FULL PAPER *Virology*

Attachment and Penetration of Canine Herpesvirus 1 in Non-Permissive Cells

Kazuo NAKAMICHI, Kentaro OHARA, Yasunobu MATSUMOTO and Haruki OTSUKA*

Department of Global Animal Resource Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan

(Received 16 February 2000/Accepted 26 May 2000)

ABSTRACT. Canine herpesvirus 1 (CHV-1) has a relatively narrow host cell range when compared to other alphaherpesviruses. The early events of CHV-1 infection in a permissive Madin-Darby canine kidney (MDCK) and non-permissive cell lines. In order to quantify attachment and penetration, were investigated quantitative competitive PCR (QCPCR) method was established for quantitation of CHV-1 DNA. In all non-permissive cells tested, no significant decrease in viral attachment was observed. When CHV-1 was treated with heparin, viral attachment to MDCK cells was reduced by 25% of the input CHV-1 attached to MDCK cells even in the presence of 50 μg /m*l* heparin. However, the attachment of CHV-1 to non-permissive cells was severely impaired by heparin treatment. In permissive MDCK cells, about 80% of attached CHV-1 penetrated into cells. However, only 4–10 % of CHV-1 attached to non-permissive cells penetrated into cells. Our data indicated that CHV-1, like other herpesviruses, attached to permissive MDCK cells through two mechanisms: the first one is through the interaction mediated by heparan sulfate (HS) on the cell surface and the second involves unidentified viral component and the cellular receptor. In contrast, the non-permissive cells lacked the cellular receptor for the second attachment mechanism and the defect in viral penetration into non-permissive cell might be related to the lack of the cellular receptor.

KEY WORDS: attachment, CHV-1, penetration, QCPCR.

J. Vet. Med. Sci. 62(9): 965–970, 2000

Canine herpesvirus 1 (CHV-1) is a member of the alphaherpesvirus subfamily [23] and causes a fetal hemorrhagic disease in puppies less than 2 weeks of age and upper respiratory tract infections in adult dogs [4]. CHV-1 has a relatively narrow host cell range *in vivo* and *in vitro* when compared to some other members of alphaherpesvirus such as herpes simplex virus (HSV) and pseudorabies virus (PrV). HSV and PrV can grow in many tissue culture cell lines derived from diverse vertebrate species while CHV-1 can grow only in canine derived cells, such as Madin-Darby canine kidney (MDCK) cells, and most of the cell lines are non-permissive to CHV-1. In several alphaherpesviruses, the attachment and penetration stages have been shown to be major determinants of host cell specificity [20, 27, 28]. Recently, quantitative competitive polymerase chain reaction (QCPCR) analysis of the early events in bovine herpesvirus 1 (BHV-1) infection has suggested that virus attachment and penetration were reduced in hamster lung (HmLu-1) cells, which are semi-permissive to this virus [19]. In this study, we have analyzed the attachment and penetration of CHV-1 in several tissue culture cells by using QCPCR to see whether the attachment and penetration characteristics of CHV-1 might determine the permissive and non-permissive nature of the cells.

MATERIALS AND METHODS

Virus and cells: MDCK, MDBK (Madin-Darby bovine kidney), RK13 (rabbit kidney), CPK (porcine kidney) and HmLu-1 cells were cultivated in Eagle's minimum essential medium (EMEM, Nissui, Japan) supplemented with 7.5% fetal calf serum (FCS) and 60 μg/m*l* of kanamycin. The DFD-6 strain of CHV-1 has been described previously [18].

One-step growth analysis: Cells in 12-well culture dishes were infected with CHV-1 at a multiplicity of infection (MOI) of 1 (1 PFU / cell). After incubation period for 2 hr at 37°C, cells were treated with low-pH solution for 2 min to inactivate any remaining extracellular virus [30], washed with PBS twice and overlaid with prewarmed medium. At different times after infection, cells and supernatants were harvested, and progeny virus was titrated on MDCK cells.

Rapid-cycle PCR and QCPCR: For amplification of CHV-1 DNA, rapid-cycle PCR was performed according to the method described previously [6]. Oligonucleotide primers termed ORF2F (5' ATATTCCCCTCGAAGATGTG 3') and ORF2R (5' CCAAGGGTAACACATAAACA 3') were used to amplify the 106-bp sequence located in the ORF2 gene. The amplification reactions were in glass capillary tubes (Idaho Technology, Idaho, U.S.A.) and were performed in a total volume of 10 μ*l* containing 0.5 U of *Taq* DNA polymerase (Sigma), 0.5 μM each primer, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 μg of bovine serum albumin, 2% sucrose, 0.1 mM cresol red, 0.2 mM each dNTP, 5.5 mM $MgCl_2$, and approximately 100 *n*g of each DNA extract. PCR was carried out in an air thermocycler (the Rapidcycler, Idaho Technology) with the following program: one cycle of 15 sec at 96°C; 30 cycles each consisting of 0 sec at 94°C, 0 sec at 42°C, and 15 sec at 72°C; and a final extension step of 45 sec at 72°C.

