Properties and Functions of Feline Herpesvirus Type 1 Glycoproteins

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ABSTRACT. Feline herpesvirus type 1 (FHV-1) is a causative agent of feline viral rhinotracheitis and belongs to the subfamily *Alphaherpesvirinae* of the family *Herpesviridae*. Since first isolated in 1958 by Crandell and Maurer, FHV-1 is distributed worldwide and is the most clinically significant agent for respiratory infections in cats. In this review, we describe the recent findings with properties and functions of FHV-1 glycoproteins, especially hemagglutinins. — KEY WORDS: feline herpesvirus type 1, glycoprotein, hemagglutinin.

In 1958, Crandell and Maurer [13] isolated an agent from kittens (*Felis catus*) with an acute upper respiratory tract disease in the United States. The agent causes cytopathic effects in feline kidney cell cultures characterized by polykaryocytosis and formation of intranuclear inclusion bodies in fixed-stained preparations. The designation of "feline viral rhinotracheitis (FVR)" was proposed for the disease [12]. Subsequent works supported Crandell's view that the virus should be classified in the *Herpesviridae* [5, 16] and defined as feline herpesvirus type 1 (FHV-1) [69]. Since the original isolation, FHV-1 has been shown to be the most clinically significant pathogen for respiratory infections in cats [63]. In Japan, the outbreak of the infection was first reported in a colony of cats used for an experimental purpose in 1974 [17].

FVR caused by FHV-1 is a typically acute, febrile, contagious disease of felids characterized by sneezing with ocular and nasal discharges. The incubation period is 2–4 days, and sometimes longer. Mortality is higher in young or debilitated cats, but most of them recover within 7–10 days. The disease is common and widespread in the world. Earlier serological surveys showed that serum neutralizing antibody to FHV-1 is detectable in 50–75% of cat population [65, 79]. In Japan, 20% of the sera of cats for experimental use were positive for FHV-1 antibodies [93].

FHV-1 infection in cats is largely controlled by the use of modified live or inactivated vaccines containing FHV-1 alone or combination with feline calicivirus and feline panleukopenia virus [1–3, 7, 14, 18, 19, 21, 64, 66, 73–75, 92, 94]. None of the vaccines so far developed has been able to provide complete protection in terms of preventing viral replication on re-exposure, but clinical protection is adequately provided. It is unknown whether vaccination can prevent the establishment of a carrier state [22, 62].

FHV-1 is classified as a member of subfamily *Alphaherpesvirinae*, family *Herpesviridae* [69]. Using negative staining of the virions [16, 52, 54, 88] or ultrathin sectioning of FHV-1-infected cells [11, 54], mature virus particles are 120–180 nm in diameter, with various morphologies in shapes and sizes of the envelope surrounding the hexagonal outline of the capsid.

The genome of FHV-1 consists of approximately 126–134 kbp. It has a G+C content of approximately 50%. The genome is divided into two unique portions, the unique long (U_L) and unique short (U_S) regions, by inverted repeat segments (IR) (Fig. 1) [24, 70]. The U_L is 99–104 kbp in size and is composed of unique DNA. The adjacent short segment is approximately 27–30 kbp in size and contains a central portion of Us which is approximately 8–9 kbp in size. The Us region is bounded by inverted repeat sequences of 7–11 kbp.

We have already introduced review articles on recombinant viral vector vaccines for the veterinary use [97] and molecular interactions between retroviruses and herpesviruses [30]. Therefore, this review mainly focuses on some of the recent developments regarding properties and functions of FHV-1 glycoproteins including hemagglutinin.

