



AU9335127

(12) PATENT ABRIDGMENT (11) Document No. AU-B-35127/93
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 664597

(54) Title
PROCESS FOR THE EXPRESSION OF HERPES SIMPLEX VIRUS TYPE 1 GLYCOPROTEIN I AND METHODS OF USE

International Patent Classification(s)
(51)⁵ **C12N 015/86 C07K 003/00 A61K 039/245**

(21) Application No. : **35127/93** (22) Application Date : **10.03.93**

(30) Priority Data

(31) Number (32) Date (33) Country
852999 18.03.92 US UNITED STATES OF AMERICA

(43) Publication Date : **23.09.93**

(44) Publication Date of Accepted Application : **23.11.95**

(71) Applicant(s)
CEDARS-SINAI MEDICAL CENTER

(72) Inventor(s)
ANTHONY BART NESBURN; STEVEN LEWIS WECHSLER; HOMAYON GHIASI

(74) Attorney or Agent
SPRUSON & FERGUSON, GPO Box 3898, SYDNEY NSW 2001

(56) Prior Art Documents
AU 660528 33959/93 C12N A61K C07K

(57) Claim

1. A recombinant baculovirus wherein the genetic construct comprises:
 - a) a gene sequence encoding HSV-1 gI or a biologically active derivative thereof; and
 - b) a polyhedrin gene promoter sequence wherein said gI gene sequence is functionally linked to the regulatory elements of said polyhedrin promoter.
10. A biologically active HSV-1 gI obtained according to the process of any one of claims 8 or 9 and being free from other products or proteins.
11. A pharmaceutical preparation for the treatment or prevention of HSV infection containing a biologically active HSV-1 gI according to claim 10.

664597 S & F Ref: 233100

AUSTRALIA
PATENTS ACT 1990

COMPLETE SPECIFICATION

FOR A STANDARD PATENT

ORIGINAL

Name and Address
of Applicant: Cedars-Sinai Medical Center
8700 Beverly Boulevard
Los Angeles California 90048-1865
UNITED STATES OF AMERICA

Actual Inventor(s): Anthony Bart Nesburn, Steven Lewis Wechsler, Homayon
Ghiasi

Address for Service: Spruson & Ferguson, Patent Attorneys
Level 33 St Martins Tower, 31 Market Street
Sydney, New South Wales, 2000, Australia

Invention Title: Process for the Expression of Herpes Simplex Virus Type
1 Glycoprotein I and Methods of Use

The following statement is a full description of this invention, including the
best method of performing it known to me/us:-

Process For The Expression Of Herpes Simplex Virus Type 1 Glycoprotein I And Methods Of Use

Field Of The Invention

The present invention is in the fields of infectious diseases and molecular biology.

5

Background Of The Invention

A. Herpes Simplex Virus Type 1 (HSV-1) Glycoprotein I

Glycoprotein I (gI) is one of ten documented herpes simplex virus type 1 (HSV-1) glycoproteins. In HSV-1 infected cells, gI is present as an unglycosylated peptide having a molecular weight of 41kDa. The unglycosylated polypeptide is partially glycosylated to
10 produce a precursor gI of approximately 55kDa, which is then further glycosylated to the mature gI with an apparent molecular weight of 65kDa.

The ten HSV glycoproteins are located on the surface of the virus, some of which are reported to be the primary inducers and targets of both humoral (antibody) and cell-mediated immune responses to HSV-1 infection. The HSV-1 glycoproteins E and I form a
15 complex that can bind the Fc portion of immunoglobulin G. While gE alone can act as an Fc receptor, gI appears to enhance this activity; yet, no Fc receptor activities have been detected with gI alone. Moreover, Blacklaws et al. found that vaccinia expressed gI produced no protective response in immunised mice. Blacklaws, E.A., Krishna, S., Minson, A.C. and Nash, A.A. Immunogenicity of Herpes simplex virus type 1
20 glycoproteins expressed in vaccinia virus recombinants. *Virology*, 177: 727736 (1990).

Glycoprotein I has been expressed by only one other group, Sullivan and Smith, in a vaccinia expression system, and reported that the gI neutralisation anti body was completely complement dependent. Sullivan, V. and Smith, G.L. The Herpes Simplex
25 Virus Type 1 US7 Gene Product is a 66K Glycoprotein and is a Target for Complement-dependent Virus Neutralisation. *J. Gen. Virol.*, 69:859-867 (1988). However, their reported neutralisation titers were exceptionally low and were determined using unconventional assays. Furthermore, Blacklaws et al., using the same vaccinia recombinant gI constructed by Sullivan and Smith, and using standard assays, obtained neutralisation
titters of less than 1:2 in the presence of complement.

30 In contrast to these reports, we have expressed in a baculovirus system, high quantities of gI which is capable of eliciting a strong protective immune response against HSV-1 infection. Mice vaccinated with our recombinant gI induced a strong neutralising antibody response. The gI neutralisation titer was 1:167 in the presence of complement and 1:91 in the absence of complement, appearing therefore to be partially complement
35 dependent. We also found that mice vaccinated with baculovirus recombinant gI showed complete protection against lethal HSV-1 infection. To our knowledge, this is the first report to demonstrate that gI can induce protective immunity in animals against HSV-1 challenge. This ability to produce large quantities of high quality bioactive gI is critical in the development of an effective vaccine against HSV.