For QCPCR assay [19], the internal control (IC) was generated by PCR using the above conditions except that pCT-KdlEH/RV-G was used as template and that primers ORF2F

* CORRESPONDENCE TO: OTSUKA, H., Department of Global Animal Resource Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan.

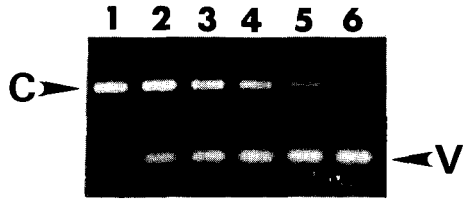

Fig. 1. Examples of QCPCR analysis. A pair of primers termed ORF2F and ORF2R was used to amplify CHV-1 DNA in the presence of competitor plasmid pO2RG DNA ranging from 1 × 10^6 (lane 1) to 244 (lane 6) copies at a 4-fold dilution series. Arrow V indicates the 106-bp PCR products from amplified viral DNA fragment, and arrow C indicates the 158-bp products from the amplified competitor DNA.

and ORF2R were replaced by primers O2RGF (5' ATATTCCCCTCGAA GATGTGATGGTTCCGCAAGCTCTT 3') and O2RGR (5' CCAAGGGTAACAC ATAAACAGATGTATATCGATCGGGCT 3'). The construction of pCTKdlEH/RV-G plasmid containing cDNA of the entire rabies virus (RV) G protein gene was described elsewhere [34]. O2RGF and O2RGR primers were designed to contain the sequence of RV G protein gene at the 3' ends of ORF2F and ORF2R, respectively. The 158-bp DNA fragment, generated by using O2RGF and O2RGR primers, was re-amplified by ORF2F and ORF2R primers and cloned into TA cloning vector plasmid pCR2.1 by using Original TA cloning Kit (Invitrogen), according to the manufacture's instruction. Resulting plasmid, designated here as pO2RG, was subjected to QCPCR assay. QCPCR assay was carried out by using primer pairs, ORF2F and ORF2R, in the same condition as described above except that the competitor plasmid pO2RG, ranging from 1 × 10^6 to 244 copies at a 4-fold dilution series, was added to each reaction mixture (Fig. 1.). Each reaction product was separated on a 1.5% agarose gel in Tris-borate-EDTA (89 mM Tris base, 89 mM boric acid, 2 mM EDTA-2Na [pH 8.0]) (TBE) buffer and the amount of viral DNA was determined from the point of equivalence of the IC and target products as described in our previous report [19].

Attachment assay: Cell monolayers in 12-well culture plates were infected with CHV-1 at an MOI of 1 (1 PFU / cell). After 2-hr incubation period at 4°C, cells were washed with PBS three times, and total DNA (viral and cellular DNA) was extracted with DNAZOL reagent (Gibco BRL) according to the manufacture's protocol. DNA pellets were dried and dissolved in 60 μl of HEPES buffer (6.8 mM NaOH, 15 mM HEPES). Viral DNA in each extract was quantified by QCPCR.

Heparin inhibition assay: Approximately 400 PFU of CHV-1 were incubated for 30 min at 37°C with soluble heparin, ranging from 50 μg to 0.78 μg/m*l* at a 2-fold dilution series. MDCK cells in 48-well culture dishes were infected with heparin-treated virus, and incubated for 2 hr at 37°C. Then, the cells were overlaid with EMEM containing 0.5% (w/v) methylcellulose (MC, Sigma) and 5% FCS, and further incubated at 37°C. After 2 days, cells were fixed and