1. HEMAGGLUTININ TO FELINE RED BLOOD CELL (RBC)

FHV-1 replicates readily in all the cells of feline origin so far tested, which include primary and secondary cell cultures or established cell lines, originated from kidney, thymus, tongue, lung, T-lymphocytes, and neurofibrosarcoma [13, 26, 32, 33, 83]. By contrast, FHV-1 does not replicate in the cells of non-feline origin. There is one report describing adsorptive infection of FHV-1 in human cells [81]. FHV-1 attaches but does not penetrate to human embryonic lung cells, which are naturally resistance to the virus, and following treatment with inactivated Sendai virus, the virus causes characteristic cytopathic effects in
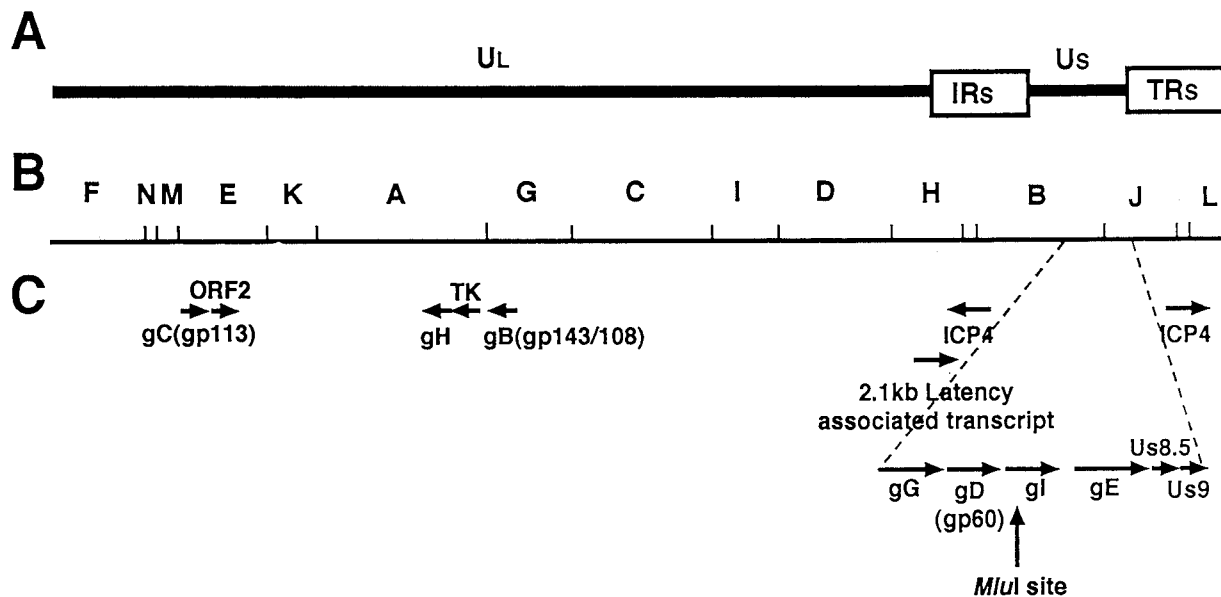
A

UL   Us

IRs   TRs

B

F   NM   E   K   A   G   C   I   D   H   B   J   L

C

ORF2
gC(gp113)

TK
gH   gB(gp143/108)

ICP4

ICP4

2.1kb Latency
associated transcript

Us8.5

gG   gD   gl   gE   Us9
(gp60)

*Mlu*I site

Fig. 1. Location of genes mapped in FHV-1. (A) Schematic diagram of the FHV-1 genome. Open boxes represent inverted repeat regions (IR$_S$=internal repeat; TR$_S$=terminal repeat) that separate the unique long (U$_L$) from the unique short (U$_S$) component. (B) *Sal*I-restriction fragment map [70]. (C) Location of genes. Horizontal arrows indicate transcriptional direction. A vertical arrow shows the heterogeneous *Mlu*I site.

Table 1. Hemagglutinins of herpesviruses

Virus	Natural host	Hemagglutinin	RBC	Inhibition of HA activity by heparin
FHV-1	feline	gD	feline	-
		gC	mouse	+
CHV	canine	gD	canine	-
		gC	mouse	+
EHV-1	equine	?	equine	?
ILTV	chicken	?	chicken	?
HSV-1	human	gC	mouse	+
BHV-1	bovine	gC	mouse	+
PRV	porcine	gC	mouse	+

the cells without releasing infectious virus [81], indicating that penetration step to the cells may regulate FHV-1 cell tropism. *In vivo* the host range appears to be confined to members of the *Felidae*, unlike other alphaherpesviruses such as herpes simplex virus 1 (HSV-1) and pseudorabies virus (PRV). However, it is unknown what factors govern the narrow host range. Here we discuss on a possible contribution of FHV-1 hemagglutinin as a candidate for determining the restricted host range.

