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Expression of the HSV-1 (F) Glycoprotein B Gene in Insect Cells Infected by HcNPV Recombinant

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Abstract The *Herpes simplex* virus type 1 (HSV-1) glycoprotein B (gB) gene in the pHLA-21 plasmid was inserted into a baculovirus (*Hyphantria cunea* nuclear polyhedrosis virus) expression vector (*lacZ*-HcNPV) to construct a recombinant virus gB-HcNPV expressing gB. *Spodoptera frugiperda* cells infected with this recombinant virus synthesized and processed gB of approximately 120 kDa, which cross-reacted with the monoclonal antibody to gB. The recombinant gB was identified on the membrane of the insect cells using an immunofluorescence assay. Antibodies to this recombinant raised in mice recognized the viral gB and neutralized the infectivity of the HSV-1 *in vitro*. These results show that the gB gene has the potential to be expressed in insect cells. They also demonstrate that it is possible to produce a mature protein by gene transfer in eukaryotic cells, and indicate the utility of the *lacZ*-HcNPV-insect cell system for producing and characterizing eukaryotic proteins. Furthermore, the neutralizing antibodies would appear to protect mice against HSV. Accordingly, this particular recombinant protein may be useful in the development of a subunit vaccine.

Key words: HSV-1, glycoprotein B, baculovirus, neutralizing antibody

The glycoproteins produced by *Herpes simplex* virus type 1 (HSV-1) are the major viral proteins embedded in the virion envelopes and cell membranes of infected cells [49, 50]. There are at least 11 known antigenically and functionally distinct species (gB- gE, gG- gM) of glycoproteins in an HSV virion [1, 41, 43, 50]. The HSV-1 glycoproteins appear to be the primary inducers and targets of humoral and cell-mediated immune responses to an HSV-1 infection [16, 38, 50].

The nucleotide sequences of the gB gene of the HSV-1 strain KOS [7, 14] and strain F [42] have already been determined. Based on the DNA sequence analysis, the gB gene encodes a polypeptide of 904 amino acids with an estimated molecular weight of 100 kDa [7, 14]. In HSV-1 infected cells, a gB precursor is synthesized as a high mannose protein of approximately 110 kDa and then processed into a mature gB with a molecular weight of up to 120 kDa [12, 13, 53]. gB plays an important role in the penetration, cell fusion, and interaction of the virus with the immune system of the host [8, 14, 21, 37, 50].

gB is an important target for both humoral and cell-mediated immune responses to an HSV-1 infection [3, 4, 9, 16, 35, 38]. The prophylactic immunization of naive animals with gB provides protection against primary infection and reduced latency [4, 9, 10, 18, 25, 38, 40]. Several expression systems have been used to express gB, and gB is a principal candidate for an HSV subunit vaccine [9, 25, 40]. However, a number of different expression vectors and cell lines expressing HSV-1 gB have been described [17, 22]. Baculovirus expression vectors have proved useful in achieving a high-level expression of a variety of foreign genes in insect cells [20, 39, 47, 51]. The current authors have constructed a baculovirus transfer and expressions vectors using the *Hyphantria cunea* nuclear polyhedrosis virus (HcNPV) [26, 28], which have proven to be useful in achieving a high-level expression of a variety of foreign genes in *Spodoptera frugiperda* cells [27, 29]. Therefore, the current study is focused on the expression and properties of the gB of HSV-1 strain F [15] as a subunit vaccine. Accordingly, using these systems, the gB coding sequence of HSV-1 strain F was recombinated into an expression vector and the resulting gB proteins were characterized. It was then demonstrated that the immunization of mice with this recombinant induced an immune response.

