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Expression and Characterization of Equine Herpesvirus 1 Glycoprotein D in *Escherichia coli*

Department of Veterinary Medicine,
Graduate School, Cheju National University,

Hyunju Ko

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Hyunju Ko
(Supervised by Professor Dusik Lee)

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This has been examined and approved.

Thesis director,
Taekyun Shin, Prof. of Veterinary Medicine

Dusik Lee, Prof. of Veterinary Medicine

Wongun Son, Prof. of Veterinary Medicine

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Abstract

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HyunJu Ko,
(Supervised by Professor Dusik Lee)

Department of Veterinary Medicine,
Graduate School, Cheju National University,
Jeju, Korea

Equine herpesvirus 1 (EHV-1) causes respiratory disease, abortion, and neurological disorders in horses. The glycoprotein D (gD) of EHV-1 is a conserved major glycoprotein that can be used to protect horses from clinical EHV-1 infection. In this study, three strains of EHV-1 were isolated from the liver samples of three of eight aborted fetuses. The Jeju isolates of EHV-1 were propagated in the equine dermal (ED) cell line, and viral DNA was extracted. The EHV-1 gD gene was amplified by PCR, cloned into pMAL-c2, and transformed into *E. coli* DH5α.

Recombinant gD was expressed as a maltose-binding (MBP) fusion protein in *E. coli*, and characterized by SDS-PAGE and Western blot analyses. The MBP-gD fusion protein reacted strongly with anti-EHV polyclonal antibody, to give bands of 101 kDa and 98 kDa.

The sera of infected horses responded to purified MBP-gD, which shows that the high-level reactivity of the MBP-gD antigen can be used to detect EHV-1 infection in horse populations.

Key words: equine herpesvirus 1; glycoprotein D; pMAL-c2; MBP-gD.

CONTENTS

I. INTRODUCTION

Equine herpesvirus 1 (EHV-1), which is the causative agent of equine viral rhinopneumonitis, is an enveloped, 150-nm diameter, double-stranded DNA virus. The virus, which is restricted to members of the family *Equidae,* is present in horse populations in many countries (Matsumoto et al., 1965; Bagust et al., 1972). Although EHV-1 is usually associated with upper respiratory tract infections, it may also cause neurological sequelae (Allen and Bryans, 1986; Crabb and Studdert, 1995; OCallaghan and Osterrieder, 1998). Importantly, EHV-1 infection of pregnant mares causes abortion.

EHV-1 is the prototype virus of the *Alphaherpesvirinae,* which also include herpes simplex virus (HSV), bovine herpesviruses 1, 2, and 5, caprine herpesvirus 1, canine herpesvirus 1, and felid herpesvirus. At least 10 structural herpesvirus glycoproteins are present on the virion envelope or appear on the surfaces of infected cells. These glycoproteins are involved in adsorption, penetration, and cell-cell fusion (Spear, 1993), and a few of these glycoproteins are conserved among all herpesviruses and appear to be essential for virus growth in cell culture. The envelope glycoproteins of Alphaherpesviruses not only play important roles in infectivity and pathogenicity, but also are major targets for the host immune system (Norrild, 1985; Stokes et al., 1989; Allen et al., 1991; Hannant et al., 1993; Babiuk et al., 1996). One of the EHV-1 glycoproteins, glycoprotein D (gD), is present in virions as a 58- to 60-kDa polypeptide (Flowers and OCallaghan 1992b; Whittaker et al., 1992; Love et al., 1993) that contains complex N-linked oligosaccharides. A neutralization epitope has been

identified at the amino terminus of EHV-1 gD between amino acids 4 and 22; antibodies to a synthetic peptide that corresponded to these residues neutralized EHV-1 in a plaque reduction assay (Flowers and OCallaghan 1992b). Several studies have investigated the immunogenicity of recombinant gD. Monoclonal antibodies that recognize EHV-1 gD neutralize virus infectivity *in vitro* and inhibit virus penetration, which suggests a role for EHV-1 in virus entry (Whittaker et al., 1992). The equine herpesvirus 1 glycoprotein D (EHV-1 gD) (Audonnet et al., 1990; Flowers et al., 1991; Whalley et al., 1991) has been shown to elicit protective immune responses against EHV-1 infection (Tewari et al., 1994; Zhang et al., 1998) using recombinant proteins that were produced in either *Escherichia coli* or the baculovirus expression system.

Although the polymerase chain reaction (PCR) has been applied to the detection of EHV-1 in biological samples (Kerstin Borchers et al., 1993), EHV-1 detection is still based mainly on virus isolation from cell cultures. Another important diagnostic method that is currently used is the serodiagnosis of EHV-1, which is based on antibody determination using the virus neutralization test. It was previously reported that mouse and equine antisera recognize the native and recombinant EHV-1 gD, gB, gC, and gH proteins in immunoblot assays (Love et al., 1993; Tewari et al., 1994, 1995; Whalley et al., 1995).

