Comparison of biological properties of feline immunodeficiency virus isolates using recombinant chimeric viruses

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The biological properties of homogeneous populations of feline immunodeficiency viruses derived from infectious molecular clones of the TM1, TM2 and Petaluma strains were compared. Differences in infectivity for Crandell feline kidney (CRFK) cells, and in syncytium formation and replication kinetics in a feline T lymphoblastoid cell line (MYA-1 cells) were observed. To investigate the basis of these differences between the TM2 and Petaluma strains, we first compared the basal promoter activity of the long terminal repeat which is a highly divergent region, but no significant difference in activities was found in CRFK cells. We then constructed two recombinant chimeric clones which carry gag, pol, vif, and ORF A from the heterologous virus. From analyses using the chimeric clones, it was revealed that efficient virus growth in CRFK cells and MYA-1 cells was regulated by the gag, pol, vif and ORF A regions, whereas viral determinants of infectivity for CRFK cells, and syncytium formation and cytopathogenicity in MYA-1 cells, were located in the env region.

Introduction

Lentiviruses belonging to Retroviridae have been isolated from many species including human, monkey, cattle, horse, sheep, goat and cat (for a review, see Narayan & Clements, 1990). Feline immunodeficiency virus (FIV) was first isolated from domestic cats with an immunodeficiency-like syndrome in the U.S.A. (Pedersen et al., 1987), and subsequently molecularly cloned (Olmsted et al., 1989a). The nucleotide sequence and genomic organization revealed that FIV is a member of the lentiviruses (Olmsted et al., 1989b; Talbott et al., 1989). Owing to the low fidelity of lentivirus-encoded reverse transcriptase (RT), genomic mutation occurs frequently during reverse transcription of viral RNA to proviral DNA (Montelaro, 1992). Further attempts to isolate FIV strains have been carried out worldwide, and comparison of nucleotide sequences of isolates has revealed that genomic heterogeneity occurs among various FIV strains isolated in the U.S.A. (Talbott et al., 1989; Olmsted et al., 1989b; Phillips et al., 1990), Switzerland (Morikawa et al., 1991), the Netherlands (Siebelink et al., 1992), Italy (Rigby et al., 1993), Australia (Greene et al., 1993a, b) and Japan (Maki et al., 1992; Miyazawa et al., 1991). These reports also noted a high diversity of biological properties including antigenicity, cell tropism and replication kinetics.

Previously we reported biological differences between the Japanese isolates of FIV (TM1 and TM2 strains) and a U.S. isolate, the prototype Petaluma strain, using uncloned wild-type viruses (Kawaguchi et al., 1992; Miyazawa et al., 1989a). FIV strains TM1 and TM2 occasionally induce ballooning and giant cell formation in feline T lymphocytes and they are unable to infect Crandell feline kidney (CRFK) cells. In contrast, the Petaluma strain can infect both feline T lymphocytes and CRFK cells productively (Olmsted et al., 1989a; Phillips et al., 1990; Yamamoto et al., 1988). In other lentiviruses, many determinants of cell susceptibility such as the envelope region of human immunodeficiency virus type 1 (HIV-1) (Cann et al., 1992; Cheng-Mayer et al., 1990; Shioda et al., 1991) or the long terminal region (LTR), gag-pol, and/or vif regions of simian immunodeficiency virus (SIV) (Shibata et al., 1991) have been reported. However the determinants in FIV are still unknown.

In the present study, we found some differences between biological properties of the TM1, TM2 and Petaluma strains using genomically homogeneous virus populations derived from the respective infectious molecular clones (Miyazawa et al., 1991; Maki et al., 1992; Olmsted et al., 1989a). Furthermore, to define the genomic determinants of phenotypic diversity, we constructed reporter plasmids containing the LTRs from each of the infectious clones of the TM2 and Petaluma strains and compared their promoter activities. Next, we constructed reciprocal recombinant chimeric viruses...
from the TM2 and Petaluma strains, and examined the infectivity of these chimeric viruses for feline T lymphoblastoid line cells (MYA-1 cells) and CRFK cells.