B. DNA Technology

Recombinant DNA and associated technologies can be applied to effectively provide the large quantities of high quality bioactive HSV glycoprotein I required for a therapeutic or prophylactic HSV vaccine.

5 DNA technology involves in part, producing a replicable expression vehicle or transplacement vector by the DNA recombination of an origin of replication, one or more phenotypic selection characteristics, an expression promoter, a heterologous gene insert and remainder vector. The resulting expression vehicle is introduced into cells by transformation allowing a transplacement of the genetic construct from the recombinant
10 baculovirus transplacement vector to the receptor baculovirus. Large quantities of the recombinant vehicle are then obtained by growing the transformant. Where the gene is properly inserted or functionally linked with reference to regulatory elements which govern the transcription and translation of the encoded DNA message, the expression vehicle may produce the polypeptide sequence for which the inserted gene codes. This process of
15 producing the polypeptide is called "expression." The resulting product may be obtained by lysing the host cell, and recovering the product by appropriate purification.

A wide range of host cells can be used, including prokaryotic and eukaryotic organisms. In addition to microorganisms, cultures of cells derived from multicellular organisms, whether vertebrate or invertebrate, may also be used as hosts. Our system
20 involved use of baculovirus, the polyhedrin promoter system and insect cells as host cells to produce high quantities of bioactive gI. To our knowledge, we are the first to demonstrate high levels of protection against lethal HSV-1 challenge using a gI vaccine.

The references cited herein are all incorporated by reference.

Summary Of The Invention

25 The present invention relates to the production of HSV-1 gI, by recombinant DNA techniques, and its use as an immunogen in a vaccine to protect against HSV-1 and/or HSV-2 infections. Vaccines made from genetically engineered immunogens should be safer than conventional vaccines made from attenuated virus because there is no risk of infection to the recipient; and specifically with the herpes virus, there should be no risk of cervical
30 cancer. Alternatively, the genetically engineered glycoprotein or protein product may be used to produce antibodies for use in passive immunotherapy. The invention also relates to the transformed cell line, which contains the subject transplacement vector, and its cultures which produce HSV-1 gI.

To this end, we constructed a recombinant baculovirus expressing high levels of
35 HSV-1 gI in Sf9 cells. We unexpectedly discovered, however, that vaccination of mice with our expressed gI, demonstrated high levels of protection against lethal HSV-1 challenge. Methods and compositions are therefore provided for the cloning and expression of HSV gI gene in single-cell host organisms. Also described are methods for culturing these novel single-cell organisms to produce the HSV gI gene product as well as methods for the

purification of the gene product.

A human host is then preferably inoculated with a vaccine comprising an immunity inducing dose of gI alone or with one or more HSV glycoproteins or proteins by the systemic route, the enteric route or by the ocular route. The vaccine may also comprise one or more adjuvants administered with, before or after the glycoprotein component of the vaccine.

The vaccine of the invention may be conveniently utilised in liquid form, freeze-dried, spray dried or lyophilised form, in combination with one or more suitable preservatives and protective agents to protect the glycoproteins or proteins during processing.

A. Antigen

The baculovirus expressed gI migrated on gels with molecular weights of 52 and 56kDa. The recombinant gI appeared to be glycosylated, as demonstrated by its susceptibility to both tunicamycin and endoglycosidase H. Indirect immunofluorescence also demonstrated that it was transported to the membrane of Sf9 cells. Mice vaccinated with our expressed gI developed high serum titers of complement-dependent and non-complement-dependent HSV-1 neutralising antibodies, which protected the mice from lethal HSV-1 challenge.

B. Adjuvants

Vaccines are often administered in an emulsion with various adjuvants. The adjuvants aid in attaining a more durable and higher level of Immunity using smaller amounts of antigen in fewer doses than if the immunogen were administered alone. The adjuvants for use in the present invention include but are not limited to alum, Freund's, MTP-PE and ISCOMs (Quil A). In addition, the vaccine may comprise a liposome or other membrane bound vesicle comprising one or more HSV-1 glycoproteins administered with or without one or more adjuvants to induce the cell mediated immune response.

C. Immunisation Routes

The vaccine can be administered by the systemic route, the ocular route either alone or in combination with systemic vaccination, or the enteric route. The systemic route includes but is not limited to subcutaneous, intramuscular or intravenous injection in one or multiple doses. The ocular route includes but is not limited to subconjunctival injection, surface drops, a slow-release device such as a collagen shield, a hydrogel contact lens or an ALZA "Ocuser" in one or multiple doses.

Doses to be administered are variable and depend on the desired effect and on the chosen administration route, with 1 to 3 doses generally comprising the vaccination. However, inoculation doses to humans by injection vary from about 1 μ g to 1000 μ g. For ocular vaccination, the human dosages vary from about 1 μ g to 500 μ g; whereas for enteric vaccination, the human dosages vary from about 1 μ g to 2000 μ g.

It is therefore a general object of the present invention to express high levels of

HSV-1 gI.

It is an object of the present invention to express high levels of HSV-1 gI from one virus strain in a single vector system.

It is also an object of the present invention to express high levels of bioactive HSV-1 gI from cells which have been infected or transformed with a recombinant baculovirus.

It is another object of the present invention to obtain a transformed cell line which produces HSV-1 gI.

