragments in strain C27. Strains Davis and C14 showed hybridization with a fragment equivalent to AD169 XbaI fragment H and to a higher Mr, fragment (21.7 × 10⁶) corresponding to the sum of AD169 XbaI fragments F and G. This pattern confirmed the colinearity of these two strains with AD169 and revealed the loss of an XbaI restriction site between AD169 fragments F and G in Davis and C14. This site was also absent from strain C31. In addition, the XbaI site between AD169 fragments H and B was also missing, giving rise to hybridization with a band of 28.3 × 10⁶ Mr, corresponding to the sum of the Mr of these two fragments. For strain C31, it can therefore be said that the colinearity with AD169 exists not only for fragments F, G, H covered by pCM1007, but also for XbaI fragment B which was not normally seen with this cosmid but became detectable here because of the restriction site missing between fragments H and B.

When pCM1050 was hybridized to XbaI digests of the five strains (Fig. 9), the patterns of hybridization with AD169, C14, C31 and C27 were practically identical. The only difference was that the lack of heterogeneity of M bands of the three isolates. It should also be noted that
Fig. 4. Hybridization of \textit{Hind}III digests of five strains of HCMV with cosmid pCM1007. Strains AD169, Davis, C14, C31 and C27 are in lanes 1, 2, 3, 4 and 5 respectively. When compared to AD169, it can be seen that some bands (\(\bigcirc\)) were absent from the other four strains. Conversely, some bands (\(\bullet\)) present in the other four strains were absent from AD169. Longer exposure revealed bands of low molecular weight.
there appeared to be slight $M_r$ differences at the level of the $XbaI$ C band. This might reflect slight variations in the position of the restriction site between fragments M and C in the three isolates. When pCM1050 was hybridized with Davis DNA digested with $XbaI$ (Fig. 9, lane 2), the AD169 $XbaI$ M bands were absent, indicating the appearance of a new site within the M fragment.

The HindIII maps which we constructed are shown in Fig. 10.
Fig. 6. Hybridization of HindIII digests of five strains of HCMV with cosmid pCM1017. Strains AD169, Davis, C14, C31 and C27 are presented in lanes 1 to 5, respectively. This cosmid hybridized with AD169 fragments J, M, N, Y and Z. Bands equivalent to fragments M, N, Y and Z were present in all strains. The AD169 J fragment was replaced by two (Davis, lane 2; C14, lane 3; C27, lane 5) or three (C31, lane 4) fragments (©) of lower $M_r$. In each case, the sum of the $M_r$ of the smaller fragments was equal to that of the AD169 J fragment.
Fig. 7. Hybridization of pCM1035 with HindIII digests of five strains of HCMV. This cosmid hybridized not only with all the submolar fragments but also with the equivalent of the AD169 V fragment of the U5 region, which shows that this band was homologous in the five strains. Lanes 1 to 5, AD169, Davis, C14, C31 and C27, respectively.
DISCUSSION

Analysis of the genomes of four strains of HCMV by DNA–DNA hybridization with cloned subgenomic fragments of strain AD169 has allowed us to ascertain that the genomes of these HCMV strains are colinear with that of AD169 used as a reference strain. This notion was first suggested by LeFemina & Hayward (1980) and later by Weststrate et al. (1983). The latter authors compared the BglII, HindIII and XbaI maps of the AD169 and SG strains by cross-hybridization. An inspection of their HindIII map of the SG strain reveals that many of the restriction sites correspond to those found in our study, particularly among HindIII sites in the Us region. Similarly, when the HindIII map of the Towne strain (LaFemina & Hayward, 1980; Thomsen & Stinski, 1981) UL section is inverted, numerous restriction sites are seen to correspond to HindIII sites of AD169. It can therefore be said that the differences between strains occurs by the loss or gain of a small number of restriction sites, although the majority remain constant from one strain to another. When a site disappears between two other sites which are conserved, the new fragment represents the sum of the two fragments of the reference strain (in our case AD169). The sum of the M_r of fragments in the UL and in the Us regions is the same from one strain to another, at least within the limits of precision of M_r determination used here. No major deletion or insertion could be detected in the unique regions of the four strains examined.

The fact that HCMV genomes appear to be colinear and to show more than 90% homology between strains makes it possible rapidly to construct restriction maps for fresh isolates using cosmid-cloned large subgenomic fragments covering several restriction sites. Our studies show that a certain number of fragments which clearly co-migrate in agarose gels are indeed homologous and have the same genome location. For example, examination of the first study by Kilpatrick et al. (1976) of the HindIII restriction analysis of HCMV DNA shows that the co-migrating bands observed correspond to current, published maps of AD169 as follows. Fragment A corresponds to the complex N/O, B to P, C and D to R and S/T, respectively, E to the complex U–V–W, and F to X. Given practice, it is no longer required to perform hybridizations in order to assign map locations to such fragments. The use of large fragments for hybridization can lead to some ambiguities in the orientation of new restriction sites, as was the case for sites in the HindIII J fragment in the four strains studied. However, these ambiguities can easily be resolved by simultaneous digestion with a second enzyme. With this construction method we cannot determine precisely to which configuration the quarter-molar joint fragments belong. Furthermore, it cannot be affirmed that very small fragments (<5 × 10^5 M_r), particularly those near the repeat regions, have not escaped detection.

The validity of our approach to map construction is seen when we compare the HindIII map we constructed for Davis with that already published by De Marchi (1981). By inverting the UL region with respect to the Us region the two maps are in excellent agreement with only a few minor discrepancies. For instance, we found that an equivalent of the AD 169 b fragment exists in Davis.

This technique, applicable to a large number of isolates simultaneously can be used to study the molecular epidemiology of HCMV. It will, hopefully, contribute to answering some of the questions posed at the beginning of this article regarding possible genomic differences existing between strains isolated in various pathological situations and at various times during the course of disease.

Fig. 8. Hybridization of HindIII cosmid pCM1007 with XbaI digests of five HCMVs. pCM1007 hybridized with the fragments F, G, and H of AD169 (lane 1) and C27 (lane 5). XbaI fragments F and G were absent from strains Davis, C14 and C31 (lanes 2 to 4, respectively) and were replaced by a fragment with a M_r equal to their sum (21 × 10^6). No hybridization with XbaI fragment H was seen for strain C31 (lane 4). Instead, hybridization occurred with a high M_r fragment (28 × 10^6) representing the sum of XbaI fragments H + B.
Fig. 9. Hybridization of XbaI digests of five HCMVs with cosm id pCM1050. Hybridization patterns with AD169 (lane 1) and strains C14, C31 and C27 (lanes 3 to 5, respectively) were the same except that with the fresh isolates (lanes 3 to 5) no size heterogeneity of the M fragment was seen. Davis (lane 2) showed hybridization with lower M, fragments, the sum of whose mol. wt. corresponded to that of the M fragment not seen in this strain.
Restriction maps of HCMV isolates

AD169

Davis

C14

C31

C27

Fig. 10. Restriction maps of strains Davis, C14, C31 and C27 deduced by DNA-DNA hybridization using cosmids containing subgenomic HindIII fragments of strain AD169 whose HindIII map was used as a reference. Fragment nomenclature is that of AD169 in order to demonstrate the colinearity and to follow more easily the appearance and disappearance of restriction sites compared to those in AD169 DNA. In the equivalent of the AD169 J fragment, there are one (Davis, C14 and C27) or two (C31) additional restriction sites. Open arrowheads indicate the possible location of these additional sites. Terminal and internal repeat regions are delineated by dotted lines since the size of these regions cannot be determined precisely.

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