Identification of the feline herpesvirus type 1 (FHV-1) genes encoding glycoproteins G, D, I and E: expression of FHV-1 glycoprotein D in vaccinia and raccoon poxviruses

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The genome of feline herpesvirus type 1 (FHV-1), the major cause of viral upper respiratory disease in cats, contains several genes encoding homologues of herpes simplex virus type 1 (HSV-1) glycoproteins. Restriction mapping studies have indicated that the group D genome of FHV-1 contains a unique short region that is 9.0 kb long. The nucleotide sequence of a 6.2 kb portion of this region was determined. Analyses of this sequence have identified five open reading frames capable of encoding homologues to HSV-1 protein kinase and glycoproteins gG, gD, gI and gE. Since gD of FHV-1 is most likely an immunologically important polypeptide, vaccinia and raccoon poxvirus recombinants expressing this glycoprotein were generated. In an indirect fluorescent antibody test these recombinants reacted strongly with a rabbit anti-FHV-1 serum. High titres of virus-neutralizing antibodies were also generated in rabbits inoculated with the vaccinia virus recombinant. A 53K viral polypeptide (gD) was detected with this antiserum on Western blots containing polypeptides from potassium tartrate-purified virions.

Introduction

Feline rhinotracheitis, a common viral infection of cats, is caused by the alphaherpesvirus feline herpesvirus type 1 (FHV-1) (Crandell & Maurer, 1958; Ditchfield & Grinyer, 1965). Serological studies have indicated that 50 to 70% of adult domestic cats have detectable antibodies to this virus (Povey, 1979; Tham & Studdert, 1987). The prevalence and seriousness of the disease are largely controlled with licensed modified-live virus and inactivated virus vaccines.

As is typical of other herpesviruses, numerous FHV-1 glycoproteins are synthesized and then incorporated into membranes of infected cells and the virion envelope (Fargeaud et al., 1984; Maes et al., 1984; Horimoto et al., 1990; Limcumpao et al., 1990). These glycoproteins have been well characterized and are conserved among related herpesviruses. Herpes simplex virus type 1 (HSV-1) glycoproteins have been shown to be involved in membrane attachment, complement binding and penetration of the virion into cells by cell-to-cell spread (Courtney, 1991; Fuller & Lee, 1992). They also play an important role in the induction of virus-neutralizing (VN) antibodies and cell-mediated immunity (Eberle et al., 1985; Blacklaws et al., 1987; Pereira et al., 1989; Hanke et al., 1991). Animals vaccinated with synthetic HSV-1 glycoprotein D peptides or eukaryotic viruses expressing glycoproteins D or B have been shown to be protected against the establishment of latency by a virulent challenge strain (Long et al., 1984; Cremer et al., 1985; Eisenberg et al., 1985; Cantin et al., 1987; Rooney et al., 1988; McDermott et al., 1989; Wachsmann et al., 1989; Blacklaws et al., 1990; Elton et al., 1990; Hanke et al., 1991; Johnson, 1991).

In order to improve currently available vaccines against feline rhinotracheitis, we decided to identify genes encoding immunologically important glycoproteins. Based on the nucleic acid sequences of the unique short (Us) regions in the genomes of HSV-1, varicella zoster virus (VZV), equine herpesvirus type 1 (EHV-1), pseudorabies virus (PRV) and Marek's disease virus (MDV), a glycoprotein gene cluster (gG, gD, gI and gE) appears to be conserved, with minor variations, throughout the subfamily of alphaherpesvirinae (Davison, 1984; McGeoch et al., 1985; Petrovskis et al., 1986b; Audonnet et al., 1990; Elton et al., 1991a; Flowers et al., 1991; Ross & Binns, 1991; Flowers & O'Callaghan, 1992;
Fig. 1. Genomic organization of the FHV-1 Uₘ genes encoding a putative protein kinase and glycoproteins gG, gD, gI and gE. (a) The 134 kb genome is represented as two unique sequences (Uₘ and Uₛ) and two inverted repeat regions (Iₙ and Tₙ) flanking the Uₛ region. (b) The SaI and EcoRI restriction maps of 13 kb of FHV-1 DNA including the Uₘ and inverted repeats (Rota et al., 1986). (c) A detailed restriction map of the Uₛ region is presented along with the position and transcriptional direction of the genes encoding the putative protein kinase (PK), gG, gD, gI and gE.