MATERIALS AND METHODS

Viruses, Cells, and Media

Herpes simplex virus type 1 (HSV-1) strain F (ATCC VR-733) was obtained from the Korean AIDS (Acquired Immune Deficiency Syndrome) Center, Seoul, Korea and

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HSV-1 KOS strain from Korean National Institute of Health, Seoul, Korea. Vero cell (ATCC-CCL 81) was obtained from the Korean Type Culture Collection (KTCC). The HSV-1 strains were grown at 37°C in the Vero cells using Eagles minimum essential medium (Gibco, Detroit, MI, U.S.A.) with 10% fetal bovine serum (Gibco), as previously described by Kang *et al.* [23]. The *lacZ-Hyphantria cunea* nuclear polyhedrosis virus (*lac*Z-HcNPV) containing a deletion in the polyhedrin gene [27] was propagated in the *Spodoptera frugiperda* cells (IPLB-SF-21) [52] obtained from L. K. Miller, University of Georgia, U.S.A., as previously described [27, 29]. The cells were routinely maintained in a TC-100 medium (Gibco) supplemented with 0.2% tryptose broth powder and 10% fetal bovine serum, and passaged every 5 days at 27°C.

Bacterial Strains and Plasmids

The pBacPAK9 plasmid (Clontech Laboratories, Palo Alto, CA, U.S.A.) and pBluescript SK(+) plasmid (Stratagene, La Jolla, CA, U.S.A.) were used for cloning or sequencing. The pBACgus4x-1 plasmid (Novagen, Madison, WI, U.S.A.) was used as the transfer vector. The *E. coli* XL1-Blue bearing the pHLA-21 clone containing the HSV-1 gB gene [23] was used as the HSV-1 gB gene source and maintained in an LB broth (Gibco, Gaithersburg, MD, U.S.A.).

Preparation of DNAs

E. coli containing the recombinant plasmids was cultured in LB broth (Difco Laboratories, Detroit, MI, U.S.A.) 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.* [27].

Oligonucleotides, Probe, and Antibody

The oligonucleotide primers (B-1, 5':5'-AGATCTCGTAG-TCCCGCCATGCGCCAG-3'; and B-2, 3': 5'-TTTGAAT-TCACAACAAACCCCCCGTCACAG-3') and probe (B-3, 5'-AGGTCGATGAAGGTGCTGA CGGTGGTGA-3') were deduced from the terminal sequences of the published sequences of the gB gene of HSV-1 strain F [42] and used for the PCR and colony hybridization, respectively. The primers (Hc-1, 5':5'-TGTTAACCTTCTCCC-3'; and Hc-2, 3': 5'-CACGTCGAG TCA ATTGTAC-3') were deduced from the terminal sequence of the published sequence of the polyhedrin gene of HcNPV [27] and used for the partial sequencing of the gene inserted in the HcNPV transfer vector. The oligonucleotides were synthesized by Korea Biotec Inc., Taejon, Korea. The HSV-1 gB-specific monoclonal antibody 52S was purchased from Medi-lab Korea (Seoul, Korea) and fluorescein isothiocyanate-conjugated sheep anti-mouse IgG was purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN, U.S.A.).

Colony and Southern Blot Hybridizations

The colony hybridization for the detection of colonies containing gB gene fragments of the HSV-1 *Bam*HI genome library [23] was carried out according to the procedure described by Grunstein and Hogness [19] using probe B-3. The gB locus in the selected recombinant plasmids was confirmed by a Southern blot analysis [33, 48].

Restriction Enzyme Digestions and Agarose Gel Electrophoresis

All restriction endonuclease digestions were performed according to the manufacturer's instructions. The DNA genome and vector DNAs were digested and electrophoresed on a 1.0% agarose gel. The molecular size of each DNA fragment was determined by comparing their mobility with *Bst*EII or *Hin*dIII-digested phage λ DNA fragments. The reactions were terminated by the addition of 1/10 volume of a stop solution [45]. The details of gel electrophoresis and visualization of the DNA fragments were previously described by Lee *et al.* [30] and Lee and Miller [32]. The DNA fragments were fractionated and purified in 1.0% low-melting-temperature agarose gels containing Tris-borate and ethidium bromide [29, 30].

