Production of infectious swine vesicular disease virus from cloned cDNA in mammalian cells

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Full-length cDNA clones of the swine vesicular disease virus (SVDV) were constructed from subgenomic cDNA clones in the expression vector pSVL (pSVLS00). The direct transfection of mammalian cells with plasmid pSVLS00 results in the production of infectious virus. The recovered virus was neutralized completely by anti-SVDV guinea-pig serum, but did show a difference in plaque morphology from the parental virus.

Swine vesicular disease (SVD) was first observed in Italy in 1966 (Nardelli et al., 1968). It is an infectious disease of pigs characterized by the appearance of vesicles on the tongue, in the mouth and on the feet and hocks. These lesions are indistinguishable from those caused by foot-and-mouth disease virus (FMDV).

The causative agent, SVD virus (SVDV) belongs to the enterovirus genus, of the Picornaviridae family and has a close serological relationship to coxsackievirus B5 (Graves, 1973). Like other picornaviruses, the genome of SVDV is a single-stranded RNA with a positive polarity. The RNA genome is 7400 nucleotides long, excluding the poly(A) tract and encodes a single polyprotein. The predicted amino acid sequence shows close homology to those of coxsackievirus B and poliovirus (Inoue et al., 1989).

Full-length infectious cDNA constructs of the RNA genomes of members of the family Picornaviridae were described first by Racaniello & Baltimore (1981). This feature, coupled with addition of a DNA-dependent RNA polymerase promoter (T7 or SP6), has significantly contributed to the molecular characterization of poliovirus and has subsequently been widely applied to many other virus genomes, mainly of human and plant pathogens. Recently, the development of engineered poliovirus chimeric vaccines using infectious poliovirus cDNA has been reported (Burke et al., 1988, Murray et al., 1988; Evans et al., 1989). The construction of infectious cDNA from the RNA genomes of livestock pathogens will enable not only the analysis of genetic functions leading to virulence but also the development of new types of vaccine.

We report here the construction and the properties of a full-length cDNA clone of SVDV from subgenomic viral cDNA clones. The constructed full-length SVDV cDNA clone generated infectious virus which was antigenically identical to the parental SVDV.

For the construction of full-length SVDV cDNA, clones MPS671, pBRS14, MPS453 and pBRS19 were used (Inoue et al., 1989). The plasmid pUCS825 containing 5' terminal cDNA (nucleotides 1 to 65) was newly prepared by the method of Geliebter (1987) with several modifications. These five clones overlap with each other. On the basis of these overlapping restriction sites, the construction steps were subdivided into three blocks: (i) from the SVDV 5' terminal end to the first SacI site (nucleotide position 747) composed of pUCS825, MPS671 and pBRS14, (ii) from the second SacI site (nucleotide position 5932) to the 3' poly(A) tract composed of MPS453 and pBRS19 and (iii), from the first SacI site to the second SacI site composed of pBRS14. The reconstruction step of each portion was performed in parallel and pSVLS00 was finally obtained (Fig. 1). Plasmid pSVLS00, based on pSVL (Pharmacia), harbours the full-length SVDV cDNA copy at the XbaI/BamHI cloning site which is located within the simian virus 40 (SV40) VP1 transcriptional unit. The orientation and composition of all inserts were confirmed by restriction digestion with several enzymes.

DNA transfection of mammalian cells was performed with the CellPhect transfection kit (Pharmacia). Three μg of pSVLS00 was transfected into Cos-7 cells or IBRS-2 cells grown to 20 to 50% confluence in 60 mm dishes. In IBRS-2 cells, distinct c.p.e. was observed 2 days after transfection of the plasmids and the infectious viruses were recovered from culture fluids. In Cos-7 cells,
infectious virus was recovered but no c.p.e. was observed. The kinetics of virus production after transfection of IBRS-2 cells or Cos-7 cells with pSVLS00 is presented in Fig. 2. Inoculated SVDV (m.o.i. of 1) showed a typical one-step growth curve in IBRS-2 cells and Cos-7 cells. When SVDV genomic RNA (1 μg) was transfected into IBRS-2 cells or Cos-7 cells, virus expression began immediately, as with the virus inoculations. In the DNA transfections, the production of viruses was delayed more than 24 h in both IBRS-2 and Cos-7 cells relative to RNA transfections or virus inoculations. However, once viruses were expressed, they multiplied efficiently in both cells. The level of virus production in Cos-7 cells seemed to lag 6 to 12 h behind in IBRS-2 cells.

The results of transfection of IBRS-2 or Cos-7 cells with the plasmids are summarized in Table 1. At least 1 μg of plasmid was necessary to produce infectious virus in IBRS-2 cells. No virus was recovered from culture fluids of Cos-7 cells transfected with the vector pSVL. When pSVLS00 was transfected into Cos-7 cells, 0.1 ng of plasmid was sufficient to produce infectious virus. This input was 1/10000 of that required for IBRS-2 cells.

