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Preliminary comparisons of the biological properties of two strains of feline immunodeficiency virus (FIV) isolated in Japan with FIV Petaluma strain isolated in the United States

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Summary. Two strains of feline immunodeficiency virus (FIV) were isolated directly from peripheral blood mononuclear cells (PBMCs) of Japanese domestic cats or indirectly from PBMCs of specific pathogen free (SPF) cats inoculated with whole blood from naturally infected cats with FIV by cocultivation with primary PBMCs from SPF cats. Two isolates, designated as FIV TM 1 and FIV TM 2, had a lentivirus-like morphology by elecron microscopy, a tropism for interleukin-2 dependent T-lymphocytes and Mg^{2+}-dependent reverse transcriptase activity. By immunoblotting the isolates gave bands at 130, 48, 44, 40, 28, 17, and 13 kDa, and these bands except 130 kDa were detected in FIV Petaluma strain when reacted with the plasma of cats infected naturally with FIV TM 1 strain.

Introduction

Feline immunodeficiency virus (FIV), formally called feline T-lymphotropic lentivirus, was first isolated in 1986 from specific pathogen free (SPF) kittens inoculated with peripheral blood or plasma from cats with an acquired immunodeficiency syndrome (AIDS)-like disease in the United States [10]. This virus has many characteristics in common with human immunodeficiency virus (HIV), such as tropism for T-lymphocytes with cytopathic effects, a virion with a cylindrical nucleoid, Mg^{2+}-dependent reverse transcriptase activity and a distinct virion protein profiles. From these properties, FIV was tentatively classified to subfamily *Lentivirinae*. Recently, molecular cloning of FIV was reported by Olmsted et al., who described there was some serological cross-reactivities in core protein of FIV with caprine arthritis-encephalitis virus and Visna virus, but not with HIV type-1 and bovine immunodeficiency virus [9].

In Japan, 43.9% of cats with some chronic diseases and 12.4% of healthy cats were seropositive to FIV [5, 6]. Ishida et al. described briefly the isolation of FIV from the seropositive cats [6], however no detailed information about biological nature of the isolate is available. In the U.K., retrospective sero-epidemiological data indicated that FIV had existed since 1975 in their country [2] and FIV was isolated from a domestic cat in Great Britain [4]. These findings suggest that FIV is widespread in the world.

The purpose of the present study is to characterize the biological properties of two strains of FIV isolated from domestic cats in Japan and to compare these FIV isolates with Petaluma strain of FIV isolated in the United States.

Materials and methods

Animals

Blood samples were taken from two anti-FIV positive cats (Cat MM and Cat PS) brought to the Veterinary Hospital of the University of Tokyo. Serological tests revealed that the 5-year-old female cat with chronic rhinitis and chronic stomatitis (Cat MM) was negative for feline leukemia virus (FeLV), feline syncytium-forming virus (FeSFV) and feline infectious peritonitis virus (FIPV), and the 3-year-old male cat with chronic stomatitis (Cat PS) was positive for FeSFV, but negative for both FeLV and FIPV. Five SPF cats (Cat 101, 102, 103, 104, 105) maintained at School of Medicine, Kitasato University were used for this study [3]. Two twelve-month-old cats (Cat 101 and Cat 102) were used as donors of PBMCs. Two female cats, one (Cat 103) at four and a half months and the other (Cat 104) at 5 months of age, were inoculated intraperitoneally (IP) with 0.5 ml of peripheral blood taken from Cat MM and Cat PS, respectively. The five-month-old cat (Cat 105) was inoculated IP with primary feline PBMCs infected with the FIV Petaluma strain.

Viruses and sera

The Petaluma strain of FIV grown in primary feline PBMCs was kindly provided by Dr. N. C. Pedersen (University of California, Davis) through Dr. T. Ishida (Nippon Veterinary and Zootechnical College) and used as a reference strain. The Coleman strain of FeSFV [7] was propagated in Crandell feline kidney (CRFK) cells [1]. Anti-FIV and anti-FeSFV reference sera were kindly provided by Dr. T. Ishida.

