A suicidal DNA vaccine based on a Semliki Forest virus (SFV) replicon was evaluated for the development of a vaccine against swine vesicular disease virus (SVDV). The 1BCD gene of SVDV was cloned and inserted into pSCA1, an SFV DNA-based replicon vector. The resultant plasmid, pSCA/1BCD, was transfected into BHK-21 cells and the antigenicity of the expressed protein was confirmed using an indirect immunofluorescence assay. Immunogenicity was studied in guinea pigs and swine. Animals were injected intramuscularly three times with pSCA/1BCD at regular intervals. Anti-SVDV antibodies were detected by ELISA, the lymphocyte proliferation response was tested by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide method and neutralizing antibodies were measured by microneutralization tests. The data showed that SVDV-specific antibodies, neutralizing antibodies and lymphocyte proliferation were induced in both guinea pigs and swine. Furthermore, after three successive vaccinations with pSCA/1BCD, half of the pigs were protected against challenge with SVDV. These results should encourage further work towards the development of a DNA vaccine against SVDV.

INTRODUCTION

Swine vesicular disease (SVD) is a highly contagious viral pig disease, characterized by the appearance of vesicles on the coronary bands, heels of the feet and less commonly on the snout and tongue. Due to the similarity of these lesions to those caused by foot-and mouth-disease (FMD), SVD is subject to international controls and is listed by the World Organization for Animal Health. SVD was first identified in Italy in 1966 (Nardelli et al., 1968) and several more outbreaks have been reported subsequently in Europe and eastern Asia (Brocchi et al., 1997). However, in the recent past, reports of SVD have been limited to Portugal and Italy.

SVD vaccines have been developed previously to control the disease, both in monovalent form (Gourreau et al., 1975) and in combination with FMD (Mitev et al., 1978), and an SVD subunit vaccine has also been described, although it was not very efficacious (Jimenez-Clavero et al., 1998). Although the inactivated virus vaccines are effective in protecting against clinical signs, there has been little, if any, assessment made of their ability to reduce wild-type virus transmission and no effective vaccine is available commercially. Once introduced, SVD could be a difficult disease to eradicate and improved methods of control would be highly beneficial, including the development of safer and more effective vaccines to protect and control this disease.

Suicidal DNA vaccines, based on the alphavirus replicon, have emerged as an important strategy to enhance immunogenicity and to improve the biosafety of conventional DNA vaccines (Berglund et al., 1998; Leitner et al., 1999; Lundstrom, 2000). Unlike the conventional DNA vaccine construct in which heterologous gene expression is driven directly by the RNA polymerase II-dependent promoter, suicidal DNA vaccines based on the replicon of alphaviruses, including Sindbis virus (SINV) (Herweijer et al., 1995), Semliki Forest virus (SFV) (Liljestrom & Garoff, 1991) and Venezuelan equine encephalitis virus (Davis et al., 1989), constitute RNA self-amplifying replicons in eukaryotic cells (Morris-Downes et al., 2001). The plasmids include a full-length human cytomegalovirus (CMV) promoter-driven expression cassette and are able to produce their replicase complex following cytoplasmic transport of the corresponding RNA. The replicase produces a full-length RNA encoding itself, as well as an abundant subgenomic mRNA encoding the heterologous protein. The RNA self-amplifying property is of considerable interest with respect to vaccine biosafety: the vector replicates at the RNA level and not at the DNA level, so the rate of foreign DNA present in vivo and possessing ‘genome integration potential’ is controlled and does not increase following vaccination (contrary to some attenuated or recombinant vaccines). Furthermore, when a suicidal DNA vaccine is transfected...
into cells, it leads eventually to apoptosis of the transfected cells (Kohno et al., 1998; Leitner et al., 2000), which is particularly important in alleviating the concerns of potential integration and cell transformation generated by the use of conventional DNA vaccines (Gurunathan et al., 2000; Lewis & Babiuk, 1999).

