JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1993, p. 494–501
0095-1137/93/030494-08$02.00/0

Vol. 31, No. 3

Detection of Feline Immunodeficiency Virus in Saliva and Plasma by Cultivation and Polymerase Chain Reaction

DONATELLA MATTEUCCI,[1] FULVIA BALDINOTTI,[1] PAOLA MAZZETTI,[1] MAURO PISTELLO,[1] PATRIZIA BANDECCHI,[2] ROSSELLA GHILARDUCCI,[1] ALESSANDRO POLI,[2] FRANCO TOZZINI,[2] AND MAURO BENDINELLI[1]*

Department of Biomedicine[1] and Department of Animal Pathology,[2] University of Pisa, I-56127 Pisa, Italy

Received 27 August 1992/Accepted 10 December 1992

The rates of feline immunodeficiency virus (FIV) isolation from saliva, plasma, and peripheral blood mononuclear cells (PBMC) of infected cats were compared; isolation rates were 18, 14, and 81%, respectively, in naturally infected cats and 25, 57, and 100%, respectively, in experimentally infected animals. There was no obvious relationship between isolation rate and clinical stage or between isolation rate and the titer of neutralizing antibody in serum. Virus could be isolated from one salivary gland as early as 1 week postinfection and, on a more regular basis, starting at 3 weeks postinfection, when, however, most other tissues were also positive. Polymerase chain reaction analysis showed that FIV genomes are present in saliva and plasma more frequently than expected on the basis of isolation data. Saliva was also found to contain viral DNA, indicating that it may harbor virus-infected cells as well as free virus. The addition of plasma but not of saliva to PBMC cultures delayed FIV growth. Isolation from plasma may be hampered by FIV neutralizing antibody and by the cytotoxic activity of this fluid for the PBMC used as a cell substrate.

The feline immunodeficiency virus (FIV) shows considerable biological and pathogenetic similarities to the human immunodeficiency virus (HIV). It is, therefore, considered a useful small-animal model for AIDS studies. In addition, it is a widespread threat to the health of domestic cats (2, 16).

Bites are believed to represent the major means of FIV transmission (16). Nonetheless, relatively few data have been published so far concerning the duration and levels of FIV shedding in saliva. Likewise, little is known about virus content in the plasma of infected cats. Precise knowledge of this matter is important not only for a better understanding of FIV epidemiology but also because virus excretion and plasma viremia levels are important parameters for evaluating the efficacies of vaccines and therapeutic interventions.

In this report, we have compared the frequencies with which FIV can be isolated from the saliva, plasma, and peripheral blood mononuclear cells (PBMC) of infected cats at different stages of clinical progression and at different times after infection. As the rates of FIV isolation from saliva and plasma were markedly lower than the rate of FIV isolation from PBMC, we examined these fluids for FIV genomes by the polymerase chain reaction (PCR) and salivary glands and other tissues for infectious virus. The possibility that saliva and plasma may interfere with FIV growth was also examined.

MATERIALS AND METHODS

Cats and FIV infection. Thirty-two naturally infected cats that had been referred to the University of Pisa Veterinary Medical Clinic and that tested positive for FIV antibody and negative for feline leukemia virus antigen by using a commercial kit (Cite Combo FIV-FeLV; Agritech Systems, Portland, Maine) were used in this study. At the time of sampling, 12 cats were asymptomatic, 10 cats had symptoms compatible with the diagnosis of feline AIDS-related complex (recurrent fever of undetermined origin, lymphadenopathy, weight loss, etc.), and 10 cats showed clinical signs of chronic opportunistic infections suggestive of feline AIDS (FAIDS) (16). Various ages and breeds and both sexes were represented. Specific-pathogen-free (SPF) cats were purchased from Iffa Credo, L'Arbresle, France, and infected at 20 to 30 weeks of age.

Virus. The Pisa-M2 isolate of FIV was originally obtained from a clinically healthy, feline leukemia virus-negative cat and has since been freed of the initially present feline syncytium-forming spumavirus by two consecutive passages of cell-free plasma in cats. The preparation used to infect cats is propagated in vivo by monthly passages in SPF cats. The inoculum used (2 ml of freshly collected blood) leads to seroconversion in 4 to 6 weeks and to a reduction in the number of circulating $CD4^+$ lymphocytes by almost two-thirds in 1 year (24). Infected SPF animals have remained symptom free through the 6 months of observation. The preparation of the Pisa-M2 isolate used for infecting PBMC cultures was produced on the MBM feline lymphoid cell line obtained in this laboratory by propagating the PBMC of a feline leukemia virus- and FIV-negative SPF cat in the presence of concanavalin A (ConA) and interleukin 2 over a period of several months (unpublished data); the supernatant harvested from such cultures 14 days after infection was centrifuged at 700 × *g* and stored at −70°C. The virus titer in this fluid was $10^{5.0}$ 50% tissue culture infective doses per ml. The Petaluma isolate used for the neutralization assay has been propagated exactly as described previously (21).

Virus isolation. Blood specimens were obtained by jugular phlebotomy and collected into tubes containing EDTA. Following Ficoll-Hypaque (Lymphoprep) separation, PBMC were cultured at concentrations of 3×10^6 to 5×10^6 cells per well in 24-well plates with 1 ml of RPMI 1640 containing 10% fetal bovine serum, 2 mM L-glutamine, 50 μg of gentamicin, and 5 μg of ConA (Sigma, St. Louis, Mo.). Three days later, the cultures were supplemented with 20 U

* Corresponding author.

of human recombinant interleukin 2 (Boehringer, Mannheim, Germany) per ml and 10^6 PBMC obtained from normal SPF cats and prestimulated with ConA 3 to 4 days earlier. Every 3 to 4 days during incubation, the cultures were examined for cell viability and 0.5 ml of each supernatant was substituted with fresh medium supplemented with 20 U of interleukin 2 per ml. With every second medium change, 5 μg of ConA was also added. When viable cell counts dropped below 10^6/ml, the cultures were supplemented with 1×10^6 to 2×10^6 normal lymphoblasts.