Cloning and Transformation

The HSV-1 gB gene was cloned using standard protocols [29, 30, 45] as detailed in Fig. 1. The gB gene (3.1 kb) in the plasmid pHLA-21 was cleaved into two *Apa*I fragments, 1.25 kb and 1.85 kb, and the two fragments were combined to create a 3.1 kb gB gene sequence which was named pBac-gB3.1. This pBac-gB3.1 was doubly digested with *Xba*I and *Eco*RI to create the 3.1 kb gB gene. This gene was then inserted into the *Xba*I and *Eco*RI sites of the baculovirus transfer vector pBACgus4X-1 bearing the gus gene encoding β-glucuronidase, which serves as a reporter for verifying recombinant viruses by staining with X-Gluc (Novagen). The resulting recombinant was named the pGus-gB3.1 plasmid (Fig. 1). Competent *E. coli* cells were prepared and transformed with the recombinant plasmids by the procedure described by Mandel and Higa [34].

Construction and Selection of HcNPV Recombinant Baculoviruses

S. frugiperda cells were seeded in 60-mm petri dishes with 2×10^6 cells per dish and then cotransfected with 2 µl (1.0 µg of DNA) of the linearized *lac*Z-HcNPV DNA with *Bsu*361 enzyme and 10 µl (10 µg) of pGus-gB3.1 plasmid DNA in a TE buffer (10 mM Tris-HCl, 1.0 mM EDTA, pH 7.5) in 40 µl H_2O to construct recombinant viruses using lipofectin-transfection, as previously described by Lee *et al.* [27, 29]. The recombinant viruses in the supernatants were selected by a 1.5% low-melting-point agarose plaque assay [27] containing X-Gluc and then incubated at 27°C for 5 days. Eight blue occlusion-negative plaques were

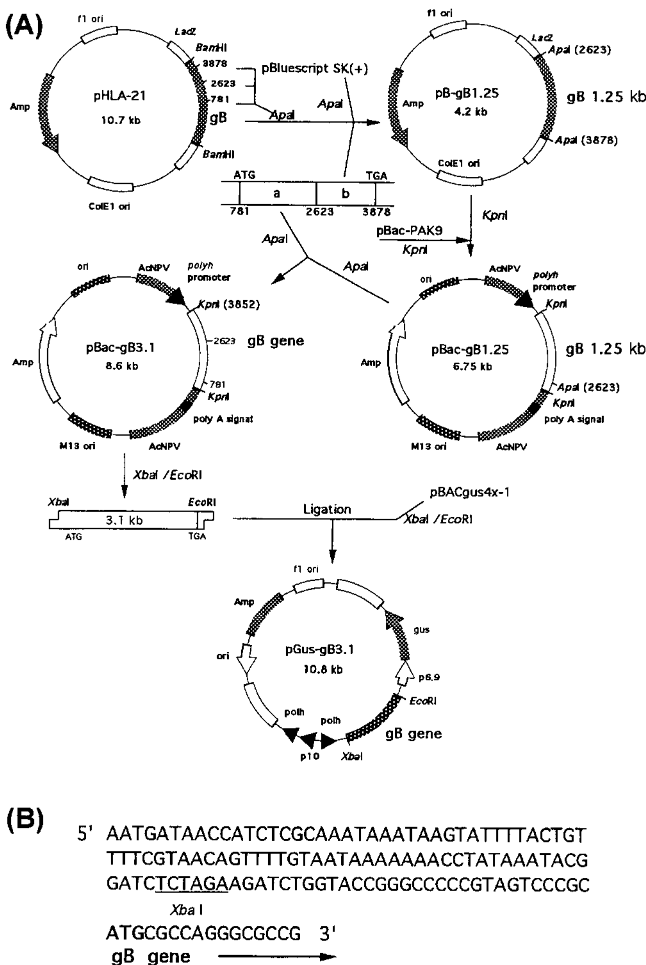

Fig. 1. Construction scheme for the pGus-gB3.1 plasmid containing the gB gene and the upstream sequence of gB.

