Characterization of the Genome of a Vaccine Strain of Canine Adenovirus Type 1

Y.-C. LIU, M.G. ABOUHAIDAR,[1] S. SIRA, and J.B. CAMPBELL

Departments of Microbiology and [1]Botany, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Requests for reprints should be addressed to Dr. J.B. Campbell, Department of Microbiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada.

Abstract

Restriction endonuclease cleavage maps have been constructed for the genome of a canine adenovirus type 1 (CAV-1) vaccine strain (CLL; Connaught Laboratories, Ltd., Willowdale, Ontario). Restriction enzyme analyses were also carried out on CAV-1 (CLL) genomes isolated from viral stocks over 8 serial passages in a dog kidney cell line (DK 6722). The right hand 20% of the genome became more heterogeneous in size with increasing passage in DK 6722 cells due to deletions up to 3–4 kb, whereas the left terminal region was stable throughout these passages. A comparative study of CAV-1(CLL) and a virulent strain of CAV-1, Glaxo, revealed that the genome of CAV-1(CLL) was the shorter, by about 480 bp, within the region covering 0.83–0.91 map units. By virtue of its location within the genome and its dispensable nature for viral growth, this region would appear to encompass a genetic sequence corresponding to the E3 region of human adenoviruses. In terms of viral attenuation, the possible importance of the observed differences between CAV-1(CLL) and CAV-1(Glaxo) is discussed.

Introduction

The adenoviral genome is a linear, double-stranded DNA molecule of 20–30 × 10⁶ D (1) with a 55 kD protein molecule covalently linked to the 5′ end (2). Canine adenovirus type 1 (CAV-1) is a member of the genus *Mastadenovirus* (3, 4). Its genome appears to have a typical adenoviral structure, with blocked 5′ ends that are sensitive to alkali (5, 6) and treatment with S1 nuclease (unpublished obser-

vations). The CAV-1 genome has been reported to possess a typical adenoviral inverted terminal repetition (ITR) of the form abc ... a'b'c' (5, 6), which region contains the origin for DNA replication (7-9).

In a previous study, we reported the cloning of the viral genome of the Connaught infectious canine hepatitis vaccine strain (CAV-1(CLL)) into a plasmid in the form of three BamHI subgenomic fragments, and of the sequencing of the viral ITRs (6). It was found that the ITRs of CAV-1(CLL) DNA molecules contained varying numbers of copies of a 40 bp sequence similar to the NFI binding site of human adenoviruses. The left hand terminus contained 2 or 3 copies of this sequence, whereas the right hand terminus had up to 7-10. One consequence of this variability of size of the right ITR was that small restriction fragments containing it migrated in agarose gels as diffuse, rather than sharp, bands (6). Such diffuse patterns were not observed with DNA from CAV-1(Glaxo), a virulent strain, indicating that the ITRs of this strain did not possess multiple reiterations similar to those of CAV-1(CLL).

In the present paper we extend our previous observations, and also report comparative restriction endonuclease analyses of the DNAs of CAV-1(CLL) and CAV-1(Glaxo). Furthermore, we show that the right hand terminal region of CAV-1(CLL) undergoes internal deletions during serial passage of the virus in dog kidney cells. We also provide some speculation as to the possible significance of the observed differences in terms of the strain characteristics of virulence and attenuation.

Materials and Methods

Virus strains

Infectious canine hepatitis virus type 1 vaccine strain (Connaught Laboratories Ltd.; CAV-1(CLL)) was provided by Dr. K.F. Lawson, Connaught Research Institute. A brief history of the vaccine was included in the report by Sira et al. (6); however, since this is incomplete, a full description is provided here. The vaccine originated in the laboratories of Cornell University, and was received by Connaught Laboratories as the "W" strain, from Mr. F. Woodruff, Gainesville, GA, at the 15th passage in primary porcine kidney cells. The virus was passaged 8 times in primary ferret kidney cultures, then readapted to primary porcine kidney (17 passages). The virus stock was received by us at the fourth passage in a continuous dog kidney cell line (DK 6722; Connaught Laboratories Ltd.). Unless otherwise specified, work described here was done with CAV-1(CLL) at the seventh and eighth passage levels in DK 6722 cells.

A virulent strain of CAV-1, Glaxo, was obtained from Dr. R.C. Povey, Langford Inc., Guelph, Ontario.

Virus culture and assay

All virus growth was carried out in DK 6722 cells which were cultivated in Eagle's minimum essential medium, alpha modification, supplemented with 10% fetal calf serum, penicillin (100 I.U./ml) and streptomycin (100 µg/ml). Monolayer cultures were infected at a multiplicity of 1–10 plaque-forming units (PFU)/cell, and were harvested when cytopathic effects (CPE) were extensive. Following 3 cycles of freezing and thawing to release cell-associated virus, the supernatants were clarified by low-speed centrifugation, and the virus concentrated by ultracentrifugation (64,000 g for 60 min) or tangential-flow membrane ultrafiltration (Pellicon system, Millipore Corp.). Virus was purified by banding in a CsCl gradient (1.34 g/cm³; 17 h at 64,000 g). The plaque assay for infectious viral titer and plaque purification of viral stocks was as described previously (6).

