Gene transfer using human polyomavirus BK virus-like particles expressed in insect cells

Antoine Touzé,1 Latifa Bousarghin,1 Céline Ster,1 Alba-Lucia Combita,1 Philippe Roingeard2 and Pierre Coursaget1

1 Laboratoire de Virologie Moléculaire, EMI-U 00-10 Protéases et Vectorisation, IFR Transposons et Virus, Faculté des Sciences Pharmaceutiques ‘Philippe Maupas’, 31 avenue Monge, 37200 Tours, France
2 Laboratoires de Biologie Cellulaire et Virologie, EA 2639, Analyse Structurale des Antigènes, IFR Transposons et Virus, Faculté de Médecine, 2 bis Boulevard Tonnellé, 37032 Tours cedex, France

The major structural protein (VP1) of the BK polyomavirus (BKV) was expressed in the recombinant baculovirus expression system. Recombinant BKV VP1 was shown to self-assemble into virus-like particles (VLPs) with a diameter of 45–50 nm. As for other polyomaviruses, BKV VP1 has the capacity to bind to exogenous DNA. Furthermore, the potential of BKV VP1 VLPs was investigated for gene transfer into COS-7 cells using three methods for the formation of pseudo-virions: disassembly/reassembly, osmotic shock and direct interaction between VLPs and reporter plasmid DNA. The latter method was shown to be the most efficient when using linearized plasmid. Gene transfer efficiency with BKV pseudo-virions was of the same order as that observed with human papillomavirus type 16 L1 protein VLPs. In addition, it is demonstrated that cellular entry of BKV pseudo-virions is dependent on cell surface sialic acid.

Human BK polyomavirus (BKV) is a member of the family Polyomaviridae, genus Polyomavirus, a family which also includes simian virus 40 (SV40), JC virus (JCV) and other animal polyomaviruses such as murine polyomavirus. Primary infection with BKV and JCV leads to lifetime persistence in the kidney, CNS and in lymphoid cells (Dorries, 1998). BKV/human cohabitation is considered to be an innocent relationship and most infections, which occur early in childhood, are asymptomatic. BKV is regarded as an opportunistic pathogen in immunocompromised patients and is frequently recovered from the urine of kidney and bone marrow transplant recipients. In such patients, BKV infection has been associated with haemorrhagic cystitis, ureteral stenosis and the development of neuroblastoma (Arthur et al., 1986; Flaugstad et al., 1999).

Polyomaviruses contain a double-stranded circular DNA molecule of about 5 kb, which is packaged with cellular histones in a capsid of 45–50 nm in diameter. The capsid of polyomaviruses contains three proteins, VP1, VP2 and VP3. X-ray crystallography of SV40 and murine polyomavirus (Liddington et al., 1991; Griffith et al., 1992) has demonstrated that the polyomavirus capsid consists of 72 pentamers arranged in a \( T = 7 \) icosahedral lattice. Each pentamer comprises five VP1 and the minor capsid protein VP2 and/or VP3 extend from the core into the cavity of the VP1 pentamer (Griffith et al., 1992).

The major structural protein (VP1) of SV40, JCV, murine polyomavirus and monkey B-lymphotropic papovavirus and the corresponding structural proteins of many papillomaviruses (L1) have been shown to self-assemble into virus-like particles (VLPs) when expressed in insect cells (Schiller & Roden, 1995; Stokrova et al., 1999; Gillock et al., 1997; Pawlita et al., 1996). Moreover, the use of papillomavirus or polyomavirus VLPs as carriers for gene transfer has been described recently (Forstova et al., 1995; Roden et al., 1996; Unckell et al., 1997; Touzé & Coursaget, 1998; Stokrova et al., 1999; Goldmann et al., 1999; Ou et al., 1999).

The aim of this study was to produce BKV VLPs by the expression of the VP1 gene in insect cells and to investigate their potential as gene transfer vectors using different methods to form pseudo-virions.