FHV-1 agglutinates only feline RBC, but not chicken, guinea pig, and canine RBCs [23, 25, 55]. Other alphaherpesviruses, canine herpesvirus (CHV), equine herpesvirus type 1 (EHV-1) and infectious laryngotracheitis virus (ILTV), also agglutinate RBCs from respective hosts [51, 56, 67]. Like FHV-1, these three viruses have very narrow host ranges. It is possible that such interaction of virus-to-RBCs may reflect characteristics of herpesvirus host ranges (Table 1).

Gillespie *et al*. [23] first reported that FHV-1, propagated

in primary feline cells or diploid feline tongue cells, shows hemagglutination (HA) activity on feline RBC at 4, 20, and 37°C, although its titer is low. Treatment of the virus with tween 80 and ethyl-ether enhances HA activity, indicating that hemagglutinin is an envelope protein. The receptors on feline RBCs are destroyed by trypsin, suggesting a protein in nature [55]. The correlation between virus-neutralization (VN) and HA-inhibition (HI) antibodies in the sera from FHV-1-infected cats is demonstrated [55]. HI test can be used for a serological survey on FHV-1 infection in cats [93]. By solubilization of virus-infected cells with detergents, such as Triton X-100, sodium deoxycholate, or 3-[(3-cholamido-propyl) dimetyl-ammonio]-1-propanesulfinate (CHAPS), high titered hemagglutinins are successfully obtained [25].

By ConA-sepharose, ion-exchange, and gel-exclusion chromatographies, an approximately 59 kDa immunogenic glycoprotein (later termed gp60) is purified as a hemagglutinin [25]. Three monoclonal antibodies (MAbs)

against FHV-1 gp60 possessed HI activities, confirming that the gp60 is the hemagglutinin [27]. A transient expression system with FHV-1 gD in COS-7 cells reveals that its product reacts with MAbs only against gp60 and agglutinates feline RBC [41]. Feline RBC adsorbs to the surface of insect cells infected with a recombinant baculovirus expressing gD. Following immunization of the insect cells expressing gD in mice, the animals produce HI antibodies in the sera [44]. Taken together, FHV-1 gD is confirmed to be the hemagglutinin to feline RBC.

Additionally, the insect cells expressing gD adhere to cell lines of feline-origin, but not to those of non-feline originated [45], indicating existence of a specific interaction between FHV-1 gD and molecules on the surface of the former. Therefore, the FHV-1 gD might be one of the factors determining host range of FHV-1.

Like FHV-1, CHV possesses narrow host range; dog is the only known natural host. The virus grows exclusively in cell types of only canine origin such as primary canine kidney cell cultures and Madin-Darby canine kidney (MDCK) cell line [6, 10, 62, 78]. CHV gD adsorbs to canine RBC, not to feline RBC [47]. Although a common epitope is demonstrated between FHV-1 and CHV gDs by an MAb, which has a complement-independent VN activity to both viruses [36], different HA properties between these two gDs provide a notion that the gD may regulate the narrow host range of each virus at the cell-receptor levels.

2. HEMAGGLUTININ TO MOUSE RBC

Culture supernatants of HSV-1-, bovine herpesvirus type 1 (BHV-1)-, and PRV-infected cells agglutinate mouse RBC, and these HA activities are inhibited by treatment of hemagglutinin with heparin or by treatment of mouse RBC with heparinase [29, 57–59, 82, 84, 85]. It was reported that gCs of these viruses are responsible for their HA activities (Table 1) [50, 58, 59, 84, 85].

Culture fluid from FHV-1-infected Crandell's feline kidney (CRFK) cells agglutinate mouse RBC. However, this HA activity to mouse RBC is not inhibited by HI MAbs

to feline RBC, which recognize FHV-1 gD, indicating the hemagglutinin to mouse RBC is not the gD [our unpublished data].