Several groups have demonstrated the ability of suicidal DNA vaccines to induce high-level humoral and cell-mediated immunity against a variety of antigens, with immunized animals developing more prominent immune responses than those receiving a conventional DNA vaccine encoding the same antigen (Berglund et al., 1998; Deshpande et al., 2002; Hariharan et al., 1998; Kirman et al., 2003; Leitner et al., 2000). In addition, it has been demonstrated that suicidal DNA vaccines could break immunological tolerance by activating innate antiviral pathways, in contrast to conventional DNA vaccines encoding the same antigen (Leitner et al., 2003). All of these advantages indicate that suicidal DNA vaccines are an attractive vaccine-delivery vehicle and an alternative strategy to conventional DNA vaccines.

The aim of this study was to assess the immunogenic properties and protection value of a suicidal DNA vaccine against SVDV.

METHODS

Cell culture and virus propagation. BHK-21 cells and IBRS-2 cells were cultured routinely at 37 °C in a 5% CO₂ atmosphere in Dulbecco’s modified Eagle’s medium (DMEM; HyClone) supplemented with 10% fetal bovine serum (FBS; HyClone), 100 U penicillin ml⁻¹ and 100 µg streptomycin ml⁻¹. SVD virus (SVDV) HK70 (Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China; Ye et al., 2005) was propagated in IBRS-2 cells cultured in DMEM. After 12 h of incubation at 37 °C, when more than 80% of the cells showed cytopathic effect, the cells were subjected to three freeze–thaw cycles. The viral suspension was clarified by centrifugation at 800 g for 10 min and stored at −70 °C.

Construction of recombinant vectors. Viral RNA was extracted from the viral suspension using an RNasy mini kit (Qiagen). A first-strand synthesis reaction was then performed using random hexamers (TaKaRa Bio) to anneal and prime the viral RNA for reverse transcription with avian myeloblastosis virus reverse transcriptase in the presence of RNasin. The cDNA was used in a high-fidelity PCR with forward primer 5'-TAGCCGACCATGGTGTCCTCATTCC-3' and reverse primer 5'-GCCATGGGGCTGGTGTTTTTCAATGG-3'. The primers were designed according to the sequence of the HK70 strain (GenBank accession no. AY429470). The forward primer contained a Kozak sequence and initiation codon (ATG) for optimal initiation of translation and the reverse primer contained a stop codon (TAG) for correct termination, as indicated by the underlined nucleotides. Following gel purification, the PCR product was cloned into the dephosphorylated SmaI site of pSCA1, the SFV DNA-based replicon vector (DiCiommo & Bremmer, 1998; kindly provided by Dr Roderick Bremmer, Vision Science Research Program, Canada) and the resultant plasmid was named pSCA/1BCD. The fidelity of the recombinant plasmid was confirmed by restriction digestion and sequence analysis. The expression plasmid was introduced into Escherichia coli DH5α and large-scale DNA production runs were performed using EndoFree Plasmid Mega kit columns (Qiagen).

In vitro plasmid expression. Expression of SVDV capsid protein from pSCA/1BCD, driven by the CMV promoter, was verified using an immunofluorescence assay (IFA). pSCA/1BCD was introduced into BHK-21 cells (in 35 mm wells) using Lipofectamine Plus reagent (Invitrogen). Two days after transfection, cells were analysed for expression of SVDV proteins. A monolayer of cells cultured on coverslips was fixed in cold 100% acetone (−20 °C for 30 min). Samples were incubated with rabbit anti-SVDV serum (37 °C for 30 min) in a humid box and then with fluorescein-conjugated goat anti-rabbit serum (Sigma) for 30 min at 37 °C (Guo et al., 2005). Fluorescence was observed under a Leica microscope.

Immunization and challenge. Guinea pigs weighing 400–500 g were obtained from the Laboratory Animal Centre of Lanzhou Veterinary Research Institute, China. The DNA vaccine was prepared by diluting the purified plasmid DNA preparation to 1 mg ml⁻¹ in Dulbecco’s PBS (DPBS: Sigma). Groups of seven guinea pigs were inoculated three times at 3-week intervals with 200 µg pSCA/1BCD DNA vaccine for primary administration and with 300 µg pSCA/1BCD DNA vaccine for booster immunization. The diluted DNA was injected into the quadriceps muscle of both rear legs using a syringe and needle. Guinea pigs inoculated with the same amount of control pSCA1 DNA, without the insert, were used as controls. Serum was collected at weeks 0, 3, 6 and 9 post-immunization (p.i.).

