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Sequencing and Baculovirus-Based Expression of the Glycoprotein B2 Gene of HSV-2 (G)

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Abstract The gene for glycoprotein B (gB2) of HSV-2 strain G was subcloned, sequenced, recombinated into the *lacZ*-HcNPV, expressed in insect cells, and compared with the homologous gene of other HSV-2 strains. The ORF of the gB2 gene was 2,715 bp. The overall nucleotide sequence homology of the gB2 gene compared with that of the two previously reported HSV-2 strains appeared to be over 98%. A recombinant virus named Baculo-gB2 was constructed, which expressed the gB2 protein in insect cells. The recombination was confirmed by a PCR and the expression was demonstrated by radioimmunoprecipitation. Insect cells infected with the Baculo-gB2 virus synthesized and processed gB2 with approximately 120 kDa in the cells, and then secreted it into the culture media, where it reacted with a monoclonal antibody to gB2. The gB2 polypeptide contained two main hydrophobic regions (a signal sequence from 1 to 23 amino acid residues, and a membrane anchor sequence from aa 745 to 798), eight *N*-glycosylation sites evenly distributed, and was rich in alanine (11.2%). Antibodies to this recombinant protein that were raised in mice recognized the viral gB2 and neutralized the infectivity of the HSV-2 *in vitro*. These results show that the gB2 protein was successfully produced in insect cells and could be used to raise a protective neutralizing antibody. Accordingly, this particular recombinant protein may be useful in the development of a subunit vaccine.

Key words: HSV-2, glycoprotein B, baculovirus, neutralizing antibody

Glycoprotein B (gB) of the *Herpes simplex* virus types 1 and 2 (HSV-1 and 2) is one of the major viral proteins embedded in the virion envelopes and cell membranes of infected cells and is one of the more abundant viral glycoproteins in infected cells [43]. HSV-1 gB1 plays an important role in the penetration, cell fusion, and interaction of the virus with the immune system of the host [7, 13, 17, 34]. gB1 [1, 3, 4, 15, 43], gB2 [3, 14, 16, 32], and gD [1, 3, 16] are important targets for both humoral and cell-mediated immune responses to an HSV infection. As such, gB1 and gB2 are useful as principal antigens for an HSV subunit vaccine.

The nucleotide sequences of the gB2 gene of HSV-2 strain 333 [45] and strain HG52 [6] have already been determined and analyzed. Stuve *et al.* [45] reported on the expression of the HSV-2 gB2 gene in Chinese hamster cells transfected with a mammalian cell expression vector containing a SV40 early promoter, however, the level of the recombinant products was low. Baculovirus expression vectors have proved to be useful in achieving a high-level expression of a variety of foreign genes in insect cells and obtaining biologically active proteins, and an allelic replacement has been adopted to insert foreign genes into the baculovirus genome [35, 42]. Current authors have previously constructed baculovirus transfer and expression vectors using the *Hyphantria cunea* nuclear polyhedrosis virus (HcNPV) [22, 25], which proved to be useful in achieving a high-level expression of a variety of foreign genes in *Spodoptera frugiperda* cells [23, 26]. Accordingly, the current study is concerned with the expression and properties of the gB2 of HSV-2 strain G as a component of a subunit vaccine. Therefore, using these systems, the gB2 coding sequence of HSV-2 strain G was recombinated into an HcNPV vector and the expressed gB2 proteins were characterized. Also, it was demonstrated that the immunization of mice with this recombinant protein induced a protective immune response.

MATERIALS AND METHODS

Viruses and Cells

The *Herpes simplex* virus type 2 (HSV-2) strain G (ATCC VR-734) was obtained from the Korean AIDS (Acquired Immune Deficiency Syndrome) Center, Seoul, Korea. The Vero cells (ATCC CCL81) were obtained from the Korean

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Type Culture Collection (KTCC). The HSV-1 strain KOS was obtained from the Korean National Institute of Health, Seoul, Korea. The HSV strains were grown at 37°C in the Vero cells using Eagle's minimum essential medium (Gibco, Middletton, WI, U.S.A.) with 10% fetal bovine serum (Gibco), as previously described by Lee *et al.* [27]. The *lac*Z-*Hyphantria cunea* nuclear polyhedrosis virus (*lac*Z-HcNPV) lacking the polyhedrin gene [23] was propagated in *Spodoptera frugiperda* cells (IPLB-SF-21) (L. K. Miller, University of Georgia, U.S.A.) at 27°C in a TC-100 medium (Gibco) [23, 30]. The cells were routinely maintained in a TC-100 medium supplemented with a 0.2% tryptose broth powder and 10% fetal bovine serum, and passaged every 5 days [25, 28]. The virus was titered by a plaque assay for infectivity [29].

Plasmids and Bacterial Strains

pBACgus4X-1 harbored in *E. coli* XL1-Blue (Novagen Inc. Madison, WI, U.S.A.) was used for subcloning and transferring the HSV-2 gB2 gene into the expression viral vector (*lac*Z-HcNPV) [23]. A pBluescript SK (+) plasmid (Stratagene, La Jolla, CA, U.S.A.) was used for both the cloning and sequencing. A pHLA2-24 plasmid containing a *Bam*HI fragment with the HSV-2 gB2 gene harbored in *E. coli* XL-blue was used [19]. The *E. coli* was maintained in an LB broth (Gibco).

Oligonucleotides

The oligonucleotide primers (gBSP-1, 5':5'-GAGCTGTA-CGTGCG-3' and gBSP-2, 3':5'-GAAATCAAGGTCGAG-AACG-3') were deduced from the terminal sequences of the published sequence (from nt 1369 for gBSP-1 and from nt 292 for gBSP-2) of the gB2 gene of HSV-2 strain 333 [45], and the T3 and T7 oligonucleotides, purchased from Stratagene Co., were used for sequencing. The primer p10-1 (5'-GTTAATAAGAATTATTATCAAATC-3') was deduced from the published sequence of the p10 promoter gene of HcNPV [37]. Primers GB-5 (5':5'-GGGGAGCG-CCCAGCGCCAACGCG-3') and GB-3 (3':5'-AAG CTC-AACCC CAACGCCAT-3') were designed from the HSV-2 strain G sequence depicted in Fig. 2 (from nt 1448 for GB-5 and from nt 1640 for GB-3) and used for detecting the inserted gB2 gene using the PCR. The oligonucleotides were synthesized by Korea Biotec Inc., Daejeon, Korea.

Monoclonal Antibody

The HSV-2 strain 333 gB specific monoclonal antibody was purchased from Biodesign International (Maine, U.S.A.) and the fluorescein isothiocyanate-conjugated sheep anti-mouse IgG was purchased from Boerhinger Mannheim Biochemicals (Indianapolis, IN, U.S.A.).