Plasma was obtained by centrifuging blood at $700 \times g$ for 20 min and then centrifuging the supernatant at $900 \times g$ for 10 min. Saliva samples were collected with two cotton swabs which were held in the mouth for about 1 min and then squeezed into a tube with the help of a plastic rod. The amount of saliva collected from each cat was approximately 200 μl as determined by weighing the swabs before and after collection. Medium (1 ml) containing penicillin (500 U/ml), streptomycin (0.5 μg/ml), gentamicin (0.5 μg/ml), neomycin (0.5 mg/ml), and amphotericin B (Fungizone) (25 ng/ml) was added to the saliva samples and allowed to act for 2 h at 4°C. Within 3 h after collection, 0.1 ml of plasma and 0.5 ml of a saliva sample were inoculated into 2×10^6 normal lymphoblasts in 24-well plates, which were then processed as described above.

The procedure used to isolate FIV from various organs varied depending on the type of tissue: lymphoid tissues were examined by treating suspended cells as described above for PBMC, while nonlymphoid tissues were examined by inoculating 0.1 ml of 20% (wt/vol) extracts into normal lymphoblasts, which were then processed as described above.

All the cultures were monitored every 3 to 4 days for Mg^{2+}- and Mn^{2+}-dependent reverse transcriptase (RT) activity. Supernatants were considered positive for FIV when Mg^{2+}-dependent RT activity was fivefold greater than that in supernatants of noninfected control cultures. FIV infection of the cultures was confirmed by assaying the supernatant fluids for FIV p24 antigen with a solid-phase enzyme assay using monoclonal antibodies which recognize different B epitopes of FIV p24 (14). Negative cultures were terminated 5 or more weeks after they had been established. In experiments not shown, the isolation procedure has been found to allow the isolation of FIV from as few as 10 PBMC obtained from donors with terminal FAIDS.

FIV neutralizing-antibody assay. Sera were tested for neutralizing antibody by using a recently described assay based on inhibition of syncytium formation (21). Briefly, twofold dilutions of heat-inactivated serum were mixed with 100 syncytium-forming units of the Petaluma FIV isolate in 24-well plates. After 1 h of incubation at room temperature, 10^4 Crandell feline kidney (CrFK) cells adapted to grow in 0.5% fetal bovine serum in 0.5 ml of medium were added to each well. Six days later, the cultures were stained and the syncytia were counted under the microscope. The titer was expressed as the highest dilution of serum which prevented the formation of syncytia.

DNA and RNA extraction. Pellets were obtained by centrifuging 1 ml of plasma passed through 0.45-μm-pore-size filters or 100 μl of whole saliva diluted in a phosphate buffer solution at $20,000 \times g$ for 2 h. For DNA extraction, such pellets or 10^6 PBMC were dissolved in 200 μl of DNA lysis buffer (10 mM Tris HCl [pH 7.5], 100 mM NaCl, 10 mM EDTA, 1% sodium dodecyl sulfate [SDS], and 200 μg of proteinase K per ml), incubated for 2 h at 55°C, and extracted with phenol-chloroform (1:1) and then with chloroform-isoamyl alcohol (24:1). DNA was precipitated with NaCl-ethanol and then dissolved in 20 μl of water. Negative and positive DNA controls were extracted as described above from noninfected and persistently infected CrFK cells (23).

For RNA extraction, all steps were conducted in ice and in the shortest possible time to reduce degradation. Pellets prepared as described above were dissolved in 200 μl of RNA lysis buffer (4 mM guanidium thiocyanate, 25 mM sodium citrate [pH 6.5], 0.5% sarcosyl, 0.2 M sodium acetate [pH 4], 0.1 M 2-mercaptoethanol) and extracted with phenol at pH 4 and chloroform-isoamyl alcohol (49:1). RNA was precipitated with cold isopropanol and then dissolved in 20 μl of 10 mM Tris HCl (pH 7.5)–1 mM EDTA–6 mM $MgCl_2$ (3). To remove contaminant DNA, the samples were incubated with 20 U of RNase-free DNase I from a bovine pancreas (Promega, Madison, Wis.) at 37°C for 15 min and then heated at 95°C for 5 min to stop the reaction. Negative and positive RNA controls were obtained as described above from cell-free supernatants of uninfected and FIV-infected CrFK cells. All precautions to avoid contamination were taken.

