Genetic diversity of Argentine isolates of feline immunodeficiency virus

Marcelo R. Pecoraro,1 Keizo Tomonaga,1† Takayuki Miyazawa,1 Yasushi Kawaguchi,1‡ Shigeo Sugita,2 Yukinobu Tohya,1 Chieko Kai,1 Maria E. Etcheverrigaray3 and Takeshi Mikami1

1 Department of Veterinary Microbiology, Faculty of Agriculture, The University of Tokyo 1-1-1, Yayoi, Bunkyo-ku, Tokyo 113, Japan
2 Epizootic Research Station, Equine Research Institute, Japan Racing Association, 1400-4 Shiba, Kokubunji-machi, Shimotsuga-gun, Tochigi 329-04, Japan
3 Department of Virology, Faculty of Veterinary Sciences, La Plata University, 1900-La Plata, Buenos Aires, Argentina

We report the nucleotide sequence and genetic diversity of part of the envelope (env) gene of four strains of feline immunodeficiency virus (FIV) isolated from Argentine domestic cats. The DNA encoding the V3 to V5 regions of the env gene of the FIV isolates were amplified by PCR, cloned and sequenced. Phylogenetic analysis revealed that the Argentine isolates did not cluster into a single group; one isolate clustered with subtype B FIV isolated in the USA and Japan, whereas the others formed a new cluster of FIV which might represent a prototype sequence for subtype E.

The feline immunodeficiency virus (FIV), a T-lymphotropic feline lentivirus associated with immunodeficiency and opportunistic infection in infected domestic cats, was first isolated in 1986 (Federsen et al., 1987). The considerable genetic diversity of lentiviruses is one of the major difficulties in vaccine development. The envelope (Env) glycoprotein has been recognized as a dominant antigen in immune responses to diversity of lentiviruses is one of the major difficulties in vaccine development. The envelope (Env) glycoprotein has been recognized as a dominant antigen in immune responses to FIV (Pancino et al., 1993a; Rigby et al., 1993; Sodora et al., 1994) and therefore the study of FIV diversity has lately focused on the env gene. Both human immunodeficiency virus (HIV) and FIV sequences have been separated into phylogenetically distinct env subtypes (Sodora et al., 1994; Louwagie et al., 1995; Kakinuma et al., 1995). Based on the differences found in env sequences, FIV subtypes A, B, C and D have been reported so far (Sodora et al., 1994; Kakinuma et al., 1995). Recent studies indicated inefficient vaccine protection against distinct heterologous strains of FIV (Johnson et al., 1994), suggesting that more detailed information about the diversity of FIV isolates will be necessary for the design of prophylactic vaccines.

FIV infection is prevalent worldwide (for a review see Miyazawa & Mikami, 1993). Preliminary seroepidemiological studies carried out on clinical cases suggested that FIV infection is widespread in the domestic cat population of Argentina (Tohya et al., 1994). In the present work we describe the amplification and cloning of part (657 bp) of the env gene by PCR and the degree of sequence variation of four Argentine FIV isolates. We compared our isolates with those of subtypes A, B, C and D by constructing a phylogenetic tree based on synonymous substitutions in the variable V3 to V5 regions of the env gene.

Argentine FIV isolates LP3, LP9, LP20 and LP24 were isolated from peripheral blood mononuclear cells (PBMCs) of four domestic cats brought to the Veterinary Medical Hospital of La Plata University (Buenos Aires, Argentina; M. Pecoraro, T. Miyazawa, G. Oliva, E. Nosetto, M. Castellano, Y. Tohya, J. Norimine, M. Etcheverrigaray & T. Mikami, unpublished results). The PBMCs that showed Mg2+-dependent reverse transcriptase activity in the culture supernatant were cocultured with MYA-1 cells (Miyazawa et al., 1989) and then the extrachromosomal DNA of the cells was obtained by the Hirt method (Hirt, 1967).