Preparation of DNAs

E. coli containing the recombinant plasmids was cultured in an LB broth containing proper antibiotics at 37°C. The plasmid DNA was purified according to the procedure described by Birnboim and Doly [2]. The *lac*Z-HcNPV DNAs were purified, based on the procedure described by Lee *et al.* [23].

SDS-PAGE Analysis

The proteins were fractionated on 10% polyacrylamide gels as described by Bollag *et al.* [5]. Briefly, *S. frugiperda* cells (1.5×10^7 cells per flask) were seeded in flask (75 cm^2), allowed to attach in a monolayer overnight at 27°C, infected with the recombinant baculoviruses expressing gB2 at a multiplicity of infection of 2 pfu/cell, and incubated with 4 ml of a TC-100 medium for 96 h at 27°C. The infected cells and media at 96 h p.i. were separated by centrifugation at 12,000 ×g for 5 min at 4°C. The media and pellets were then separated. The pellets were resuspended in 5 ml of PBS (pH 6.2) and freeze-thawed, then the suspensions were centrifuged for 10 min at 4°C. The supernatants and pellets of the lysates were reseparated, and the pellets were resuspended and incubated in 5 ml of a lysis buffer (0.1% SDS, 1.0% NP-40, 0.5% Na-deoxychlolate, 500 mM Tris-HCl, 150 mM NaCl and 1 mM PMSF) on ice for 30 min. After 30 min, the two lysates were combined, recentrifuged to remove any cell debris, then dialyzed to a final concentration of 1.0 ml. The media were also dialyzed to the final concentration. Normal *S. frugiperda* cells and cells infected with the wild-type *lac*Z-HcNPV were treated in the same manner, with the infected cells as the control. Five-hundred ml of the cell lysates and media samples were separately mixed with 500 µl of a 2× PAGE sample buffer, then 10 µl of aliquots of the diluents were loaded for a PAGE analysis.

Amplification of DNAs

DNAs were amplified by a polymerase chain reaction (PCR) according to the procedure described by Chung *et al.* [9]. The PCR analysis was conducted with 1.0 µg of template DNA, 0.25 µM of each primer, 200 µM deoxynucleoside triphosphates, and 2 µl of DMSO. The mixture was heated to 100°C for 7 min, placed on ice for 10 min, then 1 unit of Taq polymerase was added. Thirty cycles of amplification were performed on a thermal cycler (Perkin-Elmer Thermocycler). Aliquots of the amplification products (1/10 volume) were analyzed on 0.8% agarose gels.

Restriction Enzyme Digestions and Agarose Gel Electrophoresis

All restriction endonuclease digestions were performed according to the manufacturer's instructions. The DNAs were digested and electrophoresed on a 1.0% agarose gel [29, 40].

Molecular Subcloning of gB2 Gene

The HSV-2 gB2 gene in the plasmid pHLA2-24 was cleaved out with *Nru*I and *Bam*HI, inserted into the *Eco*RV

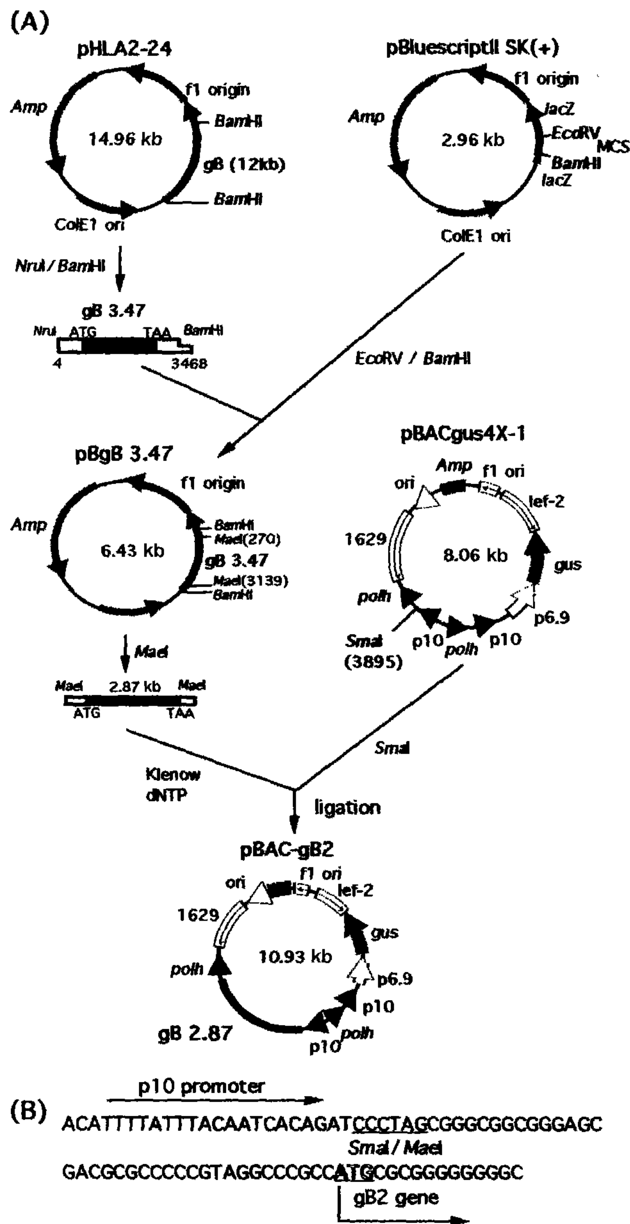

Fig. 1. Construction scheme for plasmid pBAC-gB2 containing the gB2 gene sequence of HSV-2 (A) and upstream sequence of gB2 (B).

(A): The 3.47 kb fragment containing the gB2 gene sequence in the pHLA2-24 plasmid was digested out with *Nru*I and *Bam*HI, which were then cloned into the *Eco*RI and *Bam*HI sites of the pBluescript II SK (+) vector to construct recombinant plasmid (pBgB3.47). The 2.87 kb fragment containing the gB2 gene was digested with *Mae*I and cloned into the *Sma*I site of pBACgus4X-1 to construct recombinant plasmid (pBAC-gB2). (B): Partial nucleotide sequence of the cloning site downstream of the polyhedrin promoter region. The part downstream of the promoter, the ligated *Sma*I/*Mae*I site, and the translation start codon ATG of the gB2 gene were all apparent.

and *Bam*HI sites of the pBluescriptII SK (+) vector, and named a recombinant plasmid (pBgB3.47). Thereafter, the gB2 gene in the recombinant plasmid was digested out with *Mae*I to create a 2.87 kb fragment containing the gB2 sequence, which was then cloned into the *Sma*I site of the baculovirus transfer vector pBACgus4x-1 to construct a recombinant plasmid, pBAC-gB2, using standard protocols [23, 24, 26] (shown in Fig. 1A). Competent *E. coli* cells were prepared and transformed using the procedure described by Mandel and Higa [31].