Telford et al., 1992). Glycoprotein D is the only glycoprotein encoded by a gene from the Uₛ region of most alphaherpesviruses that is essential for virion production.

In this paper, we report the nucleotide sequence of a 6.2 kb fragment from the Uₛ region of the FHV-1 genome and the identification of five open reading frames (ORFs). Four of these ORFs display homology to those of HSV-1 gG, gD, gI and gE and partial sequencing analysis of the fifth ORF reveals significant homology to the 3' terminus of the HSV-1 Uₛ protein kinase gene. The identification of these Uₛ FHV-1 genes will be of significant value in assessing the immunological roles of these glycoproteins in cats, the natural host. To this end, recombinant vaccinia and raccoon poxviruses expressing gD of FHV-1 were generated. Selection of raccoon poxvirus as a vector was based upon a report by Scott (1988) that this virus replicated to high titres in cats and induced high neutralizing antibody titres. Adverse reactions to raccoon poxvirus were not observed in any of the vaccinees. Since raccoon poxvirus is present in the wildlife population and therefore does not have the concerns associated with vaccinia virus-based vectors it should be an excellent vaccine vector for use in household cats (Moss & Flexner, 1987; Esposito et al., 1988; Knight et al., 1992).

Methods

Cells, viruses and media. Crandell Reese feline kidney (CRFK), Rat-2 thymidine kinase-negative (TK-) and human 143B TK⁺ cells were cultured in MEM containing 100 units/ml of penicillin, 100 µg/ml of streptomycin and 10% heat-inactivated fetal bovine serum (FBS). Escherichia coli strains JM101 and JM109 were grown in L-broth medium and used to propagate recombinant phage M13 mp18 and mp19 clones.

FHV-1 (strain C-27) was obtained from the American Type Culture Collection and propagated in CRFK cells (Maes et al., 1984). Lysates of FHV-1-infected cells were used as the source of viral DNA. Vaccinia virus strain WR, raccoon poxvirus and recombinant viruses derived from each were propagated in 143B cells and plaque-purified in Rat-2 cells in the presence of 25 µg/ml of 5-bromo-2'-deoxyuridine (BUDR).

DNA isolation, cloning and DNA sequencing. Viral DNA was prepared as described previously (Rota et al., 1986). Plasmid DNA was isolated from bacteria by the alkaline lysis method (Sambrook et al., 1989). Single-stranded DNA from M13 phage was isolated by the procedure described by Ausubel et al. (1988). The complete nucleotide sequence of a 6208 bp portion of the Uₛ region was determined (Fig. 1 and 2). The 4.3 kb EcoRI-EcoRI fragment and the adjacent 1.9 kb EcoRI-SalI fragment located at the right terminus (Fig. 1) of the SalI B fragment were chosen for DNA sequence analysis. Hybridization analyses have indicated that these two restriction fragments contain only Uₛ region DNA.

Single-stranded DNA from recombinant M13 phage was sequenced by the standard dideoxynucleotide chain termination method (Sanger et al., 1977) with the modified T7 polymerase Sequenase (US Biochemical). [35S]dATP (New England Nuclear) was used as the label, and dITP was used to resolve band compressions. Synthetic primers were used to generate sequencing data rapidly. The oligonucleotides used in sequencing and PCR were synthesized in a 380B automated DNA synthesizer (Applied Biosystems) with a three column upgrade. Sequencing reaction products were separated by electrophoresis and visualized by autoradiography of the dried 8% acrylamide-7 M-urea gels using Kodak X-AR film. The sequences of both strands of viral DNA were determined at least twice from individual clones.