(A) The 3.1 kb gB gene sequence in the plasmid pHLA-21 was cleaved into two *Apa*I fragments, 1.85 kb (a) and 1.25 kb (b), which were separately cloned into pBluescript SK(+) and then transferred into pBac-PAK9 vectors, which were combined together and then transferred into the *Xba*I and *Eco*RI sites of the pBACgus4x-1 transfer vector to construct the pGus-gB3.1 recombinant plasmid.

(B) Partial nucleotide sequence of the cloning site downstream of the polyhedrin promoter region. The translation start codon, ATG, transcription initiation motif, TAAG, and the ligated *Xba*I site were apparent.

selected and added to a serum-free medium. The insertion of the gB gene in the recombinant virus was analyzed by a PCR using primers B-1 and B-2 [11] and a Southern blot [48].

Nucleotide Sequencing

The dideoxy chain termination sequencing [46] was performed on a polyacrylamide sequencing gel using approximately 8 µg/ml of double-stranded DNA templates and 100 pmol/µl of a limiting primer. The DNA fragments were labeled with ^{35}S-ATP and sequenced with a 7-deaza-dGTP sequencing kit with a Sequenase version 2.0 T7 DNA polymerase (United State Biochemical, Cleaveland, OH, U.S.A.) using the pBluescript T3 primer, T7 DNA primer, and M13 reverse primer.

Radioimmunoprecipitation Assay for Detection of gB Protein

The radioimmunoprecipitation assay was carried out using a modification of the procedures described by Kessler [24]. *S. frugiperda* cells (1×10^6 cells per dish) were seeded on 35-mm petri dishes and allowed to attach in a monolayer overnight at 27°C. The cells were all infected with the recombinant virus. After 1 h of adsorption at 27°C, the monolayers were washed with PBS (pH 6.2) [32], 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 Chemicals Co., St. Louis, MO, U.S.A.) and incubated in 0.5 ml of a leucine-deficient basal medium (Sigma) for 1 h at 27°C. The medium was removed and replaced with 0.5 ml of a leucine-deficient basal medium containing 20 µCi L-[^{3}H]-leucine (Amersham-Pharmacia Biotech Korea, Seoul, Korea). The cells were incubated for 1 h at 27°C with gentle rocking. The cells were washed twice with 2 ml PBS and cooled on ice for 5 min. The cells were then incubated in 0.5 ml of an ice-cold cell lysis buffer consisting of 500 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP40, 0.1% SDS, 0.5 Na-deoxycholate, and 1.0 mM phenylmethylsulfonyl fluoride (PMSF) for 30 min on ice with gentle rocking. The lysate was transferred to a chilled eppendorf tube and centrifuged at 10,000 ×g for 5 min at 4°C [24]. The supernatant was transferred to a fresh chilled eppendorf tube. The lysate was collected and 500 µl aliquots were reacted for 1 h at 4°C on a rock platform with gB and 52S monoclonal antibodies, separately, at a final dilution of 1:2. Twenty microliters of a protein A-agarose (10 mg/ml) (Boehringer Mannheim Biochemicals) were added to the mixture and rotated at 4°C for 3 h. The agarose was pelleted for 1 min, washed twice in washing solution I (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP40, 0.5% Na-deoxycholate, 1 mM PMSF, 1 mM EDTA), washed once in washing solution II (50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 0.1% NP40, 0.05% Na-deoxycholate) and then washed once in washing solution III (50 mM Tris-HCl, pH 7.5, 0.1% NP40, 0.05% Na-deoxycholate) for 20 min at 4°C on a rock plate [24]. The agarose pellet was then resuspended in a 2× SDS gel loading Tris buffer with 4% SDS, 125 mM Tris-Cl, pH 6.7, 30% (v/v) glycerol, 0.002% bromphenol blue, boiled for 5 min, and then centrifuged in a microcentrifuge (Han-il Instruments, Seoul, Korea). The supernatants were collected and loaded onto a 7.5% SDS-polyacrylamide gel (Sigma) [5]. At the completion of the electrophoresis, the gels were transferred into several volumes of Coomassie brilliant blue solution in a fresh flat dish and incubated for 1 h at room temperature. After destaining, for fluorography, the gels were equilibrated with

