

## Immunogenicity and Efficacy of Baculovirus Derived Glycoprotein D of Herpes Simplex Virus Type-1 in Mice

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### Summary

The recombinant glycoprotein D (gD) of herpes simplex virus type-1 (HSV-1) in baculovirus expression system was produced. Spodoptera Frugiperda cell, clone 9 (Sf9) was cultured in modified Grace's medium and inoculated with 5-7 multiplicity of infection of HSV-1 recombinant baculovirus carrying gD gene. Inoculated cells were harvested 96h postinoculation and treated with 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and phenylmethylsulfonyl fluoride (PMSF) and sonicated at 20KHZ for 5 times. A western blotting was developed and applied to detect the prepared protein. Three groups of BALB/c mice each of 7 mice were inoculated with the recombinant gD, sublethal dose of challenge virus ( $10^{4.5}$ TCID<sub>50</sub>) and PBS respectively. All inoculated mice were challenged with 10MLD<sub>50</sub> ( $10^{6.5}$ TCID<sub>50</sub>) of the wild HSV-1. All mice who had received the recombinant gD survived while 14.3% and 71.5% of mice inoculated respectively with either sublethal dose of the virus or PBS died.

**Keywords:** herpes simplex virus gD, recombinant protein, western blotting, immunization.

### Introduction

Herpes simplex virus (HSV) is an enveloped, double stranded DNA virus, which is an important human pathogen (Handler *et al* 1996). It contains at least eleven

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glycoproteins in its envelope, five of which function in viral entry. Glycoprotein D presents in great amount and involves in virus infection and pathogenesis (Handler *et al* 1996, Ghiasi *et al* 1991). Glycoprotein D-1 is also a major inducer and target of humoral and cell mediated immune responses to HSV-1 infection (Ghiasi *et al* 1991, Johnson *et al* 1990, Norrild 1985). Purified gD-1 of the virion stimulates production of high titer neutralizing antibodies to the virus (Rogers *et al* 2000). GD-1 has been a principal candidate for an HSV-1 subunit vaccine (Flo *et al* 2000, Berman *et al* 1983). A wide range of expression systems has been used to express this protein.

The baculovirus expression system (Ghiasi *et al* 1996, Krishna *et al* 1989) is one of the most powerful available eukaryotic expression systems (Berman *et al* 1983, Bourne *et al* 1996, Kidd & Emery 1993). This system is a helper-independent viral system, which has been used to express heterologous genes from many different sources including viruses in insect cells (O'Reilly *et al* 1992). Because of HSV-1 is an important pathogen of human being and attempts to produce an attenuated viral vaccine or a killed one have not been successful, therefore it has been proposed repeatedly to use other sort of vaccines such as subunit or DNA vaccine to immunize the susceptible person. An appropriate vaccine should be able to protect the vaccine as well as prevention of the virus transmission (Norrild 1985, Krishna *et al* 1989, Rotenberg 2000).

In this study recombinant baculovirus carrying gD-1 gene of HSV-1 was used to express this gene in cultured Sf9 cells for producing large amount of the recombinant gD. A western blotting methods was developed and applied to observe special reaction between the protein and HSV polyclonal and monoclonal antibodies with positive result. The recombinant gD-1 was then used as a subunit vaccine to immunize susceptible BABL/c mice against 10MLD<sub>50</sub> ( $10^6$  TCID<sub>50</sub>) of the wild virus. Compared with the results obtained from control mice it was concluded that the vaccine could protect the immunized mice properly.

### **Materials and Methods**

**Virus and cells.** Plaque purified recombinant baculovirus containing full-length gD-1 gene with titer of  $10^7$ - $10^8$  pfu/ml donor by Dr. Ghiasi (UCLA, USA). *Spodoptera frugiperda* clone 9 (Sf9) insect cells were obtained from Pasteur Institute of Iran. The cells were grown in Grace's medium containing 10% fetal bovine serum. In order to get a suitable culture of the cells supplementation of the growth medium with bovine fetal serum as well as 3.3g/L yeast extract and 3.3g/L lactalbumine hydrolysate was necessary. Gentamicin sulfates (50mg/ml) and penicillin (100IU/ml) were added. The pH was adjusted to 6.2 (Figure 1). To maintain healthy cultures, Sf9 cells were sub cultured 1:3 when they reach confluence on plates (three times a week usually).