Nine 2-month-old pigs were purchased from a conventional breeding/finishing farm. Six pigs were immunized intramuscularly with pSCA/1BCD and three with pSCA1 as controls. All pigs were immunized three times with a 2-week dose interval with 300 µg DNA vaccine for primary administration and 500 µg DNA vaccine for booster immunization. Three weeks after the final immunization, all pigs were challenged subcutaneously with a 10⁷ median mouse lethal dose of SVDV strain HK70. All pigs were housed in an isolation facility and examined for 15 days after challenge.

ELISA for SVDV-specific antibodies. Serum samples from guinea pigs were evaluated by an indirect ELISA test using the recombinant VP1 protein of SVDV, produced in E. coli, as antigen. The VP1 protein was expressed in E. coli using the pGEX expression system (Amersham Pharmacia Biotech) and the recombinant product was purified by glutathione S-transferase agarose affinity chromatography. Ninety-six-well flat-bottomed plates (Nunc) were coated with recombinant VP1 protein in 0.1 M carbonate/bicarbonate buffer (pH 9.6) and incubated overnight at 4 °C. After blocking with 5% BSA in PBS, plates were incubated with duplicate twofold serial dilutions of test sera for 1 h at 37 °C. Rabbit anti-guinea pig IgG peroxidase conjugate (Sigma) at a 1:2000 dilution was then added for 1 h at 37 °C, followed by the addition of the substrate 3,3’,5,5’-tetramethylbenzidine. Absorbance was determined at 450 nm using a Bio-Rad microtitre plate reader.

SVDV-specific antibodies in pig serum were detected using a commercial competitive sandwich ELISA kit (Ceditest; Cedi-Diagnostics B.V.) following the manufacturer’s instructions.

Serum neutralization assay. Prior to testing, sera were incubated for 30 min at 56 °C to inactivate complement. Sera were diluted twofold serially in 96-well microtitre plates, mixed with 200 TCID₅₀ SVDV strain HK70 in a 100 µl volume and incubated for 1 h at 37 °C. After incubation, 100 µl IBRS-2 cell suspension containing 10⁵ cells was added and plates were incubated for 3 days at 37 °C in 5% CO₂. Thereafter, cells were examined for SVDV-specific
cytopathic effect and neutralization titres were calculated as 
\(-\log_{10}\) of the reciprocal of the highest dilution resulting in 50% 
normalization.

**Lymphocyte proliferation assay.** Blood was collected from 
immunized animals in blood-collecting tubes containing heparin. 
Peripheral blood mononuclear cells (PBMCs) were isolated by cen-
trifugation in Ficoll-Paque Plus (density 1.077; Amersham 
Biosciences) for 30 min at 18°C. Mononuclear cells were collected 
from theuffy coat and centrifuged, and residual red blood cells 
were lysed by incubation in water for 1 min followed by the addi-
tion of 2 nM Eagle’s solution. After two washes in PBS, the cells 
were resuspended in complete medium (RPMI 1640 supplemented with 
25 mM HEPES, 2 mM glutamine, 10% FBS, 5 x 10^{-5} M 2-mercap-
toethanol and penicillin/streptomycin). PBMCs were added to 96-
well flat-bottomed plates at a concentration of 100 µl per well 
(2 x 10^{5} cells per well). Subsequently, 100 µl per well of medium 
with or without inactivated SVDV was added and mixed. Phyto-
haemagglutinin (50 µg ml^{-1}; Sigma) was used as a positive control. 
Each sample was tested in triplicate. The plates were incubated at 
37°C for 45 h in 5% CO₂ followed by incubation with 3-(4,5-
dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium-bromide (MTT) 
for 3 h and then 10% SDS/0.01 M HCl was added to every well 
until the deposit was diluted. Absorbance was determined at 
570 nm.

**Statistical analysis.** Data were analysed using Student’s t-test. P 
values of less than 0.05 were considered statistically significant.