Reverse transcription and PCR. The primers and probe used were derived from the published sequence (20) of the FIV p24 region (sense primer, GGCATATCCTATTCAAA CAG [nucleotides 1025 to 1044]; antisense primer, AAGA GTTGCATTTTATATCC [nucleotides 1680 to 1699]; probe, GAAGCAAGATTTGCACCAGCTAGGATGCAGTGTAGA [nucleotides 1362 to 1397]). For DNA amplification, 10 μl of a sample or 5 ng of the DNA control was added to a mixture containing 50 pmol of each primer, 200 μM deoxynucleoside triphosphates (dNTPs), and 2 U of *Taq* polymerase (Promega) in *Taq* polymerase buffer (50 mM KCl, 10 mM Tris HCl [pH 9.0], 1.5 mM $MgCl_2$, 0.1% Triton X-100). Reverse transcription was performed on 10-μl RNA samples or RNA controls in a mixture containing *Taq* polymerase buffer, 50 pmol of the antisense primer, 7.5 mM $MgCl_2$, 1 mM dNTPs, and 7.5 U of avian myeloblastosis virus RT (Promega). After 1 h of incubation at 42°C, 50 pmol of the sense primer and 2 U of *Taq* polymerase were added to the mixtures. Amplification was carried out for 40 cycles (1 min at 94°C for denaturation, 1 min at 50°C for annealing, and 2 min at 72°C for elongation) in a thermal cycler (Savant Instruments, Farmingdale, N.Y.). Amplified products (20 μl each) were run on 1% agarose gels, denatured with alkali, and transferred onto Nytran membrane filters (Schleicher & Schuell, Dassel, Germany). After electrophoresis, the membranes were prehybridized (6× SSC [1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate]–5× Denhardt's solution–100 μg of *Saccharomyces cerevisiae* tRNA per ml–0.5% SDS) at 42°C for 1 h and hybridized (6× SSC–1× Denhardt's solution–100 μg of yeast tRNA per ml–0.05% sodium PP_i) overnight at 42°C with the ^{32}P-end-labeled oligonucleotide probe. Filters were washed at 60°C with 5× SSC and 0.1% SDS and autoradiographed with NIF-100 films (Du Pont, Bad Hamburg, Germany) at −80°C for 4 to 30 h.

The lower limit of sensitivity of PCR for FIV DNA was 12 proviral molecules, as determined by examining serial 10-fold dilutions of the p34TF10 plasmid containing the whole FIV genome of the Petaluma isolate (gift of J. E. Elder, La Jolla, Calif.) in DNA from an FIV-negative cat. The lower limit of sensitivity of PCR for FIV RNA was 15 genome molecules, as evaluated by amplifying cDNA obtained from serial twofold dilutions of RNA extracted from purified FIV

TABLE 1. Isolation of FIV from saliva, plasma, and PBMC of naturally infected cats and its correlation with clinical status

Clinical status[a]	No. of positive cultures/total no. of cultures[b]		
	Saliva	Plasma	PBMC
2 (latency)	2/12 (31)	0/11	9/12 (18)
3 (LAS)[c]	2/8 (37)	1/8 (24)	9/10 (15)
4 (FAIDS)	1/8 (9)	3/10 (14)	8/10 (8)
Total	5/28	4/29	26/32

[a] According to the system in reference 16.
[b] The average number of days before detection of the first positive culture is given in parentheses.
[c] LAS, lymphadenopathy syndrome.

(isolate Pisa-M2) grown on MBM cells in RNA from uninfected cells.

RESULTS

Isolation of FIV from naturally infected cats. FIV was successfully isolated from saliva for 5 of 28 field cats examined, from plasma for 4 of 29 cats, and from PBMC for 26 of 32 cats. Usually, detection of virus in positive saliva cultures required a longer incubation than that required for detection of virus in PBMC cultures (Table 1). Clinical stage (Table 1) and the neutralizing-antibody titer in serum (Table 2) showed no significant influences (Fisher's exact test) on isolation rates. However, the cultures of samples from animals with FAIDS consistently became virus positive earlier (Table 1) and reached higher peak levels of RT activity than those of samples from asymptomatic cats (means ± standard deviations, 37,024 ± 4,178 and 5,755 ± 2,181 cpm/30 μl, respectively; $P < 0.01$).

TABLE 2. Isolation of FIV from naturally infected cats and its correlation with the neutralizing-antibody titer

Neutralizing-antibody titer	No. of positive cultures/total no. of cultures[a]		
	Saliva	Plasma	PBMC
<1:4–1:256	2/10 (21)	1/10 (13)	6/10 (11)
1:512–1:8192	2/11 (31)	0/9	13/14 (14)

[a] The average number of days before detection of the first positive culture is given in parentheses.

Isolation of FIV from experimentally infected cats. Figure 1 summarizes the results obtained for a group of nine SPF cats examined at various intervals after experimental infection. Whereas all the PBMC examined yielded positive cultures, FIV was isolated from only four saliva samples, in each case with low levels of RT production. With these animals, plasma cultures performed considerably better than with naturally infected cats (54 versus 14% positive cultures). The mean times until detection of a positive culture were 26, 19, and 9 days for saliva, plasma, and PBMC, respectively (data not shown).

Table 3 shows the results obtained by examining two SPF cats at closer intervals and for a longer period of time. While PBMC cultures were constantly positive, saliva was positive only at 18 weeks postinfection (p.i.) for both cats and plasma was positive at 1 week p.i. and then on several occasions from the 10th to the 22nd week p.i. In these cats, the presence of FIV neutralizing antibody in serum was also monitored. Actively produced neutralizing antibody became evident at 4 to 6 weeks p.i. and reached high levels within 3 to 4 months. Again, there was no evidence that the neutralizing-antibody titer correlated with the likelihood of isolating FIV.