Computer analyses of the DNA sequence. DNA sequences were compiled on a VAX computer using versions 6.2 and 7.0 of the University of Wisconsin GCG package (UWGGC; Deveroux et al., 1984). Computer management of the sequences was used to verify that both strands of the 6.2 kb fragments were sequenced. Hydropathy analyses of individual predicted translation products were generated by the method of Kyte & Doolittle (1982). Amino acid homology searches of the Swissprot (Release 18.0, 5/91) databases were conducted using the GAP and FASTA programs (UWGGC).

PCR amplification of the FHV-1 gene encoding gD and donor plasmid construction. The complete coding sequence of FHV-1 gD was amplified using flanking oligonucleotides specific for the 5' and 3' termini of the gene. To amplify the gene encoding gD of FHV-1 two oligonucleotides, 5' CATCTCGAGTAAATGATGACACGTCTACA 3' and 5' TGTG-AATTCAAGGATGGTGAGTTGTA 3', were used. The latter oligonucleotide contained an XhoI recognition site, and the former contained an EcoRI recognition site. Incorporation of these two restriction sites into the amplified PCR product facilitated directional cloning. The PCR buffer contained 20 mM-Tris-HCl pH 8.2, 10 mM-KCl, 60 mM-Na₂SO₄, 20 mM-MgCl₂, 0.1% Triton X-100 and 10 µg/ml BSA. The PCR conditions were: 1 min at 60 °C, 2 min at 72 °C and 1 min at 95 °C for 37 cycles with one unit of Pfu polymerase (Stratagene).

The gD PCR-amplified product was digested with XhoI and EcoRI and cloned into the plasmid pKG19 (a gift from Dr Keith Gould). The resulting plasmid (pKGgD) contained the gD gene under the control of the vaccinia virus P₮ promoter and flanked by the 5' and 3' termini of the vaccinia virus TK gene.

Transfections and selection. Recombinant plasmid DNA was purified from transformed DH5 alpha cells using alkaline lysis (Ausubel et al., 1988). The DNA was further purified by centrifugation in caesium
Fig. 2. For legend see p. 1239.
Fig. 2. For legend see opposite.
chloride-ethidium bromide gradients. Human 143B TK cells were then transfected with purified plasmid DNA using the lipofection method (BRL). One hour prior to transfection 35 mm plates of 75% confluent cells were washed with 2 x Opti-MEM 1 reduced serum medium (Gibco-BRL). The cells were then infected with either vaccinia or raccoon poxvirus using an m.o.i. of < 1.0. Lipid-DNA complexes were created by mixing 25 µl of H2O, 25 µl of lipofectin (approximately 30 µg) and 20 µg of recombinant plasmid DNA in a volume of 50 µl. This mixture was incubated for 15 min at room temperature before being added to the infected cells. After adsorption at 37 °C for 4 to 6 h in a 4% CO2 atmosphere, the cells were fed with MEM containing 10% FCS and incubation was then continued for 48 h. Transfected cells were then pelleted by low-speed centrifugation and resuspended in 1.0 ml of MEM. Serial 10-fold dilutions of the viral supernatants were made and Rat-2 cells were infected for 1 h at 37 °C. Following this, 1% low melting point agarose at 45 °C containing MEM and 25 µg/ml BUdR was used to overlay the cells. After an incubation at 37 °C of 48 h for the vaccinia virus recombinants or 56 h for the raccoon poxvirus recombinants, the cells were stained for 3.5 h with 0.5% neutral red. Visible plaques were picked and resuspended in 500 µl of MEM. Recombinants were plaque-purified three times, always in the presence of BUdR (25 µg/ml).

**Immunofluorescence.** Indirect immunofluorescence tests were carried out on cells infected with wild-type poxviruses and transfected with recombinant donor plasmids. Cells were cytosenrifuged and fixed with cold absolute methanol and then blocked with 5% low-fat milk powder in PBS for 1 h. The cells were incubated with a 1/100 dilution of rabbit anti-FHV-1 serum in PBS. After incubation for 1 h at room temperature, the cells were washed twice and a goat anti-rabbit fluorescein-labelled conjugate, diluted in PBS containing 0.1% Evans Blue, was applied to the cells for 30 min. The cells were then washed and fluorescence was observed under a Zeiss u.v. microscope. Photographs were taken with Kodak Ektachrome daylight 1000 ASA film.