Extraction of viral DNA

DNA was extracted from purified virions as described by Pettersson and Sambrook (10). Viral DNA was extracted from infected cells as described by Hirt (11) with some modifications. Briefly, the infected cells in 6 cm tissue culture dishes were harvested when maximum CPE was observed. The cell pellets were resuspended in 370 µl of TE (10 mM Tris-HCl, pH 8 and 1 mM EDTA). Two µl proteinase K (35 µg/µl) and 25 µl NaDodSO$_4$ (10%) were added successively. After incubation at 37°C for 20 min, 100 µl 5M NaCl was added and the mixture was kept overnight at 4°C. The chromosomal DNA was removed by centrifugation and viral DNA was precipitated with ethanol. Following resuspension in TE buffer and extraction with phenol and chloroform, the viral DNA was ethanol-precipitated, and resuspended in 50 µl TE.

Restriction endonuclease analysis

Restriction endonucleases were mainly purchased from Boehringer Mannheim, Bethesda Research Laboratories, and New England Bio-Labs and were used as recommended by the suppliers.

Southern hybridization

The DNA bands were transferred from agarose gels to nitrocellulose filters as described by Southern (12). The DNA probes were labelled with ³²P-dATP by a random primer labeling technique (13). Hybridization was carried out overnight at 45°C. Filters were then washed successively in 2 × SSC (1 × SSC = 0.15 M NaCl in

0.015 M sodium citrate, pH 7.0) with 0.1% NaDodSO$_4$ twice for 10 min at room temperature, 1 × SSC with 0.1% NaDodSO$_4$ for 10 min at 65°C, 0.2 × SSC with 0.1% NaDodSO$_4$ for 30 min at 65°C and finally in 0.1 × SSC with 0.1% NaDodSO$_4$ for 30 min at 65°C. Filters were dried and exposed to x-ray film (Kodak XAR5) at room temperature.

Results

Restriction endonuclease analyses

Restriction endonuclease analyses were carried out on the complete viral DNA (proteinase K-treated) or on cloned BamHI subgenomic fragments covering the entire genome (6). In determining physical maps, we took advantage of the fact that, even following proteinase K treatment, the residual 5′-terminal peptide linkage of the viral DNA prevented the cloning into plasmids of digestion fragments containing the termini. Since it was possible to clone BamHI B and SalI A directly from viral DNA, this confirmed that they were internal fragments. Double-enzyme digestions showed that BamHI, ClaI, SalI and SmaI maps agreed well with the data of Darai et al. (14) for strain Behring H.c.c. 269. The SacI map, however, was quite different from that reported by these authors.

The SacI and SacI/BamHI digestions of cloned BamHI A, B and C fragments of CAV1(CLL) are shown in Fig. 1. The map order of SacI C and E was identified by SacI/MluI digestion of cloned BamHI A. The order of SacI A and B was determined by the fact that only SacI B hybridized with a ³²P-labelled cloned BamHI C probe (data not shown). In addition, the order of SacI F and G was determined by SacI/BglII digestion of a cloned BamHI C fragment. The right terminal fragment was identified by the SacI/BamHI digestion of cloned BamHI C fragment. Using a similar methodology, physical maps for AsuII, BglII, EcoRI, KpnI, MluI, SnaBI, SpeI and XhoI were constructed for CAV-1(CLL) (Fig. 2).

Because of the variable number of repeated sequences (6) in the right hand ITR of DNA from nonplaque-purified stocks of CAV-1(CLL), small DNA fragments containing this terminal sequence migrate in agarose gels as diffuse bands. This phenomenon was not observed in restriction fragments representing the left hand terminus. Diffuse banding patterns were not observed in fragments from either terminus of CAV-1(Glaxo) DNA (data not shown).

