Multiple JC Virus Genomes from One Patient

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SUMMARY

JC virus was previously isolated from the urine and from diseased brain of a patient with progressive multifocal leukoencephalopathy. The DNAs of these isolates, MAD-7 and MAD-8 respectively, were shown in this study to be mixtures of several different full-length genomes. By differences in their restriction endonuclease cleavage patterns, the genomes were categorized into two types. Type I DNA was defined as that typified by the prototype virus, MAD-1; Type II, that typified by viral DNA molecularly cloned from brain tissue of the MAD-7/8 patient (BrC-8 DNA). MAD-7 DNA was an approximately equal mixture of Type I and Type II; MAD-8 DNA appeared to be homogeneously Type II. These DNAs were molecularly cloned for further studies. Of 17 clones of recombinant MAD-7 (rMAD-7) DNA, nine were Type I and were identical to each other and to MAD-1 DNA. The other eight clones comprised five different species of Type II, none of which was identical to MAD-8 or to BrC-8 DNA. Among 17 recombinant MAD-8 (rMAD-8) clones there were two which contained Type I DNA, which had not been detected in the original viral DNA. The remaining rMAD-8 clones were of Type II. The differences among all these DNAs were primarily in the 'extra' PvuII sites and in the insertions and deletions in the hypervariable regulatory region of the genome (0·67 to 0·72 map unit). Possible explanations for multiple genomes in separate isolates from the same patient are discussed. It is concluded that the observed variation originated in vivo and not during isolation in vitro.

The human polyomavirus JC (JCV) is regularly associated with the lesions in brains of persons who have succumbed to the slowly demyelinating disease progressive multifocal leukoencephalopathy (Padgett et al., 1976; Walker, 1978; Padgett & Walker, 1983). Isolates of JCV have been obtained by cultivation of independent brain homogenates through a few passages at low multiplicities of infection in primary cultures of human foetal glial cells (Padgett et al., 1971, 1976; Grinnell et al., 1982). The genomes of 14 isolates differ from each other in their restriction endonuclease cleavage patterns, demonstrated with viral DNAs prepared from infected cells (Grinnell et al., 1983a; J. Martin, unpublished observations) and molecularly cloned from diseased brains (Rentier-Delrue et al., 1981; Grinnell et al., 1983b). Variation is localized primarily, but not exclusively, in the region 0·67 to 0·72 map unit (m.u.) as defined for prototype (MAD-1) DNA. This region includes the origin of replication and presumptive regulatory sequences (Law et al., 1979; Frisque, 1983; Grinnell et al., 1983b; Miyamura et al., 1983). The hypervariability of this region is reflected by 'extra' PvuII sites and short deletions and insertions.

Isolates of the related polyomaviruses BK of humans and SV40 of rhesus monkeys also have regulatory regions which differ from one another (Reddy et al., 1978; van Heuverswyn & Fiers, 1979; Yang & Wu, 1979). Ruley & Fried (1983) have found that no two of several polyoma virus isolates have identical regulatory regions. Although hypervariability of an important control region might be unexpected, it appears to be a regular feature of the polyomavirus group. The origin of this hypervariability is not known. There may be many natural, genetically stable strains of polyomaviruses which circulate, or there may be only one or a very few unstable
strains. In the latter case the observed variety of isolates may be a consequence of rapid evolution of the viral genome during latency, reactivation, and/or the course of disease in the natural host.