dimethylsulfoxide (DMSO), thereafter impregnated with 2,5-diphenyoxazole (PPO) (Sigma) by immersion in 4 volumes of 22.5 w/w PPO in DMSO, soaked in water, dried, and exposed to HyperfilmTM-MP (Amersham) film at -70°C for 48 h [6]. The exposed films were developed for 3 min at 20°C in a developer.

Immunofluorescence Assay

An immunofluorescence assay (IFA) for detecting the proteins in the cells was carried out using a modification of the procedures described by Ghiasi *et al.* [17] and Lee *et al.* [31]. *S. frugiperda* cells (2×10^6 cells per dish) were seeded on a glass cover slip (18×18 mm) in 60-mm dishes, infected with recombinant baculoviruses expressing gB at a multiplicity of infection of 10 pfu/cell, and incubated for 72 h. The infected cells grown on the glass cover slip were fixed with acetone for 15 min. The fixed cells were then washed in PBS (pH 6.2) for 10 min and incubated for 1 h at room temperature with the gB specific monoclonal antibody 52S (Medilab Korea) diluted in a 0.5% BSA solution in PBS at 1:500 and 1:2, respectively. The cells were rinsed three times for 5 min with PBS and then incubated for 1 h with anti-mouse immunoglobulin fluorescein (Boehringer Mannheim) diluted 1:50 with a 0.5% BSA solution. The cells were washed twice for 5 min with PBS, mounted in a mounting buffer (1 glycerol: 5 PBS), and then examined at a 100-1,000x magnification through a fluorescent microscope (AO Fluoro-star 20, U.S.A.).

Preparation of Cell Lysates for Immunization

S. frugiperda cells (1×10^6 cells 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 gB at a multiplicity of infection of 0.1 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 postinfection were freeze-thawed, lyophilized and resuspended in 2 ml of PBS buffer (pH 6.2) at a final concentration of 8×10^6 cells. *S. frugiperda* cells infected with the wild-type *lac*Z-HcNPV were treated in the same manner with the infected cells as control.

Immunization of Mice

Immunization to mice was carried out using a modification of the procedures described by Ghiasi *et al.* [17, 38]. 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 whole cell lysates of *S. frugiperda* cells infected with the recombinant baculovirus expressing gB. Two-hundred-and fifty microliters (10^6 cells) of cell lysates was injected subcutaneously with Freund's complete adjuvant at 1:1 ratio on Day 0 and an identical preparation with Freund's incomplete adjuvant on Days 21 and 42, and intraperitoneal (ip) injections were given concurrently with 250 μl (10^6 cells) of the lysates in PBS. Ten mock-vaccinated mice were inoculated with the *S. frugiperda* cells lysates infected with the wild-type baculovirus using the same regimen. A positive control group of 10 mice was immunized three times ip with 2×10^5 pfu of the nonvirulent HSV-1 strain KOS. The sera were collected at 3 weeks after the final vaccination and pooled for each group.

Serum Neutralization Assay

A virus neutralization assay was performed using a modification of the procedure described by Martin and Rouse [36]. Briefly, 100 μl of heat-inactivated serum was added in serial dilution on a 96-well flat-bottom microtiter plate (Falcon, Lincoln Park, NJ). One-hundred microliter of 200x $TCID_{50}$ of live HSV-1 strain F was added to the plate, sealed, and incubated for 18 h at 4°C. Then, 100 μl of 5×10^3 viable vero cells were added to the plates, which were incubated at 37°C for 5 days. The serum dilution factor that neutralized 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 Muench [44].

