The effects of treatment with chemical agents or infection with feline viruses on protein-binding properties of the feline immunodeficiency virus long terminal repeat

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Abstract

The effects of treatment with chemical agents or infection with feline viruses on protein-binding properties of the feline immunodeficiency virus (FIV) long terminal repeat (LTR) were examined by gel-mobility-shift assays using oligonucleotides designed to represent putative AP-1 or ATF motif from the FIV LTR. Infection with FIV led to less nuclear proteins binding to the AP-1 and ATF sites, suggesting that proteins binding to the sites were consumed or suppressed by FIV-replication in FIV-infected cells. Nuclear proteins that bind to the AP-1 or ATF site were examined by using extracts from Crandell feline kidney (CRFK) cells treated with TPA (a phorbol ester; a strong activator of protein kinase C) or forskolin (an inducer of cyclic-AMP), or infection with feline herpesvirus type 1 (FHV-1). Although TPA or forskolin treatment moderately increased the level of both proteins that bound to AP-1 and ATF sites, FHV-1 infection markedly changed the protein-binding patterns of the sites. Furthermore, FHV-1-induced proteins that bind adjacent to the transcriptional initiation site of FIV promoter were also observed in FHV-1-infected CRFK cells, suggesting that the FHV-1-induced-proteins affects the transcription of FIV through the AP-1, ATF and leader sequences. © 1997 Elsevier Science B.V.

Keywords: FIV; TPA; Forskolin; FHV-1; AP-1; ATF; LBP-1

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1. Introduction

Feline immunodeficiency virus (FIV) is a member of the genus Lentivirus of the family Retroviridae and shares some biological characters with primate lentiviruses such as cell tropisms for T lymphocytes and macrophages in vivo and association with acquired immunodeficiency syndrome (AIDS)-like diseases in cats (Miyazawa and Mikami, 1993; Sparger, 1993; Miyazawa et al., 1994). Recently, it was demonstrated that cell culture-adapted strains of FIV use a CXC chemokine receptor CXCR4, which is one of the cofactors for infection of human immunodeficiency virus type 1 (HIV-1), to infect and fuse with human cells (Willett et al., 1997). Therefore, FIV infection is regarded as a useful small animal model for AIDS studies in humans.

Lentivirus gene expression is modulated by cellular factors through transcriptional regulatory sequences in the long terminal repeat (LTR) of the virus and by the virus-encoded regulatory proteins (Cullen and Greene, 1989). The presence of rev gene activity in FIV was previously demonstrated (Kiyomasu et al., 1991; Phillips et al., 1992; Tomonaga et al., 1993a). Although the trans-activator gene activity of the tat-like gene (ORF-A) remains obscure, ORF-A product was demonstrated to facilitate an efficient viral replication in vivo and in vitro (Tomonaga et al., 1993b; Inoshima et al., 1996; Tomonaga and Mikami, 1996). In the U3 region of FIV LTR, many putative binding sites for enhancer/promoter proteins such as AP-1, AP-4, C/EBP and ATF are present (Phillips et al., 1990; Miyazawa et al., 1991) and these binding sites are important for the basal promoter activity of the FIV LTR (Kawaguchi et al., 1992; Sparger et al., 1992; Thompson et al., 1994).

In HIV-1, it is reported that the expression can be stimulated by several agents, including cytokines, phorbol esters, mitogens, and infection with heterologous viruses (Ostrove et al., 1987; Cullen and Greene, 1989; Pavlakis and Felber, 1990). Members of the herpesvirus family have been shown to stimulate transcription driven by HIV-1 LTR (Margolis et al., 1992; 1993; Vlach and Pitha, 1993; Kawaguchi and Mikami, 1995). Herpes simplex virus type 1 (HSV-1) enhances expression of HIV-1 LTR and the activation of HIV-1 transcription by HSV-1 correlates with binding of the HSV-1-induced proteins to the NF-κB binding site and the LBP-1 site in the leader sequence of the HIV-1 LTR (Margolis et al., 1992, 1993; Vlach and Pitha, 1993). This untranslated leader region is also demonstrated to be critical for the cytomegalovirus-mediated induction of the HIV-1 LTR (Barry et al., 1990).