Methods

Cell cultures and viruses. Felis catus whole foetus 4 (fcwf-4) (Pedersen et al., 1981) and CRFK (Crandell et al., 1973) cells, which are feline non-lymphoid cell lines, were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS) and antibiotics. These cells were used for transfection and virus inoculation. MYA-1 cells (Miyazawa et al., 1989b) which are FIV-sensitive feline T lymphoblastoid line cells, were grown in RPMI 1640 growth medium supplemented with 10% FCS, antibiotics, 50 μM 2-mercaptoethanol, 2 μg/ml polybrene and 100 units/ml of recombinant human interleukin-2 (IL-2) at 37 °C in a humidified atmosphere of 5% CO₂ in air.

As infectious molecular clones of FIV strains TM1, TM2 and Petaluma, plasmids pFTM191CG (Miyazawa et al., 1991), pTM219 (Maki et al., 1992) and pFIV-14 (Olmsted et al., 1989a) were used, respectively. These plasmids were transfected into CRFK and fcwf-4 cells, and the 2- or 3-day culture supernatants were collected and filtered through 450 nm Millipore filters, and then used as stock viruses.

Construction of recombinant DNAs. For construction of recombinant plasmids, the LTR of pFIV-14 was subcloned to remove the 8 kbp of flanking cellular DNA of the clone as described below. A NdeI–SacI 721 bp fragment containing the full-length of LTR was constructed by joining the NdeI–Nhel fragment of the 3’ end of the genome and a Nhel–SacI fragment from the 5’ end and subcloning into the pUC18 plasmid vector. This plasmid was designated pF14LTR.

To construct reporter plasmids for the measurement of promoter activity of FIV LTRs by the chloramphenicol acetyltransferase (CAT) assay, the entire FIV LTRs (in the HincII–NarI 418 bp fragment from pTM219 and the Nhel–NarI 573 bp fragment from pF14LTR) were excised and placed upstream of the CAT gene of pHDACL (Shibata et al., 1990), and designated pTM2–LTRCAT and pF14–LTRCAT, respectively. The LTR fragments were then cut at the Nhel site, located in the U3 region, and ligated with each other to construct reporter plasmids with chimeric LTRs, pTM2F14-LTRCAT and pF14TM2-LTRCAT.

As shown in Fig. 1, reciprocal recombinant clones between pTM219 and pFIV-14 were constructed by replacing gag, pol, vif and ORF A from the heterologous plasmid by using conserved SacI and KpnI sites. Briefly, a BglII fragment of pTM219 containing one of the three KpnI restriction sites in the env region was subcloned into a pSP72 plasmid vector (Promega). Using the SacI site of the plasmid vector and the KpnI site in the subcloned BglII fragment of pTM219, the SacI–KpnI fragment of pFIV-14 was replaced. The SacI–BglII region of pTM219 was then replaced with the fragment of subcloned DNA consisting of the SacI–KpnI region of pFIV-14 and the KpnI–BglII region of pTM219. This chimeric virus plasmid was designated pC1. To construct a chimeric virus bearing the env region of pFIV-14, plasmid pFIV-14AEV was constructed by deleting the EcoRV fragment of pFIV-14. The Nhel–Nhel fragment of pFIV-14AEV was then inserted at the Nhel site of pF14LTR, and the SacI–KpnI fragment of the resultant plasmid was replaced with the SacI–KpnI fragment of pTM219. This chimeric virus plasmid was designated pC11 (Fig. 1).

Transfection of plasmid DNA. CRFK cells and fcwf-4 cells were grown to 90% confluency in six-well plastic plates, and transfected with 3 μg of LTRCAT plasmids per well for the CAT assay or 10 μg of infectious molecular clones per well for virus infection and propagation by the calcium phosphate coprecipitation method (Graham & van der Eb, 1973). Four hours after transfection, the cells were washed twice with FCS-free medium and glycerol-shocked, and then culture medium was replaced by fresh medium.