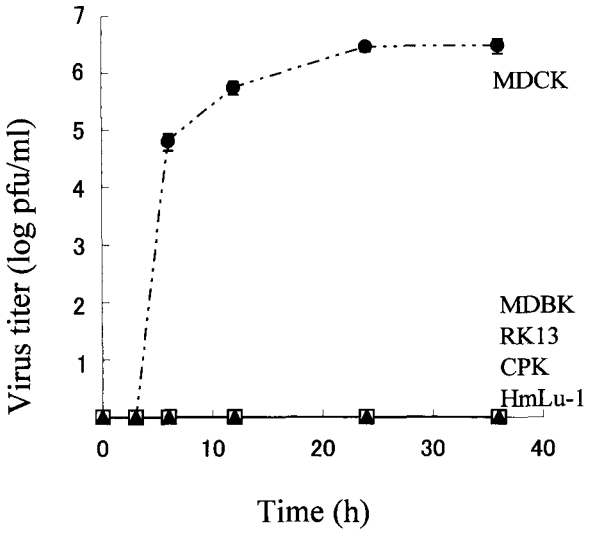

Fig. 2. One-step growth kinetics of CHV-1. MDCK, MDBK, RK13, CPK and HmLu-1 cells were infected with CHV-1 at an MOI of 1 (1 PFU/cell) for 2 hr. After low-pH inactivation of extracellular virions, the infected cells were overlaid with prewarmed medium. At time points indicated, progeny virus in supernatants was titrated on MDCK cells. Mean values and standard deviations from results of two independent experiments are shown.

stained with crystal violet, and plaques were counted as described previously [2]. For heparin inhibition on viral attachment, CHV-1 was incubated for 30 min at 37°C with soluble heparin (1, 5, 10, 50 μg/m*l*). Then, the cells were incubated with heparin-treated virus for 2 hr at 4°C. Total DNA was extracted and subjected to QCPCR as described above. Genome copy number of cell-associated CHV-1 in the absence of heparin was defined as 100% binding.

Neutralization assay: Approximately 400 PFU of CHV-1 were incubated with the heat-inactivated mouse polyclonal antisera against CHV-1 glycoprotein C (gC) (VgC2, VgC3), gB (VgB2, VgB3), or gD (VgD2, VgD3) [33] for 1 hr at 37°C. Then MDCK cells in 48-well plates were inoculated with the assay solution for 37°C. After 2-hr incubation, the inoculum was then removed, and the infected cells were overlaid with MC medium. After 2 days, plaques were counted. For neutralization in the presence of heparin, virus was incubated with 50 μg/m*l* of heparin for 30 min at 37°C before antisera treatment.

Penetration assay: Cells were incubated with CHV-1 for 2 hr at 4°C, washed with PBS and overlaid with prewarmed medium containing 400 μg/m*l* phosphonoacetic acid (PAA) to prevent the replication of viral DNA. After incubation period at 37°C for 4 hr, cells were treated with trypsin solution to remove extracellular virions attached to cell surface [19]. Then total DNA was extracted as described above and subjected to QCPCR.

RESULTS

One-step growth of CHV-1: Growth of CHV-1 in differ-

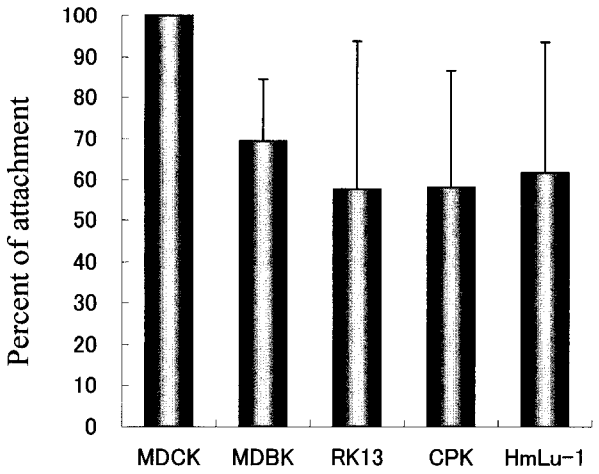

Fig. 3. Attachment of CHV-1 to non-permissive cells. Cell monolayers were incubated with CHV-1 at an MOI of 1 (1 PFU / cell) for 2 hr on ice. The cells were lysed, and total DNA was extracted. The amount of CHV-1 DNA in each extract was quantified by QCPCR, and the number of viral DNA per cell was calculated. Relative copy number of CHV-1 compared to MDCK cells are indicated. Values represent averages of triplicate samples.

ent cell types was examined. MDCK, MDBK, RK13, CPK, and HmLu-1 cells were infected with CHV-1 at an MOI of 1 (1 PFU/cell). At different times postinfection, progeny virus was plaque titrated on MDCK cells. As shown in Fig. 2, the growth of CHV-1 was observed in MDCK cells. In contrast, no infectious progeny was detected in MDBK, RK13, CPK, and HmLu-1 cells, suggesting that these cells are non-permissive to CHV-1 infection.