Construction and Selection of Baculo-gB2 Recombinant Virus

The *S. frugiperda* cells were seeded in 60-mm petri dishes with 3×10^6 cells per dish and cotransfected with five microliters (1.0 µg of DNA) of linearized *lac*Z-HcNPV DNA, *Bsu*361 enzyme, and 5 µl (100 µg) of pBAC-gB2 plasmid DNA in a TE buffer (10 mM Tris-HCl, 1.0 mM EDTA, pH 7.5) in 40 µl H_2O, and constructed recombinant viruses using lipofectin-transfection as described by Lee *et al.* [23, 26]. The recombinant viruses in the supernatants were selected using a 1.5% low-melting-point agarose plaque assay [23, 29] containing 0.2% X-Gluc and then incubated at 27°C for 5 days. The blue occlusion-negative plaques were picked as the recombinant viruses and added to a serum-free medium for further analysis. The Insertion of the gB gene in the recombinant viruses was analyzed by a PCR using primers GB-5 and GB-3.

Sequencing of HVS-2 gB2 Gene

The gB2 gene in the pBgB3.47 clone was digested with the *Mae*I enzyme, subcloned into the *Sma*I site of the pBluescript II SK (+) vector, and named pBgB2.87. The gB2 gene in the pBgB2.87 plasmid was digested with *Sma*I, then the resulting two fragments (1.46 kb and 1.41 kb) were ligated into the *Sma*I site of the pBluescript II SK (+) vector. The sequence of the gB2 gene in the resulting recombinant plasmids was determined by the dideoxynucleotide chain termination procedure [41] using 8 µg/µl of double-stranded DNA templates and 100 pmol/µl of a limiting primer. The DNA fragments were labeled with ^{35}S-ATP (Amersham-Pharmacia, Buckinghamshire, U.K.) and sequenced with a 7-deaza-dGTP sequencing kit including Sequenase version 2.0 T7 DNA polymerase (United State Biochemical, Cleaveland, OH, U.S.A.) using a pBluescript T3 primer, T7 DNA primer, and M13 reverse primer. The sequence of the gB2 gene insertion site in the pBAC-gB2 plasmid was determined using a p10-1 primer.

Radioimmunoprecipitation Assay

Radioimmunoprecipitation assay was carried out using the procedures described elsewhere [8]. *S. frugiperda* cells (1×10^6 cells per dish) were infected with a recombinant virus at a multiplicity of infection of 6 pfu/cell. After 1 h of adsorption at 27°C, the monolayers were washed with PBS (pH 6.2) [29] and the cells were incubated with 2 ml of a TC-100 medium for 48 h at 27°C. After incubation, the

monolayers were washed twice in a prewarmed leucine-deficient TC-100 basal medium (Sigma) and incubated in 0.5 ml of a leucine-deficient basal medium for 1 h at 27°C. The medium was then removed and replaced with 0.5 ml of a leucine-deficient basal medium containing 20 μCi [^{3}H]-leucine (Amersham-Pharmacia Biotech). The cells were incubated for 1 h at 27°C with gentle rocking. After labeling, the cells were lysed in an immunoprecipitation buffer [8] and incubated with an HSV-2 gB-specific monoclonal antibody for the antigen-antibody complex

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TAGCGGGCGGCGGGAGCGAGGCGCCCCCGTAGGCCCGCCATGCGCGGGGGGGCTTGATT   21
                                        M  R  G  G  G  L  I    7

TGCGCGCTGGTCGTGGGGGCGCTGGTGGCCGCGGTGGCGTCGGCGGCCCCGGCGGCCCCG   81
 C  A  L  V  V  G  A  L  V  A  A  V  A  S  A  A  P  A  A  P    27
signal sequence (1-23aa)
GCGGCCCCCCGCGCCTCGGGCGGCGTGGCCGCGACCGTCGCGGCGAACGGGGGTCCCGCC  141
 A  A  P  R  A  S  G  G  V  A  A  T  V  A  A  N  G  G  P  A    47

TCCCGGCCGCCCCCCGTCCCGAGCCCCGCGACCACCAAGGCCCGGAAGCGGAAAACCAAA  201
 S  R  P  P  P  V  P  S  P  A  T  T  K  A  R  K  R  K  T  K    67

AAGCCGCCCGAGCGGCCCGAGGCGACCCCGCCCCCCGACGCCAACGCGACCGTCGCCGCC  261
 K  P  P  E  R  P  E  A  T  P  P  P  D  A  N  A  T  V  A  A    87
                                             *
GGCCACGCCACGCTGCGCGCGCACCTGCGGGAAATCAAGGTCGAGAACGCCGATGCCCAG  321
 G  H  A  T  L  R  A  H  L  R  E  I  K  V  E  N  A  D  A  Q   107

TTTTACGTGTGCCCGCCCCCGACGGGCGCCACGGTGGTGCAGTTTGAGCAGCCGCGCCGC  381
 F  Y  V  C  P  P  P  T  G  A  T  V  V  Q  F  E  Q  P  R  R   127

TGCCCGACGCGCCCGGAGGGGCAGAACTACACGGAGGGCATCGCGGTGGTCTTCAAGGAG  441
 C  P  T  R  P  E  G  Q  N  Y  T  E  G  I  A  V  V  F  K  E   147
                            *
AACATCGCCCCGTACAAATTCAAGGCCACCATGTACTACAAAGACGTGACCGTGTCGCAG  501
 N  I  A  P  Y  K  F  K  A  T  M  Y  Y  K  D  V  T  V  S  Q   167

GTGTGGTTCGGCCACCGCTACTCCCAGTTTATGGGGATATTCGAGGACCGCGCCCCCGTT  561
 V  W  F  G  H  R  Y  S  Q  F  M  G  I  F  E  D  R  A  P  V   187

CCCTTCGAGGAGGTGATCGACAAGATTAACGCCAAGGGGGTCTGCCGCTCCACGGCCAAG  621
 P  F  E  E  V  I  D  K  I  N  A  K  G  V  C  R  S  T  A  K   207

TACGTGCGGAACAACATGGAGACCACCGCGTTTCACCGGGACGACCACGAGACCGACATG  681
 Y  V  R  N  N  M  E  T  T  A  F  H  R  D  D  H  E  T  D  M   227

GAGCTCAAGCCGGCGAAGGTCGCCACGCGCACGAGCCGGGGGTGGCACACCACCGACCTC  741
 E  L  K  P  A  K  V  A  T  R  T  S  R  G  W  H  T  T  D  L   247

AAGTACAACCCCTCGCGGGTGGAGGCGTTCCATCGGTACGGCACGACGGTCAACTGCATC  801
 K  Y  N  P  S  R  V  E  A  F  H  R  Y  G  T  T  V  N  C  I   267
          *
GTCGAGGAGGTGGACGCGCGGTCGGTGTACCCGTACGATGAGTTTGTGCTGGCGACGGGC  861
 V  E  E  V  D  A  R  S  V  Y  P  Y  D  E  F  V  L  A  T  G   287