In the case of the human polyomaviruses, the viruses isolated from different patients have different regulatory regions (Grinnell et al., 1983b; Rentier-Delrue et al., 1981; Seif et al., 1979; Yang & Wu, 1979). A unique opportunity to study variation in JCV occurred when virus was isolated from urine (MAD-7) and from the brain (MAD-8) of the same patient (Rand et al., 1977; Grinnell et al., 1982). In a previous study (Grinnell et al., 1982), the viral DNA (Br-8) purified directly from the brain of the MAD-7/8 patient was homogeneous in size and was full length. Whether or not the Br-8 DNA was homogeneous genetically is not clear. A molecular recombinant of the DNA was logically presumed to be representative of the Br-8 DNA, and it was characterized by restriction endonuclease cleavage analysis as a particular type of variant (Grinnell et al., 1983b). We report here that the DNA of MAD-7 is composed of equal amounts of prototype (Type I) DNA and several species of DNA of the general type (Type II) represented by Br-8 DNA. In contrast, the majority of MAD-8 DNA was of one species (Type II), but prototype (Type I) DNA was detectable, also. The differences among MAD-7 and MAD-8 Type II DNAs were mapped in the hypervariable regulatory region of the genome. These observations are strongly suggestive of genetic instability in the genome of JCV during its infection of the host.
Isolations of JCV MAD-7 and MAD-8 from a 46-year-old male with chronic lymphocytic leukaemia have been described previously (Rand et al., 1977; Grinnell et al., 1982). Because original specimens were no longer available, primary human foetal glial cells infected with these isolates were graciously provided by B. L. Padgett and D. L. Walker (University of Wisconsin Medical School, Madison, Wis., U.S.A.). The infected cells represented the fourth (MAD-7) and the third (MAD-8) passages of the viruses at multiplicities of infection less than 0·1 infectious unit/cell. Virus-specific DNAs were purified from lysates of frozen infected-cell pellets (Hirt, 1967) by two consecutive equilibrium centrifugations in CsCl-ethidium bromide solution.

Unlike the typically heterogeneous DNA of prototype (MAD-1) virus, the DNAs of MAD-7 and -8 were homogeneous in size (Grinnell et al., 1983a). Both EcoRI and BamHI linearized the viral DNAs, and the linear molecules migrated in electrophoretic gels as single, full-length bands (gel not shown). With respect to prototype (Type I) DNA (MAD-1 and -2; Fig. 1 a, b, e, f), MAD-8 DNA had two 'extra' PvuII sites within the prototypical PvuII fragment A (lane d) and an extra HincII site within the prototypical HincII fragment D (lane h). The extra PvuII sites were mapped in the regulatory region (0·67 to 0·72 m.u.: Martin et al., 1982; Frisque, 1983). The extra HincII site is at 0·89 m.u., in the VP2/3 codons (Law et al., 1979). The respective sets of fragments each constituted the full-length genome and in these respects MAD-8 DNA was identical to DNA cloned directly from brain tissue of the patient (Grinnell et al., 1983b). Thus, MAD-8 DNA appeared to be homogeneously Type II. In surprising contrast MAD-7 DNA contained both the prototypical PvuII fragment A and the variant subfragments A1 and A2 (Fig. 1 c). MAD-7 DNA also had both the prototypical HincII fragment D and the variant subfragments D1 and D2 (lane g). The sum of fragment sizes in each set was too large to be a single full-length genome. Visual inspection and densitometric tracings of photographic negatives suggested that MAD-7 PvuII fragments B and C and HincII fragments A, B and C were in molar excess of the remaining fragments. It was concluded that MAD-7 DNA consisted of approximately equal proportions of two full-length genomes: one of Type I DNA and one of Type II, each having apparent identity with MAD-1 and MAD-8 DNAs, respectively.

Molecular cloning experiments described below support this conclusion but identify additional complexities.

MAD-7 and MAD-8 DNAs were cloned at their unique EcoRI cleavage sites in pBR322 essentially as described by others (Israel et al., 1979; Grinnell et al., 1983b). For each isolate, recombinants having apparently full-length inserts were analysed by PvuII and HincII cleavage for the type of JCV genome present. A recombinant (BrC-1) of viral DNA purified from diseased brain tissue of the MAD-1 patient was used as a reference DNA (Grinnell et al., 1983b; Martin et al., 1982). Of the 17 MAD-7 clones (rMAD-7) that were screened, nine were identified as Type I, and eight as Type II (gels not shown). These clones were therefore representative of the dual genome of the original MAD-7 viral DNA (Fig. 1). Similar analyses of the 17 MAD-8 recombinants (rMAD-8) for genome type indicated that 15 were of Type II (gels not shown). This result was anticipated because visual inspection of electrophoretic gels of the original MAD-8 viral DNA suggested that it was homogeneously Type II (Fig. 1). Indeed, a clone of MAD-8 DNA (BrC-8) derived from diseased brain tissue was Type II (Grinnell et al., 1983b).