Recently, we demonstrated that the integrated FIV LTR was activated by treatment with 12-O-tetradecanoylphorbol-13-acetate (TPA) (a strong activator of protein kinase C (PKC)), forskolin (an inducer of cyclic-AMP (c-AMP)), or 5-azacytidine (a DNA methylation antagonist), or by infection with feline herpesvirus type 1 (FHV-1) (Ikeda et al., 1996). The AP-1 site of FIV is required for T-cell activation responses mediated by PKC and for activation by c-Fos (Sparger et al., 1992; Miyazawa et al., 1993). The ATF site of FIV is critical for c-AMP-induced response mediated by protein kinase A (PKA) (Sparger et al., 1992) and for activation by FHV-1 (Kawaguchi et al., 1991, 1992). Although specific nuclear proteins that bind to the AP-1 and ATF sites of FIV were demonstrated by the DNase I footprinting assay (Thompson et al., 1994) and we previously suggested the existence of the putative LBP-1 site near the transcriptional initiation site of FIV LTR (Kawaguchi et al., 1992; Sparger et al., 1992; Thompson et al., 1994).

In the present study, we examined the specific nuclear proteins that bind to the putative LBP-1 sequence. In the present study, we examined the specific nuclear proteins that bind to the putative LBP-1 sequence. In the present study, we examined the specific nuclear proteins that bind to the putative LBP-1 sequence.
2. Materials and methods

2.1. Cells and viruses

CRFK cells (Crandell et al., 1973) were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (FCS) and antibiotics. A feline T-lymphoblastoid cell line (MYA-1 cells) was maintained in RPMI 1640 medium supplemented with 10% FCS, antibiotics, 50 mM 2-mercaptoethanol, 2 μg/ml of polybrene, and 100 U/ml of recombinant human interleukin-2 (Miyazawa et al., 1989). As infectious molecular clones of FIV strains TM2 and Petaluma, plasmids pTM219 (Maki et al., 1992) and pFIV-14 (Olmsted et al., 1989) were used, respectively. To obtain the viruses derived from pTM219 and pFIV-14, CRFK cells were plated in 6-well plastic plates, and 7 μg of the plasmid DNAs were transfected by calcium phosphate coprecipitation method (Graham and van der Eb, 1973). Culture supernatants were collected and filtrated through 0.45-μm Millipore filters 2 days after transfection. The resulting viruses were designated as TM219 and FIV-14, respectively. Virion-associated reverse transcriptase (RT) activities of the virus stocks of TM219 and FIV-14 were measured as described previously (Willey et al., 1988). To prepare stock viruses of FHV-1 C7301 strain or feline calicivirus (FCV) F4 strain, culture supernatants of CRFK cells infected with the viruses were passed through 0.45-μm Millipore filters. The viruses were titrated in CRFK cells and the titers were expressed as 50% tissue culture infective doses (TCID₅₀).

2.2. Preparation of nuclear extracts

TM219 and FIV-14 adjusted to 2.0 × 10⁵ cpm of RT activity and FCV at a multiplicity of infection (MOI) of one were inoculated onto 1.0 × 10⁷ MYA-1 cells. The FIV-infected MYA-1 cells were incubated for 6 days and co-cultivated with uninfected MYA-1 cells for a further 3 days. The FCV-infected MYA-1 cells were incubated for 24 h. A sample of 5.0 × 10⁶ CRFK cells were inoculated with FHV-1 at an MOI of one, or treated with TPA at a final concentration of 40 ng/ml or forskolin at a final concentration of 60 μM. FHV-1-infected CRFK cells were maintained for 12 h and the TPA- or forskolin-treated CRFK cells were incubated for 24 h. Nuclear extracts from the MYA-1 and CRFK cells were prepared as described previously (Kawaguchi et al., 1995) and adjusted to 2 μg/μl of protein by a commercial kit (Protein Assay, Bio Rad).