Restriction endonuclease analysis on DNA from virus without plaque purification

CAV-1(CLL) virus was serially passaged 8 times in DK 6722 cells (passages 6–13), without dilution (i.e., at an input multiplicity of about 10 PFU/cell), from passages 6–13, and viral DNA was extracted at individual passage levels by the method of Hirt (11). Analysis of a ClaI digest of these DNAs showed that the terminal ClaI A,
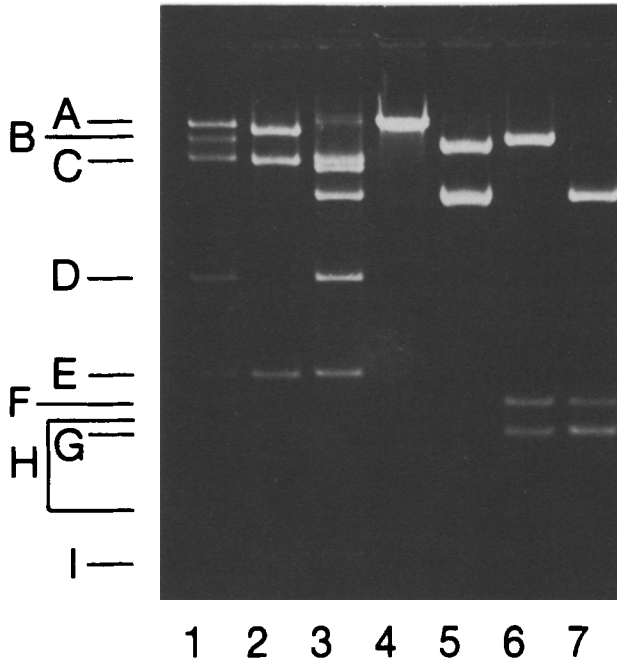
Fig. 1. Analysis of a *Sac*I digest of CAV-1(CLL) DNA. Viral DNA was extracted from purified virions. The recombinant plasmid DNA, which does not have a *Sac*I site, was purified from *E. coli* (see Materials and Methods). DNA was digested with *Sac*I and fractionated in a 1% slab agarose gel. Lane 1: *Sac*I digest of viral DNA releases 9 fragments. The right terminal band, *Sac*I H, is not visible in this picture due to the small size and diffusion of the band resulting from variable numbers of the 40 bp reiterated sequence in individual molecules (see text); 2: *Sac*I digest of cloned *Bam*HI A fragment releases intact internal *Sac*I C and E fragments from the recombinant plasmid; 3: *Sac*I/*Bam*HI digest of cloned *Bam*HI A reveals not only internal *Sac*I C and E, but also, the intact terminal *Sac*I D band, the whole plasmid (pAT153), and a portion of *Sac*I A migrating just ahead of *Sac*I C; 4: *Sac*I digest of cloned *Bam*HI B shows only one *Sac*I site in the *Bam*HI B fragment, linearizing the recombinant plasmid but not fragmenting it; 5: *Sac*I/*Bam*HI digest of cloned *Bam*HI B fragment releases three bands, the lower (plasmid) band being a doublet; 6: *Sac*I digest of cloned *Bam*HI C releases two intact internal *Sac*I F and G fragments; 7: *Sac*I/*Bam*HI digest of cloned *Bam*HI C releases, not only the *Sac*I F and G but also, the right terminal *Sac*I H (which, in the clone used, is the same size as *Sac*I G), a portion of *Sac*I B and the whole plasmid DNA band. Labelled bands on left refer to the *Sac*I digest (Lane 1).

and the *Cla*I C bands remained sharply defined throughout these passages. The *Cla*I B fragments, however, were modified with passage, resulting in a hetero-geneous pattern (Fig. 3A). A similar analysis was carried out for *Sac*I digests (Fig. 4A, B). Mobilities of all DNA fragments from the left terminus to the middle of the CAV-1(CLL) genome (*Sac*I A, C, D, and E) remained unchanged throughout the 8 passages. In particular, the small left terminal fragment resulting from *Sac*I diges-tion, *Sac*I D (2 kb), was stable throughout these passages, giving sharp bands in agarose gels, in contrast to the diffuse bands produced by the right terminal frag-
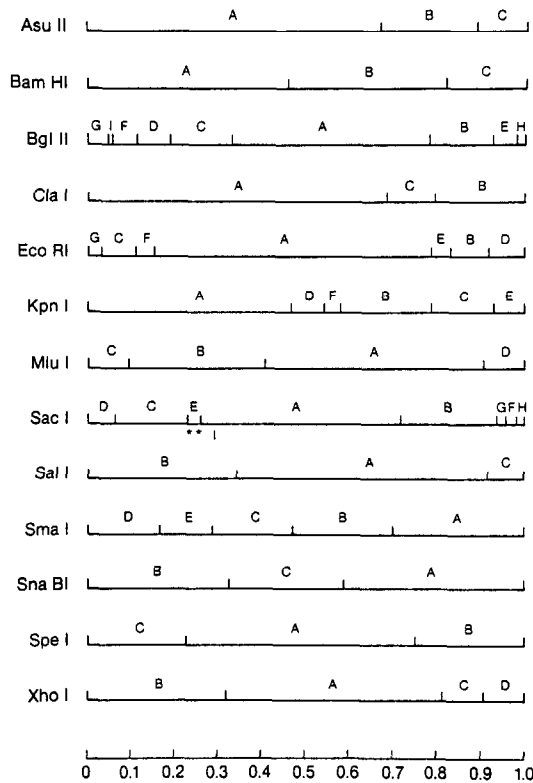
Fig. 2. Physical maps of CAV-1(CLL). Capital letters indicate restriction fragments in descending order of molecular weight. Map units are indicated on the bottom line. Map orientations were by designating *Bam*HI A as the left-hand terminus, on the basis that it has been reported to contain transforming activity (15), and the transforming (E1) region of human adenoviruses is located on the left of the genome (1). Fragments of size < 200 bp may have been excluded. The position of the 190 bp *Sac*I I fragment is not known with certainty: possible sites are noted with asterisks. Enzymes *Bcl*I, *Nde*I, *Not*I, *Nru*I, and *Pvu*I did not cut the viral DNA and/or cloned fragments.

