Natural Occurrence of Deletion Mutant of Human Papovavirus BK Capable of Inducing T Antigen

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SUMMARY

Human papovavirus BK DNA resistant to digestion with EcoRI endonuclease had the capacity to direct synthesis of T antigen in human embryonic kidney cells. The study demonstrated the natural occurrence of defective deletion mutants with intact early functions of the viral genome.

During high multiplicity passages simian virus 40 (SV40) generates defective mutants with deletion, substitution, insertion or duplication in their DNA (Tai et al., 1972; Yoshiike et al., 1974). One class of defectives, presumably containing those changes in the late region of the viral genome, can replicate their DNA in monkey cells (Watanabe, 1975), transform mouse cells (Uchida & Watanabe, 1969) and induce tumours in hamsters (Uchida & Watanabe, 1968). Such mutants in SV40 stocks are readily detected as T antigen-forming defective viruses by immunofluorescence assay (Uchida et al., 1968). In monkey cell cultures infected with non-defective SV40, T antigen-positive cells are comparable in number with virion (V) antigen-positive cells. Thus, the ratio of T positives to V positives (T/V ratio) is about 1 with the non-defective stocks. In contrast, the undiluted passage stocks enriched with defective mutants have a T/V ratio of between 2 and 10.

We prepared a series of human papovavirus BK (BKV) stocks (starting from Gardner's original strain supplied by K. K. Takemoto, National Institutes of Health, Bethesda, Md., U.S.A.) in human embryonic kidney (HEK) cells and human embryonic fibroblasts (HEF), and examined them for their T and V antigen-forming capacity in HEK cells and for oncogenicity in hamsters (Uchida et al., 1976, 1979). Among these, one stock (sample P6) had a significantly increased T/V ratio. Since defective DNA has been shown to occur in BKV stocks (Howley et al., 1975), the higher T/V ratio was considered, in analogy with SV40, as evidence that BKV can yield T antigen-forming defective mutants.

In a later study, however, we found that BKV is slightly different from SV40 in T antigen synthesis during productive infection (Watanabe et al., 1979). Two types of T antigen-positive cells can be recognized by immunofluorescence assay in HEK cells infected with BKV: one with densely stained nuclei and the other with sparsely stained nuclei. In the culture infected with non-defective wild-type BKV (grown at a low m.o.i. and with homogeneous DNA) there are as many sparsely stained nuclei as densely stained nuclei in T positive cells 4 days after infection. Since the number of V positives corresponds to that of densely stained T positives, the T(dense + sparse)/V ratio is about 2. We suggest that the cells showing weakly T positive fluorescence result from the abortive infection with non-defective BKV or from the delayed reaction of infected cells. Furthermore, a viable deletion mutant of BKV (pm-522) shows a different pattern of T antigen induction. In HEK cell cultures infected with pm-522 about one-sixth of the total T positives are densely stained and the rest sparsely stained. The V positives are comparable in number with densely stained T positives and, thus, the T(dense + sparse)/V ratio is about 6. Since DNA of pm-522 is homogeneous and the specific plaque-forming activity is one-third of the wild-type, the abortive infection of HEK cells appears to result from an intrinsic property of this mutant.
Fig. 1. Length distribution of BKV DNA. Nicked circular DNA of human wart virus (HWV) was used as the internal reference for length determination of the BKV DNA (Yoshiike & Defendi, 1977). Supercoiled BKV DNA distributed in a limited area (a single mesh hole) was measured at a final magnification of ×194 000. (a) Non-defective BKV (P35) DNA (60 molecules); (b) Undiluted passage BKV (P37) DNA (126 molecules); (c) EcoRI-resistant DNA of P37 (92 molecules). When the mean length of (a) was assumed to be 1.00 ± 0.023 (standard deviation, SD) (arrowed) the mean length of (c) was 0.91 ± 0.022 (SD) (arrowed) and reference HWV DNA had the mean length of 1.55 ± 0.015 (SD) (not shown).

The results of the above study immediately raise the question as to whether a high T/V ratio found in a prototype BKV stock indicates the presence of defective T antigen-forming mutants like SV40, or the presence of a non-defective mutant like pm-522. To answer this question we have chosen one BKV stock (Gardner’s original strain) with a high T/V ratio for this study.

One purified virion sample (P6), which had been obtained after three serial undiluted passages (input m.o.i. 0.1) in HEF cultures, had a higher T antigen-forming capacity than expected from its plaque-forming activity (Uchida et al., 1979). From this stock, the virus was passaged twice in HEK cells (at an m.o.i. of 0.3) and purified sample P37 was prepared. For comparison the plaque-cloned wild-type (wt-501) was grown at a low m.o.i. (5 × 10⁻⁵) in HEK cells and sample P35 was prepared. The yields were 4 × 10¹² virions (determined from absorbancy at 258 nm of purified samples) per 150 cm² culture for both samples P35 and P37, and 2 × 10⁸ and 3 × 10⁷ p.f.u. per culture for P35 and P37 respectively. Whereas non-defective sample P35 had a T(dense + sparse)/V ratio of 2 and a T(dense)/V ratio of 1, sample P37 had a T(dense + sparse)/V ratio of 10 and a T(dense)/V ratio of 5.