One oligonucleotide primer pair, HV3f and HV5r, was used to amplify the V3 to V5 regions of FIV env gene (Fig. 1). The primer HV3f corresponded to nucleotide position 7322 to 7344 and the primer HV5r to position 8049 to 8027, according to the published sequence of the TM2 strain (Maki et al., 1992). PCRs were performed in a total volume of 100 μl containing...
1 μg of extrachromosomal DNA, 100 pmol of each primer, 0.2 mM of each of the four dNTPs and 2.5 U Taq polymerase (Perkin-Elmer) according to the manufacturer’s instructions.

Samples were overlaid with two drops of mineral oil to prevent evaporation and then cycled in a DNA Thermal Cycler for 45 s at 94 °C, 1 min at 55 °C and 2 min at 72 °C for 30 cycles.

The PCR fragments of FIV were cloned into pUC19 or pCR cloning vectors (Invitrogen). The sequencing reaction was performed using a Taq Dye Primer Cycle Sequencing Kit (Applied Biosystems) and the sequence was resolved on Automated DNA Sequencer model 373A (Applied Biosystems).

The amino acid alignment was determined with the GENETIX-MAC program (Software Development) and the distance matrix was calculated using the OVEN Program Package for molecular evolutionary analysis (Y. Ina; National Institute of Genetics, Mishima, Japan). To compare the Argentine isolates with other FIV isolates reported previously, 39 other sequences were analysed. These sequences were representative of FIV env gene subtypes A, B, C (Sodora et al., 1994) and D (Kakinuma et al., 1995).

All the sequences were subjected to phylogenetic analysis using a total of 657 nucleotides coding for 219 amino acids. The phylogenetic analysis was carried out using the neighbor

---

**Fig. 1.** Schematic representation of the V3–V5 regions of FIV env. The locations of plus- and minus-strand PCR primers are indicated by arrows. Bars represent the position of the resultant PCR products. Open boxes represent the variable domains and shaded boxes the hypervariable regions as previously described (Pancino et al., 1993b).

**Fig. 2.** For legend see opposite.
Fig. 2. (a) Alignment of 18 deduced amino acid sequences of FIV over 657 nucleotides encompassing the V3-V5 region of env. Identical nucleotides at given positions are represented by dots (.), gaps are represented by dashes (-). The positions of cysteine residues are indicated by asterisks (*). (b) Phylogenetic tree of 43 sequences from the V3–V5 region of FIV env. The tree was constructed from synonymous substitutions by the neighbor-joining algorithm (Saitou & Nei, 1987). Multiple substitutions were corrected by the six parameter method (Gojobori et al., 1982). Branch lengths were estimated based upon minimum evolution. We examined four FIV sequences determined in this study and 39 available from GenBank (see Text).
join algorithm (Saitou & Nei, 1987) and calculations were conducted using SINCA (Fujitsu). To improve the reliability of the calculations, sites at which there was a gap in any sequence in the alignment were excluded from all comparisons.

The GenBank accession numbers for the env sequences included in this study are Amori-2 (D37817), CABCpady02C (U02392), CABCpbar01C (U02393), CABCpbar02C (U02394), CABCpbar03C (U02395), CABCpbar07C (U02397), CA.Dixon (L00608), CA.PPR (M36968), DutchK1 (M73964), Dutch4 (X69498), Dutch6 (X69499), Dutch32 (M73965), DutchUr (X60725), EngUK8 (X69496), FranceWo (L06312), Fukuoka (D37815), ItalyM1 (X69500), ItalyM3 (X69502), ItalyM4 (X69503), Petaluma (M25381), ScotUK2 (X69494), Sendai-1 (D37813), Sendai-2 (D37814), Shizuoka (D37811), SwissZ1 (X57002), SwissZ2 (X57001), USCAhnky-11A (U02420), USCAhnky12A (U02403), USCAsam_01A (U02410), USCAAtt___09A (U02413), USCAAtt___10A (U02414), USCAzepey01A (U02417), USILbmey03B (U02418), USMasboy03B (U02419), USMGlwd03B (U02420), USOK-lgrl02B (U02421), USTMxem03B (U02422), TM2 (M59418) and WalesUK14 (X69497).