ment *Sac*I H. In addition to the variable length right terminal fragment, there were a number of mutations within the interior of the right half (*Sac*I B, F and G) of the genome. *Sac*I F and G changed from sharply defined bands to a diffuse smearing. *Sac*I B changed from a single band to 3 diffuse bands (Fig. 4B).

Restriction endonuclease analysis of DNA from plaque-purified viral isolates

Viral isolates were prepared by plaque purifications from virus stocks at passages 8 and 13, and viral DNAs extracted from the cells infected by these isolates were analyzed with restriction enzymes. By *Sac*I analysis (Fig. 5), 30 out of 46 viral isolates showed homogenous genomes with a stable left terminal (*Sac*I D) and
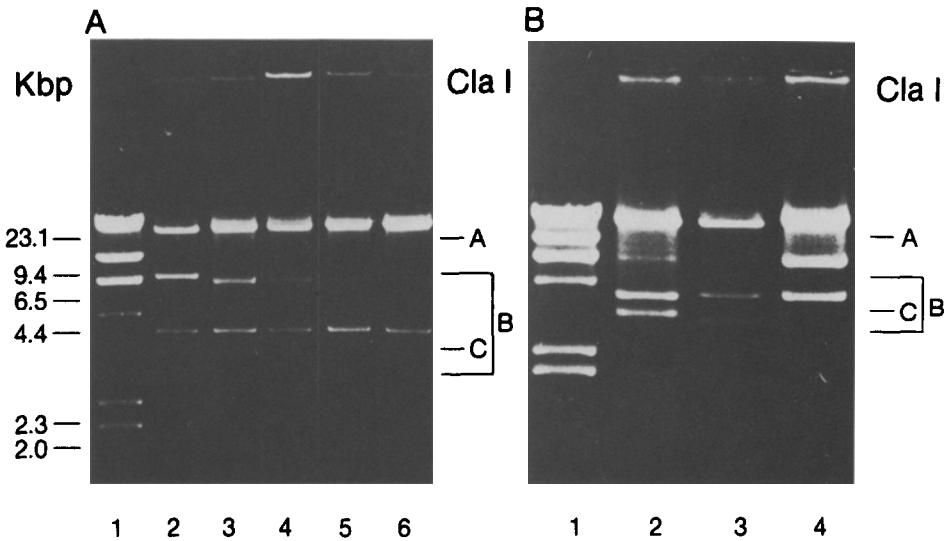
Fig. 3. Analysis of *Cla*I digests of CAV-1(CLL) DNAs from viral stocks. *A*: *Cla*I digestion of viral DNAs from nonplaque-purified viral stocks from passages 7–13. Lane 1: *Hin*dIII digest of lambda DNA; 2: *Cla*I digest of plaque-purified CAV-1(CLL) DNA (as a reference); Lanes 3–6: *Cla*I digests of DNAs from passages 7, 8, 12, and 13, respectively. Note that the *Cla*I B band became increasingly diffuse between passages 7 and 13. *B.* Lane 1: *Hin*dIII digest of lambda DNA; 2,3: *Cla*I digests of DNAs from two isolates plaque purified from stocks at passage 13; 4: *Cla*I digest of plaque-purified CAV-1(CLL). The isolates in lanes 2 and 3 both have full-length *Cla*I A and C bands. However, in addition to a small amount of full-length *Cla*I B, both exhibit well-defined shorter *Cla*I B bands.

stable internal fragments (*Sac*I A, B, C, E, F and G). Size of the right terminus *Sac*I H in isolates at passage 8 ranged from 470–800 bp (average 680). In 12 randomly selected viral isolates made at passage 13 the size of the right terminal *Sac*I H ranged from 470–773 bp (average 580). Since isolates were made from plaques, it would appear that the variable length of *Sac*I H did not affect their viability.

Of the total 46 isolates plaque purified from virus stock at passage 13, 10 appeared to show a mixture of two populations of genomes by restriction enzyme analysis. One population consisted of the full-size viral genomes while the other one consisted of genomes up to 3–4 kb shorter. Two of these isolates with heterogeneous genomes were further analyzed with restriction enzymes *Cla*I (Fig. 3B) and *Sac*I and plaque purifications. In *Cla*I digests of these two isolates, the heterogeneity of the genomes was evident as shown by the two different *Cla*I B bands displayed in each digest (Fig. 3B, lanes 2 and 3). The faint, normal size *Cla*I B band represented the full-size genome while the smaller and more abundant *Cla*I B band represented the shortened viral genomes.