Viral DNA was purified from the urine of a kidney transplant patient. The BKV VP1 gene (strain PA) was amplified by PCR using the primers upper 5′ TGA TCATGGC CCAAACTGAAGA and lower 5′ AAG CTCTT TTT AAGCA CTTTGG, each containing BclI and HindIII restriction sites, respectively. The PCR product was cloned into the pCR2.1 vector by TA cloning and sequenced using Big Dye Terminators in an ABI prism 310 sequencer (Perkin Elmer). Comparison of the deduced amino acid revealed a close relationship with the BKV Dunlop strain (Dhar et al., 1978).
Eight amino acid changes were observed in the VP1 gene of the PA strain compared to the VP1 gene of the Dunlop strain (Dunlop → PA): V^{145}L, D^{158}N, E^{159}D, S^{173}T, D^{175}E, V^{216}I, A^{219}T, and R^{370}K. Only two of these changes, positions 60 and 219, involved amino acids with different properties. Nevertheless, the point mutation detected at position 219 is also present in the MM strain of BKV (Seif et al., 1998). Analysis by SDS–PAGE and Coomassie blue staining revealed the presence of an extra 40 kDa band in the nucleus of BacBKVP1-infected cells (data not shown). In order to investigate the self-assembly of BKV VP1 into VLPs, a nuclear lysate of infected cells was analysed by isopycnic banding in CsCl gradients. Fractions with densities ranging from 1.25 to 1.35 g/ml were observed by transmission electron microscopy (TEM). VLPs were observed in fractions with densities ranging from 1.28 to 1.30 g/ml. TEM micrographs demonstrated that most of the VLPs exhibited the typical morphology of empty polyomaviruses (Fig. 1a). Positive fractions were pooled, pelleted by ultracentrifugation and resuspended in PBS. Human papillomavirus type 16 (HPV-16) L1 VLPs were purified according to the same protocol (Touze et al., 1998). Exogenous DNA binding of BKV VP1 (5 µg) was analysed by South–Western blotting using digoxigenin-labelled pBR322. Under these conditions, BKV VP1 showed strong DNA-binding activity (Fig. 1b). The nuclear localization signal (NLS) and DNA-binding domain of polyomavirus and SV40 VP1 are located in the first 12 N-terminal amino acids (Chang et al., 1993; Ishii et al., 1996; Moreland & Garcea, 1991; Moreland et al., 1991) and the amino acid sequence of BKV VP1 is closely related to that of murine polyomavirus and SV40 VP1. It is therefore reasonable to predict that the NLS and DNA-binding domain of BKV VP1 may also be located at the N terminus in the positively charged amino acid stretch (MAPTKRKGECPGAAPKKPK).

The gene transfer properties of BKV VP1 VLPs into COS-7 cells were investigated using three methods to form pseudo-virions: disassembly/reassembly, osmotic shock and direct interaction. Gene transfer experiments were carried out using a pCMV-luciferase plasmid of 1.7 kb. This plasmid was used either in the circular form or as a restriction enzyme-linearized form. A VLP:DNA mass ratio of 10 was used in all experiments. For the disassembly/reassembly method, VLPs were incubated in 50 mM Tris–HCl buffer (pH 7.5) containing 150 mM NaCl, 1 mM EGTA and 2 mM DTT at room temperature for 30 min. At this step, pCMV-luciferase diluted in 50 mM Tris–HCl buffer (pH 7.5)–150 mM NaCl was added. The preparation was then diluted in 50 mM Tris–HCl buffer (pH 7.5)–150 mM NaCl containing 2 nM CaCl₂ and 1% DMSO. CaCl₂ molarity was increased stepwise from 2 to 5 mM with an increment of 1 mM/h at 20 °C (El Mehdaoui et al., 2000). VLP–DNA complexes were also obtained by osmotic shock according to Barr et al. (1979) for polyomavirus, with some modifications. VLPs and DNA were mixed in a buffer containing 150 mM NaCl, 10 mM Tris–HCl (pH 7.5) and 0.01 mM CaCl₂ (final volume 25 µl prior to dilution). After 10 min of incubation at 37 °C, the mixture was submitted to osmotic shock by dilution in 100 µl of distilled water and incubated for 20 min at 37 °C. In the direct interaction method,
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VLPs and DNA were mixed in 40 µl of 150 mM NaCl and incubated for 30 min at room temperature.

In order to investigate if the DNA is packaged into BKV VLPs, pseudo-virions obtained with the three methods were treated with Benzonase, as described previously (El Mehdaoui et al., 2000). Results indicate that protection was observed in the three methods and that the highest level of protection was observed with the direct interaction method. In order to better characterize the interaction between DNA and BKV VLPs using osmotic shock and direct interaction, preparations were observed by TEM in dark field after positive staining with 1.5% aqueous uranyl acetate. In these conditions, VLPs were shown to decorate the circular DNA molecule (data not shown) and to be combined with the ends of linear DNA molecules (Fig. 1c), as observed previously for murine polyomavirus using osmotic shock (Stokrova et al., 1999) and for HPV-31 using both methods (L. Bousarghin, A. L. Combita, A. Touzé, S. El Mehdaoui, P. Y. Sizaret, M. M. Bravo and P. Coursaget, unpublished results). VLP–DNA complexes (10–1 µg per well, respectively) were incubated with COS-7 cells in 96-well plates. Lipofectamine (Invitrogen) was used as a positive control, according to the manufacturer’s instructions, with the same amount of DNA. After 2 days of pseudo-infection, luciferase activity was determined using the Luciferase reporter gene assay with constant light signal (Roche). The signal was integrated over 10 s (Victor®; Perkin Elmer) and results were expressed as counts per second (c.p.s.) per well. The results are the mean of two independent experiments and statistical analysis was carried out using the F-test (Epilinfo 6.04 software).