It is a further object of the present invention to develop an effective vaccine for the treatment or prevention of HSV in a host.

10 These and other objects will become readily apparent to those skilled in the art from the following description and appended claims.

D. Brief Description Of The Drawings

The present invention will be described with the help of the accompanying drawings in which:

15 FIGURE 1 is a schematic diagram of the construction of the pAc-gII recombinant baculovirus transfer vector containing the HSV-1 gI gene. The strategy for the construction of the baculovirus transfer vector (pAc-gII) containing the complete coding region of HSV-1 gI (■) under control of the baculovirus polyhedrin gene promoter (▨) is outlined herein and described in the text. At the bottom of the figure is the sequence of the region
20 near the 5' end of the cloned gI in the baculovirus transfer vector. The 3' end of the baculovirus polyhedron gene promoter, a BamHI linker, and the coding sequence of gI (preceded by five noncoding nucleotides of the gI gene) are indicated.

FIGURE 2 is a Western blot analysis of expressed gI in infected insect cells. Insect cells were infected at a multiplicity of infection of 10 with the baculovirus gI recombinant
25 (vAc-gII), and some monolayers were treated with tunicamycin (0 to 48 h postinfection) or cell extracts were treated with endoglycosidase H as recommended by the manufacturer (Boehringer Mannheim Biochemicals). Briefly, 10^5 cells were lysed in gel sample buffer, sodium acetate buffer (pH 5.0). Endoglycosidase H was then added and the samples were incubated overnight. Lanes: M, molecular size markers; 1, wild-type-baculovirus-infected
30 insect cells; 2, baculovirus-gI-infected cells 48h postinfection; 3, baculovirus-gI-infected cells 72h postinfection; 4, tunicamycin-treated, baculovirus-gI-infected cells (48h); 5, endoglycosidase H-treated, baculovirus-gI-infected cells 72h postinfection.

FIGURE 3 shows immunofluorescence of recombinant baculovirus-infected cells. Acetone-fixed or unfixed infected Sf9 cells were incubated with anti-gI monoclonal
35 antibody followed by fluorescein-conjugated goat anti-mouse immunoglobulin G antibody and examined by fluorescence microscopy as described previously and known in the art. (A) Recombinant baculovirus-gI-infected cells, total (intracellular) fluorescence; (B) baculovirus-gI-infected cells, surface fluorescence; (C) wild-type-baculovirus-infected cells, total fluorescence.

Detailed Description

The present invention utilises recombinant DNA techniques to insert a DNA sequence coding for HSV-1 gI or a portion thereof, into a DNA transplacement vector, such that the vector is capable of replicating and directing expression of the gI gene in a foreign host. 5 The resulting recombinant DNA molecule is introduced into insect cells to enable high production of gI, or a portion or molecular variant thereof by the host cells. The gI produced is then isolated and purified for use in immunotherapy against both HSV type 1 and/or type 2.

The Examples set forth below describe use of baculovirus, the polyhedron promoter 10 system and insect cells as host cells. However, it would be well within the skill of the art to use analogous techniques to construct expression vectors for expression of desired gI and gI products in alternative host cell cultures. The following detailed description is, therefore, not to be taken in a limiting sense, and the scope of the present invention is best defined by the appended claims.

15 A. Viruses and Cells

The E2 strain of *Autographa californica* nuclear polyhedrosis virus (AcNPV) and *Spodoptera frugiperda* clone 9 (Sf9) cells were grown according to procedures known in the art. Plaque purified HSV-1 (strains McKrae and KOS) and Vero cells were grown using standard techniques.

20 B. Construction of the AcNPV Recombinant Transfer Vector

Plasmid pSSI7 was double digested with Fsp I/Sph I and a 1.3 Kb fragment containing the complete coding region of gI was isolated. The resulting fragment was ligated into the Sma I site of pGem-3. Plasmid pGem-gI was linearised with Hind III, digested briefly with Bal 31 exonuclease, and ligated into the unique BamHI site of the 25 vector pAcYMI. Partial sequence analysis indicated that the cloned gI has a short noncoding region of 5 nucleotides at the 5' end and 48 HSV-1 noncoding nucleotides after the gI termination codon at the 3' end.

C. Transfection and Selection of Recombinant Viruses

Sf9 cells were cotransfected with purified infectious baculovirus (AcNPV) DNA and 30 pAc-gII plasmid DNA according to procedures known in the art. Following three cycles of polyhedrin-negative plaque purification, two recombinant viruses were obtained. Both recombinants expressed gI with similar properties as determined by Western blotting using anti-gI monoclonal antibody. One of the recombinant baculoviruses was chosen for further study and designated vAc-gII.

35 D. Preparation of Viral DNA

Sf9 cells were infected with recombinant virus at an MOI of 10PFU/cell and incubated at 28°C for 72hr. The infected cells were freeze-thawed and centrifuged for 10min. at 1000 x g to remove cell debris. The subsequent procedures used for virus isolation, DNA extraction and blotting are known in the art, and therefore will not be

repeated here.

