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Expression and purification of Suid Herpesvirus-1 glycoprotein E in the baculovirus system and its use to diagnose Aujeszky's disease in infected pigs

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ABSTRACT

Suid Herpesvirus 1 (SHV-1) is the etiological agent of Aujeszky's disease (AD), which affects swine herds worldwide and causes substantial economic losses due to animal mortality and lost productivity. In order to eradicate SHV-1, vaccination programs using viruses lacking the gene encoding glycoprotein E (gE) are ongoing in several countries. These eradication programs have generated a currently unmet demand for affordable and sensitive tests that can detect SHV-1 infection, yet distinguish between infected and vaccinated pigs. To meet this demand, we used the baculovirus-insect cell system to produce immunologically authentic full-length recombinant gE protein for use in a serum ELISA assay. As previous efforts to clone the gE gene had failed due to its extremely high GC-content (75% average), we used betaine as a PCR enhancer to facilitate amplification of the entire gE gene from the Argentinian CL15 strain of SHV-1. The cloned gE gene was expressed at high levels in recombinant baculovirus-infected insect cells and reacted strongly with sera from SHV-1 infected pigs. We used the recombinant gE protein to develop a local indirect ELISA test with sensitivity and specificity comparable to currently available commercial tests. Thus, recombinant gE produced in baculovirus-infected insect cells is a viable source of antigen for the detection of SHV-1 in ELISA tests. We also provide evidence supporting a potential application of this recombinant form of gE as a SHV-1 subunit vaccine.

Introduction

Suid Herpesvirus 1 (SHV-1[1]), also known as Pseudorabies virus (PRV), is a double-stranded DNA virus that is the etiologic agent of Aujeszky's disease (AD) in swine [1]. AD is an economically significant swine disease characterized by a range of clinical signs, including central nervous system disorders, respiratory disease, reproductive failure and death, depending on the age, reproductive status, and immune status of the host [2]. AD eradication programs are either under way or have already been successful in several countries such as the UK, Sweden and the USA [3–5]. A major development in the control of this disease has been the use of marker vaccines consisting of viral particles lacking specific structural proteins in combination with serological tests that detect serum antibodies against these structural proteins, which enable differentiation between infected and vaccinated pigs [6]. Although vaccination is effective in reducing the circulation of wild SHV-1 and preventing the illness, it generally does not prevent infection and establishment of latency [7]. In Argentina, a voluntary vaccination program was implemented in 1998, using a gE-deleted imported vaccine. However, the vaccine and differential test were discontinued between 2001 and 2002 due to an economic crisis and the use of this vaccine is currently prohibited by Argentinian authorities (Resolution 474/2009 SENASA (National Service of Animal Health) (www.senasa.gov.ar).

Glycoprotein E (gE), also known as US8, is one of the six structural glycoproteins found in the SHV-1 viral envelope [1]. Because gE was shown to be non-essential for virus replication, recombinant vaccines can be formulated with SHV-1 particles lacking gE.

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[1] *Abbreviations used:* SHV-1, Suid Herpesvirus 1; PRV, Pseudorabies virus; AD, Aujeszky' disease; gE, Glycoprotein E; BICS, baculovirus insect cell system; CPK, Continuous Porcine Kidney; BSA, bovine serum albumin; OD, optical density; Se, sensitivity; Sp, specificity; EDTA, ethylenediaminetetraacetic acid; ORF, open reading frame.

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One advantage of this vaccine formulation is that it enables serological discrimination between vaccinated or infected pigs in the field because infection with the wild-type virus always gives rise to detectable anti-gE antibodies [8,9]. Moreover, although gE is not required for virus growth, it is involved in virulence, playing a role in virus spread to the central nervous system [1,10] and in virus egression from infected cells [10].

Recombinant SHV-1 gE could be used to develop a test that can detect SHV-1 infection and distinguish between infected and vaccinated pigs. However the gE DNA coding sequence has a high guanine–cytosine content of around 75%, which makes PCR amplification extremely difficult.

In this study, we used a modified PCR method that included enhancers designed to reduce the melting temperature of GC-rich sequences [11,12] to successfully amplify the full-length gE coding sequence. We subsequently produced the recombinant gene product in the baculovirus insect cell system (BICS), which is commonly used to produce large quantities of fully processed, antigenically active foreign glycoproteins [13]. We then used the recombinant gE produced in the BICS to develop a local indirect ELISA assay with sensitivity and specificity comparable to commercially available tests. Finally, we evaluated the immunogenicity of the recombinant gE in mice to determine its potential utility as a SHV-1 subunit vaccine.

Materials and methods

DNA isolation

DNA was isolated from the Continuous Porcine Kidney cell line [14] (CPK) infected with the CL-15 SHV-1 strain [15] at a multiplicity of infection between 1 and 5, as follows. CPK cells were grown in 75 cm^2 plastic flasks, inoculated with 1 ml of supernatant from positive cell cultures and, after extensive cytopathic effect was observed, cell pellets were washed and suspended in TEN buffer (100 mM Tris–HCl; pH 7.5, 12.5 mM EDTA; pH 8.0, 150 mM NaCl, and 1% SDS). Proteinase K (Promega, Madison, WI) was added to a final concentration of 0.2 mg/ml and samples were incubated at 50° C for 4 h. The lysate was then extracted once with TE (10 mM Tris–HCl pH 8.0 and 1 mM EDTA pH 8.0) buffer-saturated phenol and once with a mixture of phenol–chloroform–isoamyl alcohol (25:24:1). Finally, total DNA was precipitated with two volumes of 99% ethanol, rinsed twice with 70% ethanol, dried and dissolved in 40 μl of sterilized distilled water. DNA was quantified and its purity was assayed by measuring absorbance at an OD_{260}/OD_{280} ratio in a nanoVue Plus™ spectrophotometer (GE Healthcare, Piscataway, NJ).

