

High Genetic Stability of TM1 and TM2 Strains of Subtype B Feline Immunodeficiency Virus in Long-Term Infection

Yasuhiro IKEDA^{1,6)}, Takayuki MIYAZAWA^{2,4)*}, Yorihiro NISHIMURA⁵⁾, Kazuya NAKAMURA²⁾, Yukinobu TOHYA¹⁾ and Takeshi MIKAMI^{1,3)}

¹⁾Department of Veterinary Microbiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, ²⁾Department of Veterinary Public Health, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, ³⁾Laboratory of Veterinary Public Health, Department of Veterinary Medicine, College of Bioresource, Nihon University, Fujisawa, Kanagawa 252-0813, ⁴⁾Host and Defense, PRESTO, Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, ⁵⁾Department of Virology II, National Institute of Infectious Diseases, Gakuen 4-7-1 Musashimurayama, Tokyo 208-0011, Japan and ⁶⁾Department of Immunology and Molecular Pathology, Windeyer Institute of Medical Sciences, University College London, London W1T 4JF, UK.

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ABSTRACT. To know the genetic changes of feline immunodeficiency virus (FIV) in long-term infection in cats, we inoculated three specific pathogen-free cats with FIV isolates and determined a partial *env* sequence covering the V3-V5 region. In 2 cats infected with subtype B strains TM1 and TM2, only one amino acid change in region V3 was observed at 9 years post infection (y.p.i.), and no nucleotide substitutions were observed between 9 and 10 y.p.i., indicating that these strains are genetically stable. On the other hand, in a cat infected with subtype A strain Petaluma at 8.7 y.p.i., 3 nucleotide insertions (one amino acid insertion) in region V5, and 1 synonymous nucleotide substitution and 2 non-synonymous nucleotide substitutions in region V5, were observed.

KEY WORDS: FIV, mutation, subtype.

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A hallmark of human immunodeficiency virus (HIV) infection is the rapid generation and turnover of viral variants, resulting in a high degree of sequence diversity within and between infected individuals [14]. Immune surveillance [15] and viral cell tropism [10] are examples of plausible selective forces that may be shaping HIV diversity *in vivo*.

Feline immunodeficiency virus (FIV) infection in cats is an important animal model for lentiviral vaccine development and antiviral therapy since FIV causes selective loss of the CD4⁺ T cell subset and acquired immunodeficiency syndrome (AIDS) in naturally infected host species [2]. Similar to HIV, FIV has considerable sequence variation in the *env* gene, and the third to fifth variable regions (V3 to V5) of *env* contain an immunodominant neutralization domain and a determinant of cell tropism [3, 12, 13]. Based on the sequence diversity in V3 to V5, FIV isolates have been classified into 5 subtypes, A to E [8, 11]. In the present study, we estimated the mutation rates of the V3-V5 region in long term infections of over 8 years duration. Our results showed remarkable genetic stability among subtype B FIV isolates in cats.

Three specific pathogen-free (SPF) cats aged 5.5 months were injected intraperitoneally with primary peripheral blood mononuclear cells (PBMCs) infected with the FIV subtype A strain Petaluma (Cat 105) which was isolated from a cat with an immunodeficiency-like disease [9], or 0.5 ml of the peripheral blood of cats naturally infected with

subtype B strains TM1 and TM2 (Cats 103 and 104, respectively) [7]. These cats were kept separately in isolation units during the experiment. As we reported previously [6], Cat 105 died from immunodeficiency-like diseases with remarkable decrease in the CD4/CD8 ratio at 8 years and 8 months after infection. FIV was isolated from Cats 103, 104 and 105 all through the experimental period.

PBMCs were isolated from Cats 103 and 104 at 3 weeks post infection (w.p.i.) and 9 and 10 years p.i. (y.p.i.). PBMCs of Cat 105 were isolated at 3 w.p.i. and 8.7 y.p.i., a week before the death. The isolated PBMCs were stored in liquid nitrogen until the genomic DNA was isolated. For sequencing analyses, total cellular DNA was extracted from the PBMCs with a QIAamp blood kit (QIAGEN, Hilden, Germany) and the part of the *env* gene encompassing the region from V3 to V5 was amplified using the primers HV3f and HV5r and subjected to direct sequencing analysis as described previously [8]. Three independent PCR amplifications were carried out for each of the DNA templates. Each PCR amplification yielded identical results.