## RESULTS

### Expression of pSCA/1BCD plasmid in BHK-21 cells

In order to demonstrate expression of the SVDV capsid 
proteins, transfected BHK-21 cells were analysed by IFA. 
Cells transfected with pSCA/1BCD showed specific green 
fluorescence but the negative control, which was transfected 
with the same amount of pSCA1, without the insert, and 
non-transfected cells did not show any fluorescent emission 
(data not shown).

### Immunogenicity of suicidal plasmid pSCA/1BCD in guinea pigs

To evaluate the immunogenicity of the recombinant repli-
con plasmid pSCA/1BCD, it was inoculated into guinea pigs 
as described in Methods. Blood was collected at week 0 
(prior to vaccination), week 3 (21 days after the first 
vaccination), week 6 (21 days after the second vaccination) 
and week 9 (21 days after the third vaccination) to test for 
the presence of anti-VP1 antibody, neutralization antibody 
and lymphocyte proliferation.

Total anti-VP1 antibody response was determined by an 
indirect ELISA. The mean antibody level of the pSCA/1BCD-vaccinated group was significantly higher (0.01 < P < 0.05) than those of the pSCA1 and DPBS control groups (Fig. 1). After the booster immunization, the mean antibody level increased, but this was not statistically significant (P > 0.05). Serum samples were evaluated further in an 
SVDV serum neutralization test (SNT) (Table 1). The 
results were similar to the ELISA antibody responses.

Compared with the control group, the vaccinated group 
showed a significant lymphocyte proliferation response 
(P < 0.01) after the first vaccination, which increased after 
the booster vaccination (Fig. 2).

### Immunogenicity test in swine

Following the positive results of the guinea pig experiment, 
further immunogenicity testing was carried out in swine.

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**Table 1.** Neutralizing antibody titres of guinea pigs

Results were measured by SNT and are shown as \(-\log\) titre 
(mean ± SD).

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 0</th>
<th>Week 3</th>
<th>Week 6</th>
<th>Week 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivated SVDV</td>
<td>&lt;0.6</td>
<td>1.4±0.1</td>
<td>1.8±0.2</td>
<td>&gt;2.1</td>
</tr>
<tr>
<td>pSCA/1BCD</td>
<td>&lt;0.6</td>
<td>0.9±0.2</td>
<td>1.1±0.1</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>pSCA1</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Anti-VP1 antibody levels in guinea pigs after immunization. Sera were tested for antibodies at a 1 : 32 dilution. The result was obtained from mean ELISA absorbance values of three sera in each group.

**Fig. 2.** Specific proliferation of PBMCs in immunized guinea pigs. This test was performed at various time points just before the primary inoculation and the booster inoculations. The data are presented as the mean absorbance value of each group.
Blood samples were collected before the primary inoculation (day 0), booster inoculations (days 14 and 28) and viral challenge (day 49). SVDV-specific antibodies were measured by ELISA and SNT, and lymphocyte proliferation was tested using the MTT method.

Total anti-SVDV antibody responses determined by a commercial competitive ELISA are presented in Table 2. Two out of six animals inoculated with suicidal DNA vaccine were seropositive on day 14 p.i. The antibody level was enhanced by booster immunization and an additional two animals were seropositive on day 28 p.i. (day 14 after the second immunization), whilst the remaining two animals were still seronegative after three immunizations. No animals seroconverted in the control group inoculated with plasmid pSCA1.

Induction of neutralizing antibody was similar to that observed for the ELISA antibody (Table 2). Briefly, neutralizing antibody was detected in four out of six immunized animals, whilst all of the control animals remained negative.

For the proliferative PBMC responses, the stimulation index of the immunized group was higher than that of the control group (Fig. 3), although this was not statistically significant ($P > 0.5$).