Recently, economic losses due to EHV-1 infections have become more acute in the horse population in Jeju. Although attenuated and killed vaccines are available, they provide incomplete protection against EHV-1. Moreover, whilst there have been many studies in other countries, only two studies have emerged from Jeju in Korea. In 1995, the characteristics and immunogenicity of equine herpesviruses from horses in Korea were reported (Cho et al.,

1995a, b, c). In 1998, the gD of EHV-1 Korea strain LC-1 was expressed successfully in recombinant baculoviruses (Yang et al., 1998).

Therefore, we attempted to isolate EHV-1 from the tissues of aborted equine fetuses, and to express the various viral glycoproteins as maltose-binding (MBP) fusion proteins in the prokaryotic expression vector pMAL-c2. We then used these fusions to develop diagnostic methods and to generate protecting immunity against the Jeju strains of EHV-1. The recombinant MBP-gD fusion proteins were tested in Western blots for reactivity with various horse sera.

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II. MATERIALS AND METHODS

1. Virus Isolation

The isolation of EHV-1 viruses was attempted from eight aborted horse fetuses (from five farms) that 4were submitted to the Veterinary Microbiology Laboratory of the Department of Veterinary Medicine, Cheju National University, Korea, during 2001. The lungs and livers of the aborted fetuses from eight horses that had naturally acquired infection were obtained. The tissues were homogenized in Dulbeccos Modified Eagle Medium (D-MEM; Gibco, Palo Alto, CA, USA) that contained 10% antibiotic-antimycotic agents (Gibco), so as to prepare pooled (10% w/v) homogenates. The homogenates were centrifuged, and the resulting supernatants were passed through a 450-nm membrane filter to isolate the virus. ED cells were grown in D-MEM that was supplemented with inactivated 10% fetal bovine serum (FBS; Gibco). In order to passage the cells, the medium was aspirated, and the cells were washed twice with trypsin solution (Gibco). The cells were incubated with traces of trypsin for 5 min at 37ºC. When single cells were observed under a microscope, the cells were resuspended in D-MEM. The cells were then inoculated at 1 ml/well in 24-well plates (Costar, NY, USA). After 16-24 h of incubation at 37ºC, the medium was discarded, and 200 l of homogenized tissue sample was added. Control cultures were inoculated with the same volume

of medium in place of the viral inoculum. After adsorption at 37ºC for 1 h, the inoculum was removed, and 1 ml of D-MEM that contained 10% FBS was added to the cell cultures, followed by three washes with 1 ml of D-MEM. After an incubation period of 2-3 days, we examined the cytopathic effect (CPE) of each sample under the microscope. Virus was collected from wells that showed CPE, and the samples were stored at -70ºC for further testing. When CPE was absent for 5 days, a third blind passage was performed using the cell culture supernatants, in the same manner as with the original samples.

2. Immunofluorescence Assay for EHV Detection

Following 24 h of infection with isolated viruses, the ED cell cultures were placed on coverslips and fixed in 4% formaldehyde for 10 min. The slides were incubated with the polyclonal antibody at 37ºC for 60 min, washed three times with phosphate-buffered saline (PBS, pH 7.4), and stained with FITC-conjugated anti-horse IgG (Sigma, St. Louis, MO, USA) at 37ºC for 50 min. After washing, the cells were mounted in buffered glycerol and examined under a fluorescence microscope.

3. Polymerase Chain Reaction (PCR) for Genotypic Analysis of EHV

PCR was carried out using gB-specific primers for equine herpesvirus-1, according to the method described previously (Moon, 2001) The oligonucleotide primers that were used for the detection of EHV-1 DNA in infected cells included: P1, 5'-TCTACCCCTACGACTCCTTC-3' (forward primer); and P4, 5'-CCTTTGTTGTTATGGGGTAT-3' (reverse primer). The primers were synthesized by Bioneer (Daejan, Korea) at a synthesis scale of 50 nmol, and desalted. DNA samples from EHV-1 (strain Aust IV) and EHV-4 (strain T252) were used as the positive and negative controls, respectively. Total DNA samples were extracted from the supernatants of ED cell cultures that were inoculated with virus, which was isolated using the QIAmp Mini Kit (Qiagen GmbH, Hilden, Germany), in accordance with the manufacturers instructions. PCR was carried out using the PCR premix [40 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl2 250 M dNTPs, 2.5 U *Taq* DNA polymerase (Bioneer)] in a final reaction volume of 50 l. Following an initial denaturing step at 94ºC for 4 min, 33 amplification cycles that each consisted of denaturation at 94ºC for 2 min, annealing at 56ºC for 2 min, and extension at 72ºC for 2 min were performed. The reaction was completed by a final extension step at 72ºC for 10 min. The amplified products were analyzed by electrophoresis in a 0.7% agarose gel, and detected by staining with ethidium bromide.