Although the viability of the viral isolates with shortened genomes was not tested, some observations with those two isolates suggested that they are defective. Following plaquing of the individual isolates and random plaque purifications,
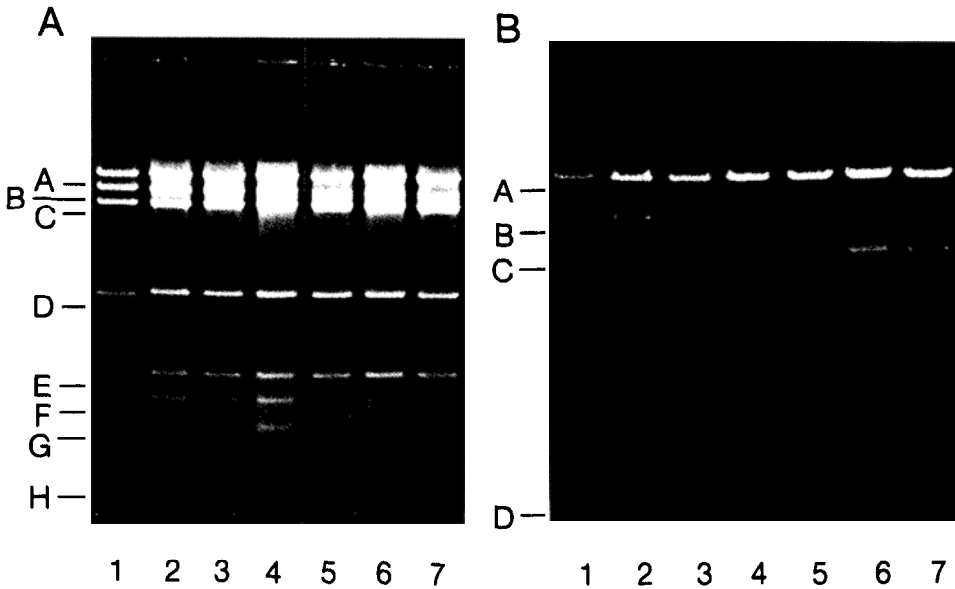
Fig. 4. Analysis of *Sac*I digests of CAV-1(CLL) DNA from nonplaque-purified viral stocks at passages 7–13. The viral DNA was first extracted from DK 6722 cells infected with virus at different passage levels, digested with *Sac*I and fractionated in 1% slab agarose gels. *A*. Lane 1: *Sac*I digest of CAV-1(Glaxo) DNA as a reference; 2–7: *Sac*I digests of CAV-1(CLL) DNAs at passages 7, 8, 9, 11, 12, and 13, respectively. *B*. More prolonged electrophoresis of the same gel shown in *A*.

some of the second generation isolates showed only full-length genomes (i.e., with full sized *Cla*I B bands). Others contained a mixture of the full-sized and shorter *Cla*I B fragments as in the original isolates. In these latter cases, the shorter genomes were always accompanied by some full-length DNA. Additionally, *Sac*I digestion of the two isolates exhibiting shortened genomes did not display *Sac*I F and G bands (data not shown) indicating that deletions were close to the viral extreme right end.

Comparison of the genomes of the CLL and Glaxo strains

Analysis of their restriction digestion profiles showed that differences existed between these two strains. Variations in the right terminal fragments were observed in all CAV-1(CLL) digests (Fig. 6). Differences in the left terminal fragments were also observed in the *Eco*RI and *Bgl*II digests, due to the increased size of the CAV-1(CLL) left ITR (containing 2–3 copies of the 40 bp sequence) and the small sizes of the left terminal *Bgl*II G and *Eco*RI G fragments (Figs. 2 and 6). Although not ascertained by direct sequence analysis, it would appear from molecular weight
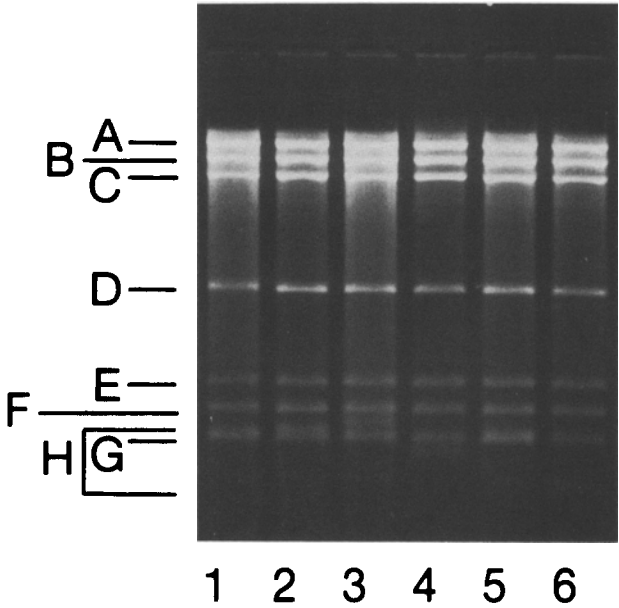
Fig. 5. Analysis of *Sac*I digests of CAV-1(CLL) DNA from isolates plaque purified twice from passage 8 stock. Viral DNA was extracted from DK 6722 cells infected with these isolates. Lanes 1–6: digests of six randomly selected isolates. Note that the *Sac*I H bands are sometimes larger (lanes 1, 2, 3, 5), sometimes smaller (lanes 4, 6) than *Sac*I G (cf. Fig. 4).

comparisons that CAV-1(Glaxo) has only one copy of the reiterated 40 bp sequence (6) in each of its ITRs.

