Characterization of Tissue Culture-induced Heterogeneity in DNAs of Independent Isolates of JC Virus

By JONATHAN D. MARTIN,* BILLIE L. PADGETT¹ AND DUARD L. WALKER¹

Tulane University, Delta Regional Primate Research Center, Covington, Louisiana 70433 and ¹Department of Medical Microbiology, University of Wisconsin Medical School, Madison, Wisconsin 53706, U.S.A.

(Accepted 28 June 1983)

SUMMARY

After several serial passages at low multiplicities of infection in primary human foetal glial cells at 37 °C, the DNA of prototype (MAD-1) JC virus and that of MAD-2 and MAD-3 are typically heterogeneous in size, but DNAs of MAD-4 and MAD-6 are relatively homogeneous. A similar dichotomy was observed in the DNAs of six isolates propagated more recently in glial cultures at 39 °C under similar conditions of brief passage in vitro at low multiplicities of infection: the DNAs of two (MAD-9 and -10) were heterogeneous, but the DNAs of four others (MAD-8, -11, -12 and -14) were homogeneous. Therefore, the propensity of the viral genome to sustain deletions was an intrinsic property of each isolate. However, actual induction and maintenance of the presumably defective DNAs was influenced by the relative proportions of permissive spongioblasts and semi-permissive astrocytes in the glial cultures and by the multiplicity of infection. Deletions in MAD-1 DNA were confined to the presumptive early region and spanned the BamHI cleavage site (map position 0.505). The heterogeneity was more complex in the DNAs of MAD-2 and MAD-3, but again most of the deletions, which ranged up to 12% of full-length DNA, spanned the BamHI site. We propose that the differential susceptibility to deletion among isolates is a consequence of natural genetic variation in JC virus.

INTRODUCTION

JC-type viruses, which are associated with the fatal disease progressive multifocal leukoencephalopathy (PML), are isolated and grown regularly in primary cultures of human foetal glial (PHFG) cells (Padgett et al., 1971, 1977). JC virus (JCV) replicates slowly in this heterogeneous population of cells, and only those cultures containing at least 50% spongioblasts are suitable for JCV production. After several passages in such cultures, the DNA of prototype JCV (MAD-1) is typically heterogeneous in size (Osborn et al., 1974; Howley et al., 1976; Martin et al., 1979). The heterogeneity seems to be a consequence of deletions from the full-length genome (Osborn et al., 1974). An interesting feature of this phenomenon is that the deletions were generated and maintained under conditions in which the multiplicity of infection (m.o.i.) must have been low. Although the proportion of spongioblasts varies considerably among independent cultures of PHFG cells, the total number of cells per culture (75 cm²) is consistently greater than 10⁶ (J. D. Martin et al., unpublished observations). Such PHFG cultures are routinely infected with 100 to 200 haemagglutinating units (HAU; Osborn et al., 1974), representing 1 × 10⁵ to 2 × 10⁵ fluorescent-cell units, which are units of infectivity (Frisque et al., 1979).

In the related simian polyomavirus SV40, such heterogeneity typically arises only upon undiluted passage of the virus in permissive cells (Uchida et al., 1968; Yoshiiki, 1968; Tai et al., 1972; Brockman et al., 1973; Sol et al., 1975; Norkin & Tirrell, 1982), i.e. at a comparatively high m.o.i. Recently, however, O’Neill and his colleagues have demonstrated that SV40 and the
human polyomavirus BK (BKV) produce heterogeneous DNAs when passed at low m.o.i. in human glioblastoma cell lines (Carroll et al., 1981; O’Neill & Carroll, 1981).

One possible interpretation of these observations is that glial cells are different from other cell types, somehow inducing deletions in polyomavirus DNAs independently of m.o.i. Consequently, stocks of homogeneous, undeleted JCV would be difficult or impossible to maintain, since efficient growth of the virus appears to be limited to PHFG cultures. This interpretation is supported by the finding that DNAs of MAD-2 and MAD-3 (Padgett et al., 1976) were heterogeneous, and also by the observations of Frisque et al. (1979). In opposition is the finding that the DNA of MAD-4, which had been propagated in the same manner, was homogeneous. Whereas glial cells themselves may induce deletions in JCV DNAs, another contributing factor may be the presence, in particular isolates, of specific nucleotide sequences that are readily deleted and/or altered. It has been difficult, however, to examine these possibilities experimentally because growth of JCV is limited practically to PHFG cultures, in which no plaque assay has been developed, and because the status of the JCV DNA prior to isolation was unknown.