4. *E. coli* Strain and Expression Plasmid

E.coli strain DH5 (Invitrogen, Palo Alto, CA, USA) was used for plasmid maintenance and transformation. All of the bacterial clones were grown in LB agar (per liter: 10 g tryptone, 5 g yeast extract, 10 g NaCl, 15 g agar) or LB broth (per liter: 10 g tryptone, 5g yeast extract, 10 g NaCl).

The expression vector pMAL-c2 was purchased from NEB (New England Biolabs, Portland, ME, USA). The pMAL-c2 plasmid contains the *malE* translation initiation signal, which allows high-level expression of cloned sequences. The vector expresses the *malE* gene fused to the *lacZ*-gene.

5. Construction of pMAL-C2-Derived Expression Vectors That Contain Fragments of EHV-1 gD

Standard procedures were used for *E. coli* transformation and DNA restriction endonuclease digestion and ligation (Sambrook et al., 1989) during the cloning of the EHV-1 gD gene. The glycoprotein D gene was amplified by PCR. The PCR templates were extracted from the supernatants of EHV-1 strain Jeju-inoculated ED cell cultures using the QIAmp Mini Kit (Qiagen), in accordance with the manufacturers instructions. The sequences of the synthetic primers were derived from the

nucleotide sequence of the EHV-1 gD gene (GenBank accession no. NC001491). The gD-specific PCR primers had the following sequences: forward primer, 5'- GCTGGATCTAGAAATAAACGAAGCATGTCTACCT-3' (the *Xba*I site underlined): reverse primer, 5'-AACTGCAGTTACGGAAGCTGGGTATATTTAACATCC-3' (*Pst*I site and stop codon underlined). The amplifications were carried out in a DNA thermal cycler (Biometra UNO-Thermoblock, Göttingen, Germany) with an initial denaturation at 94℃ for 5 min, followed by 30 cycles of 94C for 1 min, 57℃ for 1 min, 72℃ for 1 min, and a final extension step of 72C for 10 min. The PCR mixture contained 10 ng target DNA, 0.4 M of each primer, 0.2 mM dNTPs, 2.5 U *Taq* DNA polymerase, and 1 reaction buffer (Super Bio, Suwon, Gyeonggi, Korea). The PCR product was phenol-extracted, ethanol-precipitated, and digested with *Xba*I and *Pst*I (Gibco). The DNA fragment that corresponded to the expected size of gD was purified using the QIAquick Gel Extraction Kit (Qiagen), and ligated into the *Xba*I- and *Pst*I-digested pMAL-c2 vector using the standard T4 ligase procedure. Thus, the pMBPgD construct was generated, and subsequently transformed into *E. coli* DH5 (Fig. 1). The transformants were grown in LB agar that contained 100 g/ml carbenicillin.

6. Expression and Purification of MBP-gD

The expression and purification of the MBP-gD fusion protein were performed in accordance with the manufacturers instructions for pMAL-c2

(New England Biolabs). Briefly, the *E. coli* DH5α strain that harbored the pMBPgD plasmid was propagated overnight in LB medium that contained 100 g/ml carbenicillin. The overnight culture was inoculated at a 1:10 dilution into 1000 ml LB broth and incubated at 37C until the culture reached an OD600 value of 0.5. The cultures were induced with IPTG (isopropyl--D-thiogalactopyranoside; Gibco) at a final concentration of 0.5 mM. After 4 h of induction, the cells were harvested by centrifugation (4000 rpm for 10 min at 4C) and resuspended in 50 ml of column buffer [CB; 20 mM Tris-HCl (pH 7.4), 200 mM NaCl, 1 mM EDTA, 1 mM azide, 10 mM -mercaptoethanol]. The cells were frozen overnight at -20C, thawed at 4C, and then sonicated with 15-s pulses of 50 W for 2 min. The cell homogenate was centrifuged (9000 *g* for 20 min at 4C), and the supernatant was recovered (soluble fraction). The pellet was resuspended in 25 ml of CB (insoluble fraction). An amylose resin column (New England Biolabs) with a bed volume of 10 ml was prepared for the purification of the fusion protein. The soluble fraction was loaded onto the column at a flow rate of 1 ml/min, and the column was washed with CB. The fusion proteins were eluted with CB that contained 10 mM maltose. The protein concentration was determined using the Bio-Rad Protein Assay Reagent (Bio-Rad, Hercules, CA, USA). The proteins were analyzed by SDS-PAGE.

7. Characterization of MBP-gD

The MBP-gD fusion protein was characterized by SDS-PAGE and Western blot analysis. The samples were heated for 5 min at 95C in 50 l of SDS sample buffer, and the proteins were separated on 0.1% SDS-8.5%-polyacrylamide gels. The protein bands were visualized by staining with 0.05% Coomassie blue (Laemmli, 1970). For Western blots, the proteins were separated by SDS polyacrylamide gel electrophoresis using a 5% stacking gel and an 8.5% separating gel, then transferred to nitrocellulose membranes (Towbin et al., 1979), and blocked with Tris-buffered saline [TBS; 20 mM Tris (pH 7.4), 200 mM NaCl] that contained 5% skim milk. The membranes were probed using polyclonal horse antiserum against EHV-1. The bound antibody was visualized using the anti-horse IgG-peroxidase conjugate (Sigma), followed by HRP color development using the substrate 4-chloro-1-naphthol (Bio-Rad).

