

Production and Characterization of Monoclonal Antibody to Glycoprotein D Antigen of *Herpes simplex* Virus Type 2

CHOI, YOUNG-SOOK¹, TAE-UN KIM², HYUNG-HOAN LEE, AND MYUNG-HWAN CHO*

¹Department of Biological Science and ²Department of Clinical Pathology, Konkuk University, Seoul 143-701, Korea
²Department of Clinical Lab. Science, Catholic University of Pusan, Pusan 609-757, Korea

Received: September 3, 1999

Accepted: November 23, 2000

Abstract A monoclonal antibody (mAb) to the glycoprotein D (gD) of *Herpes simplex* virus type 2 (HSV-2) was successfully generated by hybridoma technology and characterized. The mAb, SKS2v, recognized a gD antigen with an apparent molecular mass of 60 kDa in a Western blot analysis. The isotype was determined by a sandwich ELISA to be IgG2a. HSV-2 exhibited major antigens of 36, 43, 46, 47, 60, 69, 81, 96, 109, 112, 159, and 227 kDa among 25 protein profiles in SDS-PAGE, and among these antigens, those of 60, 112, 125, and 227 kDa were immunodominant in a Western blot analysis using antisera, thereby indicating that they play a role in inducing neutralizing antibodies in HSV-2 infection. When reacted with Vero cells infected with HSV-1 and HSV-2, SKSv2 showed a reactivity to the surface of the infected cells, and a gD antigen of 60 kDa appeared to be expressed in both types of HSV.

Key words: *Herpes simplex* virus type 2, glycoprotein D, monoclonal antibody

Herpes simplex viruses were the first human herpesviruses to be discovered and are the most intensively investigated of all viruses. They have served as models and tools for the study of translocation of proteins, synaptic connections in the nervous system, membrane structure, gene regulation, and a myriad of other biological questions, both general to viruses and specific to HSV [25]. There are two serologically and genotypically distinct types of *H. simplex* viruses, designated HSV types 1 and 2 [15]. HSV-1 is primarily associated with ocular lesions and is transmitted in oral and respiratory secretions, whereas HSV-2 is isolated primarily from genital and anal lesions and is passed through sexual contact [4, 6, 9].

The significant rise in the incidence of *H. simplex* virus type 2 infections has evoked public demand for vaccine development [24]. However, progress has been impeded by the relatively poor understanding of the role of virus-specific immunity in protection against infection. The glycoproteins encoded by HSV represent the major virus-specific components present on the virion envelope and infected cell surfaces. The envelope glycoproteins are also important in immune recognition since they are displayed on the surface of infected cells allowing them to be antigenic in infected hosts [28].

The eleven glycoproteins have been designated as gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, and gM [1], and are believed to be particularly important in viral pathogenesis because they are the first viral component to interact with the host cell [7]. The glycoproteins gB, gD, gH, and gL are known to be required for productive infection *in vitro* and all appear to have a critical function in virion penetration [3, 8, 14, 19] and are expressed in both HSV-1 and HSV-2 [30].

HSV elicits both cellular and humoral immune responses. Neutralizing antibody (CD4+ T-cell mediated) and CD8+ T-cell mediated responses both appear to be important in controlling HSV infection. HSV glycoproteins are the major target of immune attack by the host [14]. gB, gC, gD, gE, gG, and gH can induce neutralizing antibodies [23]. For example, antibodies of the IgM, IgG, and IgA classes are elicited and recognize the surface glycoproteins [11]. gD is one of the most potent inducers of neutralizing antibodies and is likely related to viral infectivity [13], and it has been implicated in adsorption and penetration to cells [20].

Accordingly, this study was performed to develop a monoclonal antibody specific to the gD antigen of *H. simplex* virus type 2. Production of a mAb specific for a neutralizing antibody-inducing antigen could be a valuable tool in diagnosing HSV infection, and studying vaccine development and antigenic determinants of HSV.