Viral attachment of CHV-1 to non-permissive cells: We next analyzed the attachment of CHV-1 to these non-permissive cells. Cells were incubated with CHV-1 for 2 hr on ice. Then, total (cellular and viral) DNA was extracted, and viral DNA was quantified by using QCPCR. As shown in Fig. 3, significant reduction in CHV-1 attachment to non-permissive cells tested here was not observed. Therefore, it is unlikely that the inability to attach to cells is the cause of non-permissiveness in MDBK, RK13, CPK and HmLu-1 cells.

Effect of heparin on infectivity and attachment of CHV-1: In order to determine the role of cellular heparan sulfate (HS) for infectivity and attachment of CHV-1, we assessed the inhibition effect by heparin. CHV-1 was treated with various concentrations of heparin for 30 min, and subjected to plaque assay. As shown in Fig. 4 A, 25 μg/ml of heparin reduced the number of plaques formed by CHV-1 on MDCK monolayers by about 75% and further increasing the concentration of heparin up to 50 μg/ml did not result in stronger inhibition. Therefore, it would appear that approximately 25% of plaque formation was not mediated by cellular HS.

Effect of heparin on the attachment of CHV-1 to permissive and non-permissive cells was examined. CHV-1 was

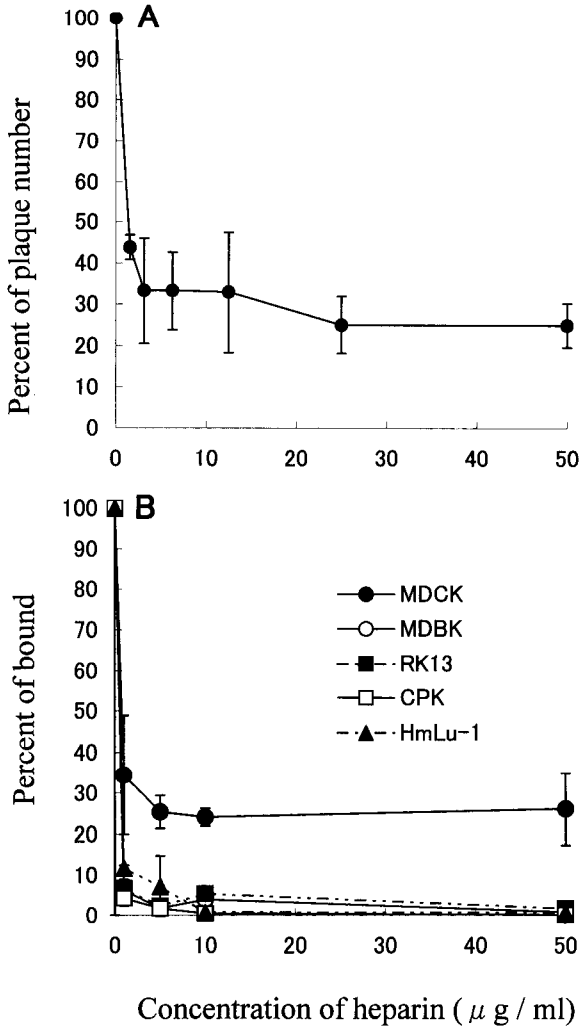

Fig. 4. Heparin inhibition on infectivity and attachment of CHV-1. (A) MDCK cells were infected with CHV-1 treated with serial concentrations of heparin and overlaid with MC medium. After 2 days, the number of plaques was counted. Plaque number in the absence of heparin was defined as 100%. Data are averages of three independent experiments. Vertical lines represent standard deviations. (B) MDCK, MDBK, RK13, CPK, and HmLu-1 cells were incubated with heparin-treated CHV-1 for 2 hr at 4°C. Cells were washed, and total DNA was extracted. The amount of viral DNA in each extract was quantified by using QCPCR method. Values are percentages of viral attachment compared to an untreated control. Data are averages of triplicate samples, and vertical lines indicate standard deviations.

treated with heparin and incubated with permissive MDCK and several non-permissive cell types for 2 hr on ice. Then CHV-1 attached to cells was quantified by measuring viral DNA by QCPCR (Fig. 4B). In MDCK cells, the heparin treatment reduced the CHV-1 attachment to the same extent as plaque formation, and approximately 25% of viral attachment was not blocked by the addition of 50 μg/ml heparin suggesting that CHV-1 can attach to MDCK cells by an