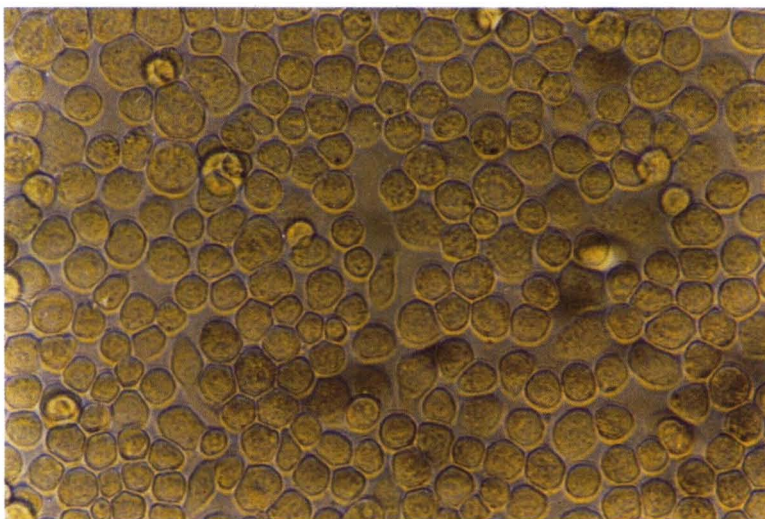


Figure 1. *Uninfected Sf9 cells*

Sf9 cells were inoculated with purified recombinant baculovirus carrying full length of gD-1 gene at multiplicity of infection (moi) of 0.1 and incubated at 27°C for 5 days (Figure 2). Then medium of the cells was collected, filtered through a 0.45  $\mu$ m pore size filter and stored at -80°C. In order to produce recombinant gpD-1,

Sf9 cells were inoculated with the purified virus at moi of 5-7 and incubated at 26.5-27°C for up to 96h. The cell were harvested and washed 3 times with ice-cold PBS and sonicated at 20KHZ for 5 times after being frozen and thawed for 3 times (Ghiasi *et al* 1994, Handler *et al* 1996). The lysate was first treated with CHAPS (0.1mM) and PMSF and then electrophoreses under denaturing condition (Ghiasi *et al* 1994, Ghiasi *et al* 1991).

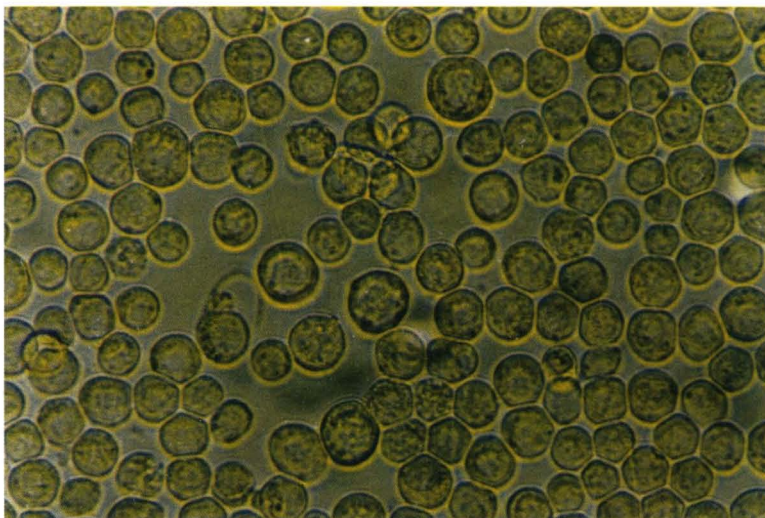


Figure 2. *Sf9* cells infected with recombinant BAC gD-1

**Western blotting.** Western immunoblot analyses were carried out under denaturing conditions through sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE ). The electrophoresed proteins were then transferred to nitrocellulose paper (Ghiasi *et al* 1991). After transferring, the nitrocellulose blots were blocked in Tris-buffered saline-Tween20 containing 0.5% gelatin and then reacted with anti-HSV-1 polyclonal antibody or murine monoclonal anti gD-1 for 2h at room temperature separately. Bounded antibody was detected by reacting with HRP-conjugated anti human or anti mouse for 2h at room temperature then the reaction was developed using 3,3' Diaminobenzidine (DAB).

**Preparation of vaccine.** Working amount of the recombinant gD-1 was prepared and the concentration of the protein measured by Bradford method. The protein was mixed with equal volume of commercial Freund's complete adjuvant (FCA) or Freund's incomplete adjuvant (FIA) (Biogene) properly, and used as vaccine separately.

**Vaccination.** BALB/c mice were obtained from Razi Institute (Karaj). All mice were first bled and a virus neutralization test was done in order to select the susceptible ones. Susceptible mice of 3-week-old were put in three groups with 7 mice in each one. The first group was immunized three times with 100 $\mu$ l of inoculums containing 10 $\mu$ g of the protein mixed with FCA or FIA at 3-week intervals respectively. The second group received sublethal doses of the wild virus three times at 3-week intervals, while the control group received only PBS with the same manner.