Recently, JCV DNA extracted from brain tissue of each of 10 cases of PML, including brain from which MAD-1 was originally isolated, was shown to be homogeneous (Grinnell et al., 1982). Therefore, the heterogeneity described above is induced by passage in vitro. We have investigated the development of heterogeneity by examining the status of the DNAs of 11 JCV isolates passed in PHFG cultures under various conditions. We present evidence that certain isolates readily sustain deletions in their DNAs during brief passage at low m.o.i., whereas others do not. Furthermore, the observed deletions in the genomes of MAD-1, -2 and -3 were localized on the physical map (Law et al., 1979; Martin et al., 1979, 1982) to a region within the presumptive major coding sequence for T antigen and therefore were not random.

**METHODS**

*Media, cells and viruses.* Cultures of PHFG cells were prepared from abortuses and were grown as described previously (Padgett et al., 1977; Martin et al., 1979). JCV MAD-1 was propagated routinely by infection of typical PHFG cultures (75 cm²; approx. 50% spongioblasts) with 100 to 200 HAU of virus at 37 °C (Osborn et al., 1974). Spongioblasts were identified from the description given by Shein (1965) and Padgett et al. (1977). Exceptional, spongioblast-rich cultures (85 to 95% spongioblasts) were infected successfully with 10 to 40 HAU (Martin et al., 1979). Each 75 cm² culture was infected with 1.0 ml of an appropriate dilution of a virus pool which had been titrated for HAU in a 0-4 ml assay system. The isolation and propagation of JCV MAD-2, -3 and -4 in PHFG cultures incubated at 37 °C are described elsewhere (Padgett et al., 1976). MAD-6 was isolated and propagated similarly (B. L. Padgett & D. L. Walker, unpublished results). The more recent isolates (MAD-8, -9, -10, -11, -12 and -14) were isolated and propagated at 39 °C (Grinnell et al., 1982). Virus pools were partially purified as described below.

*Partial purification of JC virions.* Infected cultures were frozen at −20 °C and were thawed at room temperature. Pooled contents of 10 to 20 cultures were acidified with HCl until the methyl red in the medium turned yellow, and were stirred at 4 °C for 16 to 18 h. The fluid was centrifuged at 12,000 g (11,000 rev/min; Type 19 rotor) for 1 h at 4 °C. The pellets were resuspended in 9 ml of supernatant. One ml of receptor-destroying enzyme (200 U/ml; Microbiological Associates, Walkersville, Md., U.S.A.) was added, and the suspension was stirred at 37 °C for 20 to 22 h. One ml of 1% trypsin and 10% deoxycholate (both in phosphate-buffered saline) were added, and the mixture was incubated at 37 °C for 1 h. Virus was sedimented through 3 ml cushions of 20% bovine serum in 50 mM-Tris–HCl, pH 7.5, at 95,000 g (38,000 rev/min; Type 40 rotor) for 1 h at 4 °C. The pellets were resuspended in a final volume of 5 ml of phosphate-buffered saline.

*Preparation of viral DNA.* Superciled (form I) viral DNA was purified from lysates of partially purified virions (Frisque et al., 1979) or from infected cell lysates (Hirt, 1967) by two consecutive equilibrium centrifugations in CsCl-ethidium bromide gradients as previously described (Martin et al., 1979).

*Analysis of viral DNA with restriction endonucleases.* Bacterial restriction endonucleases were purchased from New England Biolabs and Bethesda Research Laboratories. Treatment of viral DNA with restriction enzymes, analyses of intact DNA and restriction fragments in agarose–ethidium bromide gels, and measurements of sizes of DNAs have been described (Martin et al., 1979, 1982).

