Persistence of BK Virus in Human Foetal Pancreas Cells

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SUMMARY

High multiplicity BK virus (BKV) infection of primary cells derived from human foetal pancreas resulted in massive cytopathology and subsequent outgrowth of cells. Intranuclear BKV T-antigen was present in all cells and viral antigen was detected in 10 to 30% of these cells. The subcultured cells yielded BKV in the supernatant (approx. 10⁵ TCID₅₀/ml) and in the cells free viral DNA was present (approx. 10% of total cellular DNA content). Analysis of the viral DNA indicated the presence of deleted and rearranged BKV DNA molecules. Although all cells continuously expressed BKV T-antigen they did not exhibit the transformed phenotype. This persistent infection of human foetal pancreas cells represents a novel type of in vitro interaction between BKV and human cells which may correspond to the in vivo findings on BKV tropism for pancreatic cells.

After the initial isolation of BK virus (BKV) from the urine of an immunosuppressed transplant patient by Gardner et al. (1971), the virus has subsequently been isolated by many others (Coleman, 1980) from immunodeficient and immunosuppressed humans. Serological studies showed that BKV infections are very common since the majority of adults have antibodies to BKV. Seroconversions to BKV have been shown to occur mostly in childhood and have sometimes appeared to be associated with upper respiratory tract diseases (Gardner, 1973; Van der Noordaa & Wertheim-van Dillen, 1977). Initially, it was reported that BKV DNA occurred in a variety of human tumours (Fiori & DiMayorca, 1976) but these results could not be confirmed in extensive studies by others (Israel et al., 1978; Wold et al., 1978; Grossi et al., 1981). A role for BKV in the aetiology of human neoplasia remains to be elucidated. On the other hand, it has clearly been shown by many investigators that BKV has in vitro transforming potential in rodent cells (Portolani et al., 1978). Human cells also, albeit at a much lower frequency, can be transformed in vitro with BKV DNA or BKV DNA fragments containing the early region (Purchio & Fareed, 1979; Takemoto et al., 1979; Grossi et al., 1982). The state of the viral DNA in the transformed cells, whether of rodent or human origin, varies greatly. Some cell lines contain mainly non-integrated free viral DNA whereas in others mainly integrated viral DNA can be detected.

BKV or BKV DNA has been shown to be oncogenic in the hamster, mouse and rat (van der Noordaa, 1976; Costa et al., 1976; Noss & Stauch, 1984). Also, in hamster tumour-derived cell lines the presence of free or integrated viral DNA could be shown (ter Schegget et al., 1980; Corallini et al., 1982). From various experiments on the oncogenicity of BKV it has emerged that besides ependymomas, malignant insulinomas are also frequently induced (Corallini et al., 1978; Uchida et al., 1979). It has been shown that intracerebral inoculation of a viable deletion mutant of BKV into newborn hamsters frequently induces insulinomas whereas the wild-type does not (Watanabe et al., 1979).

More recently, it has been shown by Caputo et al. (1983) that free circular BKV DNA was present in a human adenoma of pancreatic islets. The viable rescued virus was found to have a
deletion in the coding region for the small T-antigen and an insertion in the non-coding regulatory sequences. These observations have led us to carry out experiments to study the interaction in vitro between BKV and human pancreatic cells.

Primary human cells derived from the pancreas of a 14 week old foetus were inoculated with plaque-purified BKV (strain Gardner) at a m.o.i. of 10 p.f.u./cell. Three weeks after inoculation extensive cytopathology was noted and viable cells could hardly be recognized any more. Six weeks after inoculation focal outgrowth of cells was observed. These cells could be subcultured weekly at a 1:2 ratio and appeared to enter crisis after 12 subsequent passages. The subcultured cells showed focal cytopathology and intranuclear inclusions were clearly visible in a minority of the cells (Fig. 1).

Intranuclear BKV T-antigen was present in 95 to 100% of the cells as detected by indirect immunofluorescence. Viral capsid antigen, detected by indirect immunofluorescence, was present in many cells, ranging from 10 to 30% of the cells in different preparations. The growth pattern of the cells appeared normal and in soft agar no growth of colonies was observed. In the cell culture fluids, infective virus was present in titres as high as $10^4$ to $10^5$ TCID$_{50}$/ml as determined by inoculation of secondary foetal human lung cells. Total intracellular DNA was isolated from passage 6 cells and analysed by (1%) agarose gel electrophoresis. Visualization of the DNA within the gel by ethidium bromide staining showed that besides the high molecular weight cellular DNA a large quantity of DNA was present with the electrophoretic mobility of covalently closed circular BKV DNA (form I, FI) and open circular BKV DNA (FII) (Fig. 2a). Restriction enzyme analysis (see below) confirmed that this DNA was BKV DNA. Based on ethidium bromide staining it was estimated that at least 10% of the total extracted DNA consisted of BKV sized DNA. Total cellular DNA was fractionated by the Hirt procedure (Hirt, 1967) and after electrophoresis of the Hirt supernatant DNA (HS DNA) many bands could be visualized in the gel (Fig. 2b). Three bands could be observed that migrated slightly faster than BKV FI DNA and besides the bands of cellular DNA, BKV DNA (FII and FIII) several other bands could be observed. After extensive BamHI digestion of HS DNA, an enzyme for which
Fig. 2. Electrophoresis of DNA isolated from BKV-infected cells through a 1% agarose gel. (a) Approximately 3 μg of total cellular DNA isolated from the sixth passage of BKV-infected human pancreatic cells. (b) Approximately 2 μg of DNA derived from the Hirt supernatant fraction of BKV-infected human pancreatic cells at the sixth passage. (c) Same as (b) but treated with BamHI. (d) 1.5 μg BKV DNA from BKV-infected human diploid cells and purified by CsCl-ethidium bromide isopycnic gradient ultracentrifugation. FI, FII and FIII designate the covalently closed, the open circular, and the linear forms of BKV DNA respectively. Ethidium bromide staining was used.