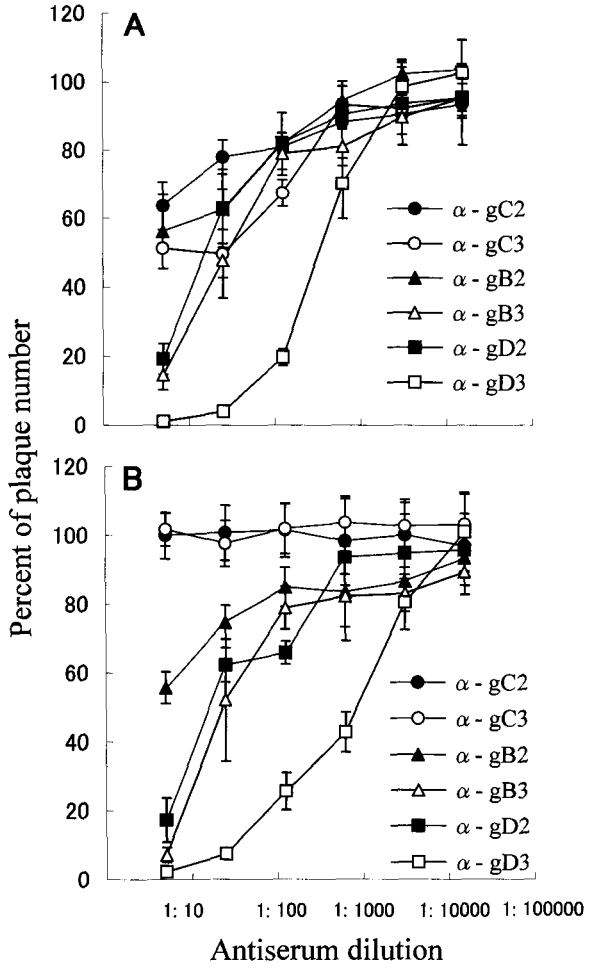

Fig. 5. Neutralization assay. (A) CHV-1 was incubated with the heat-inactivated mouse polyclonal antisera against CHV-1 gC (VgC2, VgC3), gB (VgB2, VgB3), or gD (VgD2, VgD3) for 1 hr at 37°C and subjected to titer determination on MDCK cells. (B) CHV-1 was pretreated with heparin (50 μg/ml) and subjected to neutralization assay. Values are percentages of plaque number compared to an antiserum-untreated control. Data are averages of triplicate samples, and vertical lines indicate standard deviations.

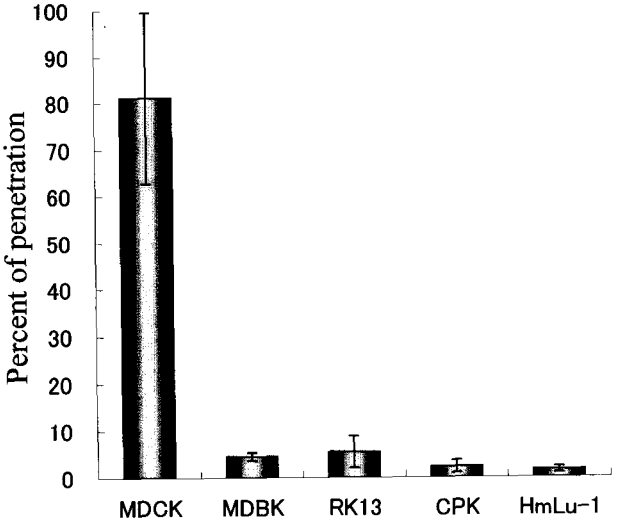

Fig. 6. Penetration of CHV-1 into non-permissive cells. Cells were incubated with CHV-1 at an MOI of 1 (1 PFU/cell) for 2 hr on ice, washed, overlaid with prewarmed medium containing PAA and further incubated at 37°C. At 4 hr after temperature shift, cells were treated with trypsin and washed with PBS. Viral DNA in each DNA extract was quantified by using QCPCR. The percentage of genome copies of CHV-1 surviving the trypsin treatment was calculated with reference to the values for virus attachment for 2 hr at 4°C and is shown as percent penetration. Values represent averages of triplicate samples, while the vertical lines indicate the standard deviation.

interaction, which is independent of cellular HS. On the other hand, in non-permissive cells more than 95% of virus attachment was impaired by the treatment of 50 μg/ml of heparin.