**Production of anti-vaccinia virus recombinant and anti-FHV-1 sera.** Female New Zealand white rabbits were injected intradermally with 106 p.f.u. of the vaccinia virus recombinant (VVgD) in 500 µl of PBS. Serum was collected 14 days after inoculation and analysed on immunoblots containing wild-type vaccinia virus. The rabbits were then boosted with 106 p.f.u. of the respective recombinant and bled 2 weeks later. In parallel, rabbits were injected intraperitoneally with 103 TCID50 of FHV-1 C-27 and boosted 3 weeks later.

**Virus neutralization assay.** Antiserum against VVgD were assayed for the presence of VN antibodies in a microtitre virus neutralization assay. Heat-inactivated (56 °C for 30 min) sera were used to make a twofold dilution series. Approximately 100 TCID50 of FHV-1 C-27 was added to each serial dilution. The virus–serum mixtures were incubated for 1 h at 37 °C. CRFK cells (15000) were added to each plate and the plates were incubated at 37 °C in an atmosphere of 5% CO2. The VN titre were expressed as the reciprocal of the highest serum dilution resulting in complete inhibition of cytopathic effect.

**Western blot analyses.** FHV-1 virions from infected CRFK cells were purified by rate zonal centrifugation through 10 to 40% potassium tartrate gradients (Talens & Zee, 1976). Purified virions were resuspended in PBS and separated by SDS-PAGE under denaturing conditions. The blocking agent was 5% low-fat milk powder in 10 mM-Tris-HCl pH 8.0 and 150 mM-NaCl. A mouse anti-rabbit alkaline phosphatase-labelled conjugate, along with the chromogens 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium, was used to visualize the bands.

### Results

**Restriction map of the U5 region of the FHV-1 genome**

As shown in Fig. 1, the 14.5 kb SalI B fragment contains three EcoRI restriction sites. The complete restriction maps of the 4.3 kb EcoRI–EcoRI and the 1.9 kb EcoRI–SalI subfragments were generated by digestions with the restriction endonucleases EcoRI, BamHI, XhoI and EcoRV.

**DNA sequence analysis of a 6.2 kb portion of FHV-1 U5 DNA**

Nucleotide sequence data obtained from the 6.2 kb region of the SalI B fragment are presented in Fig. 2. Examination of the nucleotide sequence revealed the presence of four complete and one partial ORFs.

**Analysis of the major ORFs**

Major features of the genes encoding gG, gD, gI and gE of FHV-1 are summarized in Table 1. The first ORF, extending from the EcoRI site at position 1 to position 211, encodes the last 69 amino acid residues of a suspected protein kinase. A search for amino acid similarities using FASTA and the Swissprot database has shown that this ORF contains the sequence RPSA, a sequence found in all known U5 protein kinases. No evidence for a polyadenylation site was found downstream of the termination codon TAG (Birnstiel et al., 1985).

The second ORF has several features in common with that of gG of HSV-1 and gX of PRV. Two possible initiation codons (AAAATGG and CCAATGG) were located at positions 340 and 415. However, only the initiation codon at 340 is favoured by Kozak's rules (purine residue in the +3 position; Kozak, 1986). No major cis-acting transcription sites (TATA-like elements) were found 5' to the gene, although three CAAT boxes were apparent (Corden et al., 1980). A polyadenylation signal AATAAA was found 3' to the stop codon TAA.