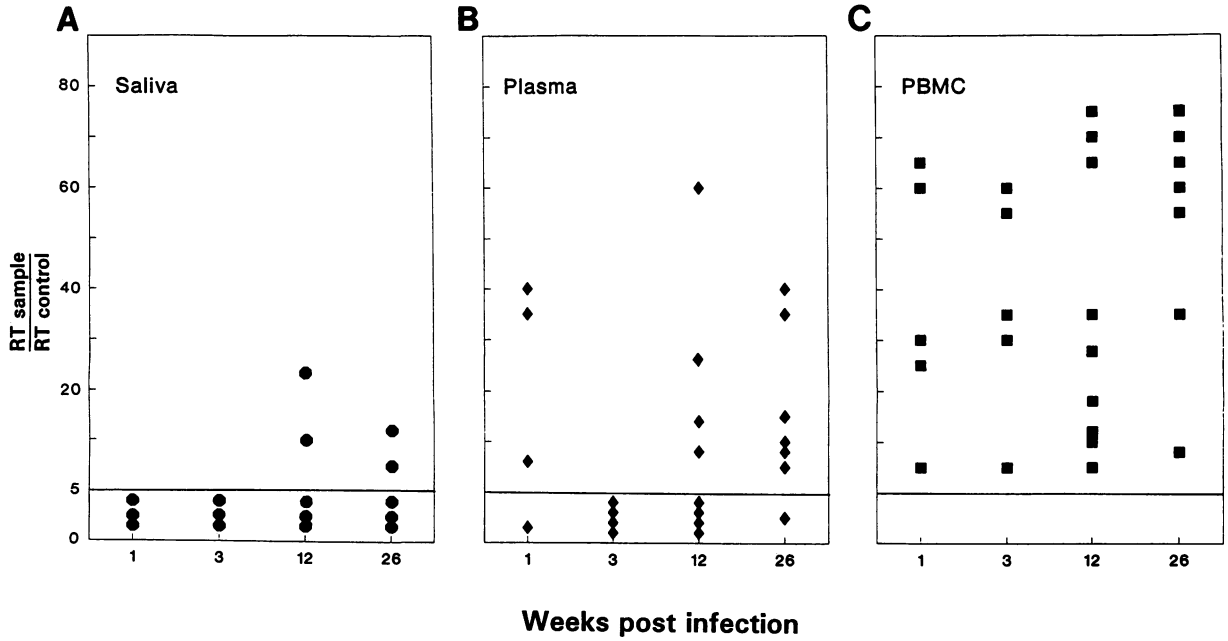

FIG. 1. Isolation of FIV from saliva (A), plasma (B), and PBMC (C) of SPF cats at various times p.i. Results are expressed as ratios of Mg^{2+}-dependent RT activities exhibited by inoculated cultures to those exhibited by control cultures. The mean number of counts per minute produced by control uninfected cultures ± standard deviation was 204 ± 74 cpm. Ratios lower than 5 are considered negative; this threshold is indicated by a horizontal line.

TABLE 3. Isolation of FIV from saliva, plasma, and PBMC of two SPF cats and its correlation with the neutralizing-antibody titer

Cat	Wk p.i.	Result for culture[a]			Neutralizing-antibody titer
		Saliva	Plasma	PBMC	
M2-VIIa	1	−	+ (42)	+ (15)	<1:8
	2	−	−	+ (7)	1:8
	3	−	−	+ (7)	1:8
	4	−	−	+ (3)	1:8
	6	−	−	+ (7)	1:32
	10	ND	+ (13)	+ (9)	1:256
	14	ND	+ (11)	+ (7)	1:1,024
	18	+ (34)	+ (34)	+ (4)	1:4,096
	22	−	−	+ (7)	1:4,096
	28	−	−	+ (3)	1:1,024
	36	−	−	+ (6)	1:8,192
	44	−	−	+ (7)	1:4,096
M2-VIIb	1	−	+ (42)	+ (8)	1:8
	2	−	−	+ (7)	1:8
	3	−	−	+ (7)	<1:8
	4	−	−	+ (7)	1:16
	6	−	−	+ (7)	1:32
	10	ND	+ (9)	+ (9)	1:256
	14	ND	+ (11)	+ (7)	1:1,024
	18	+ (34)	+ (15)	+ (4)	1:1,024
	22	−	+ (23)	+ (7)	1:2,048
	28	−	−	+ (6)	1:1,024
	36	−	−	+ (10)	1:4,096
	44	−	−	+ (7)	1:512

[a] + and −, FIV-positive and -negative cultures, respectively. The first day that the culture was positive is given in parentheses. ND, not done or contaminated.

Isolation of FIV from salivary glands and other tissues. Table 4 summarizes the results obtained by culturing various organs of experimentally infected SPF cats. All the lymphoid organs examined yielded positive cultures at 1 week p.i. The only nonlymphoid organ culture positive at this time was the submandibular salivary gland. At 3 weeks p.i., other nonlymphoid organs were positive. At the two subsequent examination times (9 and 13 weeks p.i.), FIV was isolated from all organs except the brain and the liver.

PCR analysis. The presence of viral DNA and RNA in samples obtained from SPF cats infected for various time intervals was examined by using PCR and hybridization. As shown in Fig. 2, the majority of saliva and plasma samples tested positive, usually with strong signals. They included numerous samples that remained culture negative. However, while filtered plasma was positive only for viral RNA (unfiltered plasma was occasionally DNA positive, most likely because of the presence of residual cells or cell debris [data not shown]), saliva was found to contain both viral RNA and viral DNA. As expected, all the PBMC preparations tested positive for both viral RNA and viral DNA. All tests were repeated at least once, with the same results.