*Blot hybridization analyses.* JCV-specific DNA was detected in small-scale infections by Southern blot hybridization (Southern, 1975). Infected cells from duplicate cultures (75 cm²) were pooled and lysed with 1% SDS in 10 mM-Tris–HCl pH 7.4, 150 mM-NaCl, 10 mM-EDTA. After three extractions with phenol and three with chloro-
Heterogeneity in DNAs of JCV isolates

Table 1. Source of JCV DNA preparations examined

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No. of passages in vitro</th>
<th>Input HAU*</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAD-1</td>
<td>8</td>
<td>200</td>
<td>Infected cells</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>200</td>
<td>Virions</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>10</td>
<td>Infected cells</td>
</tr>
<tr>
<td>MAD-2</td>
<td>&gt;5†</td>
<td>160</td>
<td>Infected cells</td>
</tr>
<tr>
<td></td>
<td>&gt;5 + 3†</td>
<td>10</td>
<td>Infected cells</td>
</tr>
<tr>
<td>MAD-3</td>
<td>~5†</td>
<td>160</td>
<td>Infected cells</td>
</tr>
<tr>
<td>MAD-4</td>
<td>~5†</td>
<td>160</td>
<td>Infected cells</td>
</tr>
<tr>
<td>MAD-6</td>
<td>8</td>
<td>80</td>
<td>Infected cells</td>
</tr>
<tr>
<td>MAD-8</td>
<td>2</td>
<td>16</td>
<td>Infected cells</td>
</tr>
<tr>
<td>MAD-9</td>
<td>3</td>
<td>40</td>
<td>Infected cells</td>
</tr>
<tr>
<td>MAD-10</td>
<td>6</td>
<td>64</td>
<td>Infected cells</td>
</tr>
<tr>
<td>MAD-11</td>
<td>5</td>
<td>42</td>
<td>Infected cells</td>
</tr>
<tr>
<td>MAD-12</td>
<td>3</td>
<td>10</td>
<td>Infected cells</td>
</tr>
<tr>
<td>MAD-14</td>
<td>2</td>
<td>15</td>
<td>Infected cells</td>
</tr>
</tbody>
</table>

* Haemagglutinating units (0.4 ml assay) per 1.0 ml per 75 cm² culture.
† Exact passage history beyond brain extract not known.

form, total nucleic acids were precipitated with ethanol and dissolved in 0·2 ml of 10 mm-Tris-HCl pH 7·4, 1 mm-EDTA. Samples were fractionated in 1% agarose gels. DNA fragments were transferred to nitrocellulose as described by Wahl et al. (1979). Recombinant MAD-1 DNA (Martin et al., 1982), labelled in vitro with [32P]dCTP (5 × 10⁷ ct/min/µg; Rigby et al., 1977), served as probe. Blots were pretreated for 6 h at 68 °C in 6 × SSC (1 × SSC is 150 mM-NaCl, 15 mM-sodium citrate), 10 × Denhardt’s solution (Denhardt, 1966), 0·1% SDS. Hybridizations were carried out for 21 h at 68 °C in the same solution but containing 0·5% SDS, 100 µg of heat-sheared, alkali-denatured salmon sperm DNA per ml, and 2 × 10⁵ ct/min of alkali-denatured probe per ml. The hybridized blots were washed three times at room temperature for 15 min each in 2 × SSC/0·1% SDS and three times at 68 °C for 30 min each in 0·2 × SSC/0·1% SDS. The washed blots were air-dried and were exposed to Kodak XAR-5 film between DuPont Cronex Lightning Plus intensifying screens for 16 h at 4 °C.

Passage histories of 11 JCV isolates. Table 1 lists the sources of the JCV DNAs used in this study. Unless otherwise stated, isolates MAD-1, -2, -3, -4 and -6 were passed serially in typical PHFG cultures (approx. 50% spongioblasts) at 37 °C. The first one or two passages were initiated with quantities of virus not detected in haemagglutination assays. Each subsequent passage was initiated with 80 to 200 HAU. Since one HAU is equivalent to 1 × 10³ fluorescent-cell units (Frisque et al., 1979), it can be deduced that in no passage was the m.o.i. greater than 0·1 infectious unit/cell. Isolates MAD-8, -9, -10, -11, -12 and -14 were propagated at 39 °C in spongiosblast-rich cultures (85 to 95% spongiosblasts; Grinnell et al., 1982). For these isolates, no passage was initiated with more than 40 HAU, and generally an inoculum of 10 to 16 HAU was used. MAD-1, -6, -8, -9, -10 and -11 were isolated from brain tissues shown to contain homogeneous JCV DNA (Grinnell et al., 1982).