CAT assay. CRFK and fcwf-4 cells transfected with LTRCAT plasmids were harvested by scraping after 48 h incubation. After washing with PBS once, the cells were lysed by freezing and thawing four times in 100 μl of 250 mm-Tris–HCl pH 7.8. Cell debris was centrifuged for 5 min at 4 °C, and each extract was assayed for CAT activity by the solvent partition method (Neumann et al., 1987). In brief, a 240 μl reaction mixture containing 100 mm-Tris–HCl pH 7.8, 1.0 mm-chloramphenicol, 3.7 kBq of [¹⁴C]acetyl coenzyme A (DuPont) and cell extract was overlaid with 5 ml of scintillation fluid (Econoﬂuor; DuPont). Reactions were carried out at 37 °C and the production of radioactively labelled acetylchloramphenicol was monitored by counting in a liquid scintillation counter. The CAT activity of each reporter plasmid was presented as the net d.p.m. of product formed/h. All the CAT assay data reported are from points in the linear range of the assay.

Virus infection. CRFK cells and fcwf-4 cells were transfected with plasmid DNAs of infectious molecular clones. A one-fifth volume of the cells were passaged at the indicated time and maintained in DMEM growth medium. Cell-free culture supernatants were also collected for the RT activity assay at the indicated times.

MYA-1 cells (1-8 x 10⁶ cells) were infected with FIV stocks derived from the infectious molecular clones with the same amount of RT activity. After 2 h adsorption, the cells were washed twice with RPMI 1640 medium, and seeded at 3 x 10⁶ cells/ml in growth medium. Thereafter, the cell numbers were adjusted to 3 x 10⁶ cells/ml in fresh growth medium, virus production was monitored by the RT activity assay, and numbers of viable MYA-1 cells were counted by the dye-exclusion method at the indicated times.

RT activity assay. The Mg²⁺-dependent RT activities in the cell culture supernatants were assayed as described previously (Ohki et al., 1992). Briefly, 10 μl of the culture supernatant was mixed with a reaction mixture containing poly(rA)-oligo(dT) and [α-³²P]dTTP (3000 Ci/mmol, DuPont). After incubation for 3 h at 37 °C, the mixture was dotted on DEAE filter paper (Whatman, DE81). RT activity was counted by scintillation.

Immunoprecipitation. FIV-infected and uninfected MYA-1 cells were labelled for 16 h with 50 μCi of [3H]methionine (1140 Ci/mmol, DuPont) per ml in RPMI 1640 medium containing 5% FCS. The cell lysate prepared in lysis buffer (0.5% N P40, 0.5% sodium deoxycholate, 0.05 mm-Tris–HCl pH 7.2, 0.1 mm-NaCl, 1 mm-EDTA, 1 mm-
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Fig. 2. Replication kinetics of infectious molecular clones from FIV. Mg\(^{2+}\)-dependent RT activity in culture supernatants was monitored for virus production in CRFK (a) and fcwf-4 (b) cells after transfection with infectious molecular clones. The 3-day culture supernatants of CRFK cells (a) were transferred to MYA-1 cells (1.8 x 10^6 cells), and virus production was then monitored by an RT activity assay (c). Cytopathogenic activity in MYA-1 cells was examined by dye-exclusion (d). The RT activity and viable cell ratio of cells infected with FIVs derived from pTM219 (△), pFTM191CG (▲) or pFIV-14 (●), and those of mock-infected cells ( ▲ ) are shown. These results are representative of one of the three independent experiments.

PMSF) was immunoprecipitated with the plasma from cat MM which was infected with FIV TM1 (Miyazawa et al., 1989a). The plasma had a titre of 1:256 by the indirect immunofluorescence test. Immune complexes were then precipitated with Protein A-Sepharose CL-4B beads (2 mg/5 μl of antiserum; Pharmacia) for 1 h at 4 °C. The immunoprecipitates were washed with lysis buffer, and solubilized in SDS-PAGE sample buffer described previously (Ikuta et al., 1989). The FIV proteins were separated by SDS-PAGE using a 7.5 to 15% linear gradient polyacrylamide gel and then visualized on a Bio-Imaging analyzer (BAS2000; Fuji Photo Film Co.).

Results

Biological characteristics of FIV infectious molecular clones

To compare the biological characteristics of TM1, TM2 and Petaluma strains of FIV, infectious molecular clones of the respective viruses were transfected into CRFK (Fig. 2a) and fcwf-4 cells (Fig. 2b), and the cell-free culture supernatants from the transfected cells were collected at the indicated times. The virus production was then monitored by measuring Mg\(^{2+}\)-dependent RT activity.