Effects of saliva and plasma on FIV isolation. Figure 3 shows the effects of adding saliva and plasma on the growth of cell-free virus in PBMC cultures. While the addition of saliva had no detectable effects, the addition of plasma substantially delayed FIV isolation. The effect was more pronounced with plasma obtained from infected cats than with plasma from noninfected animals, and its extent appeared to be related to the neutralizing-antibody titer of the plasma used. Plasma, but not saliva, also diminished the viabilities of the PBMC cultures (data not shown). Figure 4 shows the effect of a similar experiment, in which saliva or plasma was added to cultures of PBMC obtained from one infected cat in order to study their action on cell-associated virus. Again, saliva had no effect, while plasma was inhibitory. In this case, however, the inhibitory effects exerted by plasma from infected cats and plasma from noninfected cats were similar, suggesting that inhibition may result from the toxicity of plasma for the PBMC cultures (Fig. 5). Such

TABLE 4. Isolation of FIV from salivary glands and other organs of SPF cats

Organ	Method[a]	Result for culture the following no. of wk p.i.[b]:			
		1	3	9	13
Salivary glands					
Parotid	Ex	−	+	+	ND
Submandibular	Ex	+	+	+	+
Lung	Ex	−	+	+	+
Ovary	Ex	−	+	+	+
Liver	Ex	−	+	−	−
Brain	Ex	−	+	−	−
Muscle	Ex	−	+	+	ND
PBMC	C	+	+	+	+
Thymus	C	+	+	+	+
Bone marrow	C	+	+	+	+
Spleen	C	+	+	ND	+
Lymph nodes					
Mesenteric	C	+	−	+	+
Submandibular	C	+	−	+	+

[a] Nonlymphoid tissues were examined by inoculating 20% (wt/vol) extracts onto fresh lymphoblasts (Ex). Lymphoid tissues were examined by stimulating suspended cells with ConA (C). When needed, fresh lymphoblasts were added to the cultures. Negative cultures were terminated 5 or more weeks after they were established.

[b] + and −, FIV-positive and -negative cultures, respectively. All results are for different animals. ND, not done.

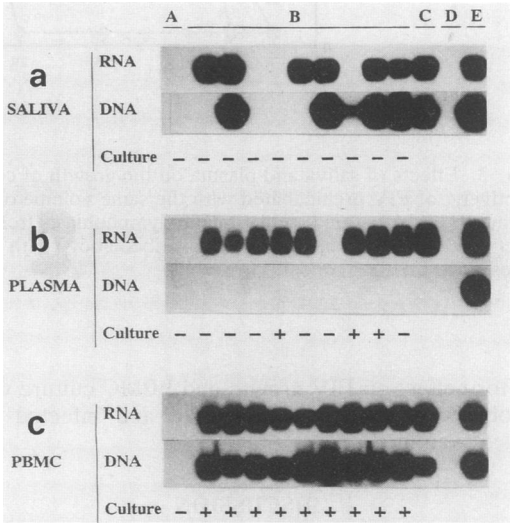

FIG. 2. Representative results of PCR analysis for FIV RNA and DNA in saliva (a), plasma (b), and PBMC (c) of SPF cats at various times p.i. A, uninfected animal; B, nine animals infected with the Pisa-M2 isolate 3 to 19 months earlier; C, animal infected with the Petaluma isolate 7 months earlier; D, uninfected CrFK cells; E, CrFK cells infected with the Petaluma isolate.