The third ORF, encoding gD, contains two adjacent initiation codons (CTAATGA and ATGATGA), al-
Table 1: Key features of the unique short region glycoproteins of FHV-1

<table>
<thead>
<tr>
<th>ORF</th>
<th>AUG context</th>
<th>Stop codon used</th>
<th>Number of amino acids</th>
<th>Predicted signal sequence cleavage site*</th>
<th>Potential N-linked glycosylation sites</th>
<th>Mr × 10^-3†</th>
</tr>
</thead>
<tbody>
<tr>
<td>gG</td>
<td>(340):~</td>
<td>(1645):~</td>
<td>435</td>
<td>Ala21, Val22</td>
<td>6</td>
<td>61.8</td>
</tr>
<tr>
<td></td>
<td>AAAUUGG</td>
<td>UUA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gD</td>
<td>(2062)</td>
<td>(3181)</td>
<td>373</td>
<td>Ala13, Val14</td>
<td>4</td>
<td>51.4</td>
</tr>
<tr>
<td></td>
<td>AUGAUGA</td>
<td>UUA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gI</td>
<td>(3310)</td>
<td>(4420)</td>
<td>370</td>
<td>Gly18, Ile19</td>
<td>9</td>
<td>62.1</td>
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<tr>
<td></td>
<td>AAUAUGU</td>
<td>UUA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gE</td>
<td>(4601)</td>
<td>(6197)</td>
<td>532</td>
<td>Ser14, Ser17</td>
<td>4</td>
<td>58.3</td>
</tr>
<tr>
<td></td>
<td>ACAUUGG</td>
<td>UGA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Putative signal sequences were determined using the –3, –1 rule (von Heijne, 1986).
† Mr were calculated for the post-translationally processed glycoproteins (Klenk & Rott, 1980).
‡ The nucleotide position of the translational start (AUG) and stop (UAA or UGA) codons (see Fig. 2).

Table 2: Homology comparison of the putative U₄ glycoproteins of FHV-1 with those of other members of the family Herpesviridae

<table>
<thead>
<tr>
<th></th>
<th>gG</th>
<th>gD</th>
<th>gI</th>
<th>gE</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHV-1</td>
<td>57/36*</td>
<td>49/28</td>
<td>56/40</td>
<td>65/47</td>
</tr>
<tr>
<td>EHV-4</td>
<td>59/36</td>
<td>NA†</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>PRV</td>
<td>56/33</td>
<td>50/29</td>
<td>49/29</td>
<td>53/30</td>
</tr>
<tr>
<td>BHV-1</td>
<td>NA</td>
<td>54/33</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>VZV</td>
<td>DC‡</td>
<td>DC</td>
<td>51/30</td>
<td>49/28</td>
</tr>
<tr>
<td>HSV-2</td>
<td>40/21</td>
<td>47/25</td>
<td>40/24</td>
<td>43/22</td>
</tr>
<tr>
<td>HSV-1</td>
<td>42/18</td>
<td>47/25</td>
<td>43/26</td>
<td>47/24</td>
</tr>
<tr>
<td>MDV</td>
<td>DC</td>
<td>DC</td>
<td>47/24</td>
<td>47/22</td>
</tr>
</tbody>
</table>

* Values are percentage similarity/percentage identity, generated using the GAP program (UWGCG).
† NA, No data available.
‡ DC, Does not contain this glycoprotein.

though the latter sequence has the critical purine at position −3. If this second initiation codon is used, then the expected polypeptide would be 373 amino acids long. A TATA-like element at positions 1908 to 1912 is the only potential cis-acting promoter element. No polyadenylation signal was found downstream of the TAA stop codon.

The fourth ORF encodes a protein with extensive homology to gI of HSV-1 and to gp63 of PRV. An initiation codon (AATATGT) is favoured by Kozak’s rules. Potential TATA-like elements at positions 3101 to 3112 and 3156 to 3162 exist as does a CAAT box at position 3056. A poly(A) addition site (AATAAA) is located downstream from the termination codon TAA.