2.3. Gel-mobility-shift assays

Double strand oligonucleotides were designated as TM2/AP-1 (5’ ATATGAGTCAGAGTTAAA-TGCT 3’), TM2/ATF (5’ TTGCCGATGACGTGTATCTTTGA 3’), TM2/LBP (5’ AACCAGTGTTTTTAAAGCTTCGAGGAGTCTCTCTTC- GTTGA 3’), TM2/LBPdF (5’ AAAAAGTGTTTTTAAAGCTTCGAGGAGTCTCTCTTTTGA 3’), TM2/LBPdB (5’ AACCAGTGTTTTTAAAGCTTCGAGGAGTCTCTCTTTTGA 3’), TM2/LBPdB (5’ AACCAGTGTTTTTAAAGCTTCGAGGAGTCTCTCTTTTGA 3’), F14/LBP (5’ AAAAAGTGTTTTTAAAGCTTCGAGGAGTCTCTCTTTTGA 3’), F14/LBPdF (5’ AAAAAGTGTTTTTAAAGCTTCGAGGAGTCTCTCTTTTGA 3’), HIV/LBP (5’ CCGTACTGGGTCTCTCTGTTGA 3’), and Cons/Sp-1 (5’ ATTCGATCGGGGCGGGGCGAGC 3’), and utilized as probes (the position of AP-1, ATF, LBP-1 and Sp-1 sites are underlined). For gel-mobility-shift assays, 4 μg of nuclear extracts were incubated at room temperature for 20 min with a binding buffer (50 μg/ml poly(dI-dC)poly(dI-dC); 10 mM Tris–HCl, pH 7.5; 50 mM NaCl; 1 mM MgCl₂; 0.5 mM DTT; 0.5 mM EDTA; 4% glycerol). After incubation, 0.175 pmol of ³²P-labeled oligonucleotide probe was added and the mixture was incubated for an additional 20 min at room temperature. Samples were then applied to a nondenaturing 4% polyacrylamide in 0.5 × TBE gel. After electrophoresis, the gel was dried and autoradiographed.

2.4. Gel-supershift assays

The AP-1 transcription factor consists of either Jun homodimers or Fos/Jun heterodimeric complexes which bind to the palindromic sequence TGA(C/G)TCA (Mermoid et al., 1988). The
ATF/CREB family consists of a series of transcription factors that function through binding to the c-AMP responsive element palindromic octanucleotide, TGACGTCA (Roesler et al., 1988). It was previously reported that the proteins binding to the AP-1 and ATF sequences of FIV in CRFK cells were recognized by anti-human c-Jun and ATF-1 antibodies, respectively (Ikeda et al., 1997). We used rabbit anti-human c-Jun and c-Fos polyclonal IgG or mouse anti-human ATF-1 monoclonal IgG (Santa Cruz Biotechnology, CA). A sample of 8 μg of nuclear extracts were incubated at room temperature for 20 min with the binding buffer. After incubation, 0.175 pmol of 32P-labeled oligonucleotide probe was added and the mixture was incubated for an additional 20 min at room temperature. Nuclear proteins binding to the labeled oligonucleotide were incubated with the anti-human c-Jun and c-Fos polyclonal IgG (anti-AP-1 antibodies) or anti-human ATF-1 monoclonal IgG (anti-ATF antibody) for 15 min at 37°C. These samples were then applied to a non-denaturing 4% polyacrylamide in 0.5 x TBE gel. After electrophoresis, the gel was dried and autoradiographed.

3. Results

3.1. The effects of FIV infection on proteins that bind to the AP-1 and ATF sites of FIV LTR

To investigate the effects of FIV infection on proteins that bind to the AP-1 and ATF sites, gel-mobility-shift assays were performed using extracts from the TM219- or FIV-14-infected MYA-1 cells. Nuclear proteins derived from uninfected and FCV-infected MYA-1 cells were used as a control. Double stranded oligonucleotides containing the putative AP-1 or ATF sequences of FIV TM2 were labeled and incubated with the nuclear extracts. By FIV infection, proteins that bound to these two sequences were reduced in MYA-1 cells (Fig. 1). It was conceivable that the proteins binding to the sites were consumed or suppressed by FIV-replication in the cells. On the other hand, FCV-infection did not affect the protein-binding patterns of the two sites.

3.2. The effects of chemical agents on proteins that bind to the AP-1 and ATF sites of FIV LTR

The effects of treatment with TPA or forskolin, or infection with FHV-1 on protein-binding properties of the AP-1 and ATF sites of FIV LTR were investigated using extracts from the CRFK cells treated with the agents or infected with the virus (Fig. 2). When competition experiments using a 100-fold molar excess of homologous and heterologous unlabeled oligonucleotide competitors were performed, the complexes observed in the untreated, or TPA- or forskolin-treated CRFK cells were almost competed out by addition of the homologous unlabeled competitors (Fig. 2a and b, lanes 5 and 8) while binding was not inhibited by the heterologous oligonucleotide containing the consensus Sp-1 binding site (Fig. 2a and b, lanes 6 and 9), indicating that binding was site-specific. Although no significant difference in protein-binding patterns of TM2/AP-1

![Probe](image-url)

![Virus](image-url)

![Lane](image-url)