GACTTTGTGTACATGTCCCCGTTTTACGGCTACCGGGAGGGGTCGCACACCGAGCACACC  921
 D  F  V  Y  M  S  P  F  Y  G  Y  R  E  G  S  H  T  E  H  T   307

AGCTACGCCGCCGACCGCTTCAAGCAGGTCGACGGCTTCTACGCGCGCGACCTCACCACG  981
 S  Y  A  A  D  R  F  K  Q  V  D  G  F  Y  A  R  D  L  T  T   327

AAGGCCCGGGCCACGTCGCCGACGACCCGCAACTTGCTGACGACCCCCAAGTTTACCGTG 1041
 K  A  R  A  T  S  P  T  T  R  N  L  L  T  T  P  K  F  T  V   347

GCCTGGGACTGGGTGCCGAAGCGACCGGCGGTCTGCACCATGACCAAGTGGCAGGAGGTG 1101
 A  W  D  W  V  P  K  R  P  A  V  C  T  M  T  K  W  Q  E  V   367

GACGAGATGCTCCGCGCCGAGTACGGCGGCTCCTTCCGCTTCTCCTCCGACGCCATCTCG 1161
 D  E  M  L  R  A  E  Y  G  G  S  F  R  F  S  S  D  A  I  S   387

ACCACCTTCACCACCAACCTGACCCAGTACTCGCTCTCGCGCGTCGACCTGGGCGACTGC 1221
 T  T  F  T  T  N  L  T  Q  Y  S  L  S  R  V  D  L  G  D  C   407
                   *
ATCGGCCGGGATGCCCGCGAGGCCATCGACCGCATGTTTGCGCGCAAGTACAACGCCACG 1281
 I  G  R  D  A  R  E  A  I  D  R  M  F  A  R  K  Y  N  A  T   427
                                                        *
CACATCAAGGTGGGCCAGCCGCAGTACTACCTGGCCACGGGGGGCTTCCTCATCGCGTAC 1341
 H  I  K  V  G  Q  P  Q  Y  Y  L  A  T  G  G  F  L  I  A  Y   447

CAGCCCCTCCTCAGCAACACGCTCGCCGAGCTGTACGTGCGGGAGTACATGCGGGAGCAG 1401
 Q  P  L  L  S  N  T  L  A  E  L  Y  V  R  E  Y  M  R  E  Q   467

GACCGCAAGCCCCGGAATGCCACGCCCGCGCCACTGCGGGAGGCGCCCAGCGCCAACGCG 1461
 D  R  K  P  R  N  A  T  P  A  P  L  R  E  A  P  S  A  N  A   487
                     *                                   *
TCCGTGGAGCGCATCAAGACCACCTCCTCGATCGAGTTCGCCCGGCTGCAGTTTACGTAT 1521
 S  V  E  R  I  K  T  T  S  S  I  E  F  A  R  L  Q  F  T  Y   507

AACCACATACAGCGCCACGTGAACGACATGCTGGGGCGCATCGCCGTCGCGTGGTGCGAG 1581
 N  H  I  Q  R  H  V  N  D  M  L  G  R  I  A  V  A  W  C  E   527

CTGCAGAACCACGAGCTGACTCTCTGGAACGAGGCCCGCAAGCTCAACCCCAACGCCATC 1641
 L  Q  N  H  E  L  T  L  W  N  E  A  R  K  L  N  P  N  A  I   547

GCCTCCGCCACCGTCGGCCGGCGGGTGAGCGCGCGCATGCTCGGAGACGTCATGGCCGTC 1701
 A  S  A  T  V  G  R  R  V  S  A  R  M  L  G  D  V  M  A  V   567

TCCACGTGCGTGCCCGTCGCCCCGGACAACGTGATCGTGCAGAACTCGATGCGCGTCAGC 1761
 S  T  C  V  P  V  A  P  D  N  V  I  V  Q  N  S  M  R  V  S   587

TCGCGGCCGGGGACGTGCTACAGCCGCCCCCTGGTCAGCTTTCGGTACGAAGACCAGGGC 1821
 S  R  P  G  T  C  Y  S  R  P  L  V  S  F  R  Y  E  D  Q  G   607

CCGCTGATCGAGGGGCAGCTGGGCGAGAACAACGAGCTGCGCCTCACCCGCGACGCGCTC 1881
 P  L  I  E  G  Q  L  G  E  N  N  E  L  R  L  T  R  D  A  L   627

GAGCCGTGCACCGTGGGCCACCGGCGCTACTTCATCTTCGGCGGGGGCTACGTGTACTTC 1941
 E  P  C  T  V  G  H  R  R  Y  F  I  F  G  G  G  Y  V  Y  F   647

GAGGAGTACGCGTACTCTCACCAGCTGAGTCGCGCCGACGTCACCACCGTCAGCACCTTC 2001
 E  E  Y  A  Y  S  H  Q  L  S  R  A  D  V  T  T  V  S  T  F   667

ATCGACCTGAACATCACCATGCTGGAGGACCACGAGATTGTGCCCCTGGAGGTCTACACG 2061
 I  D  L  N  I  T  M  L  E  D  H  E  I  V  P  L  E  V  Y  T   687
             *
CGCCACGAGATCAAGGACAGCGGCCTGCTGGACTACACGGAGGTCCAGCGCCGCAACCAG 2121
 R  H  E  I  K  D  S  G  L  L  D  Y  T  E  V  Q  R  R  N  Q   707

CTGCACGACCTGCGCTTTGCCGACATCGACACGGTCATCCGCGCCGACGCCAACGCCGCC 2181
 L  H  D  L  R  F  A  D  I  D  T  V  I  R  A  D  A  N  A  A   727

ATGTTCGCGGGGCTGTGCGCGTTCTTCGAGGGGATGGGGGACTTGGGGCGCGCGGTCGGC 2241
 M  F  A  G  L  C  A  F  F  E  G  M  G  D  L  G  R  A  V  G   747

AAGGTAGTCATGGGAGTAGTGGGGGGCGTGGTGTCGGCCGTGTCGGGCGTGTCCTCCTTT 2301
 K  V  V  M  G  V  V  G  G  V  V  S  A  V  S  G  V  S  S  F   767
Transmembrane anchor (745-798aa)
ATGTCCAACCCCTTCGGGGCGCTTGCCGTGGGGCTGCTGGTCCTGGCCGGCCTGGTCGCG 2361
 M  S  N  P  F  G  A  L  A  V  G  L  L  V  L  A  G  L  V  A   787

GCCTTCTTCGCCTTCCGCTACGTCCTGCAACTGCAACGCAATCCCATGAAGGCCCTGTAT 2421
 A  F  F  A  F  R  Y  V  L  Q  L  Q  R  N  P  M  K  A  L  Y   807

CCGCTCACCACCAAGGAACTCAAGACTTCCGACCCCGGGGGCGTGGGCGGGGAGGGGGAG 2481
 P  L  T  T  K  E  L  K  T  S  D  P  G  G  V  G  G  E  G  E   827