8. Immunoblotting

Field serum samples were collected from mares that were diagnosed with EHV-1 infection in 2001. In addition, sera from horses that were naturally infected with EHV-1 were collected in the field during 1995-1997 and stored at the Veterinary Microbiology Laboratory of the Department of Veterinary Medicine, Cheju National University, Korea. All of the tested

sera adsorbed with the *E. coli* DH5αstrain that expressed MBP, following IPTG induction of the bacteria for 2 hours at room temperature and overnight incubation at 4C to remove antibodies directed against MBP and *E. coli*. The purified MBP-gD proteins and MBP proteins were transferred from SDS-PAGE gels onto nitrocellulose membranes, and immunoblotting was performed as described above. The field serum samples from horses that were naturally infected with EHV-1 during 1995-1997 and in 2001 were used at final dilutions of 1: 150.

III. RESULTS

1. Virus Isolation and Genotypic Analyses of EHV

Cytopathic effects (CPEs), which were characterized by cell fusion and syncytial formation, were detected microscopically after 2-3 days in the liver samples of three of the eight aborted fetuses. These viral strains were designated as JK-1, JK-2, and JK-3 (Fig. 2).

Using IFA, specific brilliant fluorescence was observed in the cytoplasmic and nuclear compartments of the ED cells (Fig. 3), and the isolates were identified as EHV.

PCR was carried out using the EHV-1 gD-specific primers to analyze the genotypes of the isolated viruses. The results of the genotypic analyses of the equine herpesvirus isolates are shown in Figure 4. Amplification using the P1/P4 primer pair produced a 1735-bp DNA fragment from the viral DNA in the supernatants of the ED cell cultures that were inoculated with viral isolates.

2. Expression of Fusion Proteins

The EHV-1 gD gene was successfully inserted into the pMAL-c2 expression vector, such that the activity of the *tac* promoter was modulated by IPTG. When the bacterial cells were transformed with the pMBPgD construct and induced with IPTG for 4 h, a band of approximately 101 kDa that co-migrated with the MBP-gD fusion protein was readily observed in a Coomassie brilliant blue-stained gel (Fig. 5, lane 7). In contrast, this band was absent from cells that were not induced with IPTG (Fig. 5, lane 6). The soluble fraction was enriched using affinity chromatography with an amylose resin column and maltose, according to the protocols of the manufacturer (Fig. 5, lanes 8 and 9). However, following these purification procedures, extra protein bands of 43 kDa and 55 kDa, which corresponded to the MBP and gD proteins, respectively, were observed (Fig 5, lanes 8 and 9). These proteins bands were due to the spontaneous cleavage of a fraction of the MBP-gD fusion protein sample.

3. Immunoreactivity of the Bacterially Expressed Fusion Proteins

In order to confirm the identity of the 101-kDa protein that was expressed by the recombinant bacteria, samples of induced cultures that contained MBP and MBP-gD were run on SDS-PAGE, transferred to nitrocellulose, and immunoblotted with a polyclonal antibody that was raised against EHV-1 (Fig. 6). The polyclonal antibody reacted with both the induced MBP-gD and purified MBP-gD (Fig 6, lanes 6 and 7). Serum samples of naturally infected horse also reacted with the MBP-gD fusion protein (Fig. 7).

Based on these results, our immunoblotting procedure appears to be suitable for the detection of antibodies to EHV-1. It offers the important advantages of a plentiful supply of test antigens and a more rapid procedure at lower cost compared to the SN test.

In summary, our results suggest that the MBP-gD fusion protein may be used as a test antigen in immunoblots to detect sera that are positive for antibodies to EHV-1.