RESULTS

Status of JCV DNAs after passage in PHFG cultures

The 8th passage DNA of prototype (MAD-1) JCV was heterogeneous (Fig. 1). The heterogeneity in the DNA extracted from virions (Fig. 1 c) appeared to be identical to that of viral DNA extracted from infected cells (Fig. 1 e); therefore, the shorter DNA was encapsidated and was not an artefact of extraction from infected cells. Furthermore, the size distribution of the DNA was not a consequence of potential variation in supercoiling since the EcoRI-linearized DNA was equally heterogeneous (Fig. 1 d, f). The longest species of DNA was the most abundant and is presumed to be the full-length genome. Estimates of size by agarose gel electrophoresis were consistently 96 to 100% of the size of SV40 DNA (Martin et al., 1979, 1982). At least two other size classes were also present (Fig. 1). These classes apparently arose by deletions since they were all shorter than full-length DNA. (For convenience, the species of DNA shorter than full-length will be referred to as defective DNA.) The larger class of defective DNA was 97% of the size of full-length DNA; the smaller class, which often migrated as two bands (Fig. 1), was approximately 92 or 93%.
A clear distinction among JCV isolates became evident when DNAs of all 11 were compared (Fig. 2 and MAD-14, not shown). The DNAs could be characterized as either heterogeneous, slightly heterogeneous or homogeneous in size (Table 2) by photometric scanning of this gel and others (not shown). Thus, certain isolates (MAD-1, -2, -3, -9 and -10) produced distinctly heterogeneous DNA under conditions similar to those in which other isolates (MAD-6, -8, -11 and -14) produced distinctly homogeneous DNA. It was not possible to compare all isolates at the same passage level and in the same lot of glial cells because of the limited and sporadic supply of foetal brain tissue and because of the unavoidable differences in early passage histories. Nevertheless, MAD-1 and MAD-6 had very similar passage histories, yet they produced DNAs clearly distinguishable by heterogeneity (Fig. 2 and Table 2); MAD-9 and MAD-8 may also be compared. Furthermore, MAD-11 was passed more than MAD-9, yet the former maintained homogeneous DNA. Production of defective JCV DNA, therefore, is not an inevitable consequence of passage in glial cells.

The DNAs of each isolate in the group producing heterogeneous DNA consisted of discrete size classes of molecules, which are evident in Fig. 3. All isolates had presumably non-defective, i.e. full-length DNA (class I). However, the full-length genome was not the predominant class in DNAs of MAD-3 (Fig. 3) and MAD-10 (Fig. 2), or in some preparations of MAD-2 (Fig. 3). Certain size classes of deleted DNAs were common among the isolates producing heterogeneous DNA. For example, all five had a class of DNA (class II) the length of which is 97% of the full-length genome. MAD-1, -2, -3 and -9 also shared a class (III) which was 92 to 93% of full-length. However, only MAD-2 DNA contained molecules which were only 88% of the full size (class IV). The different size classes apparently were a consequence of deletions of variable, but discrete, lengths of the genome. That different isolates give rise to different sets of deletions may be an additional trait of the genetic variability of JCV. However, whether the same region of the
Heterogeneity in DNAs of JCV isolates

MAD DNA

Fig. 2. Heterogeneity in sizes of DNAs of JCV isolates. Covalently closed circular (form I) MAD DNAs were prepared from PHFG cultures infected with stock viruses (Table 1), as described in Methods. DNA samples (250 ng) were run in a 1.5 % agarose gel (40 mA, 14 h), numbers indicating type of MAD DNA used.