If the high T/V ratio of P37 was due to the presence of defectives, they would be eliminated from the resulting virus stock after the subsequent low multiplicity infection. We infected HEK cells with P37 at an m.o.i. of 4 × 10⁻⁵ and obtained a virus stock having a T/V ratio of about 2. This result strongly suggests that P37 contained T antigen-forming defectives.
Short communications

DNA was extracted from purified BKV samples P35 and P37 and subjected to electrophoresis in 1.2% agarose gel, as described previously (Watanabe et al., 1979). The P37 DNA formed two bands: a narrow, slow band moving together with non-defective DNA (P35), and a wide, faster moving band (data not shown). The length-distribution patterns determined by electron microscopy (Fig. 1a, b) were consistent with the gel electrophoresis patterns. P37 DNA contained a small amount of the unit-length DNA, but the majority of the molecules were shorter (Fig. 1b). Most of these shorter molecules turned out to be resistant to digestion with EcoRI endonuclease (data not shown). Therefore, the shorter EcoRI-resistant molecules could be isolated by ethidium bromide-CsCl buoyant-density centrifugation (Radloff et al., 1967) after the DNA was digested. The isolated DNA was homogeneous in length and 9% shorter than the non-defective wt DNA (Fig. 1c). The EcoRI-resistant DNA, after digestion with HindIII, yielded four fragments of 1.9, 1.1, 0.80 and 0.42 kilobases long (data not shown), whereas wt BKV DNA generated four HindIII fragments of 2.3, 1.9, 0.56 and 0.42 kilobases long, as reported by Yang & Wu (1979).

The isolated EcoRI-resistant DNA was assayed for its biological activity by the indirect immunofluorescence assay as described previously (Watanabe, 1975). T antigen production in HEK cells infected with BKV DNA was found to be different from that with BKV virions. In infection with non-defective DNA there were only a very few cells which were sparsely stained and most of the T positive cells were stained densely. The T/V ratio obtained with non-defective DNA was about 1 (Fig. 2a), whereas the P37 DNA had a T/V ratio of 4 (Fig. 2b). The EcoRI-resistant DNA induced T antigen synthesis (the positive cells were more densely stained than those cells infected with non-defective DNA, suggesting that more T antigen was produced in infection with defective DNA), but no V antigen synthesis (Fig. 2c). These results clearly demonstrate that, like SV40, T antigen-forming defective mutants of BKV were generated during high multiplicity infection.
To determine whether the T antigen-forming BKV defectives can replicate their DNA without the help of non-defective virus, we infected an HEK cell culture (20 cm²) with P37 virions at a low m.o.i. so that 0.8% of the cells were producing V antigen (3.7% of the cells were producing densely stained T antigen) 4 days after infection. For comparison, an HEK cell culture was infected with non-defective BKV sample (P35) at the same m.o.i. used for P37, so that 0.8% of the cells became V positives (0.8% of the cells dense-T positives). After a 6 h adsorption period the infected cultures were washed twice with PBS and incubated at 36 °C in Eagle’s minimum essential medium (MEM) with 2% calf serum containing anti-BKV serum. Four days after infection virus released by sonication from the cells was assayed for T antigen-inducing capacity. We obtained a similar yield of virus, 15 infectious units per V antigen-positive cell at day 4, from cultures infected with P35 and P37.

However, the yields of T antigen-inducing DNA varied between cultures (60 cm²) infected with P35 and P37. Four days after infection (under the same conditions as above) DNA was extracted by the method of Hirt (1967) from infected cultures and assayed for T antigen-forming capacity as described previously (Watanabe, 1975). The yield of infectious T antigen-inducing DNA from the P37-infected culture was four times as much as from that infected with non-defective P35 [18 × 10⁶ cell infectious units (CIU) versus 4.8 × 10⁶ CIU per 60 cm² culture]. Although the two infected cultures had similar numbers of V positives at day 4, the P37-infected culture had about five times as many T positives as the P35-infected culture. Therefore, the yield of infectious T antigen-inducing DNA per T positive cell at day 4 was comparable between the two cultures. These results are best interpreted as indicating that the defective viruses in P37 can replicate their DNA in the absence of the helper wt virus.

In infection of HEK cells with DNA, almost all T positives were densely stained and comparable in number with V positives, whereas in infection with virions half of the T positives were sparsely stained, the other half being densely stained and equal in number to the V positives. These results suggest that the process of infection is more synchronized with DNA than with virions, and that the uncoating process of virions may cause delay in the productive cycle of wt BKV in HEK cells. It should be noted, however, that pm-522 (which induces abortive infection in a larger proportion of HEK cells than does wt (Watanabe et al., 1979)) also induced abortive infection with DNA, as indicated by the presence of sparsely stained T antigen positives (the T/V ratio in infection with pm-522 DNA was about 3; two-thirds of T positives were sparsely stained and one-third, densely stained). Therefore, abortive infection caused by pm-522 appears to result from an intrinsic character of this mutant, not simply from the delayed reaction of the infected cells. The experiments similar to those done with P37 virions suggested that pm-522 DNA was not replicating in the sparsely stained T positive cells (S. Watanabe, unpublished observations).

In summary, the present study showed the natural occurrence of BKV mutants capable of directing synthesis of T antigen and virus DNA, but not V antigen. The complete nucleotide sequence of BKV DNA (strains MM and Dun) has been determined (Yang & Wu, 1979; Seif et al., 1979) and, from the analyses of mRNAs (Manaker et al., 1979) and base-sequence homology (Law et al., 1979), the genomic organization of BKV is believed to be identical with that of SV40. The presence of deletion mutant BKV DNA resistant to EcoRI digestion is consistent with the genomic organization on the physical map as determined by biochemical methods. The BKV mutants defective in the late functions (including the one with EcoRI-resistant DNA) have been cloned by the isolation of plaques. The mapping and characterization of these mutants are now in progress.
Short communications

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REFERENCES


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