E. Western Blots

Western immunoblot analyses were carried out under denaturing conditions. Samples for SDS-PAGE were disrupted in electrophoresis sample buffer containing 2% SDS and 10% 2-mercaptoethanol and heated at 100°C for 3 min. Proteins were separated by SDS-PAGE and transferred to nitrocellulose paper by electrophoresis. The nitrocellulose blots were blocked in BLOTTO (5% nonfat dry milk in PBS) and reacted with Fd69 anti-gI monoclonal antibody (a gift from Dr. S. Chatterjee) for 1hr at 4°C. Bound antibody was detected by reacting the blots with ¹²⁵I-protein A for 1hr at 25°C, followed by 10 autoradiography.

F. Endoglycosidase H (Endo-H) Treatment

Endo-H treatment was done on lysates from Sf9 infected cells (10PFU/cell; 72hr post infection) as described by the manufacturer (Boehringer Mannheim Biochemicals). Briefly, 105 cells were lysed in gel sample buffer, Na-acetate (pH5.0) buffer. Endo-H was then 15 added and the samples were incubated overnight.

G. Tunicamycin Treatment

Infected cells (10PFU/cell) were incubated in 4µg/mL tunicamycin in TNM-FH media from 0-48hr post infection and harvested for SDS-PAGE.

H. Immunofluorescence

20 Sf9 cells were infected with wild-type AcNPY or recombinant baculoviruses expressing gI (multiplicity of infection of 10PFU/cell) and incubated for 72hr. To look at total fluorescence, cells were washed with PBS, fixed with acetone, and incubated with gI monoclonal antibody for 1hr at 37°C. To examine cell surface immunofluorescence, unfixed, unpermeabilized cells were incubated with anti-gI monoclonal antibody for 1hr at 25 4°C, and then fixed with acetone. Slides for total and surface fluorescence were then stained with fluorescein-conjugated goat anti-mouse IgG for 1hr at 37°C, and examined for fluorescence.

I. Vaccination

Sf9 cells infected for 72hr with 10 PFU/cell of wild type (AcNPV) or vAc-gII were 30 collected, washed and suspended in PBS. Mice (Balb/C, 6-8 weeks old) were vaccinated three times subcutaneously and intraperitoneally (concomitantly) with freeze-thawed whole insect cells expressing gI. Subcutaneous injections were done with 1 x 10⁶ cells mixed with Freund's complete adjuvant on day 0 or mixed with Freund's incomplete adjuvant on days 21 and 42. Intraperitoneal injections were done using 1 x 10⁶ cells in PBS on the same day. 35 Mock vaccinated mice were similarly inoculated with Sf9 cells infected with wild type baculovirus. A positive control group was immunised three times intraperitoneally with 2 x 10⁵PFU of KOS. Sera were collected three weeks after final vaccination and pooled for each group.

J. Serum Neutralisation Assay

For in vitro serum neutralisation assays, heat inactivated pooled sera were diluted in MEM, and mixed with 500PFU of HSV-1 strain KOS, for 30min at 37°C. Two and one half percent of heat-inactivated or fresh guinea pig complement was added and the mixture incubated for another 30min. Duplicate samples were added to CV-1 cells in 24-well 5 microtiter plates and residual HSV-1 infectivity was assayed. The plates were incubated at 37°C for 72 hr, strained with 1% crystal violet, and plaques were counted.

K. HSV-I Challenge

Three weeks after the final vaccination, mice were challenged intraperitoneally with 2×10^6 PFU of HSV-1 (McKrae strain). Challenged mice were monitored for a period of two 10 weeks.

Results

A. Construction of Recombinant Viruses Expressing gI

The strategy for the construction of the baculovirus transfer vector containing the complete gI open reading frame is shown in figure 1. A complete DNA copy of the gI gene 15 was isolated by restriction enzyme digestion with Fsp I/Sph I. The resulting fragment containing the complete coding region of HSV-1 gI (black) was blunt-ended into the Sma I site of pGem-3. Plasmid pGem-gI was linearised with Hind III, digested briefly with Bal 31 exonuclease, and BamHI linker was added. The resulting DNA was then inserted into the BamHI site of the pAcYM1 vector. (See figure 1 and the Detailed Description above). 20 Restriction enzyme analysis and partial sequencing confirmed that this construct contains the entire sequence of the gI gene. It has a noncoding region of 5 nucleotides in front of the first ATG. This is followed by the complete coding region of 1170 nucleotides. To transfer the gI gene into the baculovirus AcNPV genome, Sf9 cells were cotransfected with pAc-gII DNA and infectious AcNPV DNA. Two putative recombinant viruses were 25 selected by three cycles of polyhedron-negative plaque purification. Both recombinant baculoviruses expressed gI with similar properties as determined by Western blotting. One was arbitrarily chosen for subsequent study and designated vAc-gII.

B. Expression of gI in Sf9 Cells

To analyse the size of the baculovirus expressed gI, confluent monolayers of Sf9 cells 30 were infected at a multiplicity of 10PFU/cell with the baculovirus recombinant vAc-gII. Total protein extracts were run on 10% SDS-PAGE and analysed by western blotting using Fd69 monoclonal antibody against gI. Two bands of 52 and 56kDa reacted strongly with the gI specific antibody (FIGURE 3, lanes 2 and 3). In addition, these bands were more prominent at 72hr post infection (lane 3) than at 48hr post infection (lane 2). None of these 35 bands was seen in wild type baculovirus infected cells (lane 1) or mock-infected Sf9 cells.

