Adhesion of Insect Cells Expressing the Feline Herpesvirus Type 1 Hemagglutinin (gD) to Feline Cell Lines

Ken MAEDA, Mitsuru ONO, Yasushi KAWAGUCHI*, Katsunori OKAZAKI1), Naoaki YOKOYAMA, Yukinobu TOHYA, and Takeshi MIKAMI**

Department of Veterinary Microbiology, Faculty of Agriculture, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113 and 1)Department of Veterinary Hygiene and Microbiology, School of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan
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ABSTRACT. Feline herpesvirus type 1 (FHV-1) gD-expressing Sf9 cells adhered to two feline cell lines, but not to the porcine, bovine, or canine cell lines tested. In addition, this adhesion activity was inhibited by a monoclonal antibody against FHV-1 gD. These results showed that the FHV-1 gD might bind to a specific-molecule(s) on the surface of feline cell lines. We discussed a possible importance of the FHV-1 gD in host cell restriction to FHV-1 infection. — KEY WORDS: adhesion, FHV-1 gD, hemagglutinin.

Feline herpesvirus type 1 (FHV-1) is a member of the Alphaherpesvirinae subfamily of Herpesviridae. Clinically, it is considered to be one of the most important pathogens in cats causing primarily an upper respiratory tract disease known as feline viral rhinotracheitis. FHV-1 genes encoding seven glycoproteins, gB, gC, gD, gG, gH, gI, and gE, were identified [14, 17, 18, 21, 22]. With the use of monoclonal antibodies (MAbs) against FHV-1, we identified three kinds of glycoproteins, gB, gC, and gD which induce virus-neutralizing (VN) antibodies [7]. Especially, some of the MAbs against FHV-1 gD had hemagglutination (HA)-inhibition (HI) and complement-independent VN activities, and the gD expressed in SF9 cells or affinity-purified gD induced VN antibody in mouse [7, 13]. Therefore FHV-1 gD is thought to be one of the important candidates for a subunit vaccine against FHV-1 infection and to play an important role in FHV-1 infection.

FHV-1 replicates readily in all the cultured cells of feline origin that have been investigated. These include primary and secondary cultures, or established cell lines originated from kidney, thymus, tongue, lung, T-lymphocytes, and neurofibrosarcoma [5, 6, 10, 13]. In vivo and in vitro the host range also seems to be confined to members of the Felidae [20]. However, it is unknown what causes the narrow host range of FHV-1 infection.

Recently it was reported that gDs of alphaherpesviruses bind to a specific cell surface molecule and are important for virus penetration to cells [1–3, 8, 9, 11]. On the other hand, we reported that the FHV-1 gD expressed in COS-7 cells or Spodoptera frugiperda (SF9) cells possessed both HA and hemadsorption activities against feline red blood cells (RBC), but not against murine RBC [16, 18]. Canine herpesvirus (CHV) gD (hemagglutinin) could agglutinate only canine RBC [15, 19]. These interactions between gD and RBCs from the respective hosts might correlate with the host range of herpesvirus infection. Therefore, we speculated that the FHV-1 gD may bind to a feline-specific cell surface molecule. In this paper, we examined adhesion of FHV-1 gD-expressing SF9 cells to several cell lines originated from cat, dog, cattle, and pig.

Two recombinant Autographa californica nuclear polyhedrosis viruses (rAcNPVs), AcfgD and AcYM [16], were grown in SF9 cells in TC100 medium (GIBCO, Grand Island, N. Y.) supplemented with 10% fetal calf serum (FCS), 0.3% of tryptose phosphate broth (Difco, Detroit, Mich.), and antibiotics. AcfgD-infected SF9 cells expressed FHV-1 gD on the surface of cells, and the expressed gD possessed HA and hemadsorption activities against feline RBC and appears to be biologically authentic [16]. AcYM was used as a control. Crandell feline kidney (CRFK) and Felis catus whole fetus (fcwf)-4 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 8% FCS. Madin-Darby bovine kidney (MDBK) and cloned porcine kidney (CPK) cells were grown in Eagle’s minimal essential medium (EMEM) with 10% calf serum (CS), and Madin-Darby canine kidney (MDCK) cells in EMEM with 5% CS.

SF9 cells were inoculated with AcfgD or AcYM at a multiplicity of infection (M.O.I.) of 10 plaque-forming units (PFU) per cell. At 48 hr p. i., the AcfgD- or AcYM-infected cells were collected and washed twice with phosphate-buffered saline (PBS) containing 3% FCS and 0.1% sodium azide (NaN3). Then the cells were counted and diluted to 1 × 105 cells per 1 ml of PBS containing 3% FCS and 0.1% NaN3. The confluent monolayers of CRFK, fcwf-4, MDCK, MDBK, and CPK cells in 35 mm well were overlaid with 1 × 105 SF9 cells infected with AcfgD or AcYM. These dishes were placed for 30 min at 4°C and washed three times with 2 ml PBS containing 3% FCS and 0.1% NaN3. Figures 1 and 2 showed that the AcfgD-infected SF9 cells bound to only cell lines originated from cat, CRFK and fcwf-4 cells, but did not bind to MDBK, MDCK, and CPK cells originated from other animals. AcYM-infected cells could not bind to any cell lines used (Fig. 2). When AcfgD-
infected Sf9 cells were preincubated with an MAb 25C9 which recognized FHV-1 gD and possessed VN and HI activities [7] for 30 min at 4°C before placing on the CRFK cell monolayer, the adhesion was specifically inhibited by the MAb (Fig. 3). However, the adhesion was not inhibited by MAbs 22F4 and 17C11 against FHV-1 gB and gC, respectively [7], when the MAbs were used as a control.
These results indicated that FHV-1 gD might involve in restrictive adhesion between FHV-1 gD-expressing cells and cells susceptible to FHV-1 infection.

The host range of FHV-1 infection is very narrow both in vivo and in vitro [20]. Similarly, the host range of CHV is also very narrow [4]. A 47 kDa glycoprotein of CHV being responsible for HA was encoded by CHV gD gene [15, 24], and cross-reacted with FHV-1 gD by an MAB 25C9 against FHV-1 gD [12]. This MAB possessed inhibition activities against both HAs by FHV-1 gD and CHV gp47 [12]. Therefore it seems that the gDs of both viruses are structurally very similar, but they agglutinate RBCs from respective host. These reports seem to support our observation that gD of FHV-1 might play an important role in restriction of host range.

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