Influence of cell culture and m.o.i. on heterogeneity

Whereas a propensity for the genome to sustain deletions during brief passage at low m.o.i. appears to be an intrinsic property of certain JCV isolates themselves, the induction and maintenance of the defective species is probably affected by the m.o.i. and the composition of PHFG cultures employed. PHFG cultures are heterogeneous, and the proportion of the two major cell types, permissive spongioblasts and semi-permissive astrocytes (Shein, 1965; Padgett et al., 1977), can vary considerably. JCV is routinely propagated in cultures which typically are about 50% spongioblasts. Occasionally, PHFG cultures consisting of approximately 90% spongioblasts can be obtained, and these can be infected successfully with 10 HAU per culture, i.e. at a multiplicity 10- to 20-fold lower than that usually required for useful yields. MAD-1 DNA derived by two passages of 7th-passage virus at the lower m.o.i. has been shown to be homogeneous (Frisque et al., 1979; Martin et al., 1979) and is contrasted with typical DNA in Fig. 4. To corroborate this result, a sample of the MAD-2 virus which yielded highly heterogeneous DNA was used to initiate a series of three passages in spongioblast-rich cultures at a m.o.i. of 0.01 infectious unit per cell.

In an attempt to separate the influences of cell type and m.o.i. on the induction of heterogeneity, small-scale infections were initiated with MAD-14, an isolate which produced distinctly homogeneous DNA during three passages in vitro (not shown). Seventy-five cm² cultures of spongioblast-rich (approx. 90% spongioblasts) and spongioblast-poor (approx. 20% spongioblasts) cell populations were infected at a comparatively high m.o.i. (1000 HAU/bottle) and at low m.o.i. (10 HAU/bottle). The infected cultures were maintained for 5 weeks at 39 °C, the optimal temperature for viral replication (Grinnell et al., 1982). In each of three experiments, cells from duplicate bottles were pooled and analysed for JCV-specific DNA as described in Methods. Fig. 5 displays a gel in which JCV-specific DNA was detected by blot
Fig. 3. DNAs of four early isolates of JCV. Covalently closed circular (form I) DNA was extracted from PHFG cells infected under routine conditions with isolates indicated in the figure. Samples containing 1 μg of DNA were analysed as outlined in the legend to Fig. 1, except that electrophoresis was for 8.5 h. The size classes correspond to those mentioned in the text.

Fig. 4. JCV DNAs obtained after brief passage at low multiplicity. Prototype (MAD-1) JCV and the isolate MAD-2 were passed twice in exceptional glial (high spongioblast) cultures at low m.o.i., as described in the text. (a) SV40 DNA, (b, c) MAD-1 DNAs (form I) from PHFG cells infected under standard conditions (b) and from partially purified virions obtained by low-multiplicity passage (LMP; two passages) (c) analysed as outlined in the legend to Fig. 1, except that electrophoresis was 8.5 h. (d, e, f) MAD-2 DNA from PHFG cells infected under standard conditions (d) or under low-multiplicity passage (three passages) (e) and MAD-1_LMP DNAs (f) analysed by electrophoresis in a 1% agarose gel at 25 mA for 3 h followed by 50 mA for 2 h.

Table 2. Characterization of JCV DNA produced in tissue culture

<table>
<thead>
<tr>
<th>MAD isolate no.</th>
<th>Passed at 37 °C</th>
<th>Passed at 39 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status of DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneous</td>
<td>1, 2, 3</td>
<td>9, 10</td>
</tr>
<tr>
<td>Slightly heterogeneous</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Homogeneous</td>
<td>6</td>
<td>8, 11, 14</td>
</tr>
</tbody>
</table>

hybridization. Equal amounts of total DNA were analysed so that the samples could be compared directly and quantitatively.

In spongioblast-poor cultures (Fig. 5c, e, and gels not shown), distinctive heterogeneity in MAD-14 DNA was effected by infection at high m.o.i. (lane c) but not at low m.o.i. (lanes e, f). In contrast, high m.o.i. had only a slight effect on MAD-14 DNA synthesized during one passage in spongioblast-rich cultures (lane b), and the DNA was only slightly heterogeneous. As expected, DNA synthesized in spongioblast-rich cultures infected at low m.o.i. was homogeneous (lane d). Thus, the astrocyte population in spongioblast-poor cultures exerted a strong pressure for deletions, provided there was ample viral DNA synthesis. In fact, at high m.o.i. there was slightly more viral DNA synthesis in spongioblast-poor (lane c) than in spongioblast-rich (lane b) cultures. Therefore, astrocytes may be as capable as spongioblasts of supporting replication of JCV DNA, but they apparently exert greater pressure for deletions. However, it is
Heterogeneity in DNAs of JCV isolates