Firstly we determined the nucleotide sequence of a 657 bp segment encompassing the V3 to V5 env regions of four Argentine FIV isolates. The predicted amino acid sequences of the LP3, LP9, LP20 and LP24 strains were aligned along with 14 previously reported sequences representing A, B, C and D env subtypes (Fig. 2a). The env sequences of the Argentine isolates had an open reading frame. The first cysteine residue present in the V3 regions was absent in the LP24 strain. The N-linked glycosylation sites were conserved among all the isolates.

Previous phylogenetic analysis of variable regions V3 to V5 of the FIV env gene demonstrated the existence of A, B, C and D env subtypes (Sodora et al., 1994; Kakinuma et al., 1995). We included the sequences of four newly isolated Argentine strains in the analysis. Table 1 shows the average distance values among and within subtypes. In this study, relatively high values of synonymous substitutions (silent substitutions) were observed in the analysed region between subtypes. N:S ratios within each subtype were 3:0, 5:9, 1:8, 2:6 and 1:8 for subtypes A, B, C, D and E, respectively (Table 1). According to these results, subtype B seems to accumulate more synonymous than non-synonymous changes, suggesting the presence of constraints against amino acid changes in the subtype. $K_A/K_S$ ratios ([proportion of potential amino acid replacement changes]/[proportion of potential silent changes]) for B subtype FIV sequences were similar to those found for non-pathogenic simian immunodeficiency virus. Sodora et al. (1994) therefore predicted a lower immune response and reduced pathogenicity for cats infected with subtype B FIV compared with cats infected by subtype A. In contrast, according to our data, subtypes C and E seem to include amino acid substitutions at much higher frequency although the numbers of isolates analysed in the subtypes were too small to draw any conclusions. The functional constraints that influence variation of the region under study might not be the same for each subtype.

A phylogenetic tree based on synonymous substitution analysis showed that the overall branching pattern was similar to that previously described (Sodora et al., 1994; Kakinuma et al., 1995). The same grouping pattern was found when the phylogenetic tree was constructed from amino acid substitutions (data not shown). env subtype A formed a highly divergent group of North American (California) and European isolates clustering with a recently described Japanese isolate, Sendai-1 (Kakinuma et al., 1995). It is also interesting to note that in group A, strain CA.PPR branched off earlier than the other American and European sequences and is the most distant in the group. env subtype B formed a cluster consisting of geographically distant isolates from Japan, North and South America (LP9 strain). This might be explained by the mobility of the cats (Sodora et al., 1994) which could have been brought by people who migrated from Asia to America or vice versa. env subtypes C and D consisted of Canadian isolates from British Columbia and Japanese isolates of Shizuoka and Fukuoka. The other Argentine isolates, LP3, LP20 and LP24, formed a fifth and new cluster designated here as subtype E. The restricted geographical representation of the cats might
explain why C, D and E subtypes, which were isolated only from Canada, Japan and Argentina, respectively, are less divergent.

It might be of interest that only subtype A was found in the phylogenetic analysis of European isolates (Rigby et al., 1993); env subtypes A and B are present in North America (Sodora et al., 1994) and env subtypes A, B and D are found in Japan (Kakinuma et al., 1995).

Taken together, the nucleotide and amino acid alignments, phylogenetic analyses and genetic distances presented in this study indicate that the isolates designated as subtype E are quite distant from other reported isolates and form a new FIV group. In a related paper, Yamada et al. (1995) described similar results in the phylogenetic analysis of nucleotide sequences of FIV long terminal repeats and found that LP9 clustered together with subtype B sequences, whereas LP3, LP20 and LP24 formed a new group. Although there are no reports on significant biological differences among the five subtypes, the analyses presented here suggested that in the design of an FIV candidate vaccine, subtypes might have to be taken into consideration.

We thank Prof. M.C. Castellano (Veterinary Medical Hospital of La Plata University, Buenos Aires, Argentina) for obtaining blood samples from Argentine cats. We are also grateful to JICA (Japan International Cooperation Agency) for supporting the veterinary research in Argentina. This work was partially supported by grants from the Ministry of Education, Science, Sports and Culture and the Ministry of Health and Welfare. M.R.P. (a visiting scientist from La Plata University) is supported by a Monbusho scholarship.

References


Received 10 November 1995; Accepted 1 April 1996