C. Glycosylation of gI

To demonstrate that the expressed gI underwent glycosylation, tunicamycin treatment was done to prevent N-glycosylation in infected Sf9 cells. Infected cells were treated with 4µg tunicamycin/mL of media from 0-48hr post infection, and total cell extracts were

analysed by Western blots using anti-gI monoclonal antibody. The tunicamycin treatment (figure 3, lane 4) increased the mobility of gI relative to the control (lanes 2 and 3), indicating that the 52 and 56kDa polypeptides both contain N-linked sugars. This result indicates that like native gI, the untreated recombinant gI was glycosylated.

5 Following Endo-H treatment, the 52 and 56kDa bands were replaced by a polypeptide with an apparent molecular weight of 50kDa (figure 3, lane 5). These results indicate that the recombinant gI was N-glycosylated and contained high mannose sugars.

D. Localization of Recombinant gI in Insect Cells

To determine whether the expressed gI was transported to the cell surface, vAc-gII
10 infected Sf9 cells were examined by indirect immunofluorescence antibody staining using monoclonal antibody to gI (figure 3). Total cell immunofluorescence was readily observed in recombinant infected cells (Panel A). To look specifically for gI on the cell surface, indirect immunofluorescence antibody staining was done on cells prior to fixation (Panel B). The surface fluorescence on vAc-gII infected cells was strong and comparable to that
15 observed for permeabilised fixed cells. Only background level immunofluorescence was seen in cells infected with the wild type baculovirus, AcNPV, (Panel C) or mock-infected Sf9 cells. This indicates that the expressed gI was transported to the cell surface.

E. Neutralising Antibody Response

Balb/C mice were immunised three times subcutaneously and intraperitoneally with
20 whole insect cells expressing gI. Three weeks after the final vaccination, mice were bled. Pooled sera from 20 mice inoculated with the recombinant gI was heat-inactivated and reacted with HSV-1 in the presence of either fresh or heat-inactivated complement. Antibody from the recombinant gI vaccinated mice neutralised HSV-1 infectivity; however, in the presence of fresh complement, the level of neutralisation was higher than in the
25 presence of heat-inactivated complement. No neutralising antibody was produced in mock (AcNPV) vaccinated animals.

F. Viral challenge

Vaccinated mice were challenged by intraperitoneal injection with HSV-1 strain McKrae (2 x 10⁶PFU) three weeks after the final inoculation. As illustrated in Table I
30 below, 60% of the mock vaccinated mice died within 14 days, while 90% of mice vaccinated with expressed gI survived. In the positive control group, 100% of mice immunised with KOS were protected (Table I). Our results suggest that the inoculation of naive animals with baculovirus expressed gI protected mice from lethal intraperitoneal challenge with HSV-1.

Table I. Immunisation of mice with a recombinant baculovirus expressing HSV-I gI

Immunization	No. of survivors/ ^a total no.	% Survival	Neutralisation titer ^b + Complement	- Complement
Baculovirus gI	18/20	90	167	91
KOS	11/11	100	> 320	> 320
Mock	7/18	39	< 10	< 10

^aSurvival rates (protection) of the baculovirus gI recombinant- and KOS-vaccinated mice were significantly different from the mock vaccination survival rate (Fisher's exact test; $P = 0.01$).

^bNeutralisation titers are expressed as the reciprocal geometric means of the dilution that produce a 50% reduction in plaque numbers.

In summary, the present invention involves the high level expression of gI in a baculovirus expression system. The gI in this system was glycosylated and transported to the cell surface. Vaccination of naive mice with recombinant gI resulted in the production of complement-dependent and complement-independent neutralising antibodies to HSV-1. In addition, mice vaccinated with gI were protected from lethal HSV-1 challenge, making gI a useful and important component in any subunit vaccine against HSV-1.

G. Purification Of gI

The baculovirus expressed gI of the present invention may be purified for human use according to standard techniques, including but not limited to, immunoaffinity chromatography and collection of secreted truncated gI from the supernatant medium of cell cultures. The gI purified by these procedures should be free from contamination by other products or proteins.

1. Immunoaffinity Chromatography

The gI protein can be purified in roller bottles by sequential steps of lentil lectin chromatography, immunoaffinity chromatography and concentration by ultrafiltration. For the first step, 2 litres of conditioned medium can be supplemented with 1mM PMSF and 0.5% aprotinin and then loaded onto a 30-mL column of lentil lectin-Sepharose-4B (Sigma Chemical Co., St. Louis, Mo.) at a flow-rate of 50mL/h. The column can be washed sequentially with 100mL of PBS and 100mL of PBS containing 0.5M NaCl. The bound fraction can be eluted with PBS containing 0.5M NaCl, 0.5M α -methylmannoside, 0.1% Triton X-100, and 0.5% aprotinin, and fractions can be assayed for gI by enzyme-linked immunosorbent assay (ELISA).