In FHV-1, addition of heparin to virus inoculum inhibits its infectivity to CRFK cells, suggesting that heparan sulfate on the surface of this cell line is a responsible factor for virus attachment to the cells, like other herpesviruses. Two FHV-1 glycoproteins, gC and gB, are able to bind to column containing heparin-agarose and are eluted with heparin, although the latter is also detected in non-binding fraction. These results indicate that the gC binds to heparan sulfate on the cell surface [48]. Mouse RBC, not feline RBC, adsorbs to the COS-7 cells expressing FHV-1 gC. This hemadsorption is inhibited by addition of heparin but not HI MAbs to feline RBC. By contrast, HA to feline RBC is not inhibited by addition of heparin [our unpublished data]. Taken together, the hemagglutinin to mouse RBC is the gC. Thus, FHV-1 contains two hemagglutinins; heparin-sensitive gC and -insensitive gD.

3. VIRAL GLYCOPROTEINS

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) analysis reveals 23 proteins in the whole FHV-1 particle [20]. Of them, 6 glycoproteins of 125, 116, 112, 83, 70, and 60 kilodalton (kDa) are included. Maes *et al.* [49] reported 17 virus-specific proteins, and 3 immunogenic glycoproteins of 105, 68, and 60 kDa, which react with a goat anti-FHV-1 serum as well as virus-infected cat sera [4]. Compton [9] described 5 glycoproteins of 107, 103, 85, 68, and 59 kDa, in association with virus-infected cell extracts or purified virions. In addition, the 107 kDa and a novel 75 kDa are observed in culture supernatants. Horimoto *et al.* [27] identified four groups of the immunogenic proteins by use of MAbs to FHV-1. MAbs directed to 60, 113, and 143/108 kDa glycoproteins have VN activities, but those to 170 kDa protein do not. Additionally, MAbs to the 60 kDa also show HI activity to feline RBC.

At least four FHV-1 glycoprotein genes are localized in

Table 2. Glycoproteins of FHV-1

Glycoproteins	Location	VN activity	Remarks
gD	U_S	+(-C'[a], +C'[b])	Hemagglutinin to feline RBC Binding to feline-originated cell lines
gB	U_L	+(+C')	A disulfide-linked complex Binding to heparin (?)
gC	U_L	+(+C')	Hemagglutinin to mouse RBC Binding to heparin
gH	U_L	?	?
gI	U_S	?	Nonessential for viral-replication in cell culture Complex with gE
gE	U_S	?	Nonessential for viral-replication in cell culture Complex with gI
gG	U_S	?	?

-C'[a]: Complement-independent virus-neutralizing activity.
+C'[b]: Complement-dependent virus-neutralizing activity.

the U_S region (Fig. 1) [41, 77, 91]. Three more glycoprotein genes are localized in the U_L region [39, 40, 46, 76]. The seven glycoproteins found in FHV-1 so far are designated gB, gC, gD, gE, gI, gH, and gG (Table 2), all of which constitute homologues of other herpesvirus glycoproteins. Of them, at least gI and gE are shown to be nonessential for viral replication in tissue cultures [80, 89].

3-1. gD (gp60)

We described HA property of gD in detail in the section of "HEMAGGLUTININ TO FELINE RBC". MAbs to gD show complement-independent VN activities [27]. A competitive enzyme-linked immunosorbent assay (ELISA) with MAbs reveals at least four different epitopes on the gD [35]. Affinity-purified gD induces VN antibodies in mice [37], and high titers of VN antibodies are also generated in rabbits inoculated with the recombinant vaccinia virus expressing gD [76]. Additionally, mice immunized with recombinant baculovirus-infected cells expressing gD, possess high titers of VN antibodies [44]. Thus, FHV-1 gD appears to be one of the most primary candidates for subunit vaccine against FHV-1 infection.

gDs of some herpesviruses are indispensable for virus growth *in vitro* and *in vivo*, but their biological functions are quite different each other. For example, in HSV-1, gD-negative mutant virus is not able to infect susceptible cells and does not spread by direct cell-to-cell transmission [34]. Conversely, although PRV gD negative mutant virus does not infect cell lines sensitive to PRV infection, the virus spreads by direct cell-to-cell transmission [68]. A recent report [72] however proved that high-passaged gD-deleted PRV can grow in cell cultures, indicating that PRV gD is not essential for viral growth. Meanwhile, varicello-zoster virus (VZV) lacks a gene of gD counterpart [15]. In Marek's disease virus (MDV), a transcriptional product of the gD gene is not obvious by northern blot analysis, although a low level expression of gD is detected in chicken embryo fibroblasts infected with a low passage of virulent strains by indirect immunofluorecence and western blot analyses [60]. Additionally, as described above, FHV-1 gD and CHV gD agglutinate feline RBC and canine RBC, respectively. Such different biological functions of alphaherpesvirus gD counterparts may result in unique characteristics of each virus.