An unexpected result, however, was that two of the rMAD-8 clones had a Type I JCV DNA insert. Thus, the original MAD-8 viral DNA in fact was heterogeneous in genome type, although the Type I DNA was a minor component.

In agarose gels (not shown) it was noted that the Type II DNA of rMAD-7 was slightly different from that of rMAD-8, even though the viruses were isolated from the same patient. Therefore, the Type II DNA restriction fragments produced by PvuII were analysed in acrylamide gels. Type I DNAs were analysed also, but were found to be identical (gels not shown). PvuII analyses were done singly and in combination with HindIII. The double digestions were useful because they produced a fragment [261 ± 6 bp (n = 5) in Type I; 0·67 to 0·716 m.u. (Martin et al., 1982)] that contained the extra PvuII sites characteristic of Type II DNA. Fig. 2 shows the 261 bp diagnostic fragment in DNA cloned directly from brain tissue of the prototype patient (BrC-1 DNA). Representative Type II rMAD-7 DNAs (7D and 7E), which had
Short communication

Fig. 2. Multiple Type II genomes of MAD-7. Recombinant Type II DNAs (1 μg) were digested with HindIII and PvuII, and the products were analysed in a 5% acrylamide gel (M = mol. wt. markers). Differences in the upper third of the photograph were due to different orientations of the JCV-specific inserts. The other differences were due to PvuII sites and not to HindIII sites. The fragments derived from the 0.67 to 0.72 region are marked, as are the adjacent, invariant 205-bp fragments (0.63 to 0.67 m.u.). The 205-bp fragment, recently shown by sequencing to be 204 bp (Frisque, 1983), migrated anomalously in acrylamide gels as 220 bp.

appeared to be identical in agarose gels, clearly differed in the diagnostic region. Compared with BrC-1, each had a single additional PvuII site in the 261 bp fragment, but it was located differently in each. The sum of the two rMAD-7E fragments, 303 ± 4 bp (n = 3), indicates an insertion of 42 bp. Unlike 7E, rMAD-7D contained a deletion of 38 bp, the region being 223 ± 2 bp (n = 3) in length. Table 1 indicates the variety in the extra PvuII sites of the eight rMAD-7 clones that were Type II. The 0.67 to 0.72 region of the majority of these clones was 222 to 275 bp in length and contained only one extra PvuII site. Both rMAD-8AA and -8BB contained the same two extra PvuII sites (Fig. 2), as did 13 other clones (Table 1). The sum of the three fragments, 297 ± 4 bp (n = 6), implies that there is an insertion of 36 bp within the region. None of the Type II DNAs produced by JCV isolated from urine (MAD-7) was identical to the Type II genomes of virus isolated from brain (MAD-8) of the same patient (Table 1).

There are at least four explanations for the mixed population of MAD-7 and -8 DNAs and their differences from DNA (BrC-8) cloned from brain tissue of the patient. First, the differences were noted in recombinant DNAs and therefore could have been artefacts of molecular cloning. We have not noted any changes in restriction endonuclease cleavage patterns of Type I or Type II recombinants continually subcultured (in broth) ten times during a period of 5 days (data not shown). The DNAs described in this work were prepared from broth cultures inoculated with bacterial transformants 'passed' twice on agar plates. Thus, there was not Type
I–Type II switching as a consequence of propagation of the DNA in bacteria. Since all of the Type I DNAs so far examined for MAD-1 and MAD-7 have identical PvuII patterns, it is doubtful that variations among the Type II DNAs were induced by molecular cloning itself.

A second explanation is the theoretical possibility that MAD-7 and, to a lesser extent, MAD-8 were contaminated with MAD-1 during isolation and propagation. This is most unlikely in view of the virological precautions employed in the laboratory in which the viruses were isolated and in view of the discontinuity of the passage histories of the isolates (Padgett et al., 1971; Rand et al., 1977; Grinnell et al., 1982). Furthermore, it is doubtful that MAD-1 virus could accidentally and successfully contaminate cultures infected with MAD-7 and -8 such that MAD-1 DNA was replicated equally in one case (MAD-7) but quite unequally in the other (MAD-8).

