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Detection of Salivary Antibodies in Cats Infected with Feline Immunodeficiency Virus

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The saliva of cats infected with feline immunodeficiency virus was examined for total immunoglobulin content and antiviral antibodies. Seropositive cats showed an increase in salivary immunoglobulin G levels, which was only partly attributable to the enhanced prevalence of oral inflammatory lesions, compared with the levels in seronegative cats. Immunoglobulin G, but not immunoglobulin M, levels in serum were also increased. Salivary antibodies were determined by indirect immunofluorescence and Western blot (immunoblot) analysis. All but 1 of the 16 seropositive cats examined were positive, while all 16 control cats were negative. The presence of oral lesions was not a prerequisite for antibody detection in saliva. It was concluded that salivary antibody might be usefully exploited for diagnostic and epidemiologic purposes.

Feline immunodeficiency virus (FIV) is a widespread lentivirus which resembles human immunodeficiency virus (HIV) in many respects. Infected cats show a progressive depletion of $CD4^+$ T lymphocytes and other immunological deficits which, after a lengthy, clinically silent phase, may culminate in severe opportunistic infections and death. Like the diagnosis of HIV infection, the diagnosis of FIV infection relies mainly on the detection of circulating virus-specific antibodies, which are considered indicative of an ongoing infection (16).

The saliva of HIV-infected patients contains antiviral antibodies that are detectable by a variety of methods and has been proposed as an alternative diagnostic specimen in situations in which blood collection is difficult (2, 4, 8, 9, 15, 22). Saliva plays a much more important role in FIV infection than it does in HIV infection, because FIV appears to spread among feline populations mainly, if not solely, through biting (16), whereas epidemiologic evidence indicates that HIV is not significantly transmitted by saliva (5, 10). In addition, isolation of FIV from cat saliva is relatively easy, while isolation of HIV from human saliva has yielded divergent results (11, 17). Nonetheless, there are no published reports on salivary antiviral antibodies in FIV-infected cats. The study described here addressed the latter point.

MATERIALS AND METHODS

Cats. The cats examined in this study were allowed to roam outside and, therefore, were considered at high risk for FIV infection (3). They were screened for FIV antibody in serum by a commercial enzyme-linked immunosorbent assay (ELISA) (CITE Combo FIV-FeLV; Agritech Systems, Portland, Maine) according to the recommendations of the manufacturer. This study included 16 FIV-seropositive and 16 FIV-seronegative subjects that were matched as much as possible for breed, age, gender, and weight. None of the cats was positive for feline leukemia virus antigen.

Serum and saliva samples. Samples were obtained after a thorough clinical examination of the donor cats. Saliva samples were collected with one or more cotton swabs which were held in the mouth for about 1 min, squeezed into tubes with the help of a plastic rod, and then clarified by low-speed centrifugation. Sera were prepared from blood obtained by venipuncture immediately after saliva collection. Unless otherwise stated, the samples were tested for FIV antibody within 4 h of the time of collection. Paired serum and saliva samples that were coded were tested in parallel.

Total Ig assays. Immunoglobulin G (IgG) and IgM concentrations were measured by a commercial radial immunodiffusion assay specific for feline IgG and IgM (Binding Site, Birmingham, United Kingdom) by following the protocol of the manufacturer. Repeated attempts to measure IgA levels in saliva by a laboratory-made radial immunodiffusion assay and a nephelometric assay using affinity-purified anti-IgA gave inconsistent results that were therefore omitted from the analysis.

Indirect immunofluorescence (IIF) assay for FIV antibody. Crandell feline kidney (CrFK) cells persistently infected with FIV (Petaluma isolate) and fixed with cold acetone on multiwell slides were used as a substrate. The cells were exposed to appropriate dilutions of saliva or serum in phosphate-buffered saline (PBS; pH 7.2) solution for 1 h at 20°C, washed thoroughly, and then incubated with a fluoresceinated rabbit anti-cat IgG serum (Sigma, St. Louis, Mo.). A sample was scored as positive when it produced a clearly evident cytoplasmic fluorescence (Fig. 1). Each test included uninfected CrFK cells as well as standard FIV-positive and -negative sera as controls.

WB assay for FIV antibody. FIV antigen was produced by persistently infected CrFK cells, gradient purified, and then disrupted by sonication followed by treatment with Triton X-100. Viral proteins were separated with a sodium dodecyl sulfate-12% polyacrylamide gel and blotted onto nitrocellulose by standard methods. The nitrocellulose sheets were then blocked with 1% bovine serum albumin, cut into 0.5-cm-wide strips, dried, and stored in the cold until use. Individual strips were incubated for 2 h with saliva or serum diluted in PBS-Tween 20 (0.05%), washed thoroughly, incubated with horseradish peroxidase-conjugated rabbit anti-cat IgG (Western blot [WB; immunoblot] [WB-IgG]) or IgA

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