The Feline Herpesvirus Type 1 ICP4 Down-Regulates Feline Immunodeficiency Virus Long Terminal Repeat (LTR)-Directed Gene Expression Via the C/EBP Site in the LTR

Yasushi KAWAGUCHI*, Ken MAEDA, Marcelo R. PECORARO, Yasuo INOSHIMA, Hyung-Kwan JANG, Mariko KOHMOTO, Kiyoko IWATSUKI, Yasuhiro IKEDA, Masahiro SHIMOJIMA, Yukinobu TOHYA, Chieko KAI, and Takeshi MIKAMI

Department of Veterinary Microbiology, Faculty of Agriculture, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan
(Received 7 June 1995/Accepted 24 August 1995)

ABSTRACT. We investigated effects of feline herpesvirus type 1 (FHV-1) ICP4 on feline immunodeficiency virus (FIV) long terminal repeat (LTR)-directed gene expression by transient transfection assay in Crandell feline kidney cells. We demonstrated that FHV-1 ICP4 significantly stimulates the FIV LTR after introduction of site-specific mutation of the C/EBP site in the LTR, and the C/EBP site is sufficient to confer inhibitory effects by FHV-1 ICP4 on a heterologous promoter. These results indicate that FHV-1 ICP4 possesses both ability to transactivate FIV LTR-directed gene expression and to down-regulate the FIV LTR via the C/EBP site. — **KEY WORDS:** FHV-1 ICP4, FIV LTR, C/EBP site.

J. Vet. Med. Sci. 57(6): 1129–1131, 1995

Feline immunodeficiency virus (FIV) belongs to the genus *Lentivirus* of the family *Retroviridae* [13, 14]. FIV shares some biological characteristics with human immunodeficiency virus (HIV) such as cell tropisms for T-lymphocytes and macrophages *in vivo* [13] and association with immunodeficiency-like diseases in cats [13]. Further, cats are the smallest animal that can be naturally infected with lentivirus. From these reasons, the infection is considered to be one of the most promising animal models for studies of acquired immunodeficiency syndrome (AIDS) in humans.

Lentivirus infection is characterized as having a long latency period before clinical signs appear [1, 13]. Mechanisms for terminating lentivirus latency leading to the progression of clinical disorders are unknown; however, herpesviruses have been implicated as potential cofactors that trigger and progress an active lentivirus infection because human and animal herpesviruses possess abilities to stimulate lentivirus long terminal repeat (LTR)-directed gene expression [3, 12]. In FIV, it has been shown that cats latently infected with feline herpesvirus type 1 (FHV-1), which is a member of the alphaherpesvirus and causes a severe respiratory tract disease in feline species, frequently developed clinical diseases typical of feline AIDS, recurrent upper respiratory diseases, 18–24 month after experimental FIV infection and high titers of FHV-1 were isolated from these cats [2]. These results suggest that FHV-1 may play a role in the development of feline AIDS *in vivo*. During the last five years, we have investigated the interaction between FIV and FHV-1 *in vitro* and demonstrated that (i) FHV-1 possesses ability to transactivate FIV LTR-directed gene expression in Crandell feline kidney (CRFK) and *Felis catus* whole fetus 4 (fcwf-4) cells [9], (ii) feline T lymphocytes are productively co-infected with both FIV and FHV-1 *in vitro* [9], (iii) the sequence between positions -63 and -23

(relative to the cap site) of the FIV LTR responds to activation by FHV-1 in fcwf-4 cells [10], and (iv) products of FHV-1 immediate-early (IE) gene encoding a homologue to the ICP4 of herpes simplex virus type 1 (HSV-1) can transactivate the FIV LTR in human colon carcinoma cells (SW480 cells) but not in the two feline cell lines (CRFK and fcwf-4 cells) [6]. In the course of the investigation, we have also found unique facts that ICP4 homologues of the other alphaherpesviruses including pseudorabies virus (PRV), HSV-1 and equine herpesvirus type 1 (EHV-1) significantly inhibit FIV replication and down-regulate FIV gene expression in CRFK and fcwf-4 cells [7] and the C/EBP site in the FIV LTR is involved in the negative regulation [11]. It is of interest that why the FHV-1 ICP4 can not have effects on FIV LTR-directed gene expression in the feline cell lines despite a high degree of homology and a similar regulatory characteristic to ICP4s of the other alphaherpesviruses [6]. In the present study, we focused on effects of the FHV-1 ICP4 on FIV LTR-directed gene expression via the C/EBP site.