GAAGGCGCGGAGGGGGGCGGGTTTGACGAGGCCAAGTTGGCCGAGGCCCGAGAAATGATC 2541
 E  G  A  E  G  G  G  F  D  E  A  K  L  A  E  A  R  E  M  I   847

CGATATATGGCTTTGGTGTCGGCCATGGAGCGCACGGAACACAAGGCCAGAAAGAAGGGC 2601
 R  Y  M  A  L  V  S  A  M  E  R  T  E  H  K  A  R  K  K  G   867

ACGAGCGCCCTGCTCAGCTCCAAGGTCACCAACATGGTTCTGCGCAAGCGCAACAAAGCC 2661
 T  S  A  L  L  S  S  K  V  T  N  M  V  L  R  K  R  N  K  A   887

AGGTACTCTCCGCTCCACAACGAGGACGAGGCCGGAGACGAAGACGAGCTCTAAGGGAGG 2715
 R  Y  S  P  L  H  N  E  D  E  A  G  D  E  D  E  L            904

GGAGGGGAGCTGGGCTTGTGTATAAATAAAAAGACACCGATGTTCAAAAATACACATGAC

TTCTGGTATTGTTTTGCCTTGGTTTTTATTTGGGGGGGGGGCGTGTGGACT
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Fig. 2. Nucleotide sequence and deduced amino acid sequence of the ORF of the HSV-2 gB2 gene (GenBank accession No. AF295528).

The sequence of the coding strand is given. The gB2 gene in the pBgB3.47 plasmid (Fig. 1A) was fragmented with restriction enzymes, subcloned, and then sequenced using the procedure of dideoxy chain termination. The translational start codon ATG and termination codon TAA are underlined. Hydropathy profile was determined using the values of Kyte and Doolite (1982). *N*-Glycosylation sites, NXT or NXS (Kornfeld and Kornfeld [20]), were underlined and marked with *, where X can be any amino acid except possibly aspartic acid. Potential hydrophobic signal and transmembrane sequences are underlined.

formation. Thereafter, the immunoprecipitation products were separated on a 10% SDS-polyacrylamide gel. The gel was dried, exposed to film, and then developed for 3 min at 20°C in a developer.

Immunization and Neutralization Assay

The cell lysate preparations and immunization of mice were carried out using the procedures described by Cha *et al.* [8]. Briefly, *S. frugiperda* cells (1×10^6 cells in 2 ml per flask) were seeded in eight flasks (60 mm), allowed to attach in a monolayer overnight at 27°C, infected with the recombinant baculoviruses expressing gB2 at a multiplicity of infection of 1.0 pfu per cell, and incubated with 4 ml of a TC-100 medium for 96 h at 27°C. The infected cells and media were freeze-thawed, lyophilized, and resuspended in 2 ml of a PBS buffer (pH 6.2) at a final concentration of 8×10^6 cells. As a negative control, *S. frugiperda* cells were infected with the wild-type *lac*Z-HcNPV, and then as a positive control, Vero cells were infected with HSV-1 strain KOS and cultured at 37°C, which were treated in the same manner as the recombinant virus-infected cells. Ten mice (Balb/c strain, 6–8 weeks old) (Daehan Laboratory Animal Research Center, Chungnam, Korea) were vaccinated three times at 3-week intervals, both subcutaneously and intraperitoneally, with freeze-thawed cell lysates of *S. frugiperda* cells infected with the recombinant baculovirus expressing gB2. The sera were collected 3 weeks after the final vaccination and pooled for each group. A virus neutralization assay was performed using a modification of the procedure described by Martin and Rouse [33] and Cha *et al.* [8]. Briefly, 100 µl of heat-inactivated serum was added in serial dilution to a 96-well, flat-bottom microtiter plate. One-hundred ml of 200× $TCID_{50}$ of the live HSV-2 strain G was also added to the plate, which was then sealed and incubated for 18 h at 4°C. Next, 100 µl of 5×10^3 viable Vero cells was added to the plate, which was then incubated at 37°C for 5 more days. The serum dilution factor to neutralize 50% of the virus was determined as the titer. The $TCID_{50}$ was 0.69 pfu, as determined by the procedure described by Reed and Munch [39].

RESULTS AND DISCUSSION

Cloning and Nucleotide Sequence Analysis of gB2 Gene

The HSV-2 gB2 gene (2.87 kb) was cloned into the baculovirus transfer vector and recombinated into HcNPV (Fig. 1A). An analysis of the pBAC-gB2 plasmid using three restriction enzymes (*Sma*I, *Sph*I, and *Xho*I) showed that the 2.87 kb fragment was inserted in the correct direction. The insertion of the gB2 fragment was also confirmed by the sequencing of the cloning site (Fig. 1B), which identified a ligated *Sma*I/*Mae*I (CCCTAG) site downstream of the p10 gene promoter. The nucleotide sequence in Fig. 1B demonstrates that the gB2 gene sequence was subcloned correctly.

The nucleotide sequence of the gB2 gene and deduced amino acid sequence are shown in Fig. 2. The entire gB2 sequence was determined for both DNA strands. The open reading frame (ORF) of the gB2 gene was 2,715 bp and coded 904 amino acid residues (Fig. 2). The translation start codon was ATG and the termination codon was TAA. These results were consistent with previous reports on HSV-2 strain 333 [6] and HG52 [45]. The ORF of the gB2 gene of HSV-2 strain G in this report was compared with the previously reported sequences of HSV strain 333 and strain HG52 (Table 1). The gB2 gene sequence of strain G was highly conserved between strain 333 and/or strain HG52. When compared with the strain 333 gB gene sequence, five nucleotides were different and only two amino acid changes were found in the coding region. When compared with strain HG52, fifteen nucleotides were different and eleven amino acid changes were found in the coding region. When strain 333 was compared with strain HG52, fourteen nucleotides were different and eight amino acid residues were changed in the HG52 strain according to the nucleotide changes, as shown in Table 1. These findings indicate that the genome of strain G is more homologous

Table 1. Comparisons of gB nucleotide and aa sequences from different HSV-2 strains.