BKV VP1 VLPs were shown to be able to transfer a gene of interest into COS-7 cells with the three methods explored (Fig. 2). Nevertheless, the level of gene transfer depends both on the method used to form pseudo-virions and on the conformation of the DNA. The more effective way to produce efficient pseudo-virions is the direct interaction method between BKV VLPs and linear DNA. The results obtained with this latter method are similar to those obtained with Lipofectamine (15 821 and 12 750 c.p.s., respectively; P = 0.35) and, when using a 7·2 kb β-galactosidase plasmid in place of the luciferase plasmid, 50% of COS-7 cells were transfected. Gene transfer results were shown to correlate protection of DNA from Benzonase. The BKV data were also compared to those obtained with HPV-16 VLPs. The levels of gene transfer with the same amount of HPV-16 L1 VLPs were higher than those observed with BKV VLPs (P = 0.019 to P < 0.001), with the exception of the direct interaction method using linear DNA (P = 0.25). HPV VLPs may have been more efficient than BKV VLPs, since the plasmid used was 7·1 kb, which is larger than the BKV genome (5·1 kb) but smaller than the size of the HPV-16 genome (7·9 kb). We have shown previously that the size of DNA that can be packaged into HPV-16 VLPs by the disassembly/reassembly method is limited to the size of the genome (Touzé & Coursaget, 1998). It could thus be expected that the size of the plasmid used might have inhibited the packaging into BKV VLPs. However, the results indicate that gene transfer and thus DNA packaging was obtained with a plasmid of a higher size than the BKV genome. This is in agreement with the observation that SV40 capsids can package plasmids that are at least 50% larger than the SV40 minichromosome of 5·2 kb (Sandalon et al., 1997).

Gene transfer using linear DNA was always better than that observed with circular DNA (P = 0·038 to P < 0·001). In contrast, gene transfer was lower with linear DNA than with
Table 1. Inhibition of gene transfer mediated by BKV VP1 into COS-7 cells by neuraminidase treatment of target cells

Pseudo-virions were generated by the direct interaction method using linear plasmid.

<table>
<thead>
<tr>
<th>Neuraminidase (IU/ml)</th>
<th>Inhibition of gene transfer (%)</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
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<tr>
<td>0.002</td>
<td>3</td>
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<tr>
<td>0.01</td>
<td>34</td>
</tr>
<tr>
<td>0.02</td>
<td>42</td>
</tr>
<tr>
<td>0.03</td>
<td>73</td>
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circular DNA when using Lipofectamine ($P = 0.28$). A similar result was reported previously by Zanta et al. (1999) using synthetic peptides as carriers and by Kreiss et al. (1999) using cationic liposomes. Zanta et al. (1999) explained the lower efficiency of linear DNA by the fact that free ends are prone to cytosolic exonuclease-mediated degradation. TEM results suggest that linear DNA is protected from such degradation when BKV (Fig. 1c) or HPV VLPs stick to the end of the DNA molecules. In addition, the specificity of the gene transfer with BKV pseudo-virions, generated by direct interaction with linear DNA, was established by pre-incubation with anti-BKV VP1 serum raised in mice (diluted 1:1000) before anti-BKV VP1 serum raised in mice (diluted 1:1000) before COS-7 cell transfection. In these conditions, gene transfer was totally abolished.

In order to characterize cellular entry of BKV pseudo-virions, COS-7 cells were treated with increasing concentrations (0-002–0-03 IU/ml) of Vibrio cholerae neuraminidase (Sigma) prior to incubation with VLP–DNA complexes generated by direct interaction between VLPs and linear DNA (Table 1). Neuraminidase treatment inhibited gene transfer mediated by BKV VLPs in a dose-dependent manner, whereas no reduction was observed on gene transfer induced by HPV-16 VLP–DNA complexes. These findings indicate that entry of BKV, but not HPV pseudo-virions, is dependent on the presence of cell surface sialic acid, like JCV and murine polyomavirus (Cahan & Paulson, 1980; Fried et al., 1981; Chen & Benjamin, 1997) and in contrast with SV40 (Haun et al., 1997) or HPV VLPs stick to the end of the DNA molecules. Zanta et al. (1999) also showed that BKV VLPs have DNA-binding activity, bind to cell surface sialic acid and are shown to be promising vehicles for gene transfer studies, especially when pseudo-virions are formed using the direct interaction of VLPs with linear DNA.

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