*Corresponding author
Phone: 82-2-450-3427; Fax: 82-2-454-7997;
E-mail: mcho@kkucc.ac.kr

MATERIALS AND METHODS

Cell Culture and Virus Infection

African green monkey kidney cells (Vero, ATCC CCI 81) were originally obtained from the Korean Type Culture Collection (KTCC). The cells were maintained by passing every three days in Eagle's minimum essential medium (EMEM, GIBCO/BRL, U.S.A.) supplemented with 5% fetal bovine serum (FBS, Hyclone, U.S.A.), 100 units of penicillin G sodium per ml, 100 µg of streptomycin sulfate per ml, 2.5 µg of amphotericin B per ml, and 0.2% sodium bicarbonate. The cells were incubated at 37°C in a humidified CO₂ incubator adjusted to a constant 5% CO₂ concentration (SANYO Electric Co., Ltd., Japan).

Virus Titration

The *H. simplex* virus type 1 (HSV-1) strain F (ATCC VR-733, Rockville, MD, U.S.A.) [10] was originally obtained from the Korean National Institute of Health (KNIH). Virus production was then carried out as previously described by Stannard *et al.* [28] with some modifications. The virus was cultured in monolayers of Vero cells in EMEM with 2% FBS, which was prepared as mentioned above. Briefly, the monolayers of Vero cells were rinsed with PBS (Dulbecco's phosphate buffered saline; KCl 0.2 g/l, NaCl 8.0 g/l, KH₂PO₄ 0.2 g/l, Na₂HPO₄ 1.15 g/l, Sigma, U.S.A.), then inoculated with 200 µl of the virus suspension at a multiplicity of infection of 0.01 to 0.1 pfu/cell for an adsorption period of 1 hour. Following the removal of the inoculum, the cells were overlaid with EMEM supplemented with 2% FBS and incubated at 37°C for 48 to 72 h. Once the cytopathic effect (CPE) stopped progressing, the culture fluids were freeze-thawed three times to release the intracellular virus, then harvested into a sterile 50-ml conical centrifuge tube (NUNC, Denmark), and centrifuged at 1,400 ×g for 20 min to pellet any cellular debris. Thereafter, the supernatants were either used immediately or stored at -70°C in sterile 50-ml conical centrifuge tubes until use.

Immunization of Mice

Seven-week-old female BALB/c mice were injected intraperitoneally with a mixture of 250 µl of the virus suspension and the same amount of complete Freund's adjuvant (GIBCO/BRL, U.S.A.), and boosted twice at 2-week intervals. The mice were tail-bled on the 10th day to determine the titer of anti-HSV-2 antibodies after the third injection. The final tail injection was given intravenously with HSV-2 antigens, four weeks after the third boosting. The antigens in the PBS were given for every injection. The hyperimmune mice were then sacrificed 3 days after the final booster, and the spleen was removed for the preparation of mono-splenocytes.

Preparation of Splenocytes

The spleen of the hyperimmune mice was used for B lymphocyte preparation for cell fusion. The spleen was

removed from the peritoneum by an aseptic technique and homogenized on a wire mesh screen using the plunger of the syringe to produce mono-splenocytes. The mono-splenocytes were then washed twice with basal media.

Cell Fusion

P3-X63/Ag 8.653 Myeloma and the splenocytes were mixed (10:1) in a 50-ml conical tube, filled with basal media to 40 ml, and centrifuged at 400 ×g for 10 min. The supernatant was removed and 1 ml of polyethylene glycol 4000 was slowly added to the pellet of cells while gently flicking for 1 min and held for 1 min. Next, 20 ml of the basal media was slowly added to the cells with gentle flicking for 4 min. These procedures were performed at 37°C in a water bath. The cell mixture was centrifuged at 300 ×g for 5 min and the supernatant was removed. Thymocyte-conditioned medium (150 ml) was then added to suspend the cell mixture. The fused cells were dispensed into 24-well tissue culture plates and cultured at 37°C in a CO₂ incubator. After 24 h of incubation, 1 ml of HAT medium was added to each well.