The peak column fractions can be pooled and applied to a 10mL immunoaffinity column prepared by linking 70mg of a rabbit anti-gI polyclonal antibody to cyanogen bromide-activated Sepharose 4B. The gI-specific rabbit anti serum was raised against gI protein, which was purified by preparative SDS-polyacrylamide gel electrophoresis from

HSV-1 infected Vero cell lysates. Prior to coupling, an IgG-enriched fraction can be prepared from the gI-specific rabbit anti serum by precipitation with 33% saturated ammonium sulfate. Following application of the lectin column eluate to the immunoaffinity column, the column can be washed consecutively with 2 mL of 10mM Tris hydrochloride, 5 pH7.5, and 10mL of LB without SDS and BSA and then with 30mL of 10mM Tris hydrochloride, pH7.5-0.5 MNaCl. The bound fraction can be eluted with 3M ammonium thiocyanate, pH7.5, and the gI protein peak can be detected by ELISA and Western analysis. The peak fractions can be concentrated and equilibrated in storage buffer (100mM NaCl, 10mM Tris hydrochloride, pH7.5, 1mM EDTA, 7.5% glycerol) by ultrafiltration 10 with a PM10 membrane (Amicon Corp., Danvers, Mass.). To remove protein absorbed to the membrane surface, the membrane can be washed with storage buffer plus 0.1% Triton X-100, and this wash can then be combined with the initial concentrated fraction.

2. Collection of the secreted truncated gI

The procedures for the collection of a secreted form of gI are known in the art and 15 will therefore not be repeated here. Essentially, however, the fragment encoding the transmembrane anchor sequence can be excised from the gI gene. The deleted gI gene can then be reconstructed by self-ligation to put in frame sequence coding for the extra membrane and C-terminal intracytoplasmic domains. The product can be detected after transfection by immuno-precipitation of the supernatant medium of cell cultures with anti- 20 gI monoclonal antibody.

H. Pharmaceutical Compositions

The gI of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation are described for 25 example in *Remington's Pharmaceutical Sciences* by Martin. The compositions will contain an effective amount of gI together with a suitable amount of vehicle in order to prepare pharmaceutically acceptable compositions suitable for effective administration to the host.

For purely descriptive and not limiting purposes, several examples of a pharmaceutical preparation of gI for parenteral administration prepared according to the 30 present invention is described.

The vaccine may be supplied as a single dose vial of lyophilised baculovirus expressed HSV-1 gI, alone or in combination with one or more HSV-1 glycoproteins, and a vial of diluent with alum. Alternatively, the vaccine may be supplied in a multidose vial, and a vial of diluent with alum.

35 The invention being described, it is clear that these methods can be modified, which modifications do not diverge from the spirit and purpose of the invention and which would be apparent to one skilled in the art. It is therefore understood that the present invention is not to be construed as limited to such, but rather to the lawful scope of the appended claims.

The claims defining the invention are as follows:

1. A recombinant baculovirus wherein the genetic construct comprises:
 - a) a gene sequence encoding HSV-1 gI or a biologically active derivative thereof; and
 - 5 b) a polyhedrin gene promoter sequence wherein said gI gene sequence is functionally linked to the regulatory elements of said polyhedrin promoter.
2. A recombinant baculovirus according to claim 1 which is capable of expressing HSV-1 gI by cell infection in a culture medium.
3. A recombinant baculovirus transplacement vector containing the genetic
10 construct as characterised in claims 1 or 2 and being capable of introducing it into the baculovirus genome.
4. A recombinant baculovirus transplacement vector which is pAc-gII.
5. A process for the preparation of a recombinant baculovirus according to any one of claims 1 or 2, comprising introducing a recombinant baculovirus transplacement
15 vector according to claims 3 or 4 and a receptor baculovirus into a host cell, allowing a transplacement of said genetic construct from said recombinant baculovirus transplacement vector to said receptor baculovirus, and isolating the recombinant baculovirus of any one of claims 1 or 2.
6. A recombinant insect cell containing a recombinant baculovirus according to
20 any one of claims 1 or 2 and being capable of expressing said HSV-1 gI.
7. The recombinant insect cell according to claim 6 which is derived from *Spodoptera Frugiperda*.
8. A process for obtaining biologically active HSV-1 gI, comprising culturing a recombinant cell according to claims 6 or 7 and recovering said HSV-1 gI from the culture.
- 25 9. The process of claim 8 wherein said HSV-1 gI is obtained without contamination by other products or proteins.
10. A biologically active HSV-1 gI obtained according to the process of any one of claims 8 or 9 and being free from other products or proteins.
11. A pharmaceutical preparation for the treatment or prevention of HSV infection
30 containing a biologically active HSV-1 gI according to claim 10.
12. A pharmaceutical preparation according to claim 11 for administration to humans or animals.
13. A pharmaceutical preparation according to claim 12 which can be administered by the parenteral route.
- 35 14. A pharmaceutical preparation according to claim 12 which can be administered by the enteral route.
15. A pharmaceutical preparation according to claim 12 which can be administered by the ocular route.
16. A recombinant baculovirus, substantially as hereinbefore described.

17. A biologically active HSV-1 gI, substantially as hereinbefore described.

18. A process for the preparation of a recombinant baculovirus, substantially as hereinbefore described.

19. A process for obtaining biologically active HSV-1 gI, substantially as 5 hereinbefore described.

20. A method for the treatment or prophylaxis of HSV infections in a mammal requiring said treatment or prophylaxis, which method comprises administering to said mammal, an effective amount of a biologically active HSV-1 gI according to claim 10 or claim 17, or of a pharmaceutical preparation according to any one of claims 11 to 15.