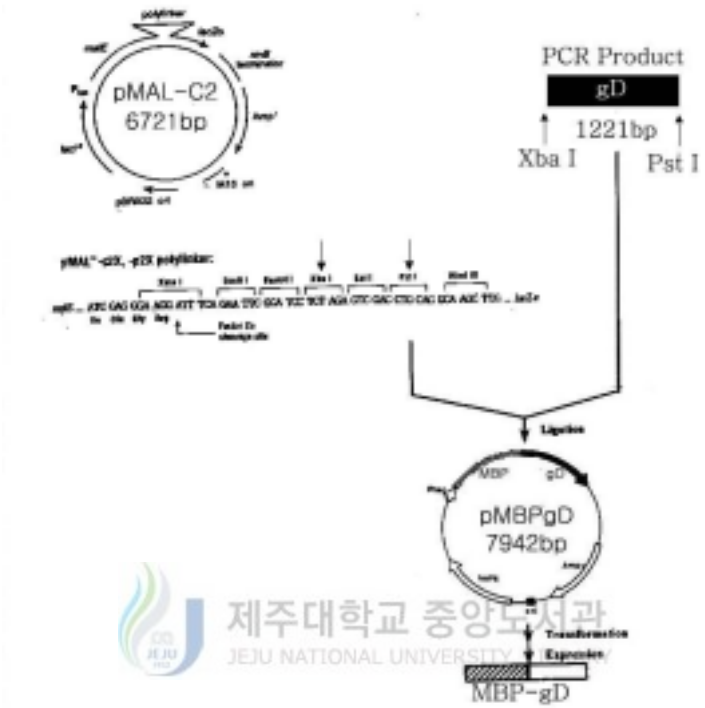

Fig. 1. Schematic diagram of the construction of plasmid pMBPgD. The 1.22-kb gD DNA fragment was amplified from the EHV-1 Jeju strain by PCR, and inserted into pMAL-c2 using the standard T4 ligase procedure. The generated pMBPgD construct was subsequently transformed into *E. coli* DH5.

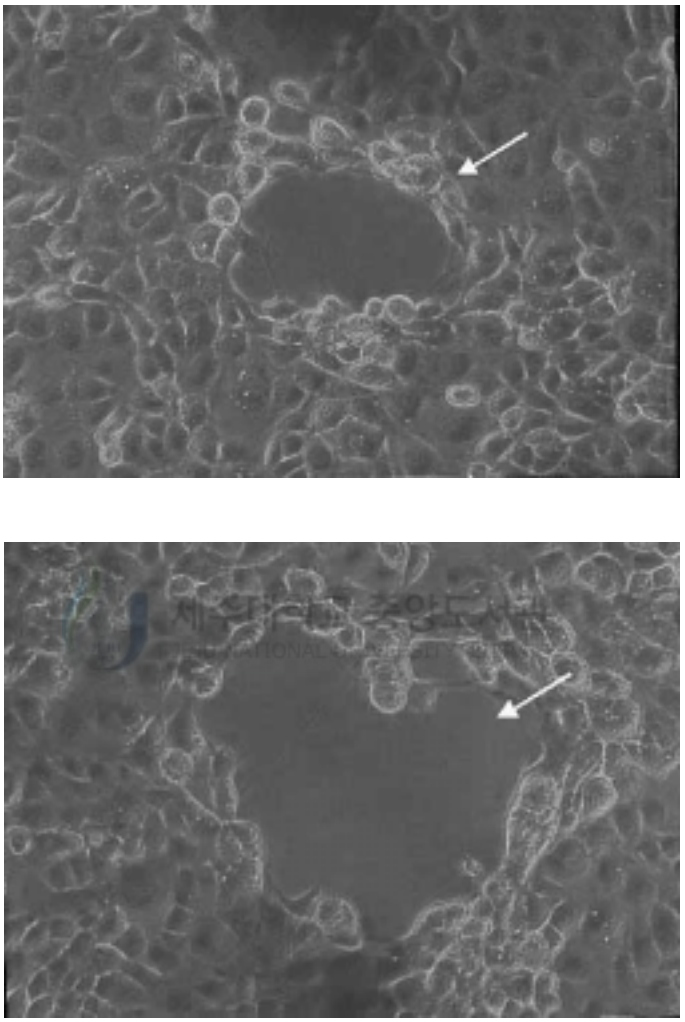

Fig. 2. Typical pattern of plaques in ED cells that are infected with the equine herpesvirus. Magnification, 200.
CPE is characterized by cell fusion and syncytial formation 2-3 days after infection.