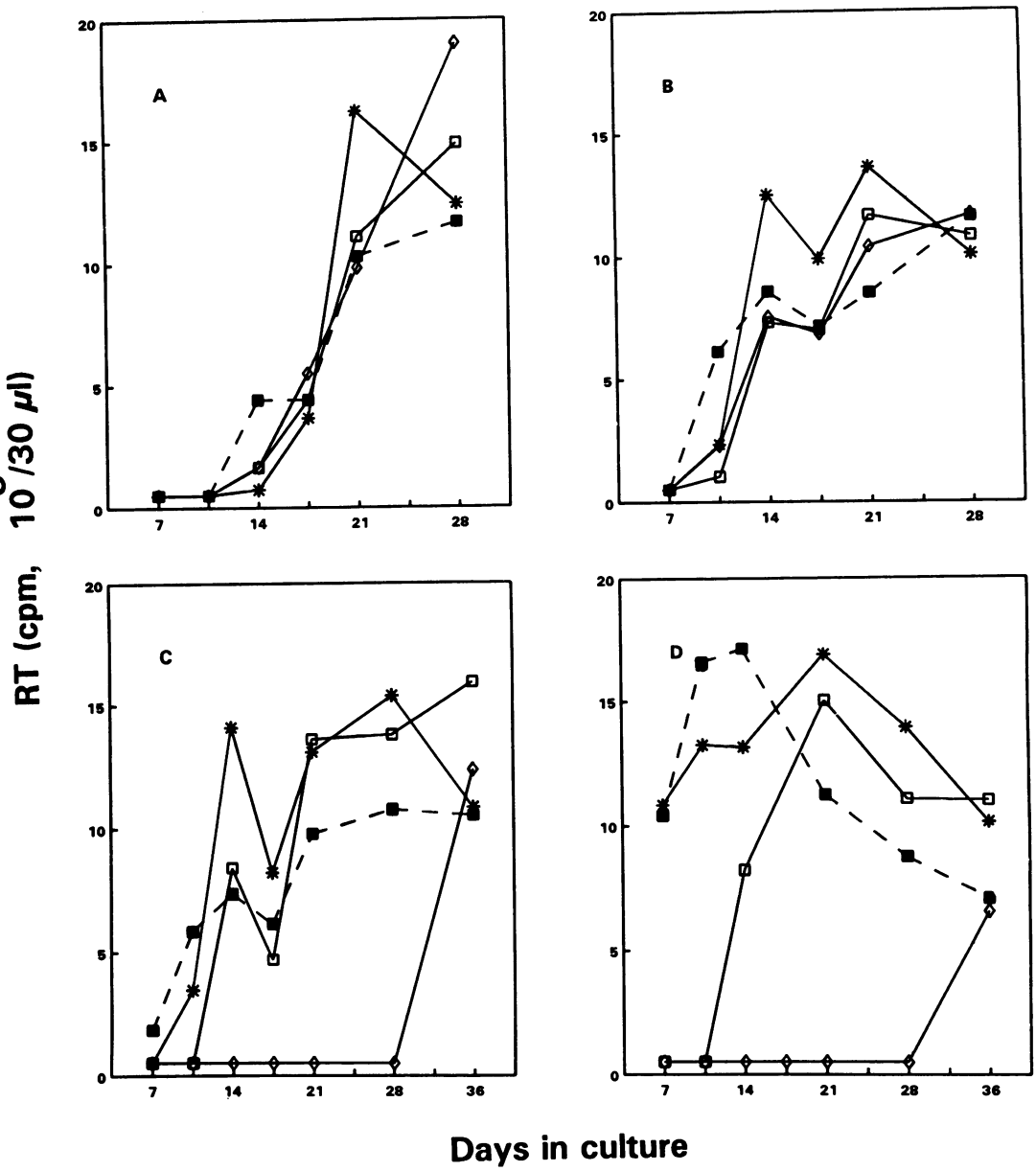

FIG. 3. Effects of saliva and plasma on the growth of cell-free FIV. (A and B) Growth of 10 and 10^3 50% tissue culture infective doses, respectively, of FIV preincubated with the same volume of saliva from an uninfected cat (*) or one of two infected cats (□ and ◇) or with medium (■) for 2 h before inoculation into lymphoblasts from normal cats. (C and D) Growth of 10^2 and 10^4 50% tissue culture infective doses, respectively, of FIV preincubated as described above with plasma samples from the same cats. Titers of neutralizing antibody in the plasma samples were 1:512 (□) and 1:4,096 (◇).

inhibitory effect on FIV growth and PBMC culture viability was observed with all noninfected and infected plasma samples examined.

DISCUSSION

Existing epidemiological data indicate that biting is the major means of FIV infection among domestic cats (16, 22, 23). Nonetheless, little is known about the extent of FIV shedding in saliva. Yamamoto et al. isolated FIV from the saliva of 2 of 5 healthy cats and of 5 of 5 unhealthy infected cats and observed that a single experimentally administered bite from an infected cat was sufficient to transmit the infection to susceptible animals (22). On the other hand, data on FIV isolation from plasma are also scanty (23).

The present findings show that FIV isolation from saliva is considerably less frequent than FIV isolation from PBMC. FIV isolation from plasma was uncommon with field cats but relatively frequent with recently infected SPF cats. The results also indicate that the likelihoods of isolating FIV from saliva and plasma are not related to neutralizing-antibody titers in serum or the animal's clinical condition.