The 627 bp nucleotide sequence covering the V3-V5 region from each of the cats was determined and the three were compared. In Cats 103 and 104, no synonymous but non-synonymous substitutions were observed (Fig. 1). The amino acid changes in both cats were located in region V3 at 9 y.p.i. The sequences from Cats 103 and 104 at 10 y.p.i. revealed no nucleotide substitutions between 9 and 10 y.p.i. On the other hand, 3 nucleotide insertions and 3 nucleotide substitutions were observed in Cat 105 at 8.7 y.p.i. One nucleotide change between V3 and V4 was a synonymous substitution, but the others resulted in two amino acid sub-

* CORRESPONDENCE TO: DR. MIYAZAWA, T., Department of Veterinary Public Health, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan.

			V3	
Cat 103 3 w.p.i.	358	QVAYNTCKWEEANVTFQCHRTQSQSGSWIRTISWQQRNRWEWRPDFESEKVKISLQCNSTKNL		
Cat 103 9 y.p.i.	358	N.....		
Cat 103 10 y.p.i.	358	N.....		
			(AAA→AAT)	
			V3	
Cat 104 3 w.p.i.	358	QVAYDTCKWEEANVTFQCHRTQSQSGSWIRTISWQQRNRWEWRPDFESEKVKISLQCNSTKNL		
Cat 104 9 y.p.i.	358	N.....		
Cat 104 10 y.p.i.	358	N.....		
			(GAT→AAT)	
			V3	
Cat 105 3 w.p.i.	372	VKFHCQRTQSQPGSWLRAISSWQQRNRWEWRPDFESEKVKISLQCNSTKNLTFAMRSSGDYGEVT		
Cat 105 8.7 y.p.i.	372	#		
			(ACG→ACA)	
			V4	
Cat 105 3 w.p.i.	437	GAWIEFGCHRNKSKLHAEAREFRIRCRWNVGSNTSLIDTCGNTQNVSGANPVDCTMYSNKMYNCSL		
Cat 105 8.7 y.p.i.	437			
			V5	
Cat 105 3 w.p.i.	502	QNGFTMKVDDLIMHFNMTKAVEMYNIAGNWSCTSDLPSSWGYMNCNCTNSSSN-SGTKMACPSN		
Cat 105 8.7 y.p.i.	502	K.....HG.....		
			(AAT→AAG) (AGT→CAT GGT)	

Fig. 1. Comparison of the amino acid sequence of the V3-V5 region of the FIV *env* gene for isolates within single hosts through long term infection. Identical amino acids are indicated by dots (•), and gaps are indicated by bars (–). The position of the synonymous substitution in Cat 105 is indicated by #. Nucleotide substitutions and insertions in the amino acid codons are indicated as underlined in parentheses. Since there are few amino acid substitutions in the three cats, completely conserved sequences in the V3-V5 region are omitted.

stitutions and one amino acid insertion in the V5 region.

As we reported previously [7], peripheral blood samples from domestic cats naturally infected with FIV strains TM1 and TM2 were used to directly inoculate SPF Cats 103 and 104, respectively. Although we had expected to find a high degree of mutation through long term infection, only one non-synonymous substitution was observed in the V3-V5 region in both Cats 103 and 104 at 10 y.p.i., suggesting remarkable genetic stability among the viruses *in vivo*.

Balfe *et al.* [1] examined a cohort of hemophiliacs who were infected with the same source of HIV type 1 (HIV-1) and estimated the mutation rate of the sequence to be 0.4% nucleotide substitutions per site per year in the V4-V5 region and 0.5% per year in the V3 region. Greene *et al.* [4] reported that the rate of mutation in the V1-V2 *env* region of a subtype A FIV isolate was 0.34% per year, which is comparable to that of HIV-1. However, in the present study, the mutation rates of both TM1 and TM2 (subtype B) were estimated to be only 0.015% per year. The nucleotide mutation rate of strain Petaluma (subtype A) (0.11% per year) was about six times that of TM strains. Cat 105 developed an AIDS-like disease 8 years after infection, whereas both Cats 103 and 104 remained asymptomatic for over 10 years, suggesting a correlation between disease progression and nucleotide substitution rates. The low genetic diversity rates may, in part, be ascribed to the long term non-progres-

sion without viremia in the infected cats even at 8 years post-infection [6]. Although it is still unknown whether this genetic stability of the strains is subtype-specific, it is of note that subtype B isolates are considered to be more host-adapted than subtype A isolates [11], leading to higher genetic stability in cats.

In conclusion, we found the mutational rates of the subtype B isolates, TM1 and TM2 strains, to be lower than expected. Because of their genetic stability and low virulence, modified TM2-type viruses [5] might be a good candidate for an attenuated live vaccine against FIV.

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