Cells and cultures

CRFK cells were obtained from Dr. T. Ishida. These cells were grown in Eagle's minimum essential medium supplemented with 10% tryptose phosphate, 8% heat inactivated fetal calf serum (FCS) and antibiotics. The FIV sensitive feline T-lymphoblastoid line cells, designated as MYA-1 cells, were also used as donor cells for isolation and propagation of FIV. The characteristics of the MYA-1 cells are described elsewhere [6a].

Virus isolations

For FIV isolation, PBMCs from cats (2×10^6/ml) were stimulated with 10 μg/ml of Con-A (Pharmacia, Uppsala, Sweden) for 72 h and then cultured 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 (rhIL-2) at 37 °C in a humidified atmosphere of 5% CO_2 in air. After cultivation for several days (usually 5 to 8 days), these cells were cocultivated with Con-A stimulated primary PBMCs from SPF cats. The medium

was replaced by fresh medium every 2–5 days. For indication of virus isolation, the cell-free culture supernatants were tested for reverse transcriptase activity, and/or the cultures were examined for the expression of FIV antigen by indirect immunofluorescein assay (IFA). In some experiments, the PBMCs obtained from the test animals and cultured in growth medium for 5 or 21 days were cocultured with primary PBMCs from SPF cats to examine the efficacy for FIV isolation. The cultures from which FIV was not isolated by cocultivation with fresh PBMCs were frozen, and later, were also cocultured with MYA-1 cells for isolation of FIV.

Serological tests

For detection of FIV or FeSFV antigens, cultured cells were smeared on a glass slide, air-dried and then fixed in acetone for 10 min at room temperature. The fixed cells were incubated at 37 °C for 30 min with the reference serum of FIV or FeSFV. After incubation, the slides were washed 3 times with phosphate-buffered saline (PBS), and then rabbit anti-cat immunoglobulin G (IgG) conjugated with fluorescein-isothiocyanate (FITC) was applied. After incubation for 30 min at 37 °C, the slides were rinsed 3 times with PBS, mounted in buffered glycerol and examined by fluorescence microscopy.

For detection of antibodies against FIV, primary feline PBMCs infected with the FIV Petaluma strain were smeared on glass slides, treated with the test cat sera and stained with FITC conjugated anti-cat IgG as described above.

For detection of antibodies against FeSFV, CRFK cells infected with the FeSFV Coleman strain were smeared on glass slides, treated with the test cat sera and stained with FITC-conjugated anti-cat IgG as described above.

For detection of the group specific antigen of FeLV, Leukassay F kit (Pitman Moore Inc., Washington Crossing, NJ) was used. The antibody to FIPV was detected by enzyme-linked immunosorbent assay.

Immunoblotting analysis

Purified virions obtained by ultra-centrifugation were mixed with sample buffer (2% SDS, 0.0625 M Tris-HCl pH 6.8, 20% glycerol, 5% 2-mercaptoethanol, 0.01% bromophenol blue) and heated at 100 °C for 3 min. The virus proteins fractionated by electrophoresis on 12% polyacrylamide slab gels were transferred to polyvinylidene difluoride filter by electro-blotting [12]. The filters were incubated with Tris-buffered saline (TBS) containing 3% gelatin for 1 h at room temperature and washed 3 times with TBS containing 0.05% Tween-20 (T-TBS). The filters were incubated with test plasma diluted with T-TBS containing 1% gelatin (1 : 300) for 1.5 h at room temperature, washed 3 times and then incubated with anti-cat IgG antibody conjugated with horseradish peroxidase for 1 h at room temperature. After washing, peroxidase activity was detected colorimetically by addition of a fresh solution of diaminobenzidine (3 mg in 10 ml of PBS supplemented with 0.03% hydrogen peroxide).

Electron microscopy

The morphology of the two FIV isolates and the FIV Petaluma strain was compared by elecron microscopy (EM). FIV-infected MYA-1 cells were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), post-fixed in 1% osmium tetroxide in the same buffer, dehydrated, and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate, and observed with JEOL's model 1200 EX electron microscope.

Reverse transcriptase activity assay

The reverse transcriptase activities in cell culture supernatants were assayed as described by Ohta et al. [8].