The fifth ORF encodes a polypeptide exhibiting similarities to gE of HSV-1 and gI of PRV. Two initiation codons, (ACAAATGG) at position 4598 and (ACGATAGA) at position 4673, were predicted. The former is favoured by Kozak’s rules. Putative transcription regulatory signals were found 5' of the initiation codon at positions 4423 and 4508. A termination codon is located at positions 6197 to 6199, three base pairs 5' of the SalI site. Since this is the limit of the sequencing analysis, no information about polyadenylation of the gE transcript is available.

Comparison of the FHV-1 U₄ glycoproteins gG, gD, gI and gE

Table 2 shows the amino acid similarities between the individual U₄ glycoproteins of FHV-1 and those of related herpesviruses. These numbers were determined with the UWGCG GAP program. Overall, extensive homology could be demonstrated between predicted translation products of the genes encoding gG, gD, gI and gE of FHV-1 and other members of the varicellosivirus genus.

Construction of recombinant vaccinia and raccoon poxviruses expressing gD of FHV-1

The gene encoding gD of FHV-1 is contained within a 1·5 kb HincII–XhoI restriction fragment from the U₄ region of the genome. In order to construct the donor plasmid (pKGgD), this fragment was gel-purified and used in PCR amplification of the gD gene. The 1·1 kb amplified products were verified by restriction endonuclease analysis (data not shown) and cloned into the vaccinia virus donor plasmid (pKG19). Recombinant donor plasmid was transfected into human 143B or Rat-2 TK− cells, previously infected with either vaccinia or raccoon poxviruses.

Expression of gD in transfected human 143B cells

Transfected cells were analysed for the expression of FHV-1 gD using an indirect fluorescent antibody assay.
Fig. 3. Indirect fluorescence antibody assay. Visualization by immunofluorescence of gD synthesized in vaccinia virus- (a) or raccoon poxvirus- (b) infected Rat-2 cells transfected with pKGgD. Cells infected with either vaccinia (c) or raccoon poxvirus (d) and then transfected with plasmid containing an inverted gene show no evidence of fluorescence. Cells infected with wild type vaccinia (e) or raccoon poxvirus (f) also lack evidence of fluorescence. Fixed cells were treated with a rabbit anti-FHV-1 antibody, followed by a goat anti-rabbit fluorescein isothiocyanate-conjugated antibody. The bar marker represents 25 μm.

As shown in Fig. 3, intense cytoplasmic staining was observed only in transfected cells previously infected with either poxvirus. No fluorescence was observed in the controls. Cells infected with wild-type vaccinia or raccoon poxvirus served as one form of negative control. The other control consisted of cells transfected with vaccinia or raccoon poxvirus donor plasmids containing a gene inverted with respect to the vaccinia virus P5 promoter.

Western blot analysis (Fig. 4) was conducted using rabbit antisera against the vaccinia virus recombinant expressing gD (VVGd) and potassium tartrate-purified FHV-1 virions. One band of 53K was detected at a 1/400 dilution of the polyclonal serum. FHV-1 proteins did not
Fig. 4. Western blot analysis of FHV-1 polypeptides with antisera against VVgD. Denatured purified virions were separated using SDS-PAGE and electrophoretically transferred to Nytran membrane (Schleicher and Schuell). A rabbit anti-FHV-1 serum was used to probe blot (a). Blot (b) was probed with a rabbit anti-VVgD serum. A mouse anti-rabbit alkaline phosphatase-labelled conjugate was used as the secondary antibody. Blot (c) contained prestained M, standards (high range; BRL).

react with the antiserum specific for wild-type vaccinia virus (data not shown). The anti-VVgD serum also had a VN antibody titre of 1024.

Discussion

FHV-1 is a leading cause of viral respiratory disease in cats. Current vaccines can protect against clinical disease, but not against reinfection and latency. It is clear from work done with other herpesviruses that viral glycoproteins are the obvious candidates for inclusion in newer vaccines. Little was known about the glycoproteins of FHV-1 and their respective genes at the onset of this study. Since gD of other alphaherpesviruses has been reported to be an essential immunodominant glycoprotein, the identification of the gene encoding gD in FHV-1 and its study, by expression in poxviruses, seemed logical.