Identification of cell surface molecules that bind specifically to these gDs must be useful for analyses of initial process for herpesvirus infection to susceptible cells contributing to determination of host ranges of herpesviruses.

3-2. gB (gp143/108)

SDS-PAGE analysis shows that gB (gp143/108) in virus-infected cells has molecular masses of 143 and 108 kDa under non-reducing conditions and those of 108, 70, 64, and 58 kDa under reducing conditions [27, 38]. Of them, 108 kDa glycoprotein is not incorporated to virion. Three of the nine MAbs against gB possess complement-dependent VN activities [27]. A competitive ELISA with MAbs reveals only one antigenic site consisting of five similar or overlapping epitopes on the gB [35]. Purified gB induces high titers of VN antibodies in mice [37]. Vaccinia virus expressing gB induces fairly high titers of VN antibodies in rabbits [76]. When the expression plasmid of FHV-1 gB is inoculated intramuscularly into mice, a specific antibody against gB is induced [38]. Therefore FHV-1 gB appears to be also one of the most important candidates for subunit vaccine against FHV-1 infection.

Processing of FHV-1 gB has not been fully elucidated. Amino acid sequence of FHV-1 gB shows that there are two potential cleavage sites (RTRR/S and RSRR/S) by intracelluler endoproteases [76]. Authentic cleavage of these two sites is unknown. However, because FHV-1 gB seems to be a heterodimer, either or both sites must be cleaved. Herpesvirus gBs are one of the most conservative proteins beyond three herpesvirus subfamilies, such as *Alpha-*, *Beta-* and *Gammaherpesvirinae*. Evolutionary trees generated by using 15 herpesvirus gBs show that FHV-1 gB is closer to those of genus *Varicello*, EHV-1, EHV-4, PRV, BHV-1, and VZV than to those of genus *Simplex*, HSV-1 and HSV-2 [39, 76], indicating that FHV-1 should be classified in the former genus.

FHV-1 gB is immunoprecipitated by monospecific rabbit anti-HSV-1 gB sera [76], and is recognized by MAbs against CHV gB [36]. Thus, FHV-1 gB shares common antigenic epitope(s) with HSV-1 gB or CHV gB. HSV-1 gB is essential for virus adsorption, penetration process to the cells, and cell-to-cell spread. Further analyses will be required to determine functions of gB for FHV-1 replication.

3-3. gC (gp113)

We described HA property of gC in detail in the section of "HEMAGGLUTININ TO MOUSE RBC". Recently, nucleotide sequences of FHV-1 gC were determined [46, 90]. The FHV-1 gC expressed in COS-7 or insect cells is recognized by a series of MAbs against FHV-1 gp113, showing that gp113 is the gC. The expressed gC in COS-7 cells is approximately 125-150 and 75 kDa. This 75 kDa protein is thought to be a precursor form of gC. By *N*-glycanase treatment, a molecular mass of gC is reduced to approximately 57 kDa, indicating that the gC possesses *N*-linked oligosaccharides [48]. Some MAbs against gC possess complement-dependent VN activities [27]. Affinity purified-gC induces VN antibody in mice [37]. A competitive ELISA with MAbs reveals two antigenic domains, a neutralizing domain consisting of three overlapping epitopes and a non-neutralizing domain on gC [35].

gC of one field isolate, 91-58, possesses molecular masses of 130 and 80 kDa instead of 113 and 75 kDa in all other isolates. Sequence analyses reveals that this isolate has additional 26 amino acids in *N*-terminal region of the gC [43, our unpublished data]. This insertion must result in an increased molecular mass of this gC. Biological effect of this insertion remains to be elucidated.