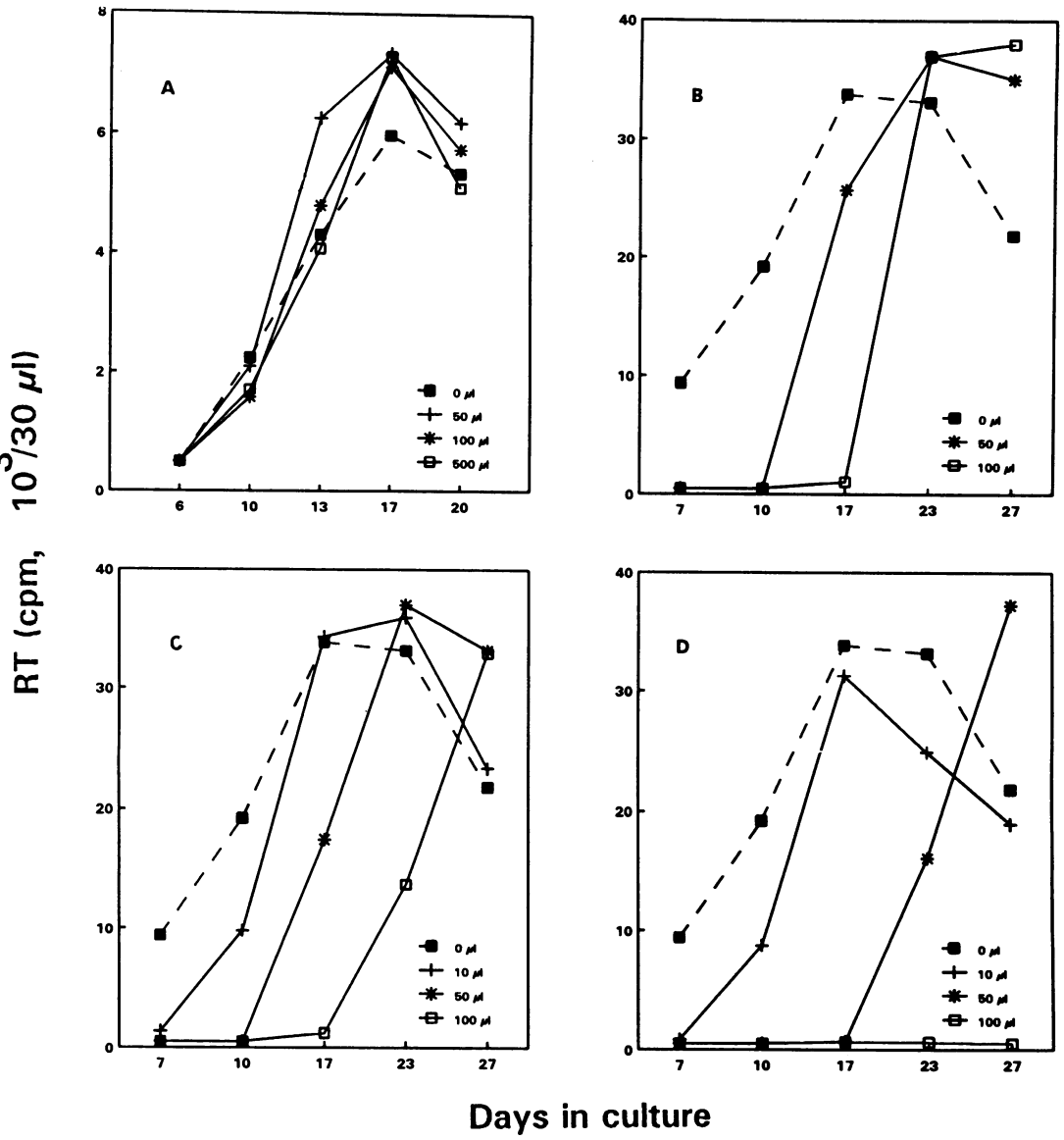

FIG. 4. Effects of saliva and plasma on the growth of cell-associated FIV. FIV growth rates in cultures of PBMC obtained from one infected SPF cat and supplemented with the indicated volumes of saliva (A) or with plasma obtained from an uninfected cat (B), the same infected cat which donated the PBMC (C), or another infected cat (D) are shown.

Cultured virus, however, was recovered more rapidly from animals with FAIDS. Similar results have been reported for HIV type 1 (HIV-1) isolation from infected individuals. In fact, there are conflicting data on the possibility of isolating HIV-1 from saliva (11, 12, 17), while HIV-1 isolation from plasma is relatively easy, especially during advanced stages of infection (5, 7) or during primary infection (4, 6).

Salivary glands were virus isolation positive from the first week p.i. throughout the period of observation, suggesting that they may represent an important and early site of FIV migration and/or replication, although definitive proof will require methods, such as in situ hybridization and quantitative PCR, capable of distinguishing between local virus replication within the tissues examined and virus present in the circulation. In addition, in a finding similar to what has been found for HIV-1-infected individuals (1, 10–12, 15), PCR analysis evidenced FIV genomes in a higher number of saliva and plasma samples and with stronger signals than expected on the basis of the isolation data. These results may appear contradictory, but it should be kept in mind that the detection of viral sequences by PCR does not prove that infectious virus is present. Although human saliva has been shown to be somewhat inhibitory for HIV-1 infectivity (8, 9, 19) and anti-FIV antibodies are present in the saliva of infected cats (18), we found no evidence that feline saliva inhibits FIV infectivity or growth. In contrast, the addition of plasma to PBMC cultures delayed FIV growth considerably. As the addition of plasma also leads to decreased viabilities of PBMC cultures, it is possible that this effect contributes to impaired FIV growth, although an effect of neutralizing antibody is also possible, as suggested by the fact that plasma of infected cats was more inhibitory for the growth of cell-free virus than plasma of noninfected animals. These findings prompted a comparison of FIV isolation rates

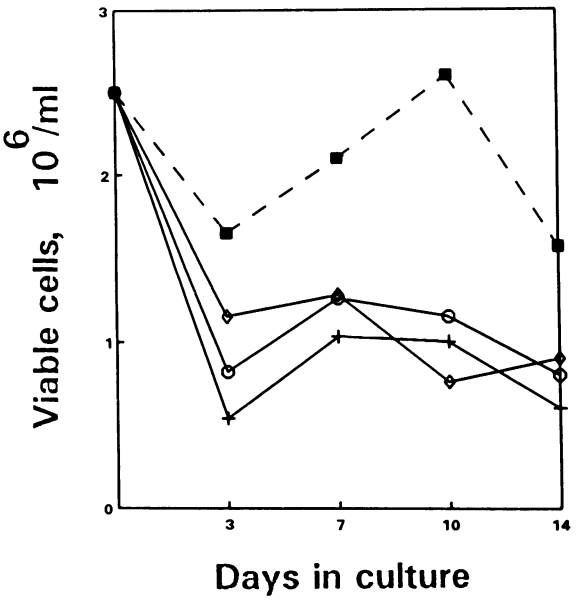

FIG. 5. Cell viabilities of PBMC cultures obtained from one infected cat and supplemented with 50 μl of plasma from an uninfected cat (+), 10 μl of plasma from the infected cat which donated the PBMC (○), 10 μl of plasma from another infected cat (◇), or 10 μl of medium (■).

with and without removal of plasma 3 to 24 h after initiation of the cultures. Removing plasma delayed the need for periodic addition of normal lymphoblasts but did not appreciably increase the rate of isolation (data not shown).

At present, we are investigating the implications of these observations. However, the low isolation rate indicates that the amount of infectious virus in saliva is usually small. This may be part of the explanation for the low efficiency of FIV transmission in nature (16). It is of interest, however, that the saliva of FIV-infected cats contains viral DNA in addition to viral RNA, suggesting that this fluid harbors virus-infected cells or cellular debris as well as free virus. Proviral sequences have also been detected in a high proportion of saliva samples of HIV-1-infected individuals and have been attributed to the presence of infected lymphocytes and macrophages that have migrated from the blood vessels into the oral cavity (10). A similar explanation for FIV seems reasonable, although the spectrum of cells susceptible to this virus is still poorly understood (16). In any case, the finding indicates that infected cells may be an important mode of transmission for FIV as well as for HIV (13). This knowledge may be important for designing strategies for vaccine development, as it indicates that induced immunity should be able to cope with FIV-infected cells as well as with cell-free virus. The present results also show that when FIV excretion with saliva and plasma viremia are used for evaluating drugs or vaccines, PCR analysis may be more informative than virus cultivation.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministero della Sanità, Istituto Superiore di Sanità, Progetto Allestimento Modelli Animali per l'AIDS and Ministero Università e Ricerca Scientifica, Rome, Italy.