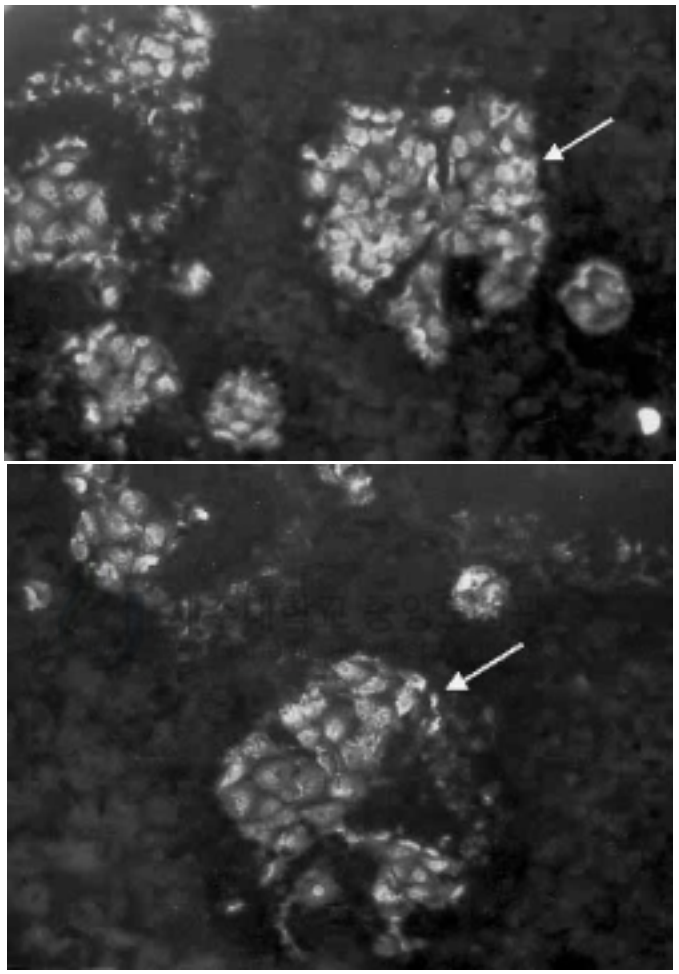

Fig. 3. Immunofluorescence assay using horse antiserum directed against the Aust IV strain. Note the specific brilliant fluorescence observed in the cytoplasmic and nuclear compartments of the infected cells.

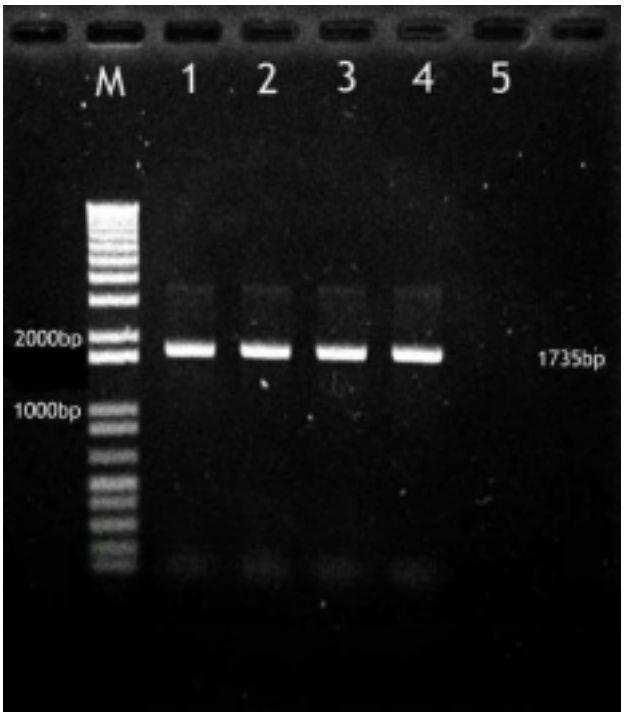

Fig. 4. Genotypic analysis using PCR amplification of purified chromosomal DNA from the Jeju strains and gDspecific primers. Lane M: molecular size ladder; lane 1: EHV-1 strain Aust IV, as the (+) control; lane 2: JK-1; lane 3: JK-2; lane 4: JK-3; lane 5: EHV-4 stain T252, as the (-) control.

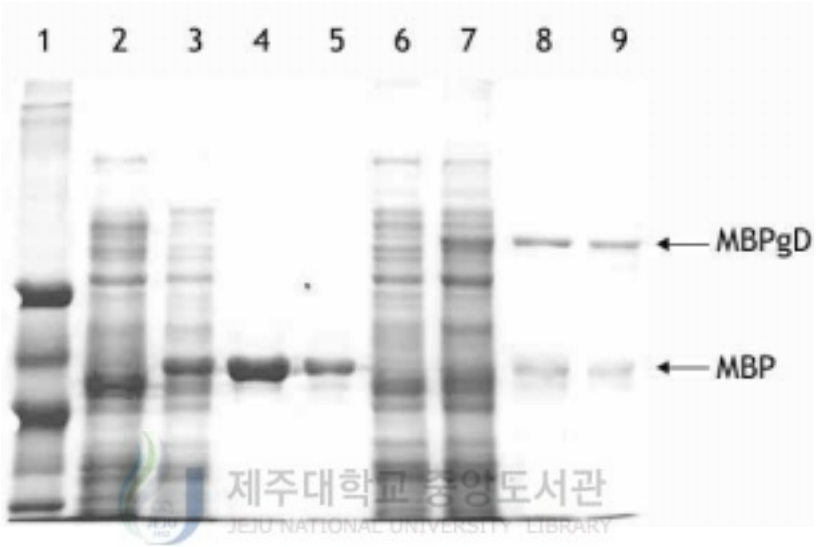

Fig. 5. The MBP-gD fusion protein was expressed intracellularly in *E. coli* DH5, and purified by affinity chromatography.

Lane 1: molecular size marker; lane 2: uninduced cells; lane 3: IPTG-induced cells with MBP; lane 4: purified MBP; lane 5: purified MBP; lane 6: uninduced cells that carry pMBPgD; lane 7: IPTG-induced cells that carry pMBPgD; lane 8: purified MBP-gD; lane 9: purified MBP-gD.