At 3 days post-transfection (p.t.), significant RT activities were detected in the culture supernatants of the cells transfected with all of the clones. However, the RT activity of TM1 and TM2 strains in CRFK cells and that of all of the FIV strains in fcwf-4 cells decreased gradually as the cells were passaged. In contrast, the RT activity of the Petaluma strain in CRFK cells gradually increased. In addition, when the cell-free supernatants from these transfected cells were transferred to CRFK cells, an increase in RT activity was observed only with the Petaluma strain (data not shown). These results indicate that the Petaluma strain from the infectious molecular clone can infect CRFK cells not only by cell-to-cell spread but also as cell-free virus.

The culture supernatants obtained from the transfected CRFK cells at 3 days p.t. were adjusted to the same amount of RT activity (150 c.p.m.) and inoculated onto 1.8 x 10^6 MYA-1 cells (Fig. 2c). Although all of the FIVs grew well in MYA-1 cells, the Petaluma strain showed a higher peak of RT activity than did the TM1 and TM2 strains (Fig. 2c). All of the FIVs could induce cell death in MYA-1 cells and the viability of the FIV-
infected MYA-1 cells decreased after 9 days post-infection (p.i.). The cytopathogenicity of the Petaluma strain was much higher than that of the TM1 or TM2 strains (Fig. 2d). In addition, the TM2 strain induced ballooning c.p.e. in the infected MYA-1 cells at 6 days p.i., whereas the Petaluma strain did not. Another two independent experiments varying slightly in sampling times and amounts of inoculum for MYA-1 cells were carried out and the results were essentially similar to those described above (data not shown).

Comparison of promoter activity of the LTRs

As some differences in the putative enhancer protein binding sites existed between the U3 region of TM2 LTR and that of Petaluma LTR (Maki et al., 1992), the LTRCAT plasmids of pTM219 and pFIV-14, and two reporter plasmids which contained the chimeric U3 region were constructed. Each of the four LTRCAT plasmids was transfected into CRFK and fewf-4 cells, and the promoter activity was assayed by the CAT assay. However, no significant difference was observed between the promoter activities of the four LTRs (data not shown). These results indicated that LTRs of two FIV strains have similar promoter activity in the cells, and might have no effect on cell tropism.

Chimeric analyses of the infectious molecular clones

To determine which FIV gene(s) regulate infectivity for CRFK cells and the different virus phenotypes in MYA-1 cells, two recombinant chimeric viruses were constructed (pC1 and pC11). pC1 contains the gag, pol, vif and ORF A regions of pFIV-14, whereas pC11 contains those of pTM219 (Fig. 1). To confirm that the chimeric viruses were constructed correctly, the viral proteins in MYA-1 cells infected with each of the viruses were compared by immunoprecipitation analysis (Fig. 3). The four FIVs gave specific bands corresponding to Mr values of 50K, 27K and 17K. Furthermore a protein with an Mr of 130K was identified for the TM2 strain (lane 1) and C1 chimera virus (lane 2) which had the env region of pTM219, and one with an Mr of 124K was identified for the C11 chimera virus which carried the env gene of pFIV-14 (lane 3), and for pFIV-14 (lane 4). These results indicate that the chimeric viruses were constructed correctly.

After transfection of these four plasmids into CRFK cells (Fig. 4a), transient increases in RT activity were detected in the culture supernatants of all the transfected cells at 3 days p.t. Thereafter, the RT activity of the Petaluma strain increased gradually whereas that of the C11 chimera virus stayed virtually constant. On the other hand, the activity of the TM2 strain and the C1 chimera decreased as passage levels increased and fell to control levels after 11 days p.t. When the cell-free supernatants from these transfected cells were transferred to CRFK cells, increases of RT activity were observed in C11 chimera virus as well as the Petaluma strain (data not shown). These results indicate that the tropism for CRFK cells was determined by the 3' half of the Petaluma genome and that the cell-free infectivity of the Petaluma strain was not impaired by exchanging the 5' half of the genome for that of the TM2 strain. Furthermore, it is suggested that the 5' half of the Petaluma strain genome is also involved in efficient virus growth in CRFK cells.