CRFK cells (ATCC CCL94) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and antibiotics. pTM1 CAT and pSA CAT which contains the bacterial chloramphenicol acetyltransferase (CAT) gene under the control of the FIV LTR and simian immunodeficiency virus_{AGM}, respectively, were described previously [9, 17]. Site-specific mutants of pTM1 CAT, pdEBP CAT and pdATF CAT (Fig. 1A), and pRVfIE expressing FHV-1 ICP4 gene which was constructed using pRVSV [16] were described elsewhere [6, 11]. pH1EBP CAT and pH1dEBP CAT (Fig. 1B) which contain the region (-106 to -65) of the FIV LTR including the C/EBP site and the region including a mutated version of the C/EBP site, respectively, were described [11].

For transfection of plasmid DNAs into CRFK cells, the cells were plated in six-well plastic microplates. Plasmid DNAs were transfected by calcium phosphate method [5]. At 4 hr after transfection, the cells were washed with DMEM

* PRESENT ADDRESS: KAWAGUCHI, Y., The Marjorie B. Kovler Viral Oncology Laboratories, The University of Chicago, 910E. St., Chicago, IL 60637, U.S.A.

A **B**

Fig. 1. (A) Construction of specific mutants of the FIV LTR. The top line shows sequence of the FIV LTR from -142 to -17. The relative positions of the AP-1, AP-4, C/EBP, and ATF sites, the imperfect direct repeat (IDR), and TATA element are indicated. The numbers below the sequence refer to nucleotide positions relative to the cap site. Also shown are positions of the linker-scanner mutant and endpoints of deletion (pdATF CAT). Box indicates the position of the *EcoRI* linker. (B) Construction of heterologous promoter fused to the FIV C/EBP site. pH1o CAT contains an *AvaI-NarI* fragment of the HIV-1 LTR. pH1EBP CAT contains the region (-106 to -65) of the FIV LTR ligated 5' to pH1o CAT. pH1dEBP CAT contains the region of the FIV LTR including a mutated version of the C/EBP site.

Reporter Plasmids

Fig. 2. Effects of FHV-1 ICP4 on deletion mutants of the FIV LTR (A) and insertion of the C/EBP site of the FIV LTR into the HIV LTR (B). CRFK cells were transfected with each reporter plasmid (0.5 μ g) along with each effector plasmid (pRVSV or pRVfIE). Fold increases were evaluated by comparison of the CAT activity associated with the target plasmid in the presence of the indicated effector plasmid versus that in the presence of pRVSV. The values are averages of three independent experiments and standard deviations are shown.

and glycerol-shocked, and then fresh medium was added. Amounts of reporter plasmids are indicated in Fig. 2. The effector plasmids were transfected in equimolar (0.9 pmol) and a final amount of DNA applied per transfection was adjusted to an equal amount by addition of pUC19. At 48 hr after transfection, cell monolayers were harvested for CAT assay. Cell extracts were prepared as described [4]. CAT activity was assayed by solvent partition method as described elsewhere [15] and was presented as the net dpm

of products formed per hour.

To investigate the effect of FHV-1 ICP4 on the FIV LTR in which the C/EBP site was deleted, cotransfection experiments were performed in CRFK cells using site-specific mutants of the FIV LTR and the FHV-1 ICP4 expression vector. As shown in Fig. 2, when pRVfIE was cotransfected with pTM1 CAT, pSA CAT or pdATF CAT, neither increase nor decrease in CAT activity was observed. To the contrary, cotransfection of pRVfIE with pdEBP CAT

resulted in an approximately 20-fold induction of CAT activity. Next, to assess further the ability of the C/EBP site in the FIV LTR to confer the negative regulation by the FHV-1 ICP4, we carried out cotransfection experiments in CRFK cells using the FHV-1 ICP4 expression vector and reporter plasmids which were placed the sequences including the C/EBP site or a mutated C/EBP site of the FIV LTR upstream of the HIV LTR (Fig. 1B). When the mutated FIV C/EBP site was inserted 5' to the HIV LTR, the FHV-1 ICP4 could induce approximately 6-fold levels of CAT activity, while the insertion of the FIV C/EBP site failed to activate the CAT expression (Fig. 2B). These results suggest that the FHV-1 ICP4 possesses not only the ability to transactivate the FIV LTR efficiently but also that to down-regulate the FIV LTR via the C/EBP site.

ICP4 of alphaherpesvirus is known to be one of the most important regulators for virus gene expression and is also characterized as a multifunctional regulator that stimulates a variety of heterologous viral promoters and down-regulates its own promoter [18]. The FHV-1 ICP4 gene product shows a high degree of homology to those of the other alphaherpesviruses at amino acid levels [6], and is also capable of both transactivating various heterologous promoters [6] and down-regulating its own promoter [8]. Although our previous experiment demonstrated that the expression of FIV LTR-directed CAT activity was not affected by the expression of the FHV-1 ICP4 in CRFK and fcwf-4 cells [6], it could be conceivable that the FHV-1 ICP4 may down-regulate FIV LTR-directed gene expression via the C/EBP site from the functional analogy to other alphaherpesvirus ICP4s. As expected, we demonstrated here that the FHV-1 ICP4 could possess the ability to down-regulate the FIV LTR via the C/EBP site as the other alphaherpesvirus ICP4s, indicating that the negative regulation via the C/EBP site in the FIV LTR could be a common characteristic among alphaherpesvirus ICP4s.