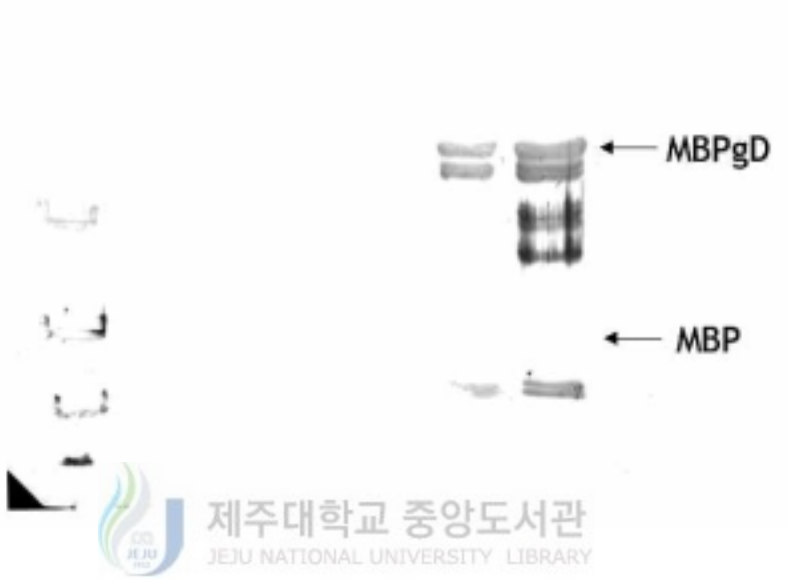

Fig. 6. The polyclonal antibody reacts with the recombinant MBP-gD and purified MBP-gD.
Lane 1: molecular size marker; lane 2: uninduced cells; lane 3: IPTG-induced cells carrying MBP; lane 4: purified MBP; lane 5: uninduced cells that carry pMBPgD; lane 6: IPTG-induced cells that carry pMBPgD; lane 7: purified MBP-gD.

Fig. 7. The serum from an infected horse reacts with the purified MBP-gD fusion protein.
Lane M: molecular size marker; lane 1: Coomassie blue-stained MBP-gD and MBP; lanes 2-5: serum from a horse that was infected a few years previously; lanes 6-9: serum from a recently infected horse.

IV. DISCUSSION

The glycoprotein D of EHV-1 offers important advantages for studying the pathogenesis and immunity of EHV-1 (Stokes et al., 1997). In order to obtain sufficient quantities of purified viral protein, the genes have been expressed in baculovirus and *E. coli* expression systems. The pMAL-c2 vector used in this study was previously adapted by Kheyar *et al* (1997) for the expression of the membrane protein of the equine arteritis virus (EAV) and the F protein of the bovine respiratory syncytial virus (BRSV) (Jordi et al., 1996). Using this approach, we cloned the gD gene of EHV-1 in pMAL-c2 and expressed it in *E. coli*.

Expression of the full-length gD in baculoviruses resulted in the production of polypeptides of 43 kDa, 55 kDa, and 58 kDa (Love et al., 1993; Flowers et al., 1995b). In this study, the 101-kDa MBP-gD fusion protein was detected in *E. coli*. Several gD-specific polypeptides were detected in Western blots of the purified gD fusion protein. The existence of several gD polypeptides in mammalian cell lines that constitutively expressed gD was previously noted by Wellington *et al*. (1996b). In addition to the polypeptides of 48-59 kDa, low molecular weight gD products of 28-30 kDa were also identified in these cell lines. These proteins, which have also been previously reported for EHV-1 expressed in *E. coli* (Love et al., 1993), are considered to be cleavage products.

Currently, diagnosis of EHV-1 infection is based on serum antibody determination, virus isolation in cell cultures, or PCR amplification of viral

nucleic acids from aborted fetuses. Virus-neutralizing antibodies have been detected by the SN test in the sera of horses that were naturally infected with EHV-1. However, these laboratory procedures are laborious and time-consuming. Residues 4 to 22 (aa 30-48) of the mature gD polypeptide of EHV- 1 contain a virus-neutralizing epitope (Flowers and OCallaghan, 1992). In this study, we produced a recombinant MBP-gD fusion protein that contained the neutralizing epitope, and we investigated the ability of this fusion protein to detect serum EHV-1 antibodies in immunoblots. The reactivity of the antisera against EHV-1 in immunoblots showed that the full-length gD protein expressed as an MBP fusion protein could act as an antigen for the detection of polyclonal antisera against EHV-1.