**Viral challenge test in swine**

Based on the results described above, direct SVDV challenge tests in swine were carried out. All animals from the control group showed lameness by day 3 p.c. and developed severe clinical signs, with lameness and vesicles in all four feet and/or the snout by day 4 p.c. Three out of six animals vaccinated with plasmid pSCA/1BCD, with the highest neutralizing antibody titres, showed no clinical signs of disease during the experimental period. In one out of six immunized pigs, SVD symptoms appeared 7 days after the appearance of SVD symptoms in the control group, whilst the other two immunized pigs showed symptoms 1 day after the appearance of SVD symptoms in the control group (Table 2).

**DISCUSSION**

SVDV is an important pathogen of swine, causing vesicular lesions that are indistinguishable from those caused by FMDV, and against which there is currently no vaccine generally available. The development of safe and effective vaccines is increasingly important to the control of this disease. DNA vaccines have been shown to induce protective cellular and humoral immune responses and can overcome many problems associated with conventional vaccination; for example, the potential danger associated with the production and distribution of attenuated live vaccines.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Animal number</th>
<th>SVDV-specific antibodies (% inhibition)</th>
<th>Neutralizing antibody (–log titre)</th>
<th>Time of appearance of SVD signs (day p.c.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSCA1</td>
<td>1</td>
<td>19.3 p.i. 26.6 p.i. 15.5 p.i. 21.3 p.i.</td>
<td>&lt;0.6 0.6 0.6 0.6 0.6 0.6 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>14.0 p.i. 18.2 p.i. 16.2 p.i. 17.1 p.i.</td>
<td>&lt;0.6 0.6 0.6 0.6 0.6 0.6 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.7 p.i. 20.7 p.i. 17.8 p.i. 10.7 p.i.</td>
<td>&lt;0.6 0.6 0.6 0.6 0.6 0.6 3</td>
<td></td>
</tr>
<tr>
<td>pSCA/1BCD</td>
<td>4</td>
<td>6.4 p.i. 7.9 p.i. 35.9 p.i. 41.1 p.i. 71.1* p.i. 0.6 0.6 0.6 0.6 0.6 0.6 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>23.8 p.i. 31.2 p.i. 51.1* p.i. 71.1* p.i. 0.6 0.6 0.6 0.6 0.6 0.6 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>18.0 p.i. 10.2 p.i. 17.8 p.i. 29.6 p.i. 0.6 0.6 0.6 0.6 0.6 0.6 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>12.7 p.i. 55.4* p.i. 68.9* p.i. 64.6* p.i. 0.6 0.7 1.0 1.0 1.0 1.0 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8.5 p.i. 58.3* p.i. 80.8* p.i. 84.7* p.i. 0.6 0.9 1.0 1.0 1.0 1.0 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>11.5 p.i. 41.2 p.i. 53.6* p.i. 89.5* p.i. 0.6 0.6 1.2 1.4 1.4 1.4 3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Inhibition ≥ 50%: SVDV-specific antibodies are present in the sample.

| NS, No clinical signs.
addition, the proteins are synthesized, processed and presented in cells, which is more similar to natural infection than administration of conventional inactivated vaccines. However, in all cases, the protection level is generally lower compared with that of attenuated or inactivated vaccines. More recently, suicidal DNA vaccines have emerged as an important strategy in enhancing immunogenicity and improving the biosafety of conventional DNA vaccines (Berglund et al., 1998; Lettner et al., 1999; Lundstrom, 2000). The goal of this study was to construct a suicidal DNA vaccine against SVDV and assess its immunogenic properties and ability to protect against challenge.

SVDV particles are composed of 60 copies each of the four capsid proteins VP1–VP4, which enclose a single-stranded, positive-sense RNA genome of about 7400 nt. Proteins VP1 (1D), VP2 (1B) and VP3 (1C) are exposed at the viral surface, whereas VP4 (1A) is in intimate contact with the RNA and thus not accessible from the outer shell surface in the intact virions. It has been shown that both conformation-dependent neutralizing sites and linear epitopes are located mainly in the outer capsid proteins (Borrego et al., 2002a, b; Jiménez-Clavero et al., 2000; Kanno et al., 1995; Nijhar et al., 1999; Rebel et al., 2000). Furthermore, a recombinant bacterially expressed SVDV polyprotein, p1, is able to induce an SVDV-specific cellular and humoral immune response in pigs (Jiménez-Clavero et al., 1998). For this reason, the 1BCD gene was chosen in this study.