Amplification of the CL-15 SHV-1 gE gene

The 1736 bp CL-15 SHV-1 gE gene was amplified in a polymerase chain reaction (PCR) with the following primers: gE-SP 5′-CACC ATG CGG CCC TTT CTG CTG C-3′ and gE-ASP 5′-AGC GGG GCG GGC ATT CAA CAG GC-3′ (IDT, Coralville, IO). The underlined CACC sequence was introduced into the forward primer to facilitate directional cloning into the pENTR™ entry vector using the Directional TOPO® Cloning Kit, according to the manufacturer's instructions (Life Technologies Corporation, Carlsbad, CA). The PCRs included 0.2 μl of Phusion DNA polymerase (New England Biolabs, Beverly, MA), 10 μl of Phusion GC Buffer, all four dNTPs at final concentrations of 0.2 μM each, primers at a final concentration of 1 μM each, and 10 μl of 5 M betaine freebase (Sigma–Aldrich, St. Louis, MO) in a total volume of 50 μl. The cycling conditions included an initial denaturation step at 98° C for 30 s, and then 30 cycles consisting of 20 s denaturation at 98° C, 15 s annealing at 58° C, and 80 s extension at 72° C, followed by a final extension for 10 min at 72° C. The PCRs were performed in a Biometra TProfessional thermal cycler (Biometra, Göttingen, Germany) and the amplification product was purified on a 1% (w/v) agarose gel in TAE buffer, stained with ethidium bromide, and extracted using a commercial kit (QIAquick Gel Extraction kit, Qiagen), according to the manufacturer's instructions.

CL-15 SHV-1 gE sequence analysis

The purified CL-15 SHV-1 PCR product was cloned into pENTR™/D-TOPO® (Life Technologies) according to the manufacturer's instructions. Three independent clones were sequenced with M13 forward and reverse primers and the sequences were analyzed using Vector NTI® 10.3 software (Life Technologies). The consensus sequence was used as the query for a BLASTP search with standard parameters at the NCBI interface. Hits representing full-length or near full-length SHV-1 gE sequences were downloaded (tabulated in Table 1), used to generate a multiple sequence alignment with ClustalX 2 [16], and exported to the PHYLIP format. Protdist (PHYLIP version 3.69 [17]) was used to generate a distance matrix from the multiple sequence alignment with the Jones–Taylor–Thornton model. An unrooted tree was then produced from the distance matrix using the neighbor-joining method (Neighbor; PHYLIP package) and drawn using the PHYLIP drawtree postscript generator.

Recombinant baculovirus production and analysis

A recombinant baculovirus encoding CL-15 SHV-1 gE was isolated in Sf9 cells using a consensus pENTR™/D-TOPO® clone and the BaculoDirect™ C-Term Baculovirus Expression System (Life Technologies) according to the manufacturer's instructions. Recombinant baculovirus clones were plaque-purified once in Sf9 cells, tested for gE expression by immunoblotting, and a clone expressing the full-length, membrane bound version of the gE protein with C-terminal 6xHis and V5 epitope tags was identified and designated

Table 1
Identities of full-length or near full-length gE protein sequences deposited in GenBank™ compared to the CL-15 strain gE protein, and the countries in which they were isolated.

Strain	Identity (residues/total)	Genbank™ accession #	Refs.	Origins
NiA1	575/577	ACU43472.1	–	Spain
NiA3	574/577	ACA97995.1	–	Spain
Consensus*	575/577	YP_068389.1	[40]	–
Rice	574/577	P08354.1	[41]	USA
75V19	572/577	ACU43469.1	–	Belgium
Kaplan	571/577	AEM64041.1	[42]	Hungary
89V87	568/577	ACU43470.1	–	Belgium
GZ-Z1	566/577	AEH93984.1	[43]	China
00V72	567/577	ACU43468.1	–	Belgium
NS374	566/577	ACU43471.1	–	Belgium
Min-A	546/579	AAO11838.1	–	China
Guangdong	531/556	AAK95640.1	[32]	China
Yangsan	555/577	AAP04400.1	–	S. Korea
Guizhou-DY	550/577	AFS64557.1	–	China
LXB6	556/579	ADN78282.1	–	China
P-PrV	554/578	ACI24005.1	–	Malaysia
HB/LF	549/579	AGF86404.1	–	China
HB/HD	550/579	AGF86402.1	–	China
Ea	554/578	AAD51327.1	–	China
HNJZ	548/579	ACB37376.1	–	China
HB/HS	548/579	AGF86403.1	–	China
HB/BD	548/579	AGF86401.1	–	China
LXB88	552/578	ADN78283.1	–	China
PRV-SH	549/577	AAF19200.1	–	China
LA	545/579	AAN65185.1	–	China
Fa	525/558	AAK95639.1	[44]	China

* Strains Kaplan, Becker, Rice, Indiana-Funkhauser, NIA-33 and TNL.