3-4. gI and gE

Genes encoding FHV-1 gI and gE are located in Us region [77, 91]. Recombinant vaccinia virus harboring gI or gE

expresses 67 kDa or 82 kDa protein, respectively. These proteins are sensitive to endoglycosidase H (endo H), suggesting that immature forms accumulate in endoplasmic reticulum. Upon co-expression of gE and gI, the expressed gE results in conversion into endo H-resistant gE forms of 95–100 kDa. In case of a single expression of each gene, neither is found on the cell surface, but in case of co-expression, both are found on the cell surface, suggesting that gE-gI interaction is required for transport from the endoplasmic reticulum [53]. A recombinant FHV-1, which has insertion of an expression cassette of β-galactosidase into C-terminal region of the gI gene, becomes attenuated in cats, and it produces smaller plaque in cell cultures than a parent virus does [89]. Sussman *et al.* [80] reported the construction of another recombinant FHV-1 which lacks C-terminal region of the gI gene and 5'-transcriptional control and 5'-encoding regions of the gE gene. The recombinant virus shows a decreased replication in one step growth kinetics and produces very small plaques in cell cultures. Cats inoculated intranasally or subcutaneously with this recombinant FHV-1 respond with only mild clinical signs and induce a strong protective immunity against subsequent virulent FHV-1 challenge [31, 80].

A comparative study among vaccine F2 strain and field isolates reveals the lack of an *Mlu*I site, which is located in the gI gene in F2 [28, 42] (Fig. 1). This mutation results in one amino acid change. It is unknown whether this change correlates with attenuation of FHV-1.

Willemse *et al.* [91] observed the presence of two messenger RNAs of 1.6 kb and 0.9 kb transcribed from the gE genes, which are translated into a polypeptide sequence comprising the C-terminus region of gE. The significance of this finding, which has not been reported previously for any other herpesviruses, is unclear. It is interesting to see whether these transcripts are actually synthesized and have some biological functions for virus replication.

3-5. gH

A gene of FHV-1 gH and its transcript were identified [40]. Although two expression systems of the gH gene; recombinant baculovirus system or transient expression system with long terminal repeat of Raus sarcoma virus as a promoter, are constructed, no gH gene product is detected [our unpublished data]. In other herpesviruses, complex formation with gL is necessary for maturation of gH, indicating that coexpression with gL counterpart may be required for expression of FHV-1 gH. gH counterparts are highly conserved among *Herpesviridae*, and are essential for viral growth *in vitro* and *in vivo*, suggesting that FHV-1 gH might play an important role in infection as well. Further analysis will be expected to clarify this issue.

3-6. gG

Spatz *et al.* [77] identified an ORF of FHV-1 gG, the predicted molecular mass of whose product is 61.8 kDa in unglycosylated form. In other herpesviruses, gGs are non-essential for viral replication *in vitro* and *in vivo*. PRV gG (gX) is secreted in the supernatant of the virus-infected cell line. gG is often used as an antigen for type-specific

serodiagnosis between EHV-1 and EHV-4, or between HSV-1 and HSV-2. A role of herpesvirus gG in viral infection is unknown.

CONCLUSIVE REMARKS

Recent studies have provided some novel information on FHV-1 glycoproteins, which might contribute for understanding of molecular basis on virus replication. Compared with other herpesviruses such as HSV and PRV, FHV-1 possesses a unique characteristics of narrow host range. In the present review, we proposed that herpesvirus gD may determine biological properties of each virus such as host range. A unique finding that FHV-1 contains two hemagglutinins, gD to feline RBC and gC to mouse RBC, would provide an experimental model for better understanding of interaction between herpesvirus and cell receptors at initial process of infection.

For prevention of cats from FVR, development of effective, safe and economical vaccines are expected. In the first step for this purpose, the immunogens that elicit VN antibodies must be identified and characterized. In this review, we described that some FHV-1 glycoproteins are included in the immunogens responsible for humoral immune responses in host animal. Additionally, cellular immunity is also important for prevention of herpesvirus infection. This issue should be studied in future.

Recently, FHV-1 recombinants accommodating some antigens of other pathogens have selected, providing a possible use as polyvalent vaccines [8, 71, 86, 87, 95–97] which could be effective to FHV-1 infection itself as well as other infectious diseases in cats. To develop more safe and effective vaccines, non-essential regions of virus replication which can be used for insertion of foreign sequences and virulence determinants whose deletion could result in attenuation of the virus must be determined. Characterization of biological properties of glycoproteins will contribute to these subjects.

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