Feline Immunodeficiency Virus Subtype C is Prevalent in Northern Part of Taiwan

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(Received 5 August 1998/Accepted 22 October 1998)

ABSTRACT. The seroepidemiological survey of cats conducted in northern part of Taiwan in 1998 revealed that the positive rate of feline immunodeficiency virus (FIV)-infection was 21.9% (7/32) and the rate was much higher than those of previous reports. We succeeded in isolation of three strains of FIV from peripheral blood mononuclear cells of the blood samples. Nucleotide sequences of the *env* variable V3 to V5 region of the strains revealed that the isolates from distinct areas belong to subtype C. These data together with our previous report (Inada *et al.* 1997. *Arch. Virol.*, 142: 1459–1467) indicate that FIV subtype C is prevalent in northern part of Taiwan.—KEY WORDS: feline immunodeficiency virus, subtype, Taiwan.

J. Vet. Med. Sci. 61(2): 197–199, 1999

Feline immunodeficiency virus (FIV) was first isolated in the United States in 1986 from a cat with an acquired immunodeficiency syndrome (AIDS)-like disease [13]. FIV infection in cats has been associated with AIDS-related diseases including stomatitis, gingivitis, anemia and neurological dysfunction [10, 13]. FIV has considerable sequence variation in the *env* region of the genome [2–4, 7, 12, 14]. Studies on the sequence variation of FIV *env* variable V3 to V5 region revealed that FIV isolates can be divided into at least five subtypes (A to E) [4, 12, 14]. Seroepidemiological surveys conducted in several countries demonstrated that FIV is prevalent worldwide, and the prevalence of FIV infection in cats varied from 1% to 15% in healthy cats and from 3% to 44% in diseased cats [10]. In Taiwan, the first survey reported in 1990 revealed that FIV-positive rate was 2.5% [5]. Previously, we reported that the FIV-positive rate in Taiwan was 4% in samples collected in 1993 and 1994 [6].

In 1995, we succeeded in isolation of four strains of FIV from cats in Taipei and determined nucleotide sequence of the *env* V3 to V5 region of the strains [3]. Quite interestingly, the sequence data revealed that all the strains belonged to subtype C which had been reported only in Canada [14]. However, it remained to be determined whether FIV in distinct areas of Taiwan also belongs to subtype C. In this study, we isolated additional three strains of FIV from cats in two distinct areas in northern part of Taiwan, and the nucleotide sequences of the *env* V3 to V5 region of the isolates were determined.

Blood samples from 21 and 11 cats in shelters in Taipei and Tam-Sui, respectively, were used in this study. Antibodies against FIV were detected by the indirect immunofluorescence (IF) assay as described previously [9]. Peripheral blood mononuclear cells (PBMCs) were purified by centrifugation over Ficoll-Paque (Amersham Pharmacia Biotech, Buckinghamshire, U.K.). The PBMCs were stimulated with 10 μg/m*l* of concanavalin-A (Amersham Pharmacia Biotech) for three days as described previously [8] and then cocultured with MYA-1 cells, an interleukin-2-dependent feline T-lymphoblastoid cell line which is highly sensitive to FIV [8]. Cultures were maintained in RPMI 1640 growth medium supplemented with 10% heat-inactivated fetal calf serum (FCS), antibiotics, 50 μM 2-mercaptoethanol, 2 μg/m*l* polybrene and 100 units/m*l* of recombinant human interleukin-2 at 37°C in a humidified atmosphere of 5 % CO_2 in air. The medium was replaced with fresh one every 2–4 days, and 12 days after cocultivation of the PBMCs with MYA-1 cells, the cells were harvested for the indirect IF assay. Antigens of FIV in the cultured cells were examined by the indirect IF assay using a serum of an FIV-infected specific pathogen-free cat. Two weeks after cocultivation, total cellular DNA was extracted from the cultures by QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany). FIV-specific DNAs were amplified by polymerase chain reaction (PCR) using a primer pair HV3f and HV5r as described previously [12]. The primer HV3f corresponds to nucleotide position 7,322 to 7,344 and the primer HV5r to position 8,049 to 8,027, according to the published sequence of FIV TM2 strain [7]. The amplified fragments were subjected to the sequencing analysis.