MAD-14

Fig. 5. Influence of PHFG culture types and m.o.i. on heterogeneity of MAD-14 DNA. Duplicate cultures of PHFG cells were infected with MAD-14, and JCV-specific DNA was detected in extracts by blot hybridization, as described in Methods. Cultures were infected at either high m.o.i. (b, c) or low m.o.i. (d, e), as described in the text. The cultures were spongioblast-rich (SR; b, d) or were spongio-
blast-poor (SP; c, e). Lane (a) contained 200 ng of typical MAD-1 DNA. Lanes (b to e) each contained 5 µg of total DNA extracted from infected cultures. The gel was 1.5% agarose (40 mA, 14 h). The auto-
radiogram was exposed for 2 h. The numbers are ratios of the amounts of virus-specific DNA in the indicated lanes as determined from photometric tracings. (f) Overexposure (16 h) of (e) to show that defective DNA was not detectable in that sample.

clear that spongioblast-rich cultures (lane d) were more efficient for replicating viral DNA after low m.o.i. than were spongioblast-poor cultures (lane e) since the former produced 20-fold more DNA than did the latter. Furthermore, viral DNA synthesis was more efficient at low m.o.i. than at high m.o.i., both in quantity (spongioblast-rich cultures; lanes b, d) and in quality (spongioblast-poor cultures; lanes c, e). These results suggest that the evolution of defective MAD-14 DNA (Fig. 5) is contingent on a threshold level of DNA synthesis in what may be semi-permissive cells (astrocytes; Padgett et al., 1977).

Localization of heterogeneity in JCV DNA

Most, but not all, of the defective DNA of typical MAD-1 JCV was resistant to cleavage with endonuclease BamHI (Fig. 6a, b). In double-enzyme digestions, BamHI did not cleave defective DNA linearized with HpaI (Fig. 6e), with PstI (Fig. 6g) or with EcoRI (gel not shown). All of the defective DNA was cleaved at one site by HpaI, PstI and EcoRI (Fig. 6d, f and gels not shown). Therefore, the deletions in MAD-1 DNA are not random throughout the genome; rather, there is a region that is susceptible to deletions which probably is localized around the BamHI site. The deletions apparently include the BamHI site, but other alterations in nucleotide sequence also could cause resistance to this enzyme. The site cleaved by BamHI has been mapped at 0.505 ± 0.015 on the JCV genome (Martin et al., 1982) and presumably is located in the major coding region for T antigen (Law et al., 1979).

endonuclease HincII cleaved typical MAD-1 DNA into four major and at least three minor fragments (Martin et al., 1979; and Fig. 6h, this paper). The defective fragments were clustered around HincII fragment A (0.355 to 0.855 map unit) and were resistant to cleavage with BamHI (Fig. 6i). Heterogeneity in MAD-1 JCV DNA, therefore, appears to be a consequence of deletions around and including the BamHI site within HincII fragment A. Other alterations in...
Fig. 6. Resistance of MAD-1 DNA to cleavage with BamHI. (a, b) Typical MAD-1 DNA (1 μg) treated with either 5 units (a) or 10 units (b) of commercially prepared enzyme. The cleavage products were analysed by electrophoresis in a 1% agarose gel for 5 h at 25 mA followed by 1-5 h at 50 mA. (c to k) Portions (0.5 μg) of the same DNA digested with the indicated enzymes as described previously (Martin et al., 1979). The products were separated in a 1% agarose gel (25 mA, 4 h; then 50 mA, 1 h). The small amounts of form I DNA in (f) and (g) are due to incomplete digestion with PstI, as repeatedly verified in other gels. To the right of (e) and (g) are indicated the positions, respectively, of the complete BamHI + HpaI, and BamHI + PstI digestion products of full-length MAD-1 DNA. The defective molecules were linearized with HpaI or with PstI but were not cleaved by BamHI. The four HincII and three HindIII fragments of full-length DNA are indicated in (h) and (j) respectively. BamHI cleaved full-length HincII fragment A (to produce A1 and A2) but not the defective 'A' fragments. Similarly, BamHI cleaved only full-length HindIII fragment A, but the cleavage site is very near one end such that A2 was run off the gel.

the defective DNA must have been present, also, since there were defective HincII fragments larger than HincII fragment A of the full-length genome. The larger two species may have been derived by deletions including a HincII site at one end of fragment A.