Besides these terminal differences, however, some differences of internal DNA fragments between the two strains were evident, most noticeably with *Bam*HI, *Bgl*II, *Eco*RI, *Kpn*I and *Xho*I digests (Fig. 7). By comparison of the molecular weights of *Bgl*II B, *Eco*RI B, *Kpn*I C and *Xho*I C fragments of both strains (Fig. 6) it can be deduced that all these fragments of the CLL strain are approximately 480 bp shorter than those of Glaxo strain. Furthermore, all these internal fragments from CAV-1(CLL) represented a common region, between 0.83 and 0.91 m.u. Although we did not construct the physical maps for these enzymes with the CAV-1(Glaxo) genome, there is some evidence that the physical map of CAV-1(Glaxo) is basically similar to that of CAV-1(CLL) despite such differences. First, CAV-1(Glaxo) has the same physical maps for *Cla*I and *Sal*I as CAV-1(CLL) (6). Second, except for the terminal restriction fragments and the internal fragments indicated above, all other fragments from both strains appeared identical. Third, despite the difference in sizes, the CAV-1(Glaxo) right terminal fragments and internal fragments *Bgl*II B, *Eco*RI B, *Kpn*I C and *Xho*I C hybridized with cloned CAV-1(CLL) *Bam*HI C probes (data not shown). Therefore, it would appear that the CAV-1(CLL) genome is shorter than that of CAV-1(Glaxo) by approximately
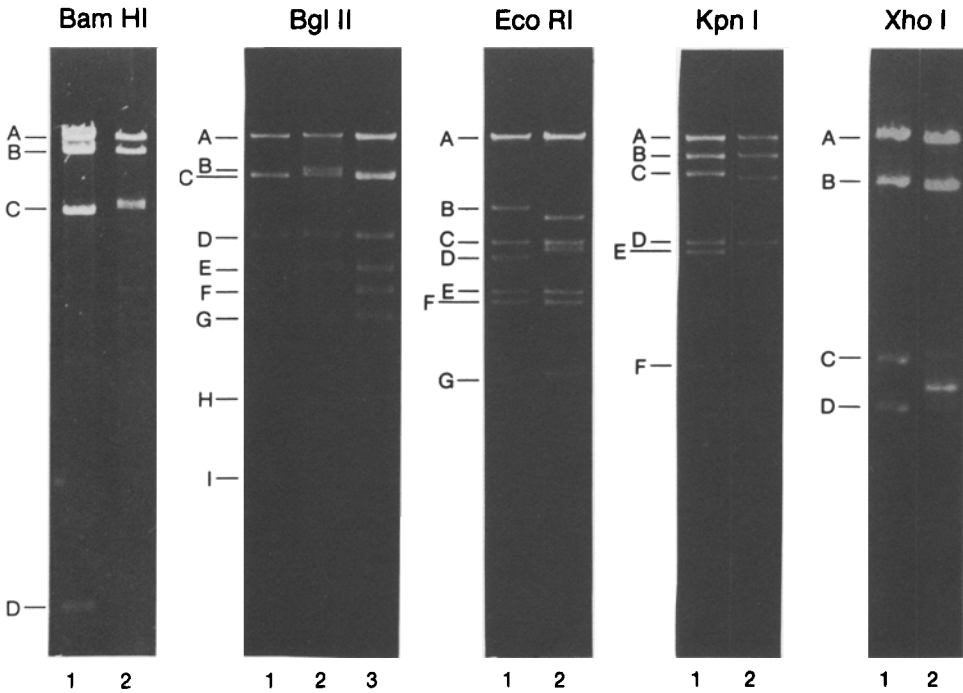
Fig. 6. Restriction endonuclease analysis of CAV-1(CLL) and CAV-1(Glaxo). Viral DNA was extracted from purified virions, digested with restriction enzymes and fractionated in 1% slab agarose gels. **BamHI.** Lane 1: CAV-1(Glaxo); 2: CAV-1(CLL). **BglII.** Lane 1: CAV-1(CLL); 2: CAV-1(Glaxo); 3: plaque-purified CAV-1(CLL), 8th passage. **EcoRI.** Lane 1: CAV-1(Glaxo); 2: CAV-1(CLL). **KpnI.** Lane 1: CAV-1(CLL); 2: CAV-1(Glaxo). **XhoI.** Lane 1: CAV-1(Glaxo); 2: CAV-1(CLL). Note that *Xho*I C and D fragments are not doublets in CAV-1(Glaxo). In the uncloned CAV-1(CLL), the D fragment is a diffuse smear above and below *Xho*I C.