Sensitivity of heparin-treated CHV-1 to anti-gC, gB, and gD antibodies: The interaction between MDCK cells and gC, gB and gD of CHV-1, which are known to be involved in viral attachment and penetration, was investigated by viral neutralization assay using antisera against these major glycoproteins. As shown in Fig. 5 A, each antiserum against gC, gB, and gD neutralized CHV-1 infectivity on MDCK cells in the absence of heparin. In the experiments shown in Fig. 5 B, CHV-1 was treated with 50 μg/ml of heparin before neutralization by antisera. The heparin treatment reduced the infectivity of CHV-1 by 75% as demonstrated in Fig. 4 A. It is seen that the antisera against CHV-1 gC did not reduce the infectivity of heparin-treated CHV-1 further down. In contrast, antisera, which recognize CHV-1 gD and gB, neutralized the infectivity of CHV-1 treated with heparin.

Reduced penetration of CHV-1 into non-permissive cells: Viral penetration of CHV-1 into permissive MDCK and several non-permissive cells was analyzed by using QCPCR method. Cells were incubated with CHV-1 for 2 hr on ice. Thereafter, cells were washed with PBS and overlaid with prewarmed medium and incubation was continued at 37°C. In order to prevent the replication of viral DNA, PAA was included in medium. At 4 hr after temperature shift, cells were trypsinized to remove extracellular virions. Then, total (cellular and viral) DNA was extracted, and viral DNA was quantified by using QCPCR. As shown in Fig. 6, in permissive MDCK cells, approximately 80% of attached CHV-1 could penetrate into cells. In contrast, penetration into non-permissive cells was greatly impaired and only a small portion (1-6 %) of attached virions could penetrate into cells.

DISCUSSION

Infection of alphaherpesviruses involves virus attachment to the cell surface membrane followed by viral penetration

and entry of the nucleocapsid into the cytoplasm [26]. In HSV, BHV-1, and PrV, initial attachment of free virions to target cells is mediated by interaction between gC and HS proteoglycans in the cell membrane [7, 16, 21]. This interaction is sensitive to competition by exogenous heparin, a structural analog of HS. HS-binding activity has also been shown for gB of HSV-1 [8], PrV [24, 25], and BHV-1 [3, 12]. A secondary interaction involves binding of gD to non-HS receptors and leads to a more stable attachment [10, 11, 13, 14, 22, 27]. At present, several cellular proteins have been reported as candidates for gD receptors required for HSV stable attachment and entry. These include a 62-kDa protein identified by anti-idiotype antibody to gD [9] and herpes virus entry mediator [5, 17, 31, 32]. Subsequently, fusion between the virion envelope and the cell membrane occurs. This penetration step requires presence of gB, gD, gH, and gL [12, 15, 26].

In this study, we demonstrated that CHV-1 attached to the surface of non-permissive MDBK, RK13, CPK, and HmLu-1 cells (Fig. 3) and this attachment was almost totally inhibited by exogenous heparin (Fig. 4 B). On the other hand, CHV-1 attached to permissive MDCK cells at a slightly higher level than that to non-permissive cells, and about 25% of CHV-1 remained to be attached to MDCK cells even in the presence of high concentration of heparin. When heparin-treated CHV-1 was incubated with anti-CHV-1 gB or gD antiserum, viral infectivity was further reduced. However, anti-CHV-1 gC antiserum did not neutralize heparin-treated CHV-1 (Fig. 5 B). These results suggested that CHV-1, like HSV and other herpesviruses, attached to permissive MDCK cells through two mechanisms: the one is through the interaction between gC and HS and the other involves the unidentified cellular receptor and the viral components other than gC. When gC-HS interaction was inhibited by heparin, CHV-1 was able to attach to permissive MDCK cells via the second mechanism. In the presence of heparin, gC is not involved in the attachment, and therefore anti-gC serum has no effect. The fact that anti-CHV-1 gB and gD antibodies reduced the infectivity of heparin treated CHV-1 would support the gD and gB are involved in the second attachment mechanism. It would appear that CHV-1 attached to non-permissive cells only through the heparin sensitive mechanism. It might be possible that these non-permissive cells lacked the cell surface receptor(s) for CHV-1 gD and/or gB. We observed here that the penetration of CHV-1 into non-permissive cells was severely impaired when compared to permissive MDCK cells (Fig. 6). This defective penetration of CHV-1 into non-permissive cells might be explained by the absence of CHV-1 gD and/or gB receptor(s) required for viral penetration.