NT sites	NT/aa differences in the strains		
	G	333	HG52
211	**G**AG/Glu	**A**AG/Lys	**A**AG/Lys
273	AC**G**/Thr	–	AC**C**/Thr
274	**C**TG/Leu	–	**G**TG/Val
850	**C**TG/Leu	**T**TG/Leu	–
923	A**G**C/Ser	–	A**C**G/Thr
924	AG**C**/Ser	–	AC**G**/Thr
1015	**T**TG/Leu	–	**C**TG/Leu
1186	**C**AG/Gln	–	**G**AG/Glu
1224	AT**C**/Ile	AT**T**/Ile	–
1313	C**T**G/Leu	–	C**A**G/Gln
1702	**T**CC/Ser	–	**G**CC/Ala
1827	GA**G**/Glu	–	GA**C**/Asp
1828	**C**TG/Leu	–	**G**TG/Val
1905	CG**G**/Arg	–	CG**C**/Arg
1906	**C**GC/Arg	–	**G**GC/Gly
2038	**A**TT/Ile	**T**TT/Phe	**T**TT/Phe
2283	GT**G**/Val	GT**C**/Val	GT**C**/Val

Nucleotide (NT) sequence was derived for the gB gene from each strain. Comparisons were made with the stain G nucleotide sequence and encoded amino acid (aa) sequence. The NT differences are bolded and underlined. The base sequence was numbered from the initiating codon. Strain 333 is from Stuve *et al.* [45] and strain HG52 from Bzik *et al.* [6].

to strain 333 than strain HG52, and the differences in the amino acid residues in the gB2 polypeptides of the HSV-2 strains may be the cause of the specific characteristic differences between the strains.

The hydropathic profile of the gB2 polypeptide sequence predicted according to the Kyte and Doolites method [21] was determined and shown in Fig. 2. The profile revealed the presence of two main hydrophobic regions. The first domain spanned from 1 to 22 amino acid residues including a signal peptidase cleavage site, which was predicted using the rules of Permian and Halvorson [38] (Fig. 2). The second hydrophobic domain was a region of 21 amino acid residues from position 745 to 798 (Fig. 2). The ORF was rich in alanine residue (11.2%). There were eight *N*-glycosylation sites along the gB2 sequence (Fig. 2). The putative *N*-glycosylation sites were predicted at positions 82, 136, 250, 393, 425, 473, 486, and 671-Asp according to Kornfeld and Kornfeld's method [20], underlined, and marked with *. The glycosylated amino acids were evenly distributed at both the N and the C terminals and in the center of the protein. The presence of a putative signal sequence and a transmembrane sequence with the *N*-glycosylation sites identified on the protein sequence strongly suggests that the ORF codes for a glycoprotein.

Construction of Baculo-gB2 Recombinant Virus Expressing gB2

A recombinant baculovirus containing the gB2 gene was constructed using the procedures outlined by Lee *et al.* [23, 27, 29]. The pBAC-gB2 transfer vector DNA containing the gB2 gene and linearized *lacZ*-HcNPV DNA with the *Bsu*361 enzyme were cotransfected into *S. frugiperda* cells according to the protocol described in Materials and Methods. The recombinant viruses were selected using a plaque assay containing X-Gluc. No polyhedral inclusion bodies were formed in the infected cells. Nine positive plaque clones of the recombinant viruses were isolated and characterized. The individual clones were named Baculo-gB2-1 to 9. The size of the plaques ranged from 2.0 mm to 4 mm in diameter (data not shown). The Baculo-gB2-1 clone, which exhibited the largest plaque size (4 mm), was used for further studies. The clones were purified twice. The presence of the HSV-2 gB2 gene DNA in the recombinant baculovirus was verified by a PCR. The correct insertion of the nine recombinants was confirmed by production of approximately 192 base-PCR products (shown in Materials and Methods). The PCR results indicate that the gB2 genes were correctly inserted in the recombinant virus genomes. The recombinant virus was multiplied in *S. frugiperda* cells and produced a high level (10^8 pfu per ml) of the recombinant viruses. Stuve *et al.* [45] cloned the *Nru*I-*Bam*HI fragment containing the gB2 gene of the HSV-2 strain 333 into a mammalian cell expression vector based on a pBR322 plasmid containing a SV40 early promoter and then expressed it in Chinese hamster cells by transfection of the recombinant plasmid. Current study used different vector systems, recombination systems, and restriction fragments with the gB2 gene.

Characteristics of gB2 Produced in Insect Cells

The expression of HSV-2 gB2 in *S. frugiperda* cells infected with the Baculo-gB2-1 recombinant virus was detected using SDS-PAGE analysis and confirmed by radioimmunoprecipitation analysis. gB2 proteins were produced in the infected cells (Fig. 3, lane A2) and then secreted into the cultured media for 5 days (Fig. 3, lane A3). The density of the gB2 band in lane A3 on the SDS-PAGE appeared to be about four times higher than that of lane A2. Other proteins in the cell lysates and media did not appear clearly on the gel due to low concentration. When a high level of specific gene products were produced, the productions of other proteins became low. The gB2 proteins on the SDS-PAGE were confirmed using radioimmunoprecipitation (Fig. 3, lanes B2' and B3'). The immunoprecipitated bands of lane 3' was about two times denser than that of the lane 2'. These densities of the immunoprecipitates were in accordance with that of the protein bands on the SDS-PAGE, because the harvest times were different in the two analyses. The sample for SDS-PAGE was collected at 5 days p.i., however, that of the immunoprecipitates were harvested at 2 days

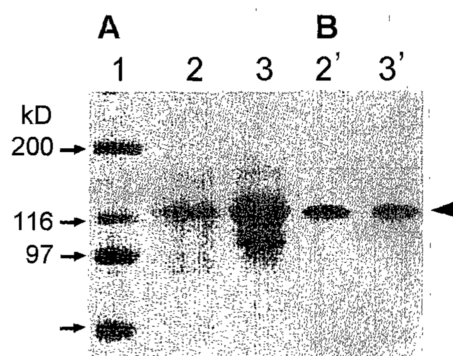

Fig. 3. Immunoprecipitation of gB2 proteins from infected cell lysates and cultured media.

(A) Cells were infected with the Baculo-gB2-1 viruses and centrifuged to separate the infected cells and cultured media for 5 days. The cell pellets were lysed by freeze-thawing in a lysis buffer. The lysates and cultured media were dialyzed to a final concentration of 1.0 ml, then 5 µl of the lysates and media was loaded in a 10% SDS-PAGE. (B) At 48 h p.i. the cells were labeled with L-^{3}H-leucine for 1 h and lysated. The soluble fractions of the lysates were mixed with HSV-2 gB2 specific Mab and the antigen-antibody complexes precipitated with protein A-Sepharose. The immunoprecipitates were analyzed by a 10% SDS-PAGE alongside samples of the cell extracts and the culture media. The dried gel was exposed to film for detection of radioactivity. Lanes A1, standard molecular weights; A2, cell lysates infected with Baculo-gB2-1; and A3, cultured media infected with the virus. Lanes B2 and B3, immunoprecipitations of lanes A2 and A3 using gB2 specific monoclonal antibody. The numbers on the left correspond to the positions of the molecular weight markers (myosin 200 kDa, β-galactosidase 116.2 kDa, phospholylase B 97.4 kDa). The positions of gB2 protein bands are indicated with an arrow.

p.i. These results indicate that a high-level of the gB2 protein with approximately 120 kDa was produced in the infected cells and secreted into the cultured media. This means that the recombinant virus Baculo-gB2-1 successfully expressed a mature gB2 in the insect cells. In HSV-1 infected cells, the gB1 precursor is synthesized as a protein of approximately 110 kDa, which is then processed into a mature gB1 with a molecular weight of up to 120 kDa [10, 11, 12, 36]. Stuve *et al.* [45] expressed the *Nru*I-*Bam*HI fragment containing the gB2 gene of the HSV-2 strain 333 in a mammalian cell (Chinese hamster cells) based on the transfection of the recombinant plasmid. They found that low-levels of the gB2 gene were expressed in cells and secreted into the culture media. The above two groups of investigators used different expression systems for the production of the gB2 gene. Kang *et al.* [18] also obtained a high-level of the HSV-1 gH protein in insect cells using the gX signal sequence-HcNPV system. This result suggests that the HcNPV expression vector system may be useful for the high-level production of glycoproteins. In these results, 110 kDa precursors were modified in insect cells into a 120 kDa matured glycoprotein, which was identified with a gB-specific monoclonal antibody.