REFERENCES

1. **Bagnarelli, P., S. Menzo, A. Manzin, M. Giacca, P. E. Varaldo, and M. Clementi.** 1991. Detection of human immunodeficiency virus type 1 genomic RNA in plasma samples by reverse transcription polymerase chain reaction. J. Med. Virol. **34**:89–95.
2. **Bandecchi, P., D. Matteucci, F. Baldinotti, G. Guidi, F. Abramo, F. Tozzini, and M. Bendinelli.** 1992. Prevalence of feline immunodeficiency virus and other retroviral infections in sick cats in Italy. Vet. Immunol. Immunopathol. **23**:337–345.
3. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162**:156–159.
4. **Clark, S. J., M. S. Saag, W. D. Decker, S. Campbell-Hill, J. L. Roberson, P. J. Veldkamp, J. C. Kappes, B. H. Hahn, and G. B. Shaw.** 1991. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. N. Engl. J. Med. **324**:954–960.
5. **Coombs, R. W., A. C. Collier, J. P. Allain, B. Nikora, M. Leuther, G. F. Gjerset, and L. Corey.** 1989. Plasma viremia in human immunodeficiency virus infection. N. Engl. J. Med. **321**:1626–1631.
6. **Daar, E. S., T. Moudgil, R. D. Meyer, and D. D. Ho.** 1991. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. N. Engl. J. Med. **324**:961–964.
7. **Ehrnst, A., A. Sönnerborg, S. Bergdahl, and Ö. Strannegård.** 1988. Efficient isolation of HIV from plasma during different stages of HIV infection. J. Med. Virol. **26**:23–32.
8. **Fox, P. C., A. Wolff, C.-K. Yeh, J. C. Atkinson, and B. J. Baum.** 1989. Salivary inhibition of HIV-1 infectivity: functional properties and distribution in men, women and children. J. Am. Dent. Assoc. **118**:709–711.
9. **Fultz, P. N.** 1986. Components of saliva inactivate human immunodeficiency virus. Lancet **ii**:1215.
10. **Goto, Y., C.-K. Yeh, A. L. Notkins, and B. S. Prabhakar.** 1991. Detection of proviral sequences in saliva of patients infected with human immunodeficiency virus type 1. AIDS Res. Hum. Retroviruses **7**:343–347.
11. **Groopman, J. E., S. Z. Salahuddin, M. G. Sarngadharan, P. D. Markham, M. Gonda, A. Sliski, and R. C. Gallo.** 1984. HTLV-III in saliva of people with AIDS-related complex and healthy homosexual men at risk for AIDS. Science **226**:447–449.
12. **Levy, J. A., and D. Greenspan.** 1988. HIV in saliva. Lancet **ii**:1248. (Letter.)
13. **Lewis, D. E., B. Yoffe, C. G. Borsworth, F. B. Hollinger, and R. R. Rich.** 1988. Human immunodeficiency virus-induced pathology favored by cellular transmission and activation. FASEB J. **2**:251–255.
14. **Lombardi, S., M. Bendinelli, and C. Garzelli.** Detection of B epitopes on the p24 *gag* protein of feline immunodeficiency virus by monoclonal antibodies. AIDS Res. Hum. Retroviruses, in press.
15. **Ottman, M., P. Innocenti, M. Thenadey, M. Micoud, F. Peloquin, and J.-M. Seigneurin.** 1991. The polymerase chain reaction for the detection of HIV-1 genomic RNA in plasma from infected individuals. J. Virol. Methods **31**:273–284.
16. **Pedersen, N. C.** Feline immunodeficiency virus infection. *In* J. A. Levy (ed.), The Retroviruses, vol. 2, in press. Plenum Press, New York.
17. **Pekovic, D., D. Ajdukovic, C. Tsoukas, N. Lapointe, J. Michaud, N. Gilmore, and M. Gornitsky.** 1987. Detection of human immunosuppressive virus in salivary lymphocytes from dental patients with AIDS. JAMA **82**:188–189.
18. **Poli, A., C. Giannelli, M. Pistello, L. Zaccaro, D. Pieracci, M. Bendinelli, and G. Malvaldi.** 1992. Detection of salivary antibodies in cats infected with feline immunodeficiency virus. J. Clin. Microbiol. **30**:2038–2041.
19. **Pourtois, M., C. Binet, N. van Tieghem, P. R. Courtois, A. Vandenabbeele, and L. Thirty.** 1991. Saliva can contribute in quick inhibition of HIV infectivity. AIDS **5**:598–600.
20. **Talbott, R. L., E. E. Sparger, K. M. Lovelace, W. M. Fitch, N. C. Pedersen, P. A. Luciw, and J. M. Elder.** 1989. Nucleotide

sequence and genomic organization of feline immunodeficiency virus. Proc. Natl. Acad. Sci. USA **86**:5743–5747.

21. **Tozzini, F., D. Matteucci, P. Bandecchi, F. Baldinotti, A. Poli, M. Pistello, K. H. J. Siebelink, L. Ceccherini-Nelli, and M. Bendinelli.** 1992. Simple in vitro methods for titrating feline immunodeficiency virus (FIV) and FIV neutralizing antibodies. J. Virol. Methods **37**:241–252.
22. **Yamamoto, J. K., H. Hansen, E. W. Hansen, T. Y. Morishita, T. Okuda, T. R. Sawa, R. M. Nakamura, and N. C. Pedersen.** 1989. Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission. J. Am. Vet. Med. Assoc. **194**:213–220.
23. **Yamamoto, J. K., E. Sparger, E. W. Ho, P. R. Andersen, T. P. O'Connor, C. P. Mandell, L. Lowestine, R. Munn, and N. C. Pedersen.** 1988. Pathogenesis of experimentally induced feline immunodeficiency virus infection in cats. Am. J. Vet. Res. **49**:1246–1258.
24. **Zaccaro, L.** Unpublished results.