Fig. 1. Gel-mobility-shift assays using nuclear extracts from the FIV-infected MYA-1 cells. End-labeled oligonucleotides were incubated with 4 μg of mock- (lanes 1 and 5), TM219- (lanes 2 and 6), FIV-14- (lanes 3 and 7) or FCV- (lanes 4 and 8) infected MYA-1 cell extracts.
Fig. 2. Gel-mobility-shift assays using nuclear extracts from the CRFK cells treated with TPA, forskolin or infected with FHV-1. Competition experiments were performed by using the labeled TM2/AP-1 (a) and TM2/ATF (b). These probes were incubated with 4 μg of untreated (lanes 1 to 3) or TPA- (lanes 4 to 6), forskolin- (lanes 7 to 9) or FHV-1- (lanes 10 to 12) treated CRFK cells. Specificity of the retarded complexes was analyzed by addition of a 100-fold molar excess of homologous (lanes 2, 5, 8 and 11) and heterologous (lanes 3, 6, 9 and 12) unlabeled competitor DNA. Quantification of the retarded complexes was performed using the labeled TM2/AP-1 (c) and TM2/ATF (d). These probes (0.35 pmol) were incubated with 8 μg (lanes 1, 4, 7 and 10), 2 μg (lanes 2, 5, 8 and 11) and 0.5 μg (lanes 3, 6, 9 and 12) of untreated (lanes 1 to 3) or TPA- (lanes 4 to 6), forskolin- (lanes 7 to 9) or FHV-1- (lanes 10 to 12) treated CRFK cells. Arrowheads indicate new additional complexes induced by FHV-1 infection.

and TM2/ATF was observed in the TPA- or forskolin-treated cells (Fig. 2a and b, lanes 4 to 9), proteins binding to the AP-1 or ATF sequence increased moderately or slightly by treatment with TPA or forskolin in CRFK cells (Fig. 2c and d, lanes 4 to 9).
3.3. The effects of FHV-1 infection on proteins that bind to the AP-1 and ATF sites of FIV LTR

FHV-1 infection markedly changed the protein-binding patterns (Fig. 2a and b, lanes 10 to 12) and increased the proteins that bound to the AP-1 and ATF sites (Fig. 2c and d, lanes 10 to 12). After FHV-1 infection, the DNA-protein complexes constitutively observed with TM2/AP-1 or TM2/ATF in uninfected CRFK cells (Fig. 2a and b, lane 1) were reduced, and new additional complexes which had higher mobility than the constitutive complexes were demonstrated (Fig. 2a and b, lane 10). Competition experiments revealed that the new FHV-1-induced proteins binding to TM2/AP-1 or TM2/ATF were not completely inhibited by addition of a 100-fold molar excess of the homologous unlabeled competitors (Fig. 2a and b, lane 11). However, the level of expression of FHV-1-induced proteins was much higher than that of the proteins binding to the AP-1 or ATF site in uninfected cells (Fig. 2c and d, lanes 1 to 3 and 10 to 12). Although the proteins induced after FHV-1 infection seem to bind to the AP-1 or ATF site with low specificity, it remains possible that the high levels of expression of FHV-1-induced proteins reduce the effects of the unlabeled homologous competitors. Similarly, when the AP-1 and ATF sequences of FIV Petaluma strain were used, similar changes in the binding patterns were observed after FHV-1 infection (data not shown).

To examine the possibility that the FHV-1-induced proteins that bound to the AP-1 or ATF site were cellular factors such as c-Jun, c-Fos and ATF-1, gel-supershift assays using anti-c-Jun and anti-c-Fos polyclonal antibodies or anti-ATF-1 monoclonal antibody were performed. When the proteins that could bind to the TM2/AP-1 in uninfected cells were untreated (Fig. 3, lane 1) or treated with the anti-c-Jun and c-Fos antibodies (Fig. 3, lane 2), a supershifted band was observed in the latter, indicating that the AP-1-binding proteins in untreated CRFK cells could react with these antibodies. Although a supershifted band was also observed, the FHV-1-induced proteins were not affected by these antibodies (Fig. 3, lane 4). Furthermore, when the proteins that bound to the TM2/ATF in uninfected cells were untreated (Fig. 3, lane 5) or treated with the anti-ATF-1 antibody (Fig. 3, lane 6), a supershifted band was observed in the latter (Fig. 3, lane 7). Although a supershifted band was shown, the FHV-1-induced proteins were not affected by addition of the antibody (Fig. 3, lane 8). These results indicated that FHV-1-induced proteins might not be c-Jun, c-Fos or ATF-1.