10

Dated 5 March, 1993
Cedars-Sinai Medical Center

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

1 1 1 1 1
1 1 1 1 1
1 1 1 1 1
1 1 1 1 1
1 1 1 1 1

Process For The Expression Of Herpes Simplex Virus Type 1 Glycoprotein I And Methods Of Use

Abstract

A process for obtaining Herpes Simplex virus type 1 (HSV-1) glycoprotein I (gI) from cells which have been infected or transformed with a recombinant Baculovirus is disclosed. The gI produced is then isolated and purified for use in immunotherapy against HSV infections.

Figure 1.



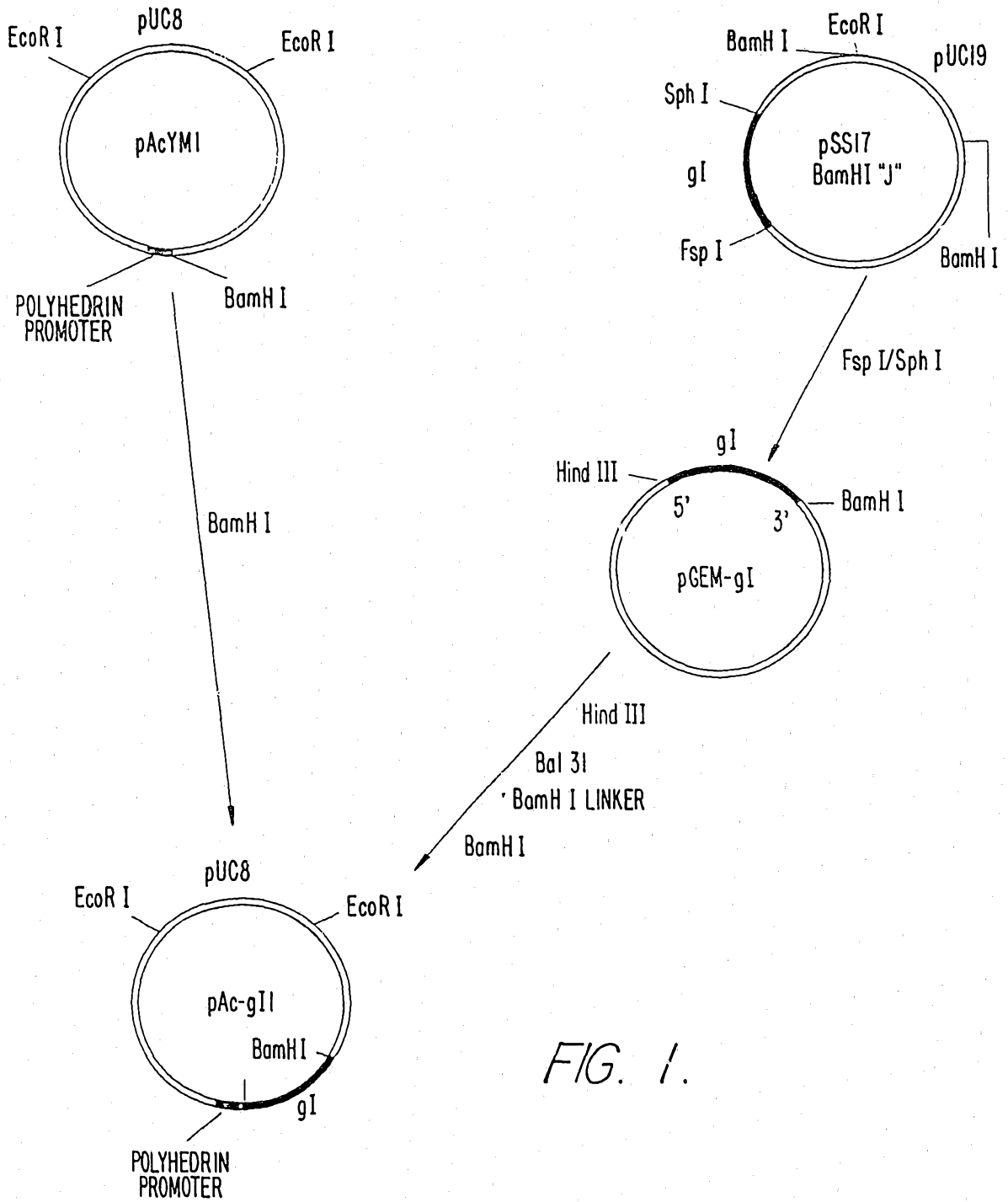
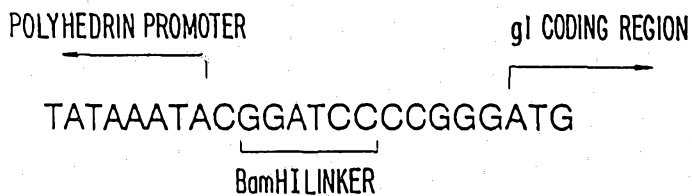


FIG. 1.