We also demonstrated that the FHV-1 ICP4 is able to efficiently transactivate FIV LTR-directed gene expression in the absence of the C/EBP site in CRFK cells. To the contrary, the PRV ICP4 induced only a slight increase in FIV LTR-directed gene expression even after deletion of the C/EBP site [11]. In addition, we previously observed that the FHV-1 ICP4 could have no effect on FIV LTR gene expression in CRFK cells [6], in which the other alphaherpesvirus ICP4s significantly down-regulate the FIV LTR. These facts suggest that the ability of the FHV-1 ICP4 to transactivate the FIV LTR could be higher in the feline cell lines than those of the ICP4s of HSV-1, PRV and EHV-1. Interestingly, the FHV-1 ICP4 as well as the ICP4s of HSV-1, PRV and EHV-1 can stimulate the FIV LTR in human cell line, SW480 cells in almost the same degree

(Kawaguchi *et al.*, unpublished observation), suggesting that the higher ability may be dependent on cell types.

ACKNOWLEDGMENTS. This work was supported in part by grants from the Ministry of Education, Science, Sports, and Culture, from the Ministry of Agriculture, Forestry and Fisheries, and from the Ministry of Health and Welfare of Japan. Y. Kawaguchi, K. Maeda, and M. Kohmoto are supported by fellowships of Japan Society for the Promotion of Science for Japanese Junior Scientist.

REFERENCES

1. Bacchetti, P. and Moss, A. R. 1989. *Nature (Lond.)* 338: 251-253.
2. English, R. V., Nelson, P., Johnson, C. M., Naisse, M., Tompkins, W. A., and Tompkins, M. B. 1994. *J. Infect. Dis.* 170: 543-552.
3. Evermann, J. F., Derse, D., and Dorn, P. L. 1991. *Microbiol. Pathogenesis* 10: 1-9.
4. Gorman, C. M., Moffat, L. F., and Howard, B. H. 1982. *Mol. Cell. Biol.* 2: 1044-1051.
5. Graham, F. L. and Van der Erb, A. J. 1973. *Virology* 52: 456-467.
6. Kawaguchi, Y., Maeda, K., Miyazawa, T., Ono, M., Kai, C., and Mikami, T. 1994. *Virology* 204: 430-435.
7. Kawaguchi, Y., Maeda, K., Miyazawa, T., Ono, M., Tsubota, K., Tomonaga, K., and Mikami, T. 1994. *J. Gen. Virol.* 75: 2783-2787.
8. Kawaguchi, Y., Maeda, K., Ono, M., Yokoyama, N., Fujita, K., Tohya, Y., and Mikami, T. (submitted for publication)
9. Kawaguchi, Y., Miyazawa, T., Horimoto, T., Itagaki, S., Fukasawa, M., Takahashi, E., and Mikami, T. 1991. *Virology* 184: 449-454.
10. Kawaguchi, Y., Norimine, J., Miyazawa, T., Kai, C., and Mikami, T. 1992. *Virology* 190: 465-468.
11. Kawaguchi, Y., Tomonaga, K., Maeda, K., Ono, M., Miyazawa, T., Kohmoto, M., Tohya, Y., and Mikami, T. 1995. *Virology* 208: 492-499.
12. Laurence, J. 1990. *J. Infect. Dis.* 162: 338-346.
13. Miyazawa, T. and Mikami, T. 1993. *J. Vet. Med. Sci.* 55: 519-526.
14. Miyazawa, T., Tomonaga, K., Kawaguchi, Y., and Mikami, T. 1994. *Arch. Virol.* 134: 221-234.
15. Neumann, J. R., Morency, C. A., and Russian, K. O. 1987. *Bio Techniques* 5: 444-447.
16. Sakai, H., Siomi, H., Shida, H., Shibata, R., Kiyomasu, T., and Adachi, A. 1990. *J. Virol.* 64: 5833-5839.
17. Shibata, R., Miura, T., Hayami, M., Sakai, H., Ogawa, K., Kiyomasu, T., Ishimoto, A., and Adachi, A. 1990. *J. Virol.* 64: 307-312.
18. Smiley, J. R., Panning, B., and Smibert, C. A. 1991. pp. 152-174. *In: Herpesvirus Transcription and Its Regulation* (Wagner, E. K. ed.), CRC Press, Boston.