3.4. The effects of FHV-1 infection on proteins that bind to the transcriptional initiation site of FIV LTR

We examined the possibility that FHV-1 infection also induced proteins that bound adjacent to
Fig. 4. The effects of FHV-1 infection on the protein-binding properties to the transcriptional initiation sequences of FIV and HIV-1. The labeled TM2/LBP (lanes 1 to 7), F14/LBP (lanes 8 and 9) and HIV/LBP (lanes 10 and 11) were incubated with 4 μg of the mock-infected (lanes 1, 8 and 10) or FHV-1-infected (lanes 2, 3, 4, 5, 6, 7, 9 and 11) CRFK cell nuclear extracts. Specificity of the retarded complexes was analyzed by addition of a 100-fold molar excess of homologous (lane 3) and heterologous (lane 7) unlabeled competitor DNA. The involvement of the putative LBP-1 sites of FIV TM2 strain was examined using unlabeled TM2/LBPdF (lane 4), TM2/LBPdB (lane 5) and TM2/LBPdFB (lane 6) as competitors. Arrowheads indicate the FHV-1-induced protein-DNA complexes.

the transcriptional initiation site of FIV promoter. The gel-mobility-shift assay was performed using the extracts from uninfected and FHV-1-infected CRFK cells. TM2/LBP-1, which contained the two LBP-1-binding motifs (WCTRG), was labeled and incubated with the nuclear extracts. Although the bands of DNA-protein complexes in uninfected CRFK cells were very faint (Fig. 4, lane 1), at least three new additional complexes were observed in FHV-1-infected CRFK cells (Fig. 4, lane 2). Similar results were also obtained when the labeled F14/LBP or HIV/LBP was used (Fig. 4, lanes 8 to 11). When competition experiments using a 100-fold molar excess of homologous and heterologous unlabeled oligonucleotide competitors were performed, the three FHV-1-induced proteins were almost competed out by addition of the homologous unlabeled competitor (Fig. 4, lane 3) while the bindings were not inhibited by the heterologous oligonucleotide containing the consensus Sp-1 binding site (Fig. 4, lane 7), indicating that the bindings were site-specific. To examine the significance of the putative LBP-1 sites in TM2/LBP, unlabeled TM2/LBPdF, TM2/LBPdB and TM2/LBPdFB, which contained identical alterations in the LBP-1 sequences, were used as competitors (Fig. 4, lanes 4, 5 and 6). However, no significant differences in effects as competitors were shown among TM2/LBP, TM2/LBPdF, TM2/LBPdB and TM2/LBPdFB, suggesting that the three FHV-1-induced proteins did not recognize the LBP-1 binding motifs. When the nucleotide sequences of TM2/LBP, F14/LBP and HIV/LBP were compared, a conserved sequence (GTCTCT) was observed among these probes. Therefore, it is likely that FHV-1-induced proteins recognize the conserved sequence.

4. Discussion

In this study, we showed that infection with FIV led to less nuclear proteins binding to the AP-1 and ATF sites and that the FHV-1-mediated activation of FIV provirus correlated with binding of regulatory proteins to at least three cis-acting elements of the FIV LTR: the AP-1, ATF and leader sequences. The stimulation of CRFK cells with TPA or forskolin was shown to lead to up-regulation of nuclear proteins that bound to the AP-1 and ATF sites. In bovine immunodeficiency virus (BIV), it was reported that the expression level of proteins binding to AP-1, AP-4, and CAAT plus ATF probes increased after BIV infection (Fong et al., 1995). The visna virus Tat protein was shown to transactivate via an AP-1 binding site in the LTR (Tomonaga and Mikami, 1996). In contrast, FIV
infection in MYA-I cells could not increase the level of proteins binding to the AP-1 and ATF probes, and other significant differences in protein-binding patterns were not observed in the FIV-infected cells (Fig. 1). Although an analysis of transactivators of FIV LTRs demonstrated very low level transactivation of FIV LTRs by two different molecular clones of FIV (Sparger et al., 1992), no transactivation ability of an infectious molecular clone of FIV, pTM219, was demonstrated and the suppressive effects of pTM219 on FIV LTR was observed (Miyazawa et al., 1993). The present results suggested that these two protein-binding sites were not involved in transactivation by FIV. Furthermore, the suppression of the activity of FIV LTR by an FIV infectious clone (Miyazawa et al., 1993) may be due to the reduction of proteins that bind to the sites.