Virus stocks prepared from the transfected CRFK cells at 2 days p.t. were adjusted to the same level of RT activity (200 c.p.m.), and inoculated into 1-8 x 10^6 MYA-1 cells. The RT activities of the TM2 strain and the C11 chimera reached a maximum at 13 days p.i. and then decreased gradually. The activity of the Petaluma strain increased rapidly and sustained the highest levels, whereas that of the C1 chimera increased gradually (Fig. 4b). The viable cell ratio of MYA-1 cells infected with each of the viruses decreased gradually and the cyto-
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Fig. 4. Replication kinetics of parent and chimeric infectious molecular clones of FIVs. Mg²⁺-dependent RT activity in culture supernatant was monitored as an indicator of virus production in CRFK cells after transfection with infectious molecular clones (a). The 2-day culture supernatants of each of the transfected CRFK cells (a) were transferred to MYA-1 cells (1.8 × 10⁶ cells), and virus production was then monitored by the RT activity assay (b). Cytopathogenic activity in MYA-1 cells was examined by dye-exclusion (c). The RT activity and viable cell ratio of the cells infected with FIVs derived from pTM219 (○), pC1 (□), pC11 (■) or pFIV-14 (●) and those of mock-infected cells (△) are shown. Values represent the average and bars represent the S.D. These results are representative of three independent experiments.

Fig. 5. C.p.e. in FIV-infected MYA-1 cells. The cells were infected with FIV strain TM2 (a) or Petaluma (b), or chimeras pC1 (c) or pC11 (d), or were mock-infected (e); the cells were photographed at 6 days p.i. Bars in (e) represent 100 μm (left and right) for panels (a) to (e).

pathogenicity of the Petaluma strain and the C11 chimera was higher than that of the TM2 strain or the C1 chimera (Fig. 4e). These results suggest that efficient virus replication in MYA-1 cells can be regulated by the 5' half of the genome.

Ballooning c.p.e. was also observed in MYA-1 cells infected with the TM2 strain (Fig. 5a) and the C1 chimera (Fig. 5c) at 6 days p.i., whereas MYA-1 cells
infected with the Petaluma strain (Fig. 5b) or C11 chimera virus (Fig. 5d) did not show this type of c.p.e. These observations suggested that syncytium formation is determined by the 3' half of the TM2 strain.

Discussion

The genetic organization of FIV is relatively simple compared with that of other primate lentiviruses (Clements & Wong-Staal, 1992; Olmsted et al., 1989b). The FIV genome contains at least three short ORFs, vif, ORF A and rev, in addition to three structural genes, gag, pol and env. The vif gene is essential for cell-free virus infectivity (Tomonaga et al., 1992), and rev for virus structural gene expression (Kiyomasu et al., 1991; Phillips et al., 1992). Recently, we demonstrated that the ORF A gene is required for efficient virus replication in peripheral blood mononuclear cells (Tomonaga et al., 1993). A high divergence of nucleotide sequence between the FIV TM2 and Petaluma strains has been reported (Maki et al., 1992; Rigby et al., 1993); the identities between the two strains in gag, pol and env were 90, 87 and 81% at the amino acid level, respectively, and the LTR was 82% identical at the nucleotide level. As the LTR and env regions are involved in early stages of virus replication and were highly divergent, they might be implicated as potential determinants of biological differences between the two strains.

When the biological characteristics of the progeny viruses derived from infectious molecular clones were compared, their infectivity for CRFK cells, growth kinetics and cytopathogenicity in MYA-1 cells (Fig. 3) were similar to those of wild-type viruses as described previously (Miyazawa et al., 1989a, b). In the present study, CRFK cells transfected with an infectious molecular clone of the Petaluma strain produced a high number of progeny viruses whereas those transfected with the TM1 and TM2 strains produced virus only transiently. In addition, the growth of the Petaluma strain was higher than that of the TM1 and TM2 strains in MYA-1 cells as observed using wild-type viruses (Miyazawa et al., 1989b). However, the cloned Petaluma strain derived from pFIV-14 could not induce ballooning c.p.e. in the present study, whereas the uncloned Petaluma strain maintained in feline lymphocytes could induce this type of c.p.e. in feline lymphocytes (Pedersen et al., 1987) and in MYA-1 cells (Miyazawa et al., 1989a). The DNA of the clone was derived from CRFK cells infected with this strain (Talbott et al., 1989; Olmsted et al., 1989a); therefore it is possible that a variant of the strain which does not induce this type of c.p.e. was selected during molecular cloning.