Screening of Hybridoma by Indirect Immunofluorescence Assay

An enzyme-linked immunosorbent assay was used to identify any hybridoma producing an antibody to HSV-2. The antigens were diluted with a coating buffer (Na₂CO₃ 1.59 g, NaHCO₃ 2.93 g, NaN₃ 0.2 g, distilled water 1 l, pH 9.6), resulting in 10⁷–10⁸ cells/ml, and then sonicated at 200 watt for 30 sec. One-hundred µl of the antigen solution was then added to each well of a 96-well EIA plate, fixed for 18 h at 4°C, treated with a 1% BSA/PBS-Tween 20 solution for 2 h at 37°C, and then washed three times with PBS-Tween. The hybridoma supernatants were then tested for their antigen-antibody reaction. Anti-HSV-2 sera and normal mouse antisera were used as the positive and negative controls, respectively. Goat anti-mouse IgG+IgM+IgA conjugated with horseradish peroxidase (Sigma Chemical Co., St. Louis, MO, U.S.A.) was diluted 4,000-fold, reacted for 2 h at 37°C, and then washed three times with 5% BSA/PBS-Tween. The substrate solution (0.1 M citric acid, 2.43 ml; 0.2 M Na₂HPO₄, 2.57 ml; distilled water, 5 ml; *o*-phenylenediamine, 50 mg; 30% H₂O₂, 5 µl) was then added and allowed to react for 30 min at 37°C. The reaction was stopped by adding 2.5 M H₂SO₄. The absorbance of each well was read at 490 nm by an ELISA reader (Molecular Devices Co., Menlo Park, California, U.S.A.).

Cloning of Hybridoma

The hybridoma producing monoclonal antibodies to HSV-2 were cloned by limiting the dilution in the 96-well plate to less than one cell per well. To clone the cells, those wells with colonies were rescreened and recloned. The cells were diluted to 1×10⁷ cells/ml into complete media after being centrifuged at 400 ×g for 10 min. One-hundred microliters

of a cell suspension with 22 cells was then dispensed into the first 3 lanes of 96-well plates containing 100 μ l of a thymocyte-conditioned medium per well. The remaining cells were diluted again with 4 ml of the complete medium so that the cell numbers were 4.4 in 100 μ l of medium and dispersed into the next 3 lanes of the plates at 100 μ l per well. Finally, 1.5 ml of the complete medium was then added to the last cells and 2.2 cells per 100 μ l were added into each well of the last 2 lanes of the plates, and all the cells were cultured in a 5% CO₂ incubator at 37°C.

Isotyping of Monoclonal Antibody

A subisotyping kit from American Qualex (San Clemente, CA, U.S.A.) was used for the detection and identification of an anti-HSV-2 monoclonal antibody subclass. One-hundred μ l of goat anti-mouse immunoglobulins was added to 10 ml of a plate coating solution. One-hundred μ l of the plate coating mixture was then added to each well of a 96-well EIA plate. The plate was sealed, incubated for 18–24 h at 4°C, washed with PBS-surfactant, and pat dried with a clean dry towel. Two-hundred μ l of diluted blocking serum (1:4 in 1 \times PBS) was added to each well. The plate was incubated at room temperature for one hour, washed with PBS-surfactant, and pat dried. Then, 50 μ l of each hybridoma supernatant was added to each of the wells in an 8-well column, and 50 μ l of diluted normal mouse serum (1:500) was added to an 8-well column as the positive control. The plate was incubated at room temperature for one hour, washed with PBS, and pat dried. This washing procedure was repeated twice. Two drops of different types of antisera were added to each row of the plate. To serve as the negative control, 100 μ l of PBS-surfactant was added to any wells that did not contain supernatant. The plate was then incubated at room temperature for one hour. The contents of the plate were shaken and washed with PBS and pat dried. One-hundred μ l of diluted conjugate was added to each well, and then incubated at room temperature for one hour, washed with PBS, and pat dried three times. One-hundred μ l of a TMB substrate reagent was added to each well, and the plate was read at 655 nm in a microtiter plate reader.