Our results clearly show that the constructed plasmid was infectious in Cos-7 cells or IBRS-2 cells. For pSVLS00, 1 μg of DNA generated infectious SVDV in
IBRS-2 cells. These results are similar to those obtained by Racaniello & Baltimore (1981) and Omata et al. (1984) with poliovirus. When plasmid pSVLS00 was transfected into Cos-7 cells, the transfection efficiency was greatly increased (0.1 to 0.01 ng of plasmid DNA could produce infectious SVDV), a result consistent with those of Semler et al. (1984) and Kean et al. (1986) using poliovirus. Plasmid pSVLS00, which is based on pSVL, harbours full-length SVDV cDNA within the SV40 VP1 translational unit and carries the SV40 origin of replication. Presumably Cos cells replicate pSVLS00. Although the mechanism of expressing infectious virus from cDNA is not understood, a delay in expression following transfection suggests some unknown steps are required for expressing infectious virus from cDNA (Racaniello & Baltimore, 1981).

Virus recovered from DNA transfections was characterized for its biological, physical and structural properties. The virus growth in IBRS-2 cells, the buoyant density in CsCl and the genomic RNA size of recovered virus were identical to those of the parental virus. To examine the identity of the virus produced in transfected cells, a virus neutralization test was performed and all of the recovered virus was neutralized with anti-SVDV serum (Table 2). The genomic RNA of recovered virus was extracted and analysed with respect to its \textit{in vitro} translated products and by sequencing the 5'-terminal region. The \textit{in vitro} translation of recovered viral RNA was performed using a rabbit reticulocyte lysate translation system (NEN) and analysed on 10% SDS-polyacrylamide gels. Autoradiography of \textit{in vitro} translation products did not demonstrate any detectable

**Table 1. Transfection of cultured mammalian cells with various plasmid DNAs**

<table>
<thead>
<tr>
<th>Template DNA</th>
<th>Cells</th>
<th>Transfected dose (ng)</th>
<th>Minimum transfected dose to yield infectious virus (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSVLS00</td>
<td>Cos-7</td>
<td>+*</td>
<td>0-01-0-1</td>
</tr>
<tr>
<td>pSVLS00</td>
<td>IBRS-2</td>
<td>+</td>
<td>0-01-0-1</td>
</tr>
<tr>
<td>pSVL</td>
<td>Cos-7</td>
<td>+</td>
<td>0-01-0-1</td>
</tr>
</tbody>
</table>

* Infectious virus can always be recovered. Cells were maintained in 12-well plastic dishes. Infectious virus was assayed at 6 days post-transfection.
† Virus can be recovered sometimes.
‡ Infectious virus cannot be recovered.

**Table 2. Virus neutralization test**

<table>
<thead>
<tr>
<th>Source</th>
<th>Virus titre (TCID\textsubscript{50}/0-05 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative serum*</td>
</tr>
<tr>
<td>pSVLS00</td>
<td>IBRS-2</td>
</tr>
<tr>
<td>pSVLS00</td>
<td>Cos-7</td>
</tr>
<tr>
<td>H/3 '76</td>
<td></td>
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* Normal guinea-pig serum (1:100 dilution).
† Anti-SVDV guinea-pig serum (1:100 dilution).
‡ Parental strain of SVDV.

**Fig. 2.** The time course of virus production in mammalian cells. Cells grown in 60 mm plastic dishes were transfected with 1 µg of SVDV genome RNA or 3 µg of the plasmid construct pSVLS00. As a control, cells were infected with the parental H/3 '76 strain of SVDV at an m.o.i. of 1. RNA transfections were performed by the calcium phosphate method.

**Fig. 3.** Comparison of plaque size. (a) SVDV strain H/3 '76. Cells were fixed and stained 2 days after inoculation with virus. (b) The recovered virus. Cells were fixed and stained 3 days after inoculation.
differences between the recovered and parental virus (data not shown). The RNA sequence of the 5’-terminal 90 sequence (nucleotides 1 to 90) of recovered virus was also identical to that of parental virus (data not shown).

The only difference observed was in plaque size. The recovered virus formed smaller plaques than the parental virus. Fig. 3 depicts typical plaques of the parental SVDV strain and the recovered virus. This phenotype was stable for multiple replications of the virus in IBRS-2 cells, so the phenotype is genetically encoded. Whether the small plaque phenotype originated by using a cDNA clone from a mutant with an unusually small plaque size and/or from mutations arising during multiple manipulations of the cDNA during the cloning and construction of full-length cDNA is unknown. Further studies are necessary to clarify the mechanisms or genes regulating plaque size.

The reported infectious cDNA of SVDV may contribute to a virus vector for developing chimeric vaccines for livestock. The cDNA may also act as a powerful tool for analysing the pathogenesis of vesicular disease caused by SVDV and FMDV.

References


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