Sparger et al. (1992) reported that the basal promoter activity of the Petaluma strain is higher than that of the PPR strain which cannot infect CRFK cells and the tissue restriction of the two FIV molecular clones did not correlate with the ability of the LTR to function as a promoter in various cell types. To compare the promoter activity of the LTRs of the TM2 and Petaluma strains, we constructed four reporter plasmids of both parent and chimeric LTRs and measured their activity in CRFK and fcwf-4 cells by the CAT assay. However there were no significant differences detected between the four LTRs. These results indicate that LTRs of these two strains were not responsible for the tropism for CRFK cells. Similarly, in HIV-1, independent researchers reported that the LTR of the virus is not a major determinant of cell tropism (Hirsch et al., 1990; Pomerantz et al., 1991).

The molecular clones pFIV-14 and pTM219 and the chimeric clones C1 and C11 were tested for replication capacity in CRFK cells and MYA-1 cells. CRFK cells transfected with the recombinant molecular clones of the Petaluma strain were able to produce the virus productively, but the cells transfected with the chimeric virus (C1) bearing gag, pol, vif and ORF A of the TM2 strain produced the virus only transiently (Fig. 4a). Since the rev trans-activator activity was detected equally in both the TM2 and Petaluma strains in CRFK cells (K. Tomonaga et al., unpublished), the tropism for CRFK cells may be determined by the env gene of FIV Petaluma strain as observed in HIV-1 (Cann et al., 1992; Cheng-Mayer et al., 1990; Shioda et al., 1991), although we cannot exclude the possibility that as yet unidentified gene(s) are involved in controlling the infectivity for these cells. Furthermore efficient virus growth in CRFK cells appears to be conferred by the 5' half of the FIV genome from evidence that the RT activity of the C11 chimera was lower than that of the Petaluma strain.

In the case of MYA-1 cells, the degree of virus replication of the Petaluma strain and C1 chimera virus was higher than that of the TM2 strain and C11 chimera virus (Fig. 4b), indicating that efficient virus growth might be controlled at a late stage of virus replication by one of the gag, pol, vif, and ORF A genes but not by the LTR of the Petaluma strain. Furthermore the 5' half of the TM2 strain genome seems to have some negative effects on efficient growth of the C11 chimera virus in the early stages of virus replication. The cytopathogenicity of the Petaluma strain and C11 chimera virus was stronger than that of the TM2 strain and C1 chimera virus (Fig. 4c), indicating that the c.p.e. might be influenced by the env region. In addition, we found that the TM2 strain and C1 chimera virus can induce ballooning c.p.e., whereas the Petaluma strain and C11 chimera virus did not induce this type of c.p.e. in MYA-1 cells (Fig. 5). This phenotype is also considered to be
determined by the env gene in HIV-1 and -2 (Freed et al., 1991; Steffy et al., 1992). Although we have gone some way towards the identification of the genetic differences between the two strains of FIV functions which control the phenotype, possible cooperative gene functions should also be considered. Therefore, further studies will be required to define the role of each gene in determining the virus phenotype.

In this study, we found that the $M_r$ of the surface envelope protein of the TM2 strain is slightly greater than that of the Petaluna strain (Fig. 3). Sequence analysis revealed that TM2 and Petaluna strains have 20 and 16 potential $N$-linked glycosylation sites in the surface envelope protein, respectively (Maki et al., 1992; Olmsted et al., 1989b). Taking into account the average $M_r$ of carbohydrate side-chains (2.1K per site; Hunter et al., 1983), the difference in sizes of the proteins is as expected. Although the tropism for CRFK cells and syncytium formation in MYA-1 cells may be determined by a few amino acid changes in the envelope protein as in the case of HIV-1 (Cheng-Mayer et al., 1991), it is also possible that the phenotype might be due to differences in the glycosylation of the envelope protein.

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References


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