Results

Isolation of FIVs from Japanese domestic cats naturally infected with FIV

Initial attempts were made to isolate FIV from PBMCs of naturally infected cats. Virus isolation was determined mainly by detection of FIV antigens using IFA and Mg^{2+}-dependent reverse transcriptase activity [RTA (Mg)] in the culture supernatants (Fig. 1). FIV was isolated from the PBMCs of Cat MM cocultured with primary PBMCs from SPF cats at day 21, but not at day 5. However, FIV was isolated from both of PBMCs of Cat PS cocultured at day 5 and day 21. We designated FIVs from Cat MM and Cat PS as FIV TM 1 and TM 2, respectively.

Experimental infection of FIV to SPF kittens

Next we attempted to reisolate FIV from SPF kittens inoculated with either peripheral blood of Cat MM or Cat PS or primary feline PBMCs infected with the FIV Petaluma strain (Table 1). By detection of FIV antigens in the cultured cells, FIV was found to be reisolated from Cat 103 at 6 weeks post inoculation (wpi) by cocultivation with primary PBMCs and at 3 wpi by cocultivation with MYA-1 cells. FIV was also reisolated from Cat 104 at 3 wpi. Similarly, the Petaluma strain of FIV was reisolated from Cat 105 at 1 wpi.

It was reported that FeSFV was isolated from buffy coat cells by cocultivation with CRFK cells [11]. In this study, Cat PS had antibody against FeSFV,

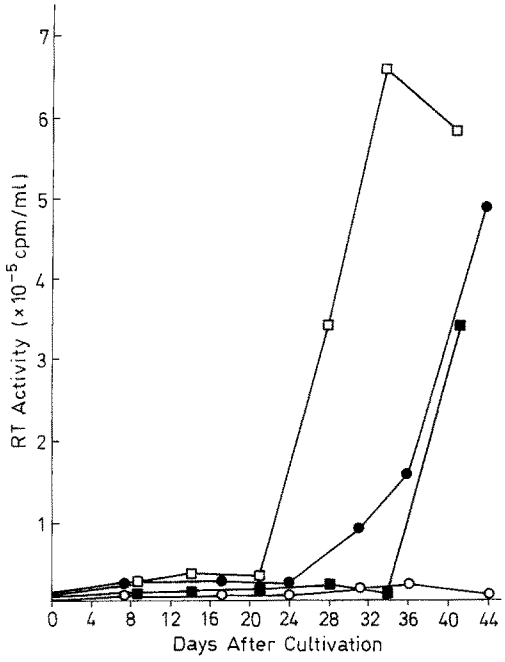

Fig. 1. Reverse transcriptase activity in supernatants of primary PBMCs from cats which were naturally infected with FIV. Cat MM cocultured at day 5 (○——○) and day 21 (●——●) after cultivation, respectively. Cat PS cocultured at day 5 (□——□) and day 21 (■——■) after cultivation, respectively