In previous studies, immunization with full-length gD that was derived from recombinant baculovirus-infected Sf9 cells or that was expressed as a GST-gD fusion protein induced higher levels of protection than synthetic peptide preparations (Zhang et al., 1998). The baculovirus-expressed gD contained virus-neutralizing epitopes, and thus could induce the formation of virus-neutralizing antibodies in mice that were inoculated with the purified gD protein (Stokes et al., 1997). Recombinant gD that was expressed as a glutathione-S-transferase (GST) fusion protein in *E. coli* elicited high titers of neutralizing antibody (nAb) and CD4 T cell proliferative responses following subcutaneous or intranasal immunization (Zhang et al., 1998). Immunization with purified gD protein induced higher levels of protection than immunization with recombinant baculovirus-infected Sf9 cells (Stokes et al., 1997). According to Stokes *et al*., it is important that the proteins are purified prior to inoculation, and that they are administered with an adjuvant.

The pMAL-c2 vector was used to express the full-length gD of EHV-1,

and the protein was purified using an amylose resin column. The purified MBP-gD fusion protein contained virus-neutralizing epitopes at residues 4 to 22 of the mature gD polypeptide of EHV-1, as reported previously by Flowers and OCallaghan. Based on the previous studies, this protein should elicit neutralizing antibodies and T-lymphocyte responses. In addition, a serological test that is based on the detection of serum antibodies that are specific for other EHV-1 gene products would be useful for the diagnosis of EHV-1 infections and for the differentiation of animals that are naturally infected with EHV-1 from animals that have been vaccinated with the recombinant gD of EHV-1.

V. CONCLUSION

Viruses were isolated from the tissues of aborted equine fetuses, to develop diagnostic methods and subunit vaccine candidates for the Jeju strains of EHV-1. Three viral strains were isolated from the tissues of eight aborted fetuses. These viruses were EHV-1 types, which were designated as JK-1, JK-2, and JK-3. The glycoprotein D genes of these viruses were cloned into the prokaryotic fusion expression vector pMAL-c2, and the MBP-gD fusion protein was expressed in *E. coli* DH5. The recombinant MBP-gD protein was easily purified from the soluble fraction by affinity chromatography. Polyclonal antibodies from horses that were naturally infected with EHV-1 reacted with purified MBP-gD, which suggests that the full-length gD protein can act as a reliable antigen for the detection of EHV-1 infection. We anticipate that MBP-gD will elicit specific antibody and T lymphocyte responses. Therefore, this purified fusion protein appears to represent a subunit vaccine candidate for Jeju strains of EHV-1.

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초 록

Equine Herpesvirus Type 1 의 Glycoprotein D 유전자의 *E. coli* 에서의 발현과 특징

고 현 주
(지도교수 : 이 두 식)

제주대학교 일반대학원
수의학과 수의미생물학 전공

Equine Herpesvirus Type 1 (EHV-1)은 말에서 호흡기 질병, 유사산, 신경증상을 유발시키는 원인체이다. EHV-1의 구조단백질인 Glycoprotein D 는 이 바이러스의 야외감염에서 숙주동물을 방어할 수 있는 주요 Glycoprotein이다. 본 연구에서는 이 Protein을 이용하여 EHV-1의 혈청학적 진단을 위한 항원을 개발하고 제주 분리주에 대한 백신을 개발하기 위한 정보를 제공하고자 하였다. 이를 위해 먼저 바이러스 분리를 시도하였다. 바이러스 분리를 시도한 8두의 유산된 태아의 장기 중 3두의 장기로부터 바이러스를 분리했다. 분리된 바이러스는 각각 JK-1, JK-2, JK-3로 명명되어졌다. 분리된 바이러스는 Equine Dermal (ED) cell에 접종하여 증식시켜 모든 실험에 사용하였다. 먼저 분리된 바이러스가 EHV-1임을 확인하기 위하여 IFA, PCR를 실시 하였

다. 그리고 glycoprotein D를 발현시키기 위하여 증식된 바이러스로부터 DNA를 분리한 다음 PCR을 이용하여 gD를 증폭시켰다. 그 후 제한효소 처리를 한 후 expression vector 인 pMAL-C2 에 T 4 ligase을 이용하여 삽입 시켰다. *E.coli*에 형질전환 시킨 후 IPTG를 첨가하였다. gD는 Maltose binding protein과 융합되어 발현 되었다. 발현된 분자량은 101 kDa으로 나타났다. 발현된 gD는 affinity chromatography 방법을 이용하여 정제되었다. 정제된gD는 anti-EHV-1 다클론성 항체와 반응을 잘 나타내었으며 분자량은 101 kDa 와 98kDa 로 나타났다. 야외에서 감염된 말의 혈청과도 잘 반응하였다. 이 결과를 통하여 발현된 후 정제된 gD는 EHV-1의 혈청학적 진단을 위해 좋은 항원으로 제공될 것이라고 생각되어진다. 또한 정제된 gD가 항원 항체 반응을 일으키는 점과 많은 연구에서 보고된 것을 미루어 볼 때 이 정제된gD는 제주의 말에서 제주 분리주에 대한 항체반응을 이끌어낼 면역원 사용될 수 있을 것이라고 생각되어진다.

중요어 : EHV-1, JK-1주, gD, p MAL-c2, MBP 융합 gD, *E.coli*

감사의글

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