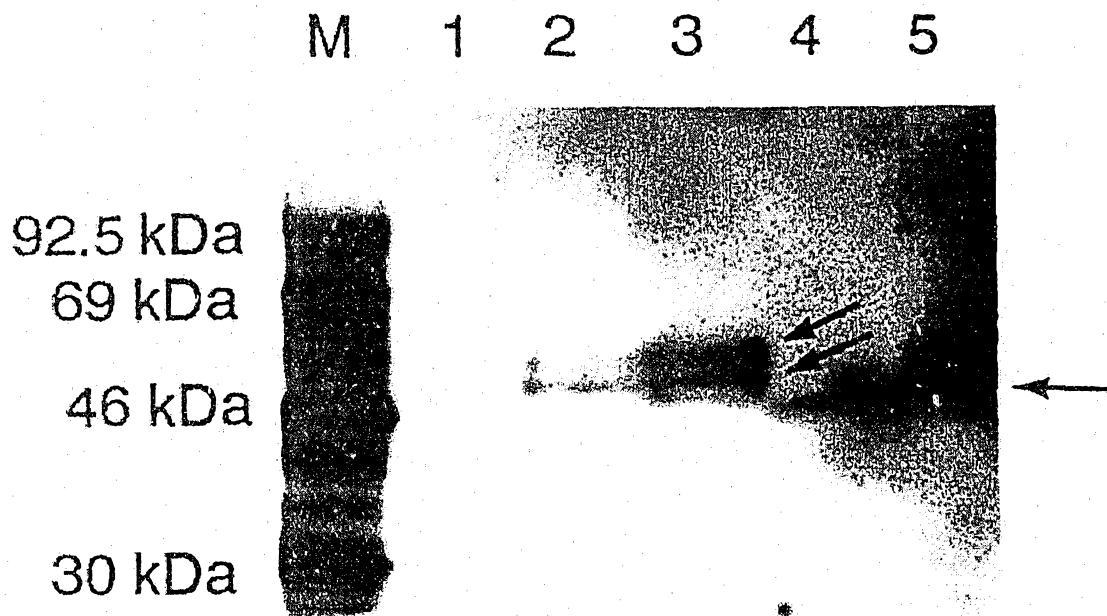


FIG. 2

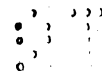
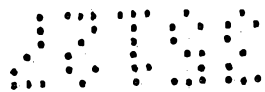


FIG. 3a.

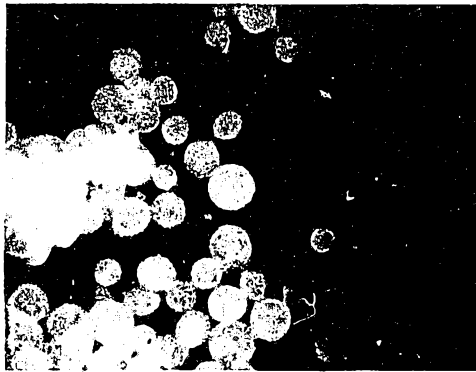


FIG. 3b.

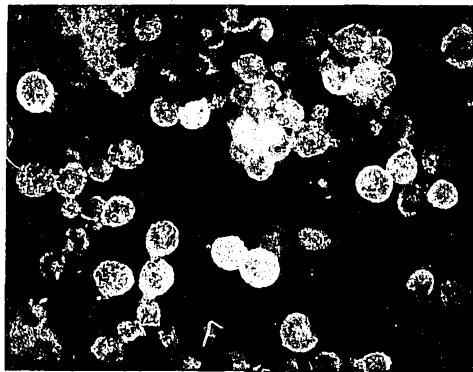


FIG. 3c.



664597

SPRUSON & FERGUSON

AUSTRALIA

PATENTS ACT 1990

PATENT REQUEST: STANDARD PATENT

I/We, the Applicant(s)/Nominated Person(s) specified below, request I/We be granted a patent for the invention disclosed in the accompanying standard complete specification.

[70,71] Applicant(s)/Nominated Person(s):

Cedars-Sinai Medical Center, incorporated in the State of California,
of 8700 Beverly Boulevard, Los Angeles, California, 90048-1865, UNITED
STATES OF AMERICA

[54] Invention Title:

Process for the Expression of Herpes Simplex Virus Type 1
Glycoprotein I and Methods of Use

[72] Inventor(s):

Anthony Bart Nesburn, Steven Lewis Wechsler, Homayon Ghiasi

[74] Address for service in Australia:

Spruson & Ferguson, Patent Attorneys
Level 33 St Martins Tower
31 Market Street
Sydney New South Wales Australia (Code SF)

Details of Basic Application(s):

[31] Appl'n No(s):

[33] Country:

[32] Application Date:

852,999

US

18 March 1992

DATED this TENTH day of MARCH 1993

Cedars-Sinai Medical Center

By:



§ 036029 10 03-93

Registered Patent Attorney

IRN: 233100

INSTR CODE: 57700

8

SPRUSON&FERGUSON

Australia

Patents Act 1990

Notice Of Entitlement

I, John Hinde, of 31 Market Street, Sydney, New South Wales, 2000, Australia, Patent Attorney for the Applicant/Nominated Person in respect of Application No. 35127/93 state the following:-

The Applicant/Nominated Person has entitlement from the actual inventors as follows:-

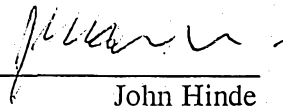
The Applicant/Nominated Person is the assignee of the actual inventors.

The Applicant/Nominated Person is entitled to rely on the basic application listed on the Patent Request as follows:

The Applicant/Nominated Person is the assignee of the basic applicants.

The basic application listed on the Patent Request is the first application made in a Convention country in respect of the invention.

Dated 15 August, 1995



John Hinde