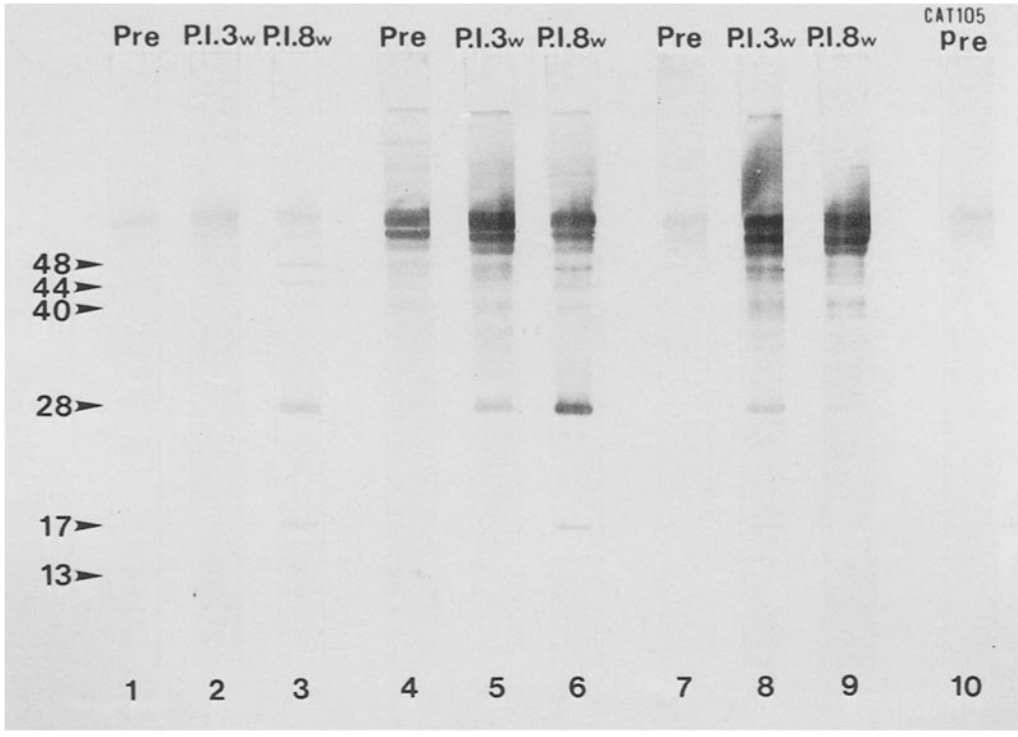

Fig. 2. Detection of anti-FIV antibodies in experimentally FIV infected kittens. Antigen: FIV TM 1 (*1–9*); FCS (*10*). Antibodies: Cat 103 (*1–3*), Cat 104 (*4–6*), Cat 105 (*7–10*); Pre (*1, 4, 7,* and *10*), 3 wpi (*2, 5,* and *8*), 8 wpi (*3, 6,* and *9*)

Table 1. Detection of FIV antigens in cultured lymphocytes from SPF kittens inoculated with FIV

Kitten No.	Inoculum	Weeks after inoculation							
		1	2	3	5	6	16	18	20
103	blood of Cat MM	−	−	(∓)[a]	(∓)	+	ND	ND	+
104	blood of Cat PS	−	ND	+	ND	+	ND	+	ND
105	Petaluma strain of FIV	+	ND	+	+	+	+	ND	ND