A third explanation is that the various genomes of MAD-7 and MAD-8 DNAs evolved during propagation in vitro. If this explanation is correct, then genomes different from that in the original brain (DNA in urine could not be examined) evolved and became predominant in three to four low-multiplicity passages. There is precedence for tissue culture-induced changes in human polyomavirus DNA (Pater et al., 1981; Watanabe & Yoshiike, 1982; Grinnell et al., 1983a). Alterations in SV40 and BK virus occur upon low-multiplicity passage in glial cells (Carroll et al., 1981; O'Neill & Carroll, 1981), but these alterations result in defective DNAs having repetitions of the origin of replication. However, the differences among the JCV MAD-7 and -8 DNA species do not involve simple repetitions of the origin of replication. Rather, there are sequence alterations upstream of the origin. Nucleotide sequencing data show that the alterations observed in rMAD-7D and rMAD-8AA (and in DNAs of five other MAD isolates) in fact occur in the 98-bp repeat region (Frisque, 1983), are quite complex, and do not involve repetitions of the origin (J. Martin & R. Frisque, unpublished results). Furthermore, both rMAD-7D and -8AA (Type II genomes) diverge from prototype MAD-1 DNA (Type I) at precisely the same point. Thus, the alterations are not random events. In view of these observations one cannot conclude that the multiple genomes of MAD-7 and -8 DNAs were strictly artefacts of tissue culture passage. In fact, the known sequence of full-length MAD-1 DNA, from 0-58 to 0-73 m.u., was not altered during eight passages in vitro; rMAD-1 and BrC-1 sequences are identical (Frisque, 1983).

The fourth explanation is the most probable: multiple species of JCV DNA were present in the MAD-7/8 patient himself (Rand et al., 1977; Grinnell et al., 1982). Multiple infection with a Type I and Type II virus could have occurred during the immunological naiveté of childhood (Padgett & Walker, 1976) or during immunosuppressive therapy for leukaemia. One consequence of either of these events would be that both viruses established an infection of some

Table 1. Variation in the 0-67 to 0-72 region* among Type II JCV inserts in recombinant MAD-7 and MAD-8 DNAs

<table>
<thead>
<tr>
<th>Fragment (bp)</th>
<th>MAD-7</th>
<th>MAD-8</th>
</tr>
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<tbody>
<tr>
<td>7.6</td>
<td></td>
<td></td>
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<tr>
<td>7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7D</td>
<td></td>
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</tr>
<tr>
<td>152 ± 2†</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>144 ± 3</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>131 ± 2</td>
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<td>116 ± 3</td>
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<td>X</td>
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<td>104 ± 2</td>
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<tr>
<td>95 ± 2</td>
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<td>85 ± 3</td>
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<tr>
<td>Total</td>
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<td>296</td>
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* Fragments produced by PvuII–HindIII.
† Fragment sizes in bp are averages of at least five determinations.
unknown target organ, and both replicated during immunosuppression. Either during latency or during the course of disease, subtypes of the genomes may have evolved. However, apparently only the Type II virus invaded the brain successfully. An alternative scenario is that the MAD-7/8 patient may have been infected with one virus which evolved into multiple types and subtypes, during either primary infection in childhood, latency in later life, or in the course of terminal disease.

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Note added in proof. During the editorial process for this paper, the authors learnt of a related paper by Kristina Dörries [Virus Research (1984), 1, 25-38]. In that paper Dörries reports that JCV DNA isolated from kidney and from virions purified from brain of the same patient differ in the hypervariable region of the viral genome. The major species of DNA from kidney had a deletion of approximately 120 bp near the origin of replication; otherwise the major species of viral DNAs from the two tissues were identical. They were of a subtype which we would consider intermediate between Types I and II described here. Interestingly, Dörries detected heterogeneous, minor species in both tissues. We would define the minor species as Type II.

REFERENCES


York: North-Holland.

WATANABE, S. & YOSHIIKE, K. (1982). Change of DNA near the origin of replication enhances the transforming 

YANG, R. C. A. & WU, R. (1979). Comparative study of papovavirus DNA: BKV (mm), BKV (wt) and SV40. Nucleic 

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