BK DNA contains one recognition site, most of the DNA was converted to the full length linear form (FIII) and at least two of the short FI size classes were also linearized (Fig. 2c). A faint band still migrated with the mobility of FI DNA, suggesting that some of the BKV DNA had lost the cleavage site for BamHI.

Viral DNA was purified from the culture medium, digested with HindIII, electrophoresed through a 1.4% agarose gel and the DNA was transferred onto a nitrocellulose filter and visualized by hybridization and autoradiography following the Southern method (Southern, 1975). Fig. 3(a) shows that when the full-size genome of BKV cloned at the BamHI site in pBR328 was used as a probe, four HindIII fragments were present that had the same size as those in the HindIII digest of the DNA of BKV used for the initial infection of the pancreas cells. When the DNA on the nitrocellulose filter was probed with a 32P-labelled cloned HindIII B fragment of BKV DNA, a fragment which represents most of the early coding region, it appeared that in the HindIII digest several fragments were present which were smaller than the HindIII B fragment but which had homology to it under stringent hybridization conditions (Fig. 3b). We consider this indicative of the presence of deleted and/or rearranged BKV DNA molecules.

To answer the question whether integration of viral DNA had occurred at a specific site, three subsequent Hirt extractions of cellular DNA from passage 6 cells were performed to remove the majority of free viral DNA. The first Hirt pellet was dissolved by gentle agitation at 37 °C in 10 mM-Tris·HCl, 1 mM-EDTA, followed by overnight incubation at 4 °C and pelleting of the high molecular weight DNA by centrifugation. This procedure was repeated once more. The
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Fig. 3. Southern blot of HindIII-digested BKV DNA purified from the culture medium of the fourth passage of persistently infected human pancreas cells (lanes 1) and HindIII-digested BKV prototype DNA (lanes 2), hybridized to either (a) a full-length genomic probe labelled with $^{32}$P to sp. act. $1 \times 10^7$ c.p.m./$\mu$g (Rigby et al., 1977) or (b) a HindIII fragment B-specific probe labelled with $^{32}$P to sp. act. $1 \times 10^8$ c.p.m./$\mu$g. After hybridization, final washings of the filters were performed in 0.1 x SSC, 0.1% SDS at 65 °C. Autoradiography was for 18 h at room temperature. Culture medium was centrifuged for 2 h at 100,000 g. The pellet was suspended in 10 mM-Tris–HCl, 10 mM-EDTA, 0.1 M-NaCl, pH 8.0, treated with SDS (1%, v/v), extracted with phenol/chloroform (50%/50%, v/v) and ethanol-precipitated. Based on ethidium bromide staining in agarose gels, the culture fluid contained about $10^{10}$ molecules of BKV FI DNA per ml. All lanes contained approx. 1 ng HindIII-digested DNA.

DNA was further purified by two phenol–chloroform extractions (50:50, v/v) followed by dialysis against 10 mM-Tris–HCl, 1 mM-EDTA, and three consecutive electrophoresis (0.8% agarose) and electroelution procedures (Maniatis et al., 1982). The DNA was incubated with several restriction endonucleases that do not cleave BKV DNA and subsequently analysed by Southern blot hybridization. For hybridization cloned BKV DNA was used. Due to the oligomeric forms of BKV DNA that co-purified with the cellular DNA and also due to the still substantial quantities of contaminating monomeric forms of BKV DNA, bands that would originate from BKV DNA integrated at a specific site were obscured by the strong hybridization signals of non-integrated DNA (results not shown). Therefore, our results on integration remain inconclusive.

The observed type of interaction in vitro between BKV and human cells has not been described before. Persistence of viable genomes for long cultivation periods has been described for papovaviruses as well as other viruses, and this has been attributed to interference by defectives in the replication cycle (Huang, 1973; O’Neill & Carroll, 1983). Although it is clear that deletion mutants were present in the pancreas-derived cells described in this
communication, they apparently did not prevent a high level of viral DNA replication and virus multiplication. Despite that, the cells could be subcultured for more than 20 generations. Furthermore, despite the continuous presence of the BKV T-antigen in essentially all cells, no transformed phenotype was observed.

We conclude that our results present a novel type of interaction in vitro between BKV and human cells which might be related to the special feature of pancreatic cells being the target for BKV in vivo as shown earlier by others (Caputo et al., 1983).

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REFERENCES


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