**Challenge.** All injected mice were challenged 21 days after the last inoculation with 10MLD<sub>50</sub> of the wild virus. The course of the infection was monitored for 20 days to observe if there were signs of the infection due to HSV-1.

**Virus isolation.** Samples of lung, brain, spleen and lymph node were collected from either dead or alive immunized mice and tested for virus isolation in bovine kidney cell.

### **Results**

The confluent monolayer of Sf9 cells was shown in figure 1. The confluent monolayer of Sf9 cells were infected at multiplicity of 5-7moi with purified recombinant baculovirus shown in figure 2, which have become enlarged, floated and their replication has stopped. Western blot analysis of the inoculated cell showed two proteins bands that reacted with HSV-1 polyclonal antibody (Figure 3). A band with an apparent molecular weight of ~43.5KDa corresponds to the immature nonglycosylated gD-1 protein that has a predicted molecular weight of

43.291Da. The larger band (~51KDa) likely represents the mature gD-1. Also western blot analysis of inoculated cell showed four distinct bands with apparent molecular weight of (~51,~43.5,~33, and 31KDa) that reacted with monoclonal anti gD-1 antibody.



Figure 3. *Western blot analysis of recombinant baculovirus expressing gD in insect cells*

Comparison of lethal rate in inoculated mice show that none of 7 mice inoculated with the recombinant gD-1 died from the lethal challenge of the virus, while 1 (14.3%) and 5 (71.5%) mice inoculated with sublethal dose of the wild virus and PBS died respectively. Samples of lung, brain, spleen and lymph node were collected from either vaccinated or control dead animals in order to understand whether death or clinical signs seen in the inoculated mice were due to the challenge

with wild HSV-1. The virus was isolated from the most inoculated animals in bovine kidney cells (Figure 4).



Figure 4. *Cytopathic effect of the isolated virus in BK cell*

### ***Discussion***

Herpes simplex virus type-1 (HSV-1) is one of the most contagious agents, which infect human being. The virus has a worldwide distribution and causes rang of different symptoms and sign from herpes labial and sore throat to fatal encephalitis (Handler *et al* 1996, Norrild 1985). Attempts have long been made to produce effective vaccines for protecting people from herpes infection of which recombinant glycoprotein D (gD-1) is one of the candidates.

In this study we tried to produce recombinant gD-1 in baculovirus expression system. The baculovirus expression vector system is one of the most powerful and available versatile eukaryotic expression systems (Kidd & Emery 1993). The baculovirus used in this project contained the gene encoding glycoprotein D of HSV-1. It has been shown that recombinant proteins have been produced in the baculovirus system at levels ranging between 0.1% and 50% of the total insect cell

protein. We were able to produce high levels of the gD-1 amounting 10% of the total cell protein. SF9 cells used in this study were adapted to the modified Grace's medium containing 10% heat inactivated fetal bovine serum. These cells were quite suitable for the virus propagation and protein production. McCarroll *et al* (1997) used these cells and produced heterologous proteins. Although they used spinner cultures or bioreactors for culturing cells but we realized that monolayer cells could produce more protein than suspension ones (data not shown).

Results of western blotting done in this study showed that there were four bands including ~51KDa, ~43KDa, ~33KDa, and ~31KDa. The first two bands correspond to partially glycosylated and nonglycosylated gD-1 respectively, and others may belong to proteolytic degradation or incomplete translation. Our results are similar to those of Ghiasi *et al* (1991). We tried to immunize a group of 7 BALB/c mice by the recombinant gD-1. A separate group of mice were also given a sublethal dose of wild HSV-1 as positive control, while negative control mice received only PBS. Although both groups of mice injected with recombinant gD or sublethal dose of the virus were protected against virus challenge but there appeared several signs of infection in the group of mice inoculated with sublethal dose of the virus and one mouse died. Vaccination with this expressed protein resulted in production of neutralizing antibodies to HSV-1 and complete protective immunity against lethal HSV-1 infection. Our results are in full agreement with what gained by Burke (1991).

Attempts were made to isolate HSV-1 virus from test or control mice, which were challenged by intraperitoneal injection of 10MLD<sub>50</sub> virus. The virus was isolated from brains and lungs of control mice that died after challenge, while no virus was isolated from immunized mice. With the large amounts of recombinant gD-1 production ability in insect cells and gD's effectiveness as subunit vaccine in mice, it could be suggested that the gD-1 protein maybe a useful and potent candidate for production of HSV-1 vaccine.



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