Size heterogeneity caused by deletions was also confined predominantly to HindIII fragment A (0.67 to 0.550 map unit) (Fig. 6j, k). [Full-length JCV DNA contains three HindIII sites (Martin et al., 1979).] Because there were more than three species of defective DNA present (compare HincII fragment A), some HindIII sites may have been reiterated at one end of fragment A. HindIII fragment C (0.63 to 0.67 map unit; Martin et al., 1979) was not heterogeneous in size and consistently appeared to be present in molar quantities with respect to full-length HindIII fragment A. Since the A–C joint (at 0.67) contains the bulk of the origin of DNA replication (R. Frisque, personal communication), the origin may or may not be reiterated.

The deletions in typical MAD-1 DNA tentatively have been localized to map positions from 0.550 (HindIII site; Martin et al., 1979) to 0.355 (HincII site 2) and slightly beyond. A definitive conclusion must await enzyme and heteroduplex mapping of molecularly cloned defective DNAs. However, from analyses of MAD-2 and MAD-3 DNAs (data not shown) it appears that this location is highly susceptible to deletions.

DISCUSSION

The observations reported here strongly suggest that the appearance of defective JCV DNA during the course of a few passages in vitro in PHFG cells is influenced by three factors: the m.o.i. employed, the constitution of the cell population in the PHFG cultures, and some intrinsic property of the JCV genome. An isolate yielding homogeneous DNA remained homogeneous after one passage in spongioblast-rich (astrocyte-poor) cultures even when a high m.o.i. was used but yielded heterogeneous DNA when passed once under the same conditions in cultures
containing a much higher proportion of astrocytes (Fig. 5). In contrast, this isolate yielded homogeneous DNA when passed once in the astrocyte-rich cultures at a low m.o.i. Therefore, the production of deleted DNA seems to occur most readily in the semi-permissive astrocyte; however, the results suggest that a certain threshold of viral DNA synthesis must occur in these cells before deletions are produced. It is also clear, though, that some isolates are intrinsically more susceptible to deletion. In this study, the simultaneous isolation and propagation of the various isolates was not possible. However, the more recent isolates, MAD-8 to MAD-14, were isolated and propagated under conditions as uniform as practicable. Even so, the DNA of some was homogeneous while that of others was distinctly heterogeneous (Fig. 2).

Passage of JCV in glial cultures does not inevitably lead to production of deleted DNA molecules. In fact, given a heterogeneous population, it was possible to select for full-length molecules by passage under appropriate conditions. In the case of MAD-2 this was possible even though the full-length molecules were not the dominant species present initially (Fig. 3 and 4). This result strongly suggests that the deleted molecules were in fact defective and incapable of independent replication. The defective molecules did not have a competitive advantage, such as would be conferred by repeated origins of replication.

The polyomaviruses SV40 and BKV also produce defective DNA upon passage at low m.o.i. in human cells of glial origin (Carroll et al., 1981; O'Neill & Carroll, 1981). However, the type of heterogeneity produced by JCV is quite different from that which has been reported for SV40. With SV40, major portions of the genome are deleted and are substituted by reiterations of the origin of DNA replication. With JCV, reiteration of the origin may or may not have occurred, but most of the deletions examined so far map downstream from the origin. For MAD-1, they map tentatively from unit 0-550 counterclockwise to 0-335 and slightly beyond. This region probably includes the amino half of the major coding sequence of JCV T antigen (Law et al., 1979).

We appreciate the technical assistance of Ms Gayle C. Foster and the secretarial help of Ms M. Ann Quiroz and Ms Mary M. Soike. This work was supported by an institutional grant RR-00164 and by Biomedical Research Support (RR-00544) and Public Health Service (CA29631) grants to J.D.M. and by Public Health Service Grant AI-11217 to D.L.W.

References


(Received 3 March 1983)