ACKNOWLEDGEMENT. This work was partly supported by the grants from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES

1. Banfield, B. W., Leduc, Y., Esford, L., Shubert, K. and Tufaro, F. 1995. Sequential isolation of proteoglycan synthesis mutants by using herpes simplex virus as selective agent: evidence for a proteoglycan-independent virus entry pathway. *J. Virol.* 69: 3290–3298.
2. Brack, A., Dijkstra, J., Granzow, H., Klupp, B. G. and Mettenleiter, T. C. 1999. Inhibition of virion maturation by simultaneous deletion of glycoproteins E, I, and M of pseudorabies virus. *J. Virol.* 73: 5364–5372.
3. Byrne, K. M., Horohov, D. and Kousoulas, K. 1995. Glycoprotein B of bovine herpesvirus-1 binds heparin. *Virology* 209: 230–235.
4. Carmichael, L. E., Squire, R. A. and Krook, L. 1965. Clinical and pathologic features of a fetal viral disease of new born pups. *Am. J. Vet. Res.* 26: 803–814.
5. Geraghty, R. J., Krummenacher, C., Cohen, G. H., Eisenberg, R. J. and Spear, P. G. 1998. Entry of alphaherpesvirus mediated by poliovirus receptor-related protein 1 and poliovirus receptor. *Science* 280: 1618–1620.
6. Heaton, P. R., McElhinney, L. M. and Lowings, J. P. 1999. Detection and identification of rabies-related viruses using rapid-cycle PCR. *J. Virol. Methods* 81: 63–69.
7. Herold, B. C., WuDunn, D., Soltys, N. and Spear, P. G. 1991. Glycoprotein C of herpes simplex virus type 1 plays a principal role in the adsorption of virus to cells and in infectivity. *J. Virol.* 65: 1090–1098.
8. Herold, B. C., Visalli, R., Susmarski, N., Brandt, C. and Spear, P.G. 1994. Glycoprotein C-independent binding of herpes simplex virus to cells requires cell surface heparan sulfate and glycoprotein B. *J. Gen. Virol.* 75: 1211–1222.
9. Huang, T. and Campadelli-Fiume, G. 1996. Anti-idiotypic antibodies mimicking glycoprotein D of herpes simplex virus identify a cellular protein required for virus spread from cell to cell and virus-induced polykaryocytosis. *Proc. Natl. Acad. Sci. U.S.A.* 93: 1836–1840.
10. Karger, A. and Mettenleiter, T. C. 1993. Glycoproteins gIII and gp50 play dominant roles in the biphasic attachment of pseudorabies virus. *Virology* 194: 654–664.
11. Lee, W-C. and Fuller, A. O. 1993. Herpes simplex virus type 1 and pseudorabies virus bind to a common saturable receptor on Vero cells that is not heparan sulfate. *J. Virol.* 67: 5088–5097.
12. Li, Y., van Drunen Littel-van den Hurk, S., Babiuk, L. and Liang, X. 1995. Characterization of cell binding properties of bovine herpesvirus 1 glycoproteins B, C, and D: identification of a dual cell-binding function of gB. *J. Virol.* 69: 4758–4768.
13. Ligas, M. W. and Johnson, D. C. 1988. A herpes simplex virus mutant in which glycoprotein D sequences bind to but is unable to penetrate into cells. *J. Virol.* 62: 1486–1494.
14. McClain, D. S. and Fuller, A. O. 1994. Cell-specific kinetics and efficiency of herpes simplex virus type 1 entry are determined by two distinct phases of attachment. *Virology* 198: 690–702.
15. Mettenleiter T. C. 1994. Initiation and spread of α-herpesvirus infections. *Trends Microbiol.* 2: 2–4.
16. Mettenleiter, T. C., Zsak, L., Zuckermann, F., Sugg, N., Kern, H. and Ben-Porat, T. 1990. Interaction of glycoprotein gIII with a cellular heparin-like substance mediates adsorption of pseudorabies virus. *J. Virol.* 64: 278–286.
17. Montgomery, R. I., Warner, M. S., Lum, B. J. and Spear, P. G. 1996. Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. *Cell* 87: 427–

436.