Recently, we demonstrated that the integrated FIV LTR was activated by treatment with TPA and forskolin (Ikeda et al., 1996) which is an activator of PKC (Castagna et al., 1982) and an inducer of c-AMP (Seamon and Daly, 1986), respectively. AP-1 and ATF sites of FIV are required for activation by TPA and forskolin, respectively (Sparger et al., 1992). From these observations, we hypothesized that TPA- and forskolin-treatments would increase the expression level of proteins binding to the AP-1 and ATF sequences, respectively. As shown in Fig. 2, treatment with TPA or forskolin moderately or slightly increased the level of both proteins binding to AP-1 and ATF sites. However, no significant change in protein-binding patterns was observed in TPA- or forskolin-treated cells. It should be noted that established cell lines often have aberrant, constitutive expression of AP-1 as a function of their transformed state (Piette et al., 1988). Since Fos/Jun and ATF/CREB families have similar protein-binding properties (Hai and Curran, 1991), it is possible that the expression of ATF/CREB family is also aberrant and constitutive in established cell lines and that the effects of TPA or forskolin on the protein-binding properties might be partly masked in established cell lines such as CRFK cells. In addition, as shown in Fig. 2, TPA- or forskolin-treatment affected both proteins that bound to the AP-1 and ATF sites (Fig. 2). The reason might be also due to the similarity of core recognition sequences between AP-1 (TGA(C/G)TCA) and ATF (TGACGTCA) or cross-family dimerization of Fos/Jun and ATF/CREB families (Hai et al., 1988; Hai and Curran, 1991; Ikeda et al., 1997). Furthermore, since no additional DNA-protein complex of TM2/AP-1 or TM2/ATF was observed by the treatments with the chemical agents (Fig. 2), it was suggested that the activation of FIV LTR by TPA or forskolin (Sparger et al., 1992; Ikeda et al., 1996) was not due to induction of new additional proteins but due to the increases of constitutively expressed proteins that could bind to the sites.

Lentivirus infection is characterized as having a long latency period before clinical signs appear (Miyazawa and Mikami, 1993). 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 production because human and animal herpesviruses possess abilities to stimulate lentivirus LTR-directed gene expression (Kawaguchi and Mikami, 1995). In FIV, it has been shown that cats latently infected with FHV-1 frequently develop recurrent upper respiratory diseases which are typical symptoms of feline AIDS and that FHV-1 is recovered from these cats with high titers (English et al., 1994). These results suggest that FHV-1 may play a role in progression of feline AIDS in vivo. 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; (ii) feline T lymphocytes are productively co-infected with both FIV and FHV-1 in vitro; and (iii) the sequence between positions −63 and −43 (relative to cap site) containing the ATF site was responsible for the activation by FHV-1 (Kawaguchi et al., 1991, 1992). In HIV, it was reported that HSV-1 infection induced expression of the HIV-1 provirus through the NF-kB and LBP-1 target sequences (Margolis et al., 1993; Vlach and Pitha, 1993). As shown in Figs. 2 and 4, changes of protein-binding patterns after FHV-1 infection were observed
in the AP-1 as well as ATF sites and FHV-1 infection also induced at least three proteins that bind adjacent to the transcriptional initiation site of FIV promoter. From these results, it was suggested that FHV-1 infection could affect the transcription of FIV through the AP-1, ATF and leader sequences. Therefore, it is possible that these FHV-1-induced factors are involved in the mechanisms for terminating the latency of FIV leading to the progression of clinical disorders. At present, it remains unknown whether the proteins induced by FHV-1 infection are cellular or viral factors, or whether the reduction of proteins constitutively binding to the AP-1 or ATF site is due to the down-regulation of the proteins or inhibition of the bindings by the FHV-1-induced proteins. However, FHV-1-induced proteins that bound to the AP-1 or ATF site did not react with anti-c-Jun, c-Fos or ATF-1 antibody (Fig. 3). In human herpesviruses, immediate early gene products such as ICP4, ICP0 and ICP27 were shown to have trans-acting ability and actually transactivate HIV LTR-directed gene expression (Kawaguchi and Mikami, 1995; Hayward, 1993). Therefore, it is possible that some of the FHV-1-induced proteins which can recognize the AP-1, ATF or leader sequence are viral factors. In addition, since the AP-1 and ATF sites are also ubiquitous in promoters of cellular genes, FHV-1 infection may influence the expression of several cellular genes as well as the virus genes directed by FIV LTR. Further characterization of the FHV-1-induced proteins will be necessary to understand the possibility that FHV-1 may play a role in progression of feline AIDS in vivo.

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