[a] FIV was isolated when cocultured with MYA-1 cells
ND Not done

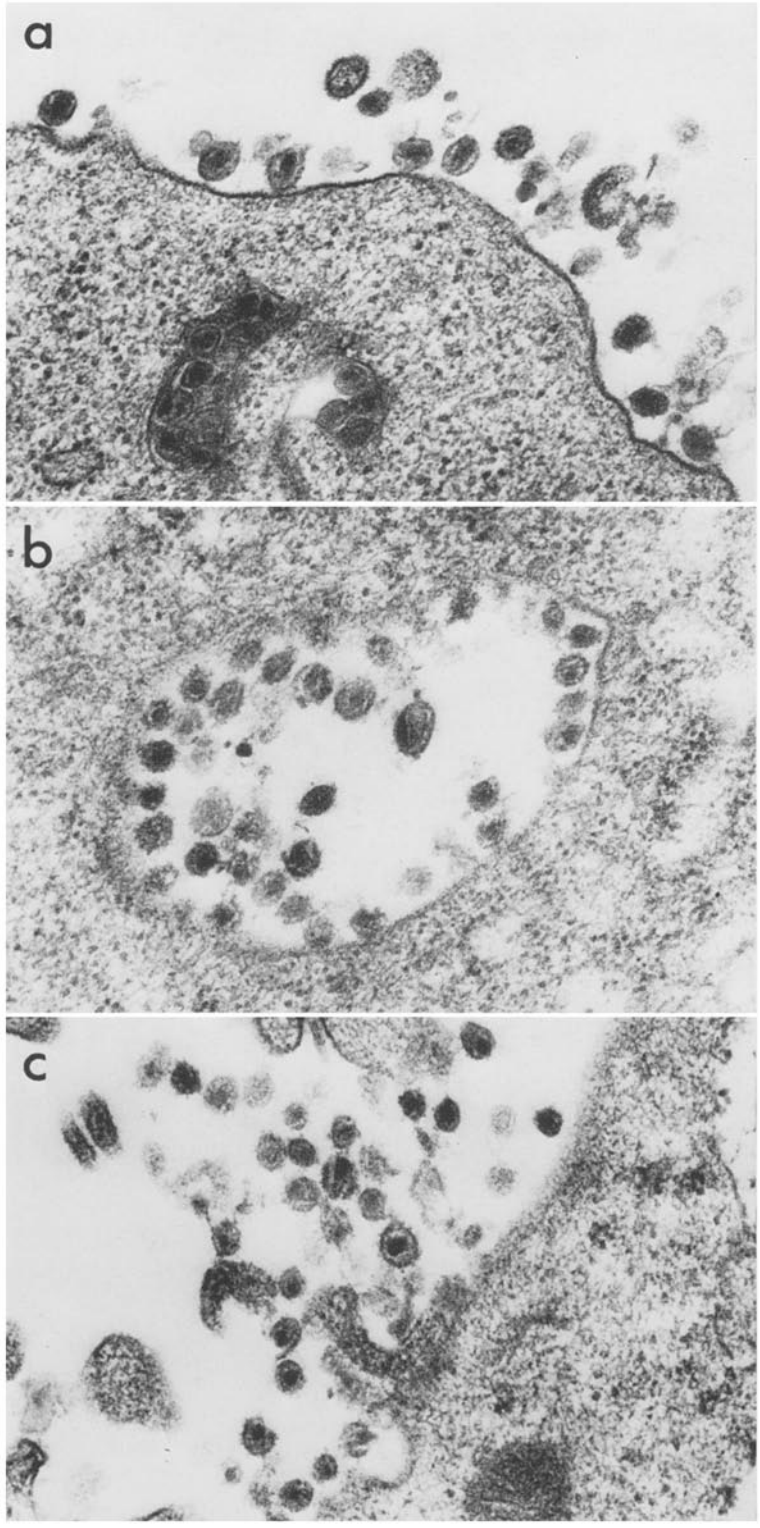

Fig. 3. Morphology of FIV infected MYA-1 cells, FIV sensitive feline T-lymphocytes (× 56,000). **a** FIV TM 1 strain, **b** FIV TM 2 strain, **c** FIV Petaluma strain

and after inoculation of the blood of Cat PS, Cat 104 was seroconverted (data not shown). During the experiments for isolation of FIV from PBMCs, FeSFV antigens were not detected in these cells. However when the cultures from Cat 104 were inoculated onto CRFK, a CPE consisting of syncytium formation was seen after the 3rd passage, and the FeSFV antigen was detected in the cells by IFA. Therefore, Cat 104 was found to be coinfected with FeSFV.

The plasma obtained from three SPF kittens inoculated with FIV were tested by immunoblotting. The TM 1 strain of FIV was used as the antigen (Fig. 2). In the case of Cat 103, distinct bands specific to FIV were detected in the plasma obtained at 8 wpi, but not 3 wpi. However, FIV-specific bands appeared in the plasma collected at 3 wpi from Cat 104 and Cat 105. The intense bands which appeared beyond 60 kDa were mainly the reaction with components of calf serum such as albumin.

Comparison of FIV isolates with Petaluma strain by electron microscopy and immunoblotting

Cultures infected with FIV TM 1, TM 2 or Petaluma strains were examined by electron microscopy (EM). All of the strains showed the characteristic morphology of lentiviruses such as budding particles on the surface of infected cells and mature extracellular enveloped particles of about 100 nm with a cylindrical nucleoid (Fig. 3).