480 bp within the region from 0.83–0.91 m.u. In addition, the *Bam*HI C fragment of CAV-1(CLL) was shown to be a fusion of the corresponding *Bam*HI C and D fragments of CAV-1(Glaxo) (6), thereby indicating one region (0.83 m.u.) of mutation.

Discussion

In this study, we report the construction of physical maps for the genome of CAV-1(CLL). Based on these maps, the size of CAV-1(CLL) DNA is about 32 kb. Other estimations of the size of CAV-1 genome are as follows: 30kb for strain H.c.c. 269 (14); 31 kb for Utrecht (16); and 34 kb for strain Woc-4 (5). Whether or not these differences are actually due to strain differences, it would appear that the genome of CAV-1 is slightly shorter than that of the human adenoviruses (34–36 kb). Some

of the physical maps derived in the present study (*Bam*HI, *Sal*I, *Cla*I and *Sma*I) are very similar to those described for the CAV-1 H.c.c. 269 strain (14). However, the *Sac*I and *Eco*RI maps are quite different from the published data (*Sac*I, ref.14; *Eco*RI, ref. 16). Derivation of the *Sac*I map is described in detail in Fig. 1. On the basis of sequencing data showing ITRs of CAV-1(Woc-4), Shinagawa et al. (5) also determined that the *Eco*RI G and D fragments of this strain were the terminal ones, which is consistent with our data.

Restriction endonuclease analyses show that serial passage of CAV-1(CLL) in DK 6722 cells results in modification of the viral genome, with deletions of up to 3–4 kb occurring mainly in the last 20% of the genome (see Fig. 3B). These observations are similar to those described for human adenovirus types 2 and 5 after serial passage in human cell lines. With human adenovirus type 5, Jones and Shenk (17) showed that there was a much higher mutation rate at the *Eco*RI site at 0.83 m.u. than at 0.76 m.u. following serial passages in HeLa cells. Because they isolated viable mutants, the mutations in other parts of the viral genome were not reported. In another study of human adenovirus type 2 deletion, and mostly defective, mutants, Challberg and Ketner (18) showed that, in a viral stock with 27 passages in some human cell lines, about 80% of the mutants had deletions mainly in the last 20% of the genome, namely within E3, E4 regions, and the fiber gene in between. The other mutants had deletions in the viral late transcription and E3 regions. These results suggest that mutations generated through limited serial passages of adenoviruses in cell cultures appear initially within the last 20% of the right terminus of the viral genome. The similarity between CAV-1(CLL) and human adenovirus might reflect some similarities in the functions of their genes and in their genomic organizations. The two further characterized viral isolates (Fig. 3B) are suggested to be defective in virus multiplication on the basis of analysis of plaque isolates (see above), the large sizes and location of the deletions that would correspond to the E4 region in the human adenoviral genome.

Differences in the restriction enzyme digest patterns between CAV-1 CLL and Glaxo strains are evident from this report. Similar differences have also been reported by Whetstone (19) with other strains (e.g., Cornell-1-66 and Cornell-1-PK) although these differences have not been located on the CAV-1 physical map. The restriction enzyme patterns of Cornell-1-66 (19) are indistinguishable from those of other CAV-1 virulent strains, including the Glaxo strain used in the present study. Cornell-1-PK (19) and CAV-1(CLL), the latter originating in a Cornell strain, have similar, although perhaps not identical, restriction profiles. It is interesting to note that Cornell-1-66 and Cornell-1-PK were derived from the same virulent viral stock (L.E. Carmichael, personal communication) the only difference being that Cornell-1-66 was grown solely in dog kidney cells while Cornell-1-PK was grown in porcine kidney cells (as was CAV-1(CLL)), indicating that differences in restriction profiles can be introduced by growing in different cells.

Our data show that both terminal fragments of CAV-1(CLL) have higher molecular weights than those of CAV-1(Glaxo). The molecular basis for the in-

crease of molecular weights in CAV-1(CLL) terminal fragments has been reported to be the multiple repeats in the ITRs (6). It may be that the increase in molecular weight at CAV-1(CLL) termini resulted from its adaptation to growth in porcine kidney cells and/or ferret kidney cells during vaccine development. In fact, human adenovirus type 12 (20–22) and the equine adenovirus (23) have been shown to gain reiterated sequences in their ITRs after culture in certain cell lines. The potential importance of these multiple repeats in ITRs in CAV-1 CLL vaccine attenuation and the vaccine development is uncertain at present.