RESULTS AND DISCUSSION

Construction of Recombinant Virus gB-HcNPV Expressing gB

From the *Bam*HI fragment library of the HSV-1 DNA genome [23] a colony containing the HSV-1 gB gene was detected by colony hybridization with the probe B-3. One colony, which strongly hybridized with the probe, was isolated. The purified DNAs were digested with *Bam*HI and separated on an agarose gel. Then, the inserted fragment was confirmed by Southern hybridization (Fig. 2). This result showed that the recombinant plasmid, pHLA-21, contained the 7.82 kb *Bam*HI DNA fragment harboring the gB gene.

For cloning the HSV-1 gB gene coding the 120 kDa polypeptide [12, 13, 53] into the baculovirus transfer vectors and constructing a recombinant baculovirus, the gB gene (3.1 kb) in the plasmid pHLA-21 was cleaved into two *Apa*I fragments, 1.25 kb and 1.85 kb, which were separately cloned into pBluescript SK(+) and pBac-PAK9 vectors, and then combined to create a 3.1 kb of the gB gene sequence which was named pBac-gB3.1. Thereafter, the gB gene was transferred into the *Xba*I and *Eco*RI sites of the pBACgus4x-1 transfer vector to construct the pGus-gB3.1 recombinant plasmid (Fig. 1A). The insertions of the gB fragments were confirmed by Southern blot analysis (Fig. 2, lane 3). These results indicate that each clone contained the gB gene fragment. For the partial sequencing of the gB gene insertion area in the pGus-gB3.1 plasmid, a 1.3 kb fragment was digested out with *Sma*I, recloned into the *Sma*I site of pBluescript SK(+), and named as the pB-

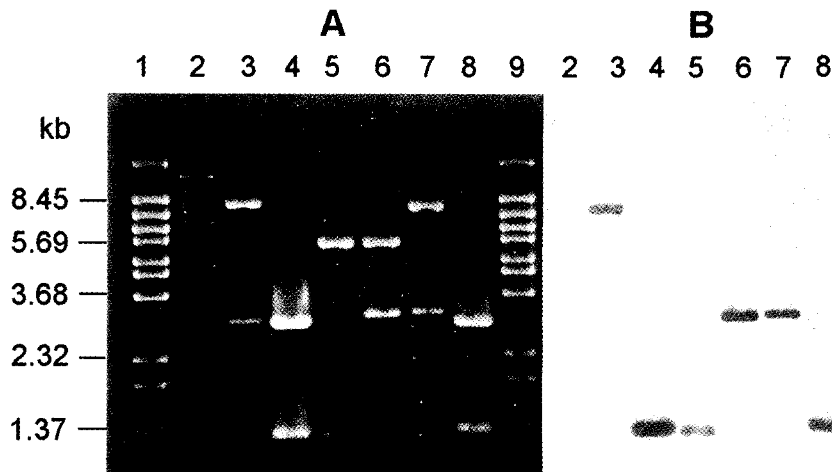

Fig. 2. Agarose gel analysis of gB gene fragments (A) and identification of gB gene fragments in the recombinant plasmids by Southern blot analysis (B).

The digested DNAs were electrophoresed for 17 h on a 1.0% agarose gel. Lanes 1 and 9, phage DNAs digested with *Bst*EII; lane 2, HSV-1 genomic DNAs digested with *Bam*HI; lane 3, pHLA-21 plasmid DNAs digested with *Bam*HI; lane 4, pB-gB1.25 plasmid DNAs digested with *Apa*I; lane 5, pBac-gB1.25 plasmid DNA digested with *Xba*I; lane 6, pBac-gB3.1 plasmid DNAs digested with *Kpn*I; lane 7, pGus-gB3.1 plasmid DNAs double-digested with *Xba*I and *Eco*RI; lane 8, pB-1.3 plasmid DNA digested with *Sma*I.