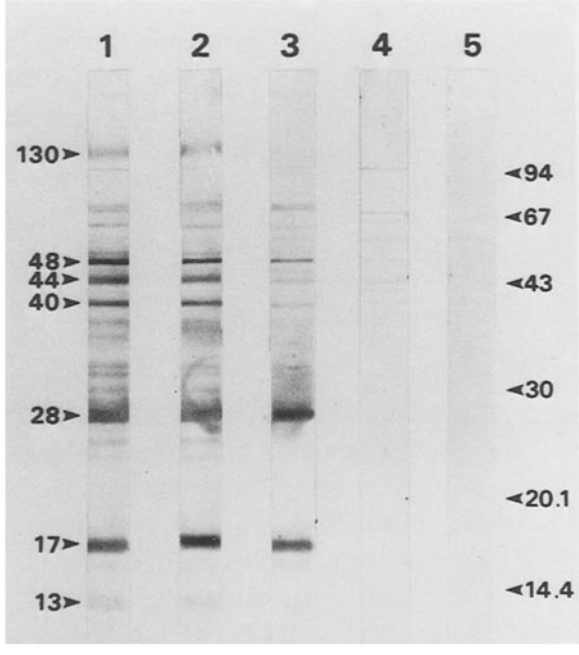

Fig. 4. Comparison of FIV isolates with Petaluma strain of FIV by immunoblotting. Antigens: FIV TM 1 (*1*); FIV TM 2 (*2*); FIV Petaluma (*3* and *5*); lysate of MYA-1 cells (*4*). *1–4* Reacted with the plasma of Cat MM, *5* reacted with the serum of SPF cat

The structural proteins of TM 1 and TM 2 isolates of FIV and Petaluma strain of FIV were compared by immunoblotting analysis (Fig. 4). The TM 1 and TM 2 strains of FIV gave bands of 130, 48, 44, 40, 28, 17, and 13 kDa when treated with the plasma of Cat MM. Almost all the major structural proteins observed in our FIV isolates were also detected in the Petaluma strain of FIV except the bands of 130 kDa and 13 kDa.

Discussion

The present study provided information on FIV isolation from Japanese domestic cats and comparisons of biological properties of these isolates with those of the reference Petaluma strain of FIV.

FIV was isolated directly from two naturally infected cats with FIV (Cat MM and Cat PS) by cocultivation with fresh PBMCs at day 21, but not at day 5 from Cat MM (Fig. 1). Further, we have not succeeded in isolating FIV from Cat 103 inoculated with the peripheral blood of Cat MM until 6 wpi by cocultivation with fresh PBMCs or detecting anti-FIV antibodies at 3 wpi. These data indicate that the population of FIV-infected cells in Cat MM were relatively few. For isolation of FIV, both the susceptibility of donor cells and the amount of FIV-infected lymphocytes in cats appear to be important. Considering the donor cells, MYA-1 cells were vigorously growing lymphocytes and found to be more susceptible for FIV replication than the fresh PBMCs from SPF cats [6a].

Induction of the balloon-type cell or syncytium formation in FIV-infected primary PBMCs were previously reported [4, 10, 13]. However, we seldom observed this type of CPE in FIV-infected primary PBMCs, and sometimes in FIV-infected MYA-1 cells. The main CPE in FIV infection which was observed in this study was of cell death type. The CPE of the balloon-type might be depend on the strains of FIV or the donor cells used.

It is reported that 72% of cats infected with FIV are positive for FeSFV infection [14]. In the present study, we isolated FeSFV from Cat 104 inoculated with the peripheral blood from Cat PS that had antibodies against FeSFV, indicating that both FIV and FeSFV could infect and grow in primary PBMCs and MYA-1 cells. Therefore, it is necessary to establish in vitro host cell systems sensitive for FIV growth but insensitive for FeSFV growth to eliminate FeSFV contamination. For isolation of FIV, we must pay attention to the contamination with FeSFV.

Immunoblotting analysis revealed that our two isolates were closely related to the Petaluma strain of FIV in structural proteins. However, several proteins which had not identified in previous reports were detected in the TM 1 and TM 2 strains of FIV. The protein at 130 kDa may be corresponding to an external envelope protein that had not been identified in previous reports [5, 10, 13]. The proteins of 48 kDa and 40 kDa which had been previously reported by Ishida et al. [5] but not in the report of Yamamoto et al. [13] were detected,

while the properties of these proteins are still unknown. The proteins of 48 kDa and 13 kDa found in the present study might be corresponding to the proteins of 52 kDa and 10 kDa detected by Yamamoto et al. [13], respectively. We failed to find the protein of 130 kDa in the Petaluma strain of FIV when reacted with the plasma from Cat MM (Fig. 4) or Cat 105 which had been inoculated with the Petaluma strain of FIV (data not shown). The reason of the failure might be due to the lack of the protein of 130 kDa in preparation of the Petaluma strain of FIV. However, there remains another possibility of the difference in antigenicity of envelope proteins between our isolates and the Petaluma strain.

Further virological studies on FIV are necessary to apply infection of FIV in cats as an animal model for human AIDS.

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