Induction of HSV-2 Neutralizing Antibody

The immunogenicity of the recombinant gB2 was studied by immunizing mice with lysates from whole insect cells infected with the Baculo-gB2-1 recombinant, *lac*Z-HcNPV-negative control group, and viral KOS-positive control group as described in Materials and Methods. Three weeks after the final vaccination, the mice were bled and their sera were tested for HSV-2-neutralizing activity: The pooled sera were heat-inactivated and then incubated with a guinea pig complement. The sera from the immunized mice exhibited a high HSV-2-neutralizing activity *in vitro* (Table 2). The level of neutralizing antibodies induced by mice vaccinated with live HSV-1 KOS strain was higher than that induced by the recombinant Baculo-gB2. This was not unexpected, since immunization with the virus allows response to other components of whole virus in addition to gB. No neutralizing antibody was detected in mock (*lacZ*-HcNPV) vaccinated animals (Table 2). Stanberry *et al.* [44] obtained a similar result, where antibodies, raised in mice to recombinants, recognized viral gB2 and neutralized the infectivity of HSV-2 *in vitro*. Also Cha *et al.* [8] obtained a similar neutralizing activity in mice with recombinant HSV-1 gB1 protein using the HcNPV. These results indicate that the glycoproteins produced by the recombinant baculoviruses exhibited an active immune response in mice.

Table 2. Neutralizing antibody titers in mice vaccinated with cell lysates infected with Baculo-gB2

Immunogens	No. of mice tested	50% Neutralization titer of antisera
Baculo-gB2	10	<1: 64
HSV-1 KOS	10	<1: 1024
Mock (*lac*Z-HcNPV)	10	<1: 8

The Baculo-gB2 is the recombinant virus vaccine. The HSV-1 KOS is the live viral vaccine. Mock is the vector virus.

A high level of secreted gB2 protein was produced in insect cells by the infection of the Baculo-gB2 recombinant virus engineered into the HcNPV vector. The DNAs described herein encoded gB2. This protein was recognized by a gB-specific monoclonal antibody, which was confirmed by immunoprecipitation. Since a high level of neutralizing antibody production may protect mice from HSV infections, this particular recombinant protein may be useful in the development of a subunit vaccine.

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REFERENCES

1. Bernstein, D. I., L. M. Frenkel, Y. J. Bryson, and M. G. Myers. 1990. Antibody response to *Herpes simplex* virus glycoprotein gB and gD. *J. Med. Virol.* **30:** 45–49
2. Birnboim, H. C. and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucl. Acids Res.* **7:** 1513–1523.
3. Blacklaws, B. A., A. A. Nash, and G. Darby. 1987. Specificity of the immune response of mice to *Herpes simplex* virus glycoprotein D and B constitutively expressed on L cell lines. *J. Gen. Virol.* **68:** 1103–1114.
4. Blacklaws, S. A. and A. A. Nas. 1990. Immunological memory to *Herpes simplex* virus type glycoprotein B and D in mice. *J. Gen. Virol.* **71:** 863–871.
5. Bollag, D. A. and S. J. Edelstein. 1991. *Protein Methods.* Wiley-Liss, New York, pp. 96–130.
6. Bzik, D. J., C. Debroy, B. A. Fox, N. E. Pederson, and S. Person. 1986. The nucleotide sequence of the gB glycoprotein gene of HSV-2 and comparison with the corresponding gene of HSV-1. *Virology* **155:** 322–333.
7. Cai, W., B. Gu, and S. Person. 1988. Role of glycoprotein B of *Herpes simplex* virus type 1 in viral entry and cell fusion. *J. Virol.* **61:** 2596–2604.
8. Cha, S. C., H. Kang, S. E. Lee, G. J. Park, and H. H. Lee. 2000. Expression of the HSV-1(F) glycoprotein B gene in insect cells infected by HcNPV recombinant. *J. Microbiol. Biotechnol.* **10:** 355–362.
9. Chung, H. K., S. H. Lee, S. Y. Kim, and H. H. Lee. 1994. Nucleotide sequence analysis of the RNA-dependent RNA polymerase gene of infectious pancreatic necrosis virus DRT strain. *J. Microbiol. Biotech.* **4:** 264–269.