Sequence reactions were performed by the dideoxynucleotide chain termination method using Big Dye Terminator cycle sequencing kit (Applied Biosystems Inc. (ABI), CA, U.S.A.) according to the manufacturer's instructions. The sequencing samples were resolved on an automated DNA sequencer (ABI model 377A). The phylogenetic analysis was carried out using GENETYX-MAC ver. 9.0 (SOFTWARE DEVELOPMENT Co., Ltd.,

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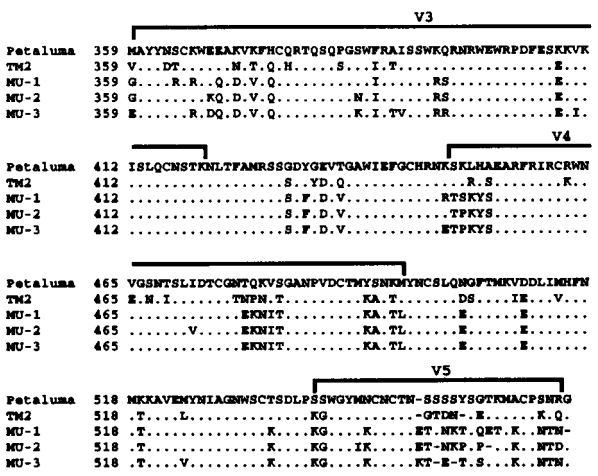

Fig. 1. Alignment of the predicted amino acid sequences encompassing the V3 to V5 region of FIV *env*. Identical amino acids at given positions are represented by dots (.), gaps are represented by dashes (–). The nucleotide sequence data will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB016666 to AB016668.

Tokyo, Japan).

The GenBank accession numbers for the FIV *env* sequences included in this study were Aomori-1 (D37816), Aomori-2 (D37817), CABCpady02C (U02392), CABCpbar01C (U02393), CABCpbar02C (U02394), CABCpbar03C (U02395), CABCpbar07C (U02397), CA.Dixon (L00608), CA.PPR (M36968), Dutch4 (X69498), Dutch6 (X69499), Dutch32 (M73965), DutchK1 (M73964), DutchUtr (X60725), EngUK8 (X69496), FrenchWo (L06312), Fukuoka (D37815), ItalyM1 (X69500), ItalyM4 (X69503), LP3 (D84496), LP20 (D84498), Petaluma (M25381), ScotUK2 (X69494), Sendai-1 (D37813), Sendai-2 (D37814), Shizuoka (D37811), SwissZ1 (X57002), SwissZ2 (X57001), TI-1 (AB016025), TI-2 (AB016026), TI-3 (AB016027), TI-4 (AB016028), TM2 (M59418), USCAalemy01A (U02404), USCAhnky02A (U02400), USCAsam_01A (U02410), USCAtt__01A (U02411), USCAtt__09A (U02413), USCAtt__10A (U02414), USCAzepy01A (U02417), USILbrny03B (U02418), USMAsboy03B (U02419), USOKlgrl02B (U02421), USTXmtex03B (U02422), WalesUK14 (X69497), and Yokohama (D37812).

Seroepidemiological survey revealed that the FIV-positive rate was 9.5% (2 of 21) and 45.5% (5 of 11) in Taipei and Tam-Sui areas, respectively. The overall FIV-positive rate in this study was 21.9%, and the rate was much higher than those of previous reports [5, 6]. Since the number of samples analyzed is small and the samples are collected in only two restricted areas, we can not conclude that the FIV-positive rate observed here is representative of the cat population in Taiwan. However, our data indicate that FIV infection is prevalent in northern part of Taiwan.