In this report, we present 6.2 kb of nucleotide sequence located within the 9.0 kb Us region of the FHV-1 genome. This sequence contains ORFs capable of encoding polypeptides with homology to the protein kinase and gG, gD, gI and gE of HSV-1. All five ORFs for these glycoproteins are encoded by the same strand of DNA and are oriented in the same direction. The gene order is identical to that of PRV. Based on these results, and extensive homology to related alphaherpesvirus proteins, we propose to designate the five putative FHV-1 gene products as a protein kinase (ORF 1) and gG, gD, gE and gI (ORFs 2, 3, 4 and 5).

FHV-1 ORF 1 encodes the last 69 amino acids of a polypeptide homologous to a serine–threonine protein kinase. Inspection of the multiple alignments of the protein kinases from the Us regions of FHV-1, HSV-1, EHV-1, PRV, VZV and MDV have shown good overall conservation of residues at the C terminus. Eight amino acids are perfectly conserved in the last 70 amino acids of the Us protein kinases. Since no poly(A) signal was found 3' to the protein kinase gene termination codon, it is probable that the protein kinase mRNA terminates at the polyadenylation signal downstream of the gG gene. Another group of suspected co-terminal transcripts encodes gD and gI. As in the case of the protein kinase–gG gene cluster, there is no AATAAA or ATTAAA polyadenylation signal between these two glycoprotein genes. A similar transcriptional organization has been reported for HSV-1, in which many families of overlapping mRNAs with unique 5' ends share common 3' ends (van Zijl et al., 1990; Rixon & McGeoch, 1985; Wagner, 1983).

Homology analyses of predicted Us glycoproteins of FHV-1 have indicated extensive similarities to corresponding glycoproteins of varicelloviruses, especially EHV-1. To add perspective to the numbers presented in Table 2, gap analyses of the Us glycoproteins of two related herpesviruses, HSV-1 and HSV-2, reveal the following percentage identities/similarities: 39/50 (gG), 82/89 (gD), 69/79 (gI) and 24/45 (gE). In multiple alignments of the known Us glycoproteins, stretches of conserved amino acids could be demonstrated at the N termini of gG, gD and gI homologues. Six cysteine residues in the N termini of gD homologues can be perfectly aligned. However, alignment of conserved amino acids of gE homologues could only be demonstrated for residues at the C termini.

The development of poxviruses as a eukaryotic expression vector capable of adequate expression of a variety of herpesvirus genes has resulted in the construction of numerous live poxvirus recombinants that are capable of protecting immunized animals against infection with HSV-1, HSV-2, PRV, EHV-1 and MDV (Cantin et al., 1987; Marchioli et al., 1987; Bell et al., 1990; Blacklaws et al., 1990; Britt et al., 1990; Yanagida et al., 1992). Furthermore, a recombinant vaccinia virus
expressing gD of HSV-1 was the first genetically engineered vaccine that could prevent the development of latency in mice by virulent virus challenge (Cantin et al., 1987). To assess the immunogenic role of the FHV-1 gD homologue, recombinant vaccinia and raccoon poxviruses expressing gD were generated. Recombinant poxviruses were identified in an indirect immunofluorescent antibody assay, using a rabbit anti-FHV-1 serum as the primary antibody. Control constructs containing an inverted PCR product did not react with the antisera (Fig. 3).

The immunogenicity of the recombinant gD polypeptide was further addressed by the response of sera from VVgD-immunized rabbits with proteins from purified FHV-1 virions. By Western blot analysis (Fig. 4), a 53K viral protein was detected by this antisera. Furthermore, a significant complement-independent neutralizing antibody response to virulent FHV-1 was demonstrated in rabbits immunized with the VVgD vaccinia virus recombinant.

The successful generation of these poxvirus recombinants expressing gD of FHV-1 will aid in assessing the role of this glycoprotein in the induction of humoral and cell-mediated immunity in immunized animals. It will also contribute to the development of better immunization strategies for cats against this important viral disease.

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