10. Claesson-Welsh, L. and P. G. Spear. 1987. Amino-terminal sequence, synthesis, and membrane insertion of glycoprotein B of *Herpes simplex* virus type 1. *J. Virol.* **61:** 1–7.
11. Compton, T. and R. J. Courtney. 1984. Evidence for posttranslational glycosylation of a nonglycosylated precursor protein of *Herpes simplex* virus type 1. *J. Virol.* **52:** 630–637.
12. Compton, T. and R. J. Courtney. 1984. Virus specific glycoproteins associated with the nuclear fraction of *Herpes simplex* virus type 1 infected cells. *J. Virol.* **49:** 594–597.
13. Deluca, N., D. J. Bzik, V. C. Bond, S. Person, and W. Snipes. 1982. Nucleotide sequence of *Herpes simplex* virus type 1 (HSV-1) affecting virus entry, cell fusion, and production of glycoprotein gB (VP7). *Virology* **122:** 411–423.
14. Fleck, M., J. Podlech, and W. D. Falke. 1994. A vaccinia virus *Herpes simplex* virus (HSV) glycoprotein B1 recombinant or an HSV vaccine overcome the HSV type 2 induced humoral immuno-suppression and protect against vaginal challenge in BALB/c mice. *Med. Microbiol. Immunol.* **183:** 87–94.
15. Ghiasi, H., R. Kaiwar, A. B. Nesburn, S. Slanina, and S. L. Wechsler. 1994. Expression of seven *Herpes simplex* virus type 1 glycoproteins (gB, gC, gD, gE, gG, gH and gI); comparative protection against lethal challenge in mice. *J. Virol.* **68:** 2118–2126.
16. Highlander, S. L., S. L. Sutherland, P. J. Gage, D. C. Johnson, M. Levine, and L. C. Gloriosol. 1987. Neutralizing monoclonal antibodies specific for *Herpes simplex* virus glycoprotein D inhibit virus penetration. *J. Virol.* **61:** 3356–3364.
17. Highlander, S. L., W. Cai, S. Person, M. Levine, and J. C. Glorioso. 1988. Monoclonal antibodies define a domain on *Herpes simplex* virus glycoprotein B involved in virus penetration. *J. Virol.* **62:** 1881–1888.
18. Kang, H., S. C. Cha, Y. J. Han, I. H. Park, M. J. Lee, S. M. Byun, and H. H. Lee. 2000. High level production of glycoprotein H of HSV-1(F) using HcNPV vector system. *J. Biochem. Mol. Biol.* **33:** 483–492.
19. Kang, H., J. K. Park, H. S. Uh, S. Y. Kim, and H. H. Lee. 1999. Detection of glycoproteins (B and D) and thymidine kinase genes of *Herpes simplex* virus type 2 strain G. *J. Kor. Soc. Virol.* **29:** 99–105.
20. Kornfeld, R. and S. Kornfeld. 1985. Assembly of asparagines-linked oligosaccharides. *Annu. Rev. Biochem.* **54:** 631–664.
21. Kyte, J. and R. F. Doolittle. 1982. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157:** 105–132.
22. Lee, H. H. 1987. Replication and cloning of *Hyphantria cunea* nuclear polyhedrosis virus in *Spodoptera frugiperda* cell line. *Hanguk J. Genet. Eng.* **2:** 3–6.
23. Lee, H. H., B. J. Kang, and K. J. Park. 1998. Construction of a Baculovirus expression system using *H. cunea* nuclear polyhedrosis virus for eukaryotic cell. *J. Microbiol. Biotechnol.* **8:** 676–684.
24. Lee, D. and D.-H. Kim. 1999. Heterologous expression of lignin peroxidase H2 in *Escherichia coli*: *In vitro* refolding and activation. *J. Biochem. Mol. Biol.* **32:** 486–491.
25. Lee, H. H. and K. W. Lee. 1988. Isolation, complementation and partial characterization of temperature-sensitive mutants of the Baculovirus *Hyphantria cunea* nuclear polyhedrosis virus. *J. Gen. Virol.* **69:** 1299–1306.
26. Lee, H. H., E. S. Moon, S. T. Lee, S. H. Hwang, S. C. Cha, and K. H. Yoo. 1998. Construction of a Baculovirus *Hyphantria cunea* NPV insecticide containing the insecticidal protein gene of *Bacillus thuringiensis* subsp. *kurstaki* HD1. *J. Microbiol. Biotechnol.* **8:** 685–691.
27. Lee, H. H., H. Kang, J. W. Jung, B. J. Kang, and J. Y. Song. 1999. Construction of a transfer vector containing gX signal sequence of Pseudorabies virus and a recombinant baculovirus. *J. Microbiol. Biotechnol.* **9:** 541–547.
28. Lee, H. H., K. K. Lee, and K. J. Lee. 1990. A stable preservation of extracellular nonoccluded virions from *Autographa californica* nuclear polyhedrosis virus infection. *Kor. J. Appl. Microbiol. Biotechnol.* **18:** 660–661.
29. Lee, H. H. and L. K. Miller. 1978. Isolation of genotypic variants of *Autographa californica* nuclear polyhedrosis virus. *J. Virol.* **27:** 754–767.
30. Lee, H. H., B. H. Min, H. K. Chung, K. K. Lee, J. Park, S. C. Cha, and N. S. Seo. 1992. Genomic structure and nucleotide sequence of the polyhedrin gene of *Hyphantria cunea* nuclear polyhedrosis virus. *Mol. Cells* **2:** 303–308.
31. Mandel, M. and A. Higa. 1970. Calcium dependent bacteriophage DNA phage infection. *J. Mol. Biol.* **53:** 154–162.
32. Mertz, G. J., R. Ashley, R. L. Burke, J. Benedetti, C. Critchlow, C. C. Jones, and L. Corey. 1990. Double-blind, Placebo-controlled trial of a *Herpes simplex* virus type 2 glycoprotein vaccine in persons at high risk for genital herpes infection. *J. Inf. Disease* **161:** 653–660.
33. Martin, S. and B. T. Rouse. 1987. The mechanism of antiviral immunity induced by a vaccinia virus recombinant expressing *Herpes simplex* virus type 1 glycoprotein D: Clearance of local infection. *J. Immunol.* **138:** 3431–3440.
34. Navarro, D., P. Paz, and L. Pereira. 1992. Domains of *Herpes simplex* virus 1 glycoprotein B that function in virus penetration, cell-to-cell spread and cell fusion. *Virol.* **186:** 99–112.
35. O'Reilly, D., L. Miller, and V. Luickow. 1994. *Baculovirus Expression Vectors: A Laboratory Manual.* Oxford University Press, London, U.K.
36. Pachl, C., R. L. Burke, L. L. Stuve, L. Sanchez-Pescador, G. V. Nest, F. M. Masiarz, and D. Dina. 1987. Expression of cell-associated and secreted forms of *Herpes simplex* virus type 1 glycoprotein gB in mammalian cells. *J. Virol.* **61:** 315–325.
37. Park, S. A., S. C. Cha, J. H. Chang, and H. H. Lee. 1996. Nucleotide sequence analyses of p10 gene and its promoter of *Hyphantria cunea* nuclear polyhedrosis virus. *J. Kor. Soc. Virol.* **26:** 131–137.
38. Perlman, D. and H. O. Halvorson. 1983. A putative signal peptidase recognition site and sequence in eukaryotic and prokaryotic signal peptidase. *J. Mol. Biol.* **167:** 391–409.
39. Reed, L. J. and H. Munch. 1938. A simple method of estimating fifty per cent endpoints. *Amer. J. Hygiene* **27:** 493–497.

40. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning; A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
41. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74:** 5463–5467.
42. Smith, G. E., M. J. Fraser, and M. D. Summers. 1983. Molecular engineering of the *Autographa californica* nuclear polyhedrosis virus genome: Deletion mutations within the polyhedrin gene. *J. Virol.* **46:** 584–593.
43. Spear, P. G. 1985. Glycoproteins specified by *Herpes simplex* viruses. *In* B. Roizman (ed.), *The Herpesviruses*, Vol. 3. Plenum Publishing Co., New York, pp. 315–356.
44. Stanberry, L. R., D. I. Bernstein, R. L. Burke, C. Pachl, and M. G. Myers. 1987. Vaccination with recombinant *Herpes simplex* virus glycoproteins: protection against initial and recurrent genital herpes. *J. Infect. Dis.* **155:** 914–920.
45. Stuve, L. L., S. Brown-Shimer, C. Pachl, R. Najarian, D. Dina, and R. L. Burke. 1987. Structure and expression of the *Herpes simplex* virus type 2 glycoprotein gB gene. *J. Virol.* **61:** 326–335.