The cocultured samples from three FIV-seropositive cats

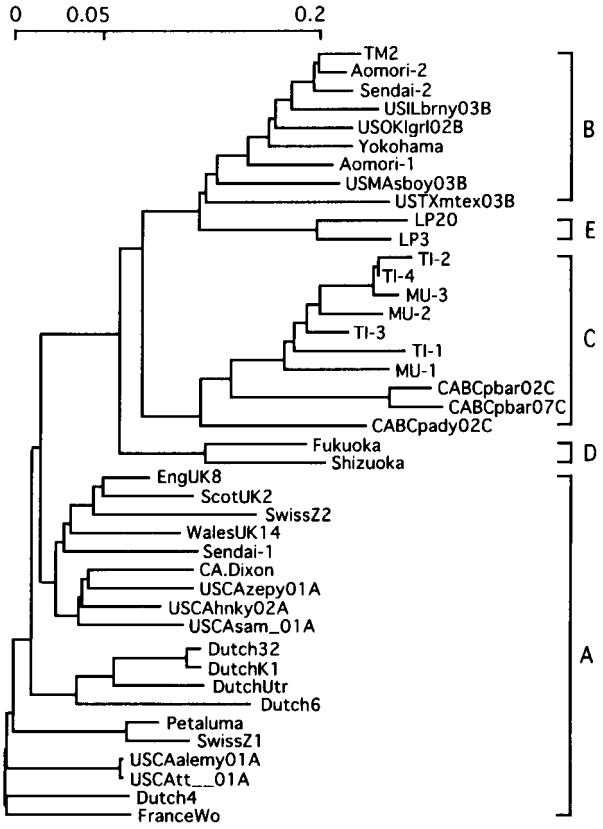

Fig. 2. Phylogenetic tree of the predicted amino acid sequences of the V3 to V5 region of FIV *env*.

were shown to be positive for FIV-specific antigens by the indirect IF assay. FIV-specific provirus was also detected in the three cultures by the PCR analysis. The FIV isolates were designated as MU-1, MU-2 and MU-3 strains. MU-1 was isolated from a cat in Taipei, while MU-2 and MU-3 were from cats in Tam-Sui.

Next, we determined the nucleotide sequences of the *env* V3-V5 region of the strains. The predicted amino acid sequences of the region of MU-1, MU-2 and MU-3 strains were aligned along with 627bp sequences representative of A and B *env* subtypes reported previously [7, 11] (Fig. 1). The cell tropism for CRFK cells depends on a single mutation of the 407th amino acid residue in *env* V3 region. The mutation from a lysine to a glutamic acid at the position converts a CRFK-tropic virus to a non-CRFK-tropic virus [15]. In this study, the predicted amino acid residue at the position of the Taiwanese isolates was glutamic acid indicating their lack of ability to infect CRFK cells. As shown in Fig. 2, phylogenetic analysis revealed that the newly isolated Taiwanese strains belonged to the subtype C group and clustered with the other Taiwanese isolates. The reason for the prevalence of the subtype C in Taiwan is still unknown, however it might be possible that subtype C FIV was introduced from Canada in recent years and is spreading in the cat population in northern part of Taiwan.

It was reported that subtype A seems more pathogenic

than subtype B according to the ratio of non-synonymous and synonymous substitutions (N:S ratio) in the amino acid sequence within the subtypes [12, 14]. However, as for subtypes C, D and E, there are little sequence data available, and it is difficult to evaluate the N:S ratios deduced from the limited number of the sequence data. It was reported that the subtype C FIV strain CABCpady00C could induce accelerated FIV disease by *in vivo* passage of acute-phase virus [1]. To date, it is unknown whether the Taiwanese isolates cause severe FIV-related diseases. Although further clinical studies on FIV-infected cats in Taiwan are necessary to know the pathogenicity of the subtype, our sequence data will be useful to calculate the N:S ratio and to know the relationship between the ratio and pathogenicity of the subtype.

ACKNOWLEDGMENTS. We are grateful to Dr. M. Hattori (Kyoto University, Kyoto, Japan) for providing recombinant human interleukin-2-producing Ltk$^-$IL-2.23 cells. We also thank Dr. Y. Nishimura (The University of Tokyo, Tokyo, Japan) for preparing this manuscript. This study was partly supported by grants from the Ministry of Education, Science, Sports and Culture of Japan.

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