The other difference between the CAV-1 CLL and Glaxo strains is an internal region (0.83–0.91 m.u.). The CAV-1(CLL) genome is 480 bp shorter than that of the Glaxo strain in this region. This deletion might explain why the vaccine developed through passages in porcine kidney cells is stably attenuated and free from the virulent revertants after several passages in animals (4). By analogy with the human adenovirus genome, the deletion in the CAV-1(CLL) strain seems within the putative E3 region because of the similar location (0.83 m.u.) and the dispensable nature for viral growth. For human adenoviruses types 3 and 5, viable mutants with deletion and substitution in the E3 region were also isolated from viral stock with serial passages in cell cultures (17, 24).

For human adenoviruses, the E3 region has been suggested to be important in viral pathogenesis (25) and tumorigenicity (26). A 19K glycoprotein encoded by E3 genes has been demonstrated to be associated with the major histocompatibility (MHC) class I antigen (27, 28) and to block its expression at the cell surface (29, 30). This glycoprotein may therefore function by protecting infected cells from elimination by the host immune system (31). In support of this, infections with adenoviruses containing mutations in the E3 region have been reported to be eliminated faster from animals than wild type adenovirus (32). Hence CAV-1(CLL) may conceivably have been attenuated by the deletions resulting in impairment of the E3 functions.

Acknowledgments

References

1. Sussenbach J.S., in Ginsberg S.H. (ed.) *The Adenovirus.* Plenum Press, New York, 1984, pp. 35–124.
2. Carusi E.A., Virology 76:380–394, 1977.
3. Wigand R., Bartha A., Dreizen R.S., Esche H., Ginsberg H.S., Green M., Hierholzer J.B., Kalter S.S., McFerran J.B., Pettersson U., Russell W.C. and Wadell G., Intervirology 18:169–176, 1982.
4. Koptopoulos G. and Cornwell H.J.C., Vet Bull 51:135–142, 1981.

5. Shinagawa M., Ishiyama T., Padmanabhan R., Fujinaga K., Kamada M. and Sato G., Virology *125*:491–495, 1983.
6. Sira S., AbouHaidar M.G., Liu, Y.-C. and Campbell J.B., Virology *159*:76–83, 1987.
7. Hay R.T., J Mol Biol *186*:129–136, 1985.
8. Nagata K., Guffenheimer R.A. and Hurwitz J., Proc Natl Acad Sci USA *80*:6177–6181, 1983.
9. Wang K. and Pearson G.D., Nucleic Acids Res *13*:5173–5187, 1985.
10. Pettersson U. and Sambrook J., J Mol Biol *73*:125–130, 1973.
11. Hirt B., J Mol Biol *26*:365–369, 1987.
12. Southern E.M., J Mol Biol *98*:503–517, 1975.
13. Feinberg A.P. and Vogelsstein B., Anal Biochem *132*:6–13, 1983.
14. Darai G., Rösen A., Delius H. and Flügel R.M., Intervirology *23*:23–28, 1985.
15. Yoshida K., Ren C.S., Yamashita T. and Fujinaga K., in Grodzicker T. and Botchan M. (eds.) *SV40, Polyoma, and Adenoviruses.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1984, p. 107.
16. Jouvenne P., Dion M. and Hamelin C., Gene *60*:21–28, 1987.
17. Jones N. and Shenk T., Cell *13*:181–188, 1978.
18. Challberg S.S. and Ketner G., Virology *114*:196–209, 1981.
19. Whetstone C.A., Intervirology *23*:116–120, 1985.
20. Schwarz E., Reinke C., Yamamoto N. and Zur Hausen H., Virology *116*:284–296, 1982.
21. Werner G. and Zur Hausen H., Virology *86*:66–77, 1978.
22. Kruczek I., Schwarz E. and Zur Hausen H., Int J Cancer *27*:139–143, 1981.
23. Ishiyama T., Shinagawa M., Sato G., Fujinaga K. and Padmanabhan R., Virology *151*:66–76, 1986.
24. Groff D.E. and Daniell E., Virology *106*: 191–194, 1980.
25. Flomenberg P.R., Chen M. and Horwitz M.S., J Virol *61*:3665–3671, 1987.
26. Signäs C., Akusjärvi G. and Pettersson U., Gene *50*:173–184, 1986.
27. Severinsson L., Martens I. and Peterson P.A., J Immunol *137*:1003–1009, 1986.
28. Signäs C., Katze M.G., Persson H. and Philipson L., Nature *299*:175–178, 1982.
29. Andersson M., Pääbo S., Nilsson T. and Peterson P.A., Cell *43*:215–222, 1985.
30. Burgert H.-G. and Kvist S., Cell *41*:987–997, 1985.
31. Burgert H.-G., Maryanski J. and Kvist S., Proc Natl Acad Sci USA *84*:1350–1360, 1987.
32. Morin J.E., Lubech M.D., Barton J.E., Conley A.J., Davis A.R. and Hung P.P., Proc Natl Acad Sci USA *84*:4626–4630, 1987.