It has been determined that alphavirus DNA-based expression vectors have broad potential application for genetic immunization (Berglund et al., 1998; Dubensky et al., 1996) and gene therapy (Herweijer et al., 1995). SINV and SFV DNA-based vectors, tested with a variety of antigens in mice, have elicited stronger protein expression and resulted in the development of humoral and cellular immune responses at higher levels than conventional promoter/reporter gene plasmids (Berglund et al., 1998; Hariharan et al., 1998). The DNA-based SFV expression vector used in this study was described by DiCiommo & BREMMER (1998), who demonstrated that this DNA-based SFV vector could produce high levels of protein rapidly.

In our study, the preliminary in vitro work indicated that the recombinant plasmid pSCA/1BCD could express the SVDV capsid protein faithfully, but the expression efficacy was not evaluated fully. The in vivo study in guinea pigs and swine showed that SVDV-specific immune responses were induced when they were injected intramuscularly with plasmid DNA. However, the humoral immunity level was relatively low, with neutralizing antibody titres (~log titre) of between 0.9 ± 0.2 and 1.2 ± 0.1 in guinea pigs and between 0.7 and 1.4 in swine. The differences in stimulation index of lymphocytes between the vaccinated and control groups were not statistically significant in swine. Although the reason for this is not clear, it may be related to the nature of the antigen itself. Previous studies have shown that different antigens can produce different results using alphavirus expression systems. For instance, it has been shown that the humoral immune responses induced by an SFV-based DNA vaccine were relatively lower than those obtained from the conventional DNA vaccine when the NS1 antigen was expressed (Vidalin et al., 2000), although this result contrasts with other reports using other antigens or other alphavirus expression systems (Berglund et al., 1998; Hariharan et al., 1998; Lettner et al., 2000). Likewise, this difference was not observed when the prME spike complex antigen was expressed (Vidalin et al., 2000). Another explanation may be related to the probability that the quantity of DNA taken up by muscle cells was low because of the large size of the plasmid (~13.8 kb), resulting in the production of only a small quantity of antigen. Alternatively, the reverse may be true: when a large amount of replicon-based DNA was transfected into muscle cells, the replicase may have produced large amounts of double-stranded RNA, which rapidly induced apoptosis, thus terminating the expression and processing of viral antigen. This latter hypothesis is supported indirectly by data reported by Kim et al. (2004), who showed that suicidal DNA vaccine potency could be enhanced by delaying suicidal DNA-induced cell death.

During the animal experiments, guinea pigs and swine were all vaccinated three times as described in Methods. Whilst the immune responses were improved by booster immunizations, this was not found to be statistically significant. The reason for this may be related to the probability that the antigen expressed in booster immunization was neutralized by antibody induced by the primary immunization. When challenged with live SVDV after three sequential vaccinations with DNA, half of the animals were protected, whilst the other half were not. However, in two of the six immunized pigs apparently without protection, the SVD clinical signs appeared later than those in the control group, even though the Ceditest ELISA and SNT were both negative before challenge. The explanation for this may be related to the contribution of the cellular immune response. Previous studies have shown that, even without detectable antiviral antibody, cytotoxic T lymphocytes induced by the DNA vaccine could protect against virus infection (Hosie et al., 1998; Zarozinski et al., 1995). Xiao et al. (2004) have also reported that the humoral responses induced by a suicidal DNA vaccine were relatively lower than those induced by a conventional DNA vaccine. However, the suicidal DNA vaccine induced higher levels of cellular immune responses and provided better protection than a conventional DNA vaccine. It is necessary to study the cellular immune responses induced by suicidal DNA vaccines carrying the capsid gene from SVDV and their role in protection against live virus attack.

In summary, a suicidal DNA vaccine carrying the capsid gene from SVDV can induce a degree of protection. These results should encourage further work towards the development of a DNA vaccine against SVDV. Therefore, we conclude that suicidal DNA technology shows promise as a tool for the development of a candidate vaccine for SVDV, although the efficacy requires further improvement.
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