1.3 plasmid. An analysis of the pB-1.3 plasmid using three restriction enzymes (*Apa*I, *Sac*II, and *Sma*I) showed that the 1.3 kb was inserted in the correct direction. The nucleotide sequence in Fig. 1B demonstrates that the gB gene was cloned correctly. An analysis of the pGus-gB3.1 recombinant using four restriction enzymes showed that the gB gene was inserted in the correct direction between the polyhedrin promoter and the gus gene. The 3.1 kb gB gene fragment was mapped using restriction enzymes.

The strategy for the construction of a recombinant baculovirus shown by Lee *et al.* [27, 29, 31] was used for the gB gene cloning. The pGus-gB3.1 transfer vector DNA containing the gB gene and the linearized *lac*Z-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 isolated by a plaque assay containing X-Gluc. No polyhedral inclusion bodies were formed in the infected cells. White-colored plaque means no insertion of the gB gene, whereas a blue-color plaque indicates a recombinant virus. Eight plaque clones of the recombinant viruses were isolated and characterized. The individual clones were named as gB-HcNPV-1 to 8 on the basis of their selected order. The size of plaques ranged from 2 to 4 mm in diameter (data not shown). The gB-HcNPV-1 clone that exhibited the largest plaque size (4 mm) was used for further studies. The plaques were plaque-purified twice. The presence of the HSV-1 gB gene DNA in the recombinant baculovirus was verified by PCR (data not shown) and Southern blot hybridization on the *Bgl*II fragments of the recombinant gB-HcNPV-1 (Fig. 3). The PCR results indicated that the gB gene was correctly inserted in the recombinant virus genome. The gB gene was located in the 12.3 kb *Bgl*II fragment of the recombinant

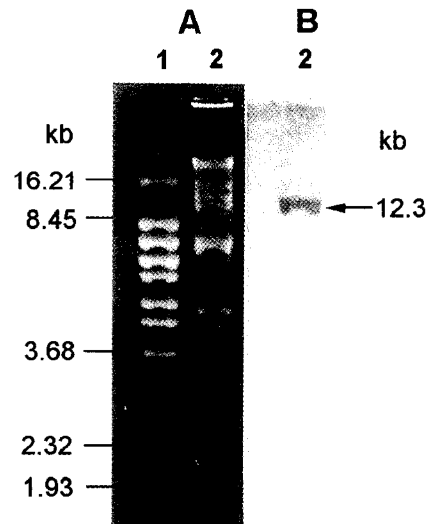

Fig. 3. Confirmation of gB gene insertion in the recombinant virus gB-HcNPV-1 DNAs by Southern blot analysis.

The *Bgl*II-digested DNAs were electrophoresed on a 1.0% agarose gel for 20 h. Panel A lane 1, phage DNAs digested with *Bst*EII; lane 2, gB-HcNPV-1 DNAs digested with *Bgl*II. Panel B shows the results of the Southern blot analysis of panel A. The arrow indicates the 12.3 kb fragment which contains the gB gene.

virus, which newly appeared. Four clones (1, 3, 4, and 8) out of the eight clones contained the gB gene sequence in their genomes. The recombinant virus was multiplied normally in *S. frugiperda* cells and produced a high level of HSV-1 gB in insect cells. One abundant gB-specific polypeptide of 120 kDa was readily detected by radioimmunoprecipitation analysis (Fig. 4). These results indicate that the baculovirus expression system (*lac*Z-HcNPV) is a useful vehicle for the production of glycoprotein gene products. Similar