Western Blot Analysis of HSV Antigens using Monoclonal Antibody

SDS-PAGE was carried out in a 10–20% gradient separating gel and 5% stacking gel, using a microslab gel electrophoresis system (SE 250-Mighty Small II, Hoefer, U.S.A.). The antigens (20–40 μ g) of the cell lysates were dissolved in a 60 mM Tris/HCl buffer (pH 6.8) containing 2% (w/v) SDS, 25% (v/v) glycerol, 14.4 mM 2-mercaptoethanol, and 0.1% bromophenol blue by heating at 100°C for 5 min. After electrophoresis, the oocyst antigens were transferred to nitrocellulose membranes using an electroblotter (Hoefer, San Francisco, CA, U.S.A.). The transfer buffer contained 15.6 mM Tris (Sigma, St. Louis, U.S.A.) and 120 mM

glycine (Sigma, St. Louis, U.S.A.), pH 8.3 at a constant current of 30 mA for 90 min at 4°C. The gel was stained with Amido black (Sigma, St. Louis, U.S.A.) to confirm a complete transfer of the proteins. The membranes were blocked with 3% BAS in Tris buffered saline (TBS 10 mM Trizma base, 150 mM NaCl, pH 7.5) for 2 h at 37°C and washed three times with TBS. The nitrocellulose was incubated with the hybridoma supernatant for 1 h at 37°C. Then, goat anti-mouse IgG+IgM conjugated with horseradish peroxidase (Jackson Immuno-Research Lab, Inc., West Grove, PA, U.S.A.) was diluted to 1:1000 in 0.5% (w/v) BAS/TBS and incubated with the blots for 1 h at 37°C. Following each step, any unbound reagents were removed with TBS using three 30 min washes. The blots were finally incubated with a substrate solution consisting of 30 mg/ml chloronaphthol, 10 ml methanol, and 30 μ l H₂O₂ (30%) in 50 ml TBS for 10 to 20 min at room temperature. The developed blots were washed with distilled water and photographed.

RESULTS AND DISCUSSION

SDS-PAGE Analysis of HSV-2 Antigens

SDS-PAGE was used to analyze the protein profiles of the soluble protein extracts of HSV-2. As shown in Fig. 1, 25 bands showed up within a molecular weight range of

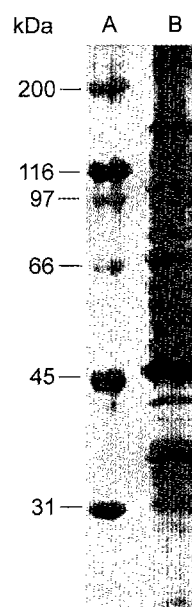


Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of HSV-2 antigens.

The 15 μ l of the concentrated viruses were subjected to electrophoresis on 10% polyacrylamide slab gel in the presence of 0.1% sodium dodecyl sulfate. The protein bands were stained with Coomassie Blue. Lane A: Standard molecular markers. Lane B: HSV-2 strain G (ATTCCVR 734, Rockville, MD, U.S.A.).

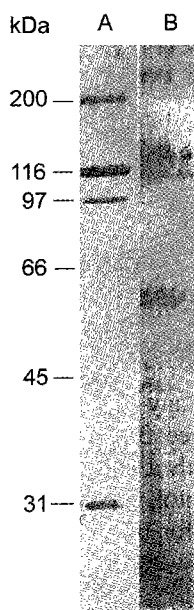


Fig. 2. Western blot analysis of HSV-2 antigen using polyclonal antibody.