18. Motohashi, T. and Tajima, M. 1966. Isolation of a herpesvirus from a diseased adult dog in Japan. *J. Vet. Sci.* 28: 307–314.
19. Murata, T., Takashima, Y., Xuan, X. and Otsuka, H. 1999. Growth behavior of bovine herpesvirus-1 permissive and semi-permissive cells. *Virus Res.* 61: 29–41.
20. Nixdorf, R., Schmidt, J., Karger, A. and Mettenleiter, T. C. 1999. Infection of Chinese hamster ovary cells by pseudorabies virus. *J. Virol.* 73: 8019–8026.
21. Okazaki, K., Matsuzaki, T., Sugahara, Y., Okada, J., Hasebe, M., Iwamura, Y., Ohnishi, M., Kanno, T., Shimizu, M., Honda, E. and Kono, Y. 1991. BHV-1 adsorption is mediated by the interaction of glycoprotein gIII with heparinlike moiety on the cell surface. *Virology* 181: 666–670.
22. Perez, A. and Fuller, A. O. 1998. Stable attachment for herpes simplex virus penetration into human cells requires glycoprotein D in the virion and cell receptors that are missing for entry-defective porcine cells. *Virus Res.* 58: 21–34.
23. Roizman, B., Desrosiers, R. C., Fleckenstein, B., Lopez, C., Minson, A. C. and Studdert, M. J. 1995. The family Herpesviridae: an up date. *Arch. Virol.* 123: 425–449.
24. Sawitzky, D., Hampl, H. and Habermehl, K.-O. 1990. Comparison of heparin-sensitive attachment of pseudorabies virus (PRV) and herpes simplex virus type 1 and identification of heparin-binding PRV glycoproteins. *J. Gen. Virol.* 71: 1221–1225.
25. Sawitzky, D., Voigt, A., Zeichhardt H. and Habermehl, K.-O. 1996. Glycoprotein B (gB) of pseudorabies virus interacts specifically with the glycosaminoglycan heparin. *Virus Res.* 41: 101–108.
26. Spear, P. G. 1993. Entry of alphaherpesviruses into cells. *Semin. Virol.* 4:167–180.
27. Subramanian, G., McClain, D. S., Perez, A. and Fuller, A. O. 1994. Swine testis cells contain functional heparan sulfate but are defective in entry of herpes simplex virus. *J. Virol.* 68: 5667–5676.
28. Subramanian, G., LeBlanc, R. A., Wardley, R. C. and Fuller, A. O. 1995. Defective entry of herpes simplex virus type 1 and type 2 into porcine cells and lack of infection in infant pigs indicate species tropism. *J. Gen. Virol.* 76: 2375–2379.
29. Svennerholm, B., Jeansson, S., Vahlme, A. and Lycke, E. 1991. Involvement of glycoprotein C (gC) in adsorption of herpes simplex virus type 1 (HSV-1) to the cell. *Arch. Virol.* 120: 273–279.
30. Van Drunen Littel-van den Hurk, S., Khattar, S., Tikoo, S. K., Babiuk, L. A., Baranowski, E., Plainchamp, D. and Thiry, E. 1996. Glycoprotein H (gII/gp108) and glycoprotein L form a functional complex which plays a role in penetration, but not in attachment, of bovine herpesvirus 1. *J. Gen. Virol.* 77: 1515–1520.
31. Warner, M. S., Geraghty, R. J., Martines, W. M., Montgomery, R. I., Whitbeck, J. C., Xu, R., Eisenberg, R. J., Cohen, G. H. and Spear, P. G. 1998. A cell surface protein with herpesvirus entry activity (HveB) confers susceptibility to infection by mutants of herpes simplex virus type 1, herpes simplex virus type 2, and pseudorabies virus. *Virology* 246: 179–189.
32. Whitbeck, J. C., Peng, C., Lou, H., Xu, R., Willis, S. H., Ponce de Leon, M., Peng, T., Nicola, A. V., Montgomery, R. I., Warner, M. S., Soulika, A. M., Spruce, L. A., Moore, W. T., Lambris, J. D., Spear, P. G., Cohen, G. H. and Eisenberg, R. J. 1997. Glycoprotein D of herpes simplex virus (HSV) binds directly to HVEM, a member of the tumor necrosis factor receptor family and a mediator of HSV entry. *J. Virol.* 71: 6083–6093.
33. Xuan, X., Kojima, A., Murata, T., Mikami, T. and Otsuka, H. 1997. Analysis of canine herpesvirus gB, gC and gD expressed by a recombinant vaccinia virus. *Arch. Virol.* 142: 1003–1010.
34. Xuan, X., Tuchiya, K., Sato, I., Nishikawa, Y., Onodera, Y., Takashima, Y., Yamamoto, A., Katsumata, A., Iwata, A., Ueda, S., Mikami, T. and Otsuka, H. 1998. Biological and immunogenic properties of rabies virus glycoprotein expressed by canine herpesvirus vector. *Vaccine* 16: 969–976.