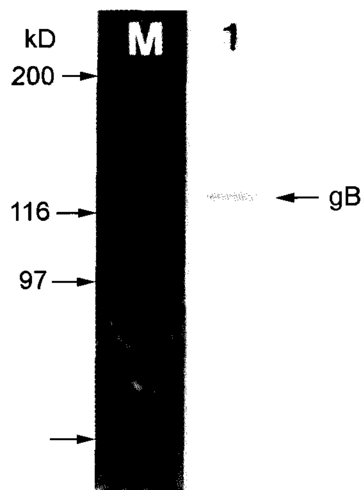

Fig. 4. Immunoprecipitation of gB protein produced from insect cells infected with recombinant virus, gB-HcNPV-1.
Forty-eight hours after infection with the recombinant viruses, the insect cells were labeled with L-[^{3}H]-leucine for 1 h and lysated. The soluble fractions of the lysates were mixed with gB Mab and the antigen-antibody complexes were precipitated with protein A-Sepharose. The immunoprecipitates were resolved by electrophoresis on a 7.5% SDS-PAGE, and the labeled protein was detected by PPO fluorography. The left side lane (M) of the figure shows standard molecular weight markers of myosin (200 kDa), β-galactosidase (116.2 kDa), phospholylase B (97.4 kDa), and bovine serum albumin (68 kDa). Lane 1 indicates the position of gB.

results were reported by Ghiasi *et al.* [17], who constructed a recombinant baculovirus that contained a *Bam*HI fragment with a HSV-1 gB gene using the *Autographa californica* NPV expression vector, which expressed the gB protein in the insect cell. The above two groups of investigators used different vector systems and restriction fragments with the gB gene.

Production and Characteristics of gB in *S. frugiperda* cells

The production of HSV-1 gB in *S. frugiperda* cells infected with gB-HcNPV-1 was also detected by radioimmunoprecipitation analysis. Figure 4 shows that the gB protein band appeared on the fluorogram. This result indicates that the gB protein was produced in the cells and was approximately 120 kDa. This means that the recombinant virus gB-HcNPV-1 expressed a glycosylated mature gB in the insect cells. In the HSV-1 infected cells, the gB precursor is synthesized by a protein of approximately 110 kDa, which is then processed into a mature gB with a molecular weight of up to 120 kDa [12, 13, 40]. The production of HSV-1 gB in *S. frugiperda* cells infected with gB-HcNPV-1 was detected by an indirect immunofluorescence analysis using a fluorescein-conjugated gB monoclonal antibody prior to fixation. No greenish immunofluorescence was seen in the cells infected with the wild-type baculovirus *lac*Z-HcNPV, however, the gB-HcNPV-1-infected cells exhibited high surface fluorescence with green color (Fig. 5).

Fig. 5. Immunofluorescence analysis of HSV-1 gB protein produced in *S. frugiperda* cells infected with gB-HcNPV-1 recombinant virus.
The virus-infected cells were fixed in acetone and treated with an anti-gB monoclonal antibody followed by a fluorescein isothiocyanate-conjugated sheep anti-mouse antibody IgG. The fluorescent cells infected with recombinant virus were observed with a fluorescent microscope (×100). The arrowed cells exhibit high surface fluorescence with a green color.

This fluorescent green color indicates that the gB protein was transported to the cell surface, in consistence with the report of Ghiasi *et al.* [17].

The immunogenicity of the recombinant gB was studied by immunizing mice with lysates from whole insect cells infected with gB-HcNPV-1, as described in Materials and Methods. Three weeks after the final vaccination, mice were bled and their sera were tested for HSV-1-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-1-neutralizing activity *in vitro* (Table 1). Accordingly, it would appear that the baculovirus-expressed gB can induce an immune response in mice that is directed against authentic gB. Cantin *et al.* [9] and Ghiasi *et al.* [17] obtained a similar result, where antibodies raised in mice to recombinants recognized viral gB and neutralized the infectivity of HSV-1 *in vitro*.

In conclusion, our results demonstrate that the gB gene in the gB-HcNPV recombinant virus produces a mature protein in eukaryotic insect cells and indicate the utility of the HcNPV-insect cell system for producing and characterizing

Table 1. Neutralizing antibody titers in mice vaccinated with gB-HcNPV-1.

Immunogens	No. of mice tested	50% neutralization titer of antisera
gB-HcNPV-1	10	<1:64
HSV-1 KOS	10	<1:1024
Mock(HcNPV)	10	<1:8

eukaryotic proteins. In this study the level of viral latency was not measured However, a high level of neutralizing antibody production may protect mice from HSV, therefore, this particular recombinant protein may be useful in the development of a subunit vaccine.

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