Viruses were prepared from HSV-2-infected Vero cells and were concentrated by ultracentrifugation. Viruses were then electrophoresed under denaturing conditions, transferred to a nitrocellulose membrane, reacted with polyclonal HSV-2 antiserum, and then reacted with an anti-goat mouse IgG+IgM+IgA conjugated with horseradish peroxidase as the secondary antibody. Lane A: Standard molecular markers. Lane B: HSV-2 strain G (ATTCCVR 734, Rockville, MD, U.S.A.).

30 kDa and 240 kDa. HSV-2 exhibited major antigens of 36, 43, 47, 60, 69, 81, 96, 109, 112, 159, and 227 kDa among 25 protein profiles.

Western Blot Analysis of HSV-2 Antigens

To determine the antigenic specificity of the polyclonal antibodies, Western blotting was performed with the HSV-2 antigens (Fig. 2). The antigens were transferred to a nitrocellulose membrane and reacted with antisera generated from BALB/c mice immunized with HSV-2 antigens. The HSV-2 antigens of 43, 48, 60, 112, 125, and 227 kDa were all recognized by polyclonal antibodies in the analysis. Among these antigens, 60, 112, 125, and 227 kDa were immunodominant (Fig. 2). Therefore, these antigens would be involved in inducing an immune response in an HSV-2 infection. Among these immunodominant antigens, the antigen with a molecular mass of 60 kDa appeared to be the mature form of HSV-2 glycoprotein D, which is known to induce a neutralizing antibody in an HSV infection. The molecular mass of a mature form of gD was previously reported to be 59–65 kDa [1].

Production and Characterization of Monoclonal Antibody SKS2v

The fusion yielded hybridoma growth in a total of 28 wells in the 24-well cell culture plates. Of the 28 hybridomas,

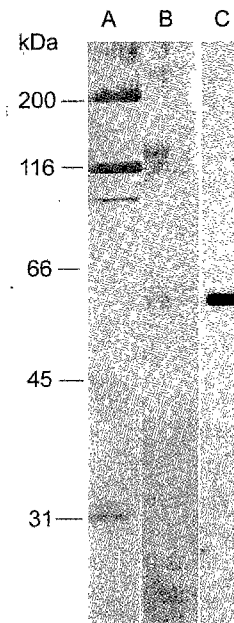


Fig. 3. Western blot analysis of HSV-2 antigens using a monoclonal antibody SKS2v, showing the recognition of the gD antigen of 60 kDa.

Viruses were prepared from HSV-2-infected Vero cells and were concentrated by ultracentrifugation. Viruses were then electrophoresed under denaturing conditions, transferred to a nitrocellulose membrane, reacted with the supernatant of a hybridoma cell line secreting SKS2v, and then reacted with an anti-goat mouse IgG+IgM+IgA conjugated with horseradish peroxidase as the secondary antibody. Lane A: Standard molecular markers. Lane B: HSV-2 antigens recognized by polyclonal antisera. Lane C: HSV-2 gD antigens recognized by a monoclonal antibody, SKS2v.

7 were obtained from the fusion of immune BALB/c splenocytes with myeloma cells (SP2/0-Ag14) and two hybridomas produced monoclonal antibodies that reacted specifically with HSV-2 antigens, when assayed by indirect immunofluorescent microscopy. One monoclonal antibody, SKS2v, was established and characterized after being cloned. The isotype of SKS2v was determined by a sandwich ELISA using a commercial isotyping kit (HYCLONE 98055) and turned out to be IgG2a.

The monoclonal antibody, SKS2v, to the gD antigen of HSV-2 was characterized by a Western blot analysis. The HSV-2 antigens were transferred to a nitrocellulose membrane and reacted with SKS2v. The results revealed that the antibody recognized a gD antigen with an apparent molecular size of 60 kDa (Fig. 3). The apparent molecular weights in an SDS-PAGE of fully mature glycoproteins and their partially glycosylated precursors were previously reported [1]. In summary, the molecular weights of the mature forms of gB, gC, gD, gE are 120, 115–130, 59–65, and 80 kDa and their precursors are 110, 86–105, 51, and 66 kDa, respectively. Therefore, the antigen recognized by mAb SKS2v would appear to be a mature form of gD. Radioimmune precipitation with ^3H glucosamine-labeled

cell lysates was also previously carried out and similar protein profiles were observed [22]. Therefore, since gD seems to have a critical function in virion penetration and is expressed in both HSV-1 and HSV-2 [30], it has been recognized as a good candidate for vaccine development.

To date, gD has been implicated in adsorption to cells. Several other studies have suggested that virion attachment to permissive cells occurs in two steps [21]. First, the virus binds to heparan sulfate proteoglycan molecules on the surface of cells [31]. The second step is thought to be the interaction of gD with a specific cell surface receptor [12]. A recent report suggested that the mannose-6-phosphate receptor may be a ligand for gD [2]. gD is also involved in penetration. The penetration of HSV into permissive cells occurs predominantly through the fusion of the virion envelope with the plasma membrane. The evidence for this is as follows. First, neutralizing mAbs to gD [12] allow HSV attachment yet prevent penetration. Second, virus mutants lacking gD are able to bind cells yet cannot penetrate them [26].

It is anticipated that the monoclonal antibody SKSv2 developed in this study will be used in the diagnosis of

HSV infection and as a tool for studying antigens and developing a vaccine against HSV.

Immunofluorescence of Monoclonal Antibody

Anti-HSV-2 monoclonal SKSv2 was examined in an indirect immunofluorescent assay for its reactivity with HSV-1 and HSV-2 (Fig. 4). When reacted with Vero cells infected with HSV-1 and HSV-2, SKSv2 showed a reactivity to the surface of the infected cells. The immunofluorescent pattern of the Vero cells infected with HSV using SKSv2 demonstrated the presence of an HSV-2 gD antigen of 60 kDa on the surface of the infected cells and showed that a 60 kDa antigen of HSV-2 was also expressed in HSV-1. Accordingly, it appears that gD plays a critical role in inducing a neutralizing antibody in HSV infection [3, 7]. In addition, since the expression of gD in transfected cells is sufficient to render cells resistant to HSV infection [16, 27], it is proposed that the presence of gD in membranes of infected cells may prevent superinfection by escaping progeny virions.

Acknowledgments

This study was supported by a grant from the Korea Science and Engineering Foundation (96-0401-11-01-3).

REFERENCES

1. Baucke, R. B. and P. G. Spear. 1979. Membrane protein specified by *Herpes simplex* viruses. V. Identification of an Fc-binding glycoprotein. *J. Virol.* **32**: 779-789.
2. Brunetti, C. R., R. L. Burke, S. Kornfeld, W. Gregory, F. R. Masiarzi, K. S. Dingwell, and D. C. Johnson. 1994. *Herpes simplex* virus glycoprotein D acquire mannose 6-phosphate residues and binds to mannose 6-phosphate receptors. *J. Biol. Chem.* **269**: 17067-17074.
3. Cai, W., B. Gu, and S. Person. 1988. Role of glycoprotein of *Herpes simplex* virus type 1 in viral entry and cellofusion. *J. Virol.* **62**: 2596-2604.
4. Cha, S. C., H. Kang, S. Y. 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.
5. Cho, J. M., G. B. Nam, S. B. Hong, and M. H. Cho. 1995. Immunological analysis of antigenic variation of *Bacillus thuringiensis* subsp. *sotto* during sporulation and crystallization. *J. Microbiol. Biotechnol.* **6**: 359-363.
6. Chung, C. C., H. H. Lee, and M. H. Cho. 2000. Immunoelectron microscopic localization and analysis of *Herpes simplex* virus type 1 antigens. *J. Microbiol. Biotechnol.* **10**: 714-720.
7. Corey, L. and P. Spear. 1986. Infections with *Herpes simplex* viruses. *N. Engl. J. Med.* **314**: 686-691.

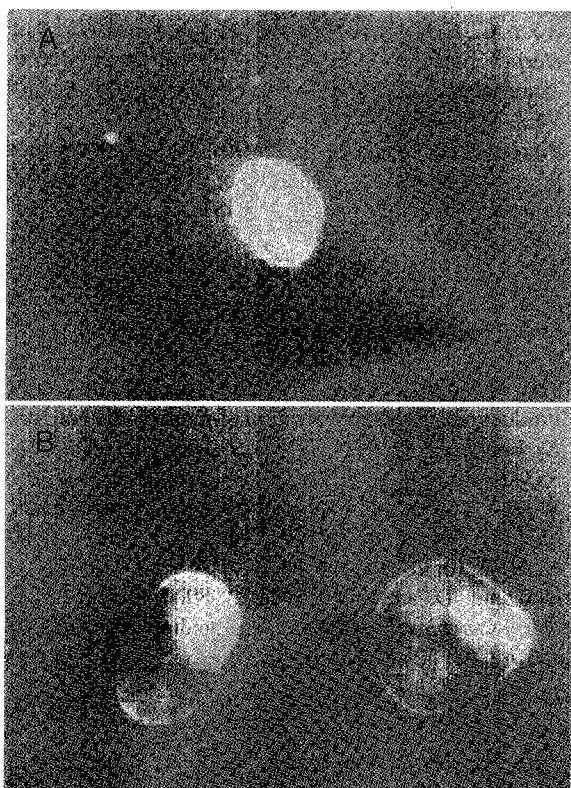


Fig. 4. Immunofluorescence of Vero cells infected with HSV-1 (A) and type 2 (B); Multinuclear giant cells formed after infection with HSV-1 and HSV-2 were reacted with a monoclonal antibody SKS2v specific for the antigen of 60 kDa of HSV-2 in a humid chamber at 37°C for 40 mins and then reacted with FITC-conjugated goat anti-mouse IgG+IgM+IgA for 20 mins ($\times 1,000$).

8. Desai, P. J., P. A. Schaffer, and A. C. Minson. 1988. Excretion of noninfectious virus particles lacking glycoprotein H by a temperature-sensitive mutant of *Herpes simplex* virus type 1: Evidence that gH is essential for virion infectivity. *J. Gen. Virol.* **69**: 1147–1156.
9. Dulbecco, R. and S. H. Ginsberg. *Virology*. 1988. p. 167. 2nd Ed, J. B. Lippincott Company, Philadelphia.
10. Ejercito, P. M., E. D. Kieff, and H. Roizman. 1968. Characterization of *Herpes simplex* virus strains differing in their effects on social behaviour of infected cells. *J. Gen. Virol.* **2**: 357–364.
11. Fuller, A. O. and P. G. Spear. 1985. Specificities of monoclonal and polyclonal antibodies that inhibit adsorption of *Herpes simplex* virus to cells and lack of inhibition by potent neutralizing antibodies. *J. Virol.* **55**: 475–482.
12. Fuller, A. O. and W. C. Lee. 1992. *Herpes simplex* virus type 1 entry through a cascade of virus-cell interactions requires different roles of gD and gH in penetration. *J. Virol.* **66**: 5002–5012.
13. Haffey, M. L. and P. G. Spear. 1980. Alterations in glycoprotein gB specified by mutants and their partial revertants in *Herpes simplex* virus type 1 and relationship to other mutant phenotypes. *J. Virol.* **35**: 114–128.
14. Hutchinson, L., H. Browne, V. Wargent, N. Davis-Poynter, S. Promorac, K. Goldsmith, A. C. Minson, and D. C. Johnson. 1992. A novel *Herpes simplex* virus glycoprotein, gL, forms a complex with glycoprotein H(gH) and affects normal folding and surface expression gH. *J. Virol.* **66**: 2240–2250.
15. Jennings, S. R., P. A. Lippe, K. J. Pauza, P. G. Spear, L. Pereiva, and S. S. Tevethia. 1987. Kinetics of expression of *Herpes simplex* virus type 1-specific glycoprotein species on the surface of infected murine, simian, and human cells: Flow cytometric analysis. *J. Virol.* **61**: 104–112.
16. Johnson, R. N. and P. G. Spear. 1989. *Herpes simplex* virus infection. *J. Virol.* **63**: 819–827.
17. Jung, J. D., J. S. Park, Y. S. Jo, S. B. Hong, H. H. Lee, and M. H. Cho. 1994. Production and characterization of monoclonal antibodies to *Bacillus thuringiensis* subsp. *canadensis*. *J. Microbiol. Biotechnol.* **4**: 290–295.
18. 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**: 674–684.
19. Ligas, M. W. and D. C. Johnson. 1988. A *Herpes simplex* virus mutant in which glycoprotein D sequences are replaced by β -galactosidase sequences binds to but is unable to penetrate into cells. *J. Virol.* **62**: 1486–1494.
20. Manservigi, R., P. G. Spear, and A. Buchan. 1977. Cell fusion induced by *Herpes simplex* virus is promoted and suppressed by different viral glycoproteins. *Proc. Natl. Acad. Sci. USA* **74**: 3913–3917.
21. McClain, D. S. and A. O. Fuller. 1994. Cell-specific kinetics and efficiency of *Herpes simplex* virus type 1 entry are determined by two distinct phases of attachment. *Virology* **198**: 690–702.
22. Manservigi, R., P. G. Spear, and A. Buchan. 1977. Passive immunization with monoclonal antibodies against *Herpes simplex* virus glycoproteins protects mice against herpetic ocular disease. *Curr. Eye Res.* **6**: 173–177.
23. Nobel, A. G., G. T. Lee, R. Sprague, M. L. Parish, and P. G. Spear. 1983. Anti-gD monoclonal antibodies inhibit cell fusion induced by *Herpes simplex* virus type 1. *Virology* **129**: 218–224.
24. Roberts, P. L., B. E. Duncan, T. J. Raybould, and D. H. Watson. 1985. Purification of *Herpes simplex* virus glycoprotein B and C using monoclonal antibody and their ability to protect mice against lethal challenge. *J. G. Virol.* **66**: 1073–1085.
25. Roizman, B. and A. E. Sears. 1996. *Herpes simplex* viruses and their replication. pp. 1043–1107. In B. N. Fields, D. M. Knipe, and P. M. Howley (eds.), *Fields Virology*, 3rd edn, Lippincott-Raven, Philadelphia, U.S.A.
26. Roop, C., L. Hutchinson, and D. C. Johnson. 1993. A mutant *Herpes simplex* type q unable to express glycoprotein L cannot entry cells, and its particles lack glycoprotein H. *J. Virol.* **67**: 2285–2297.
27. Sivropoulou, A. and M. Arsenakis. 1994. Transformed cell producing the glycoprotein D of HSV-1 are resistant to infection with clinical strains of HSV. *Arch. Virol.* **137**: 397–404.
28. Stannard, L. M., A. O. Fuller, and P. G. Spear. 1987. *Herpes simplex* virus glycoproteins associated with different morphological entities projecting from the virion envelope. *J. Gen. Virol.* **68**: 715–725.
29. Yoo, D. H., Y. H. Lee, J. D. Jung, and M. H. Cho. 1995. Production and characterization of monoclonal antibodies to *Escherichia coli* (ATCC 8739). *J. Microbiol. Biotechnol.* **5**: 353–358.
30. Ward, P. and B. Roizman. 1994. *Herpes simplex* genes: The blueprint of a successful human pathogene. *Trends in Genetics* **10**: 267–274.
31. WuDunn, D. and P. G. Spear. 1989. Initial interaction of *Herpes simplex* virus with cells is binding to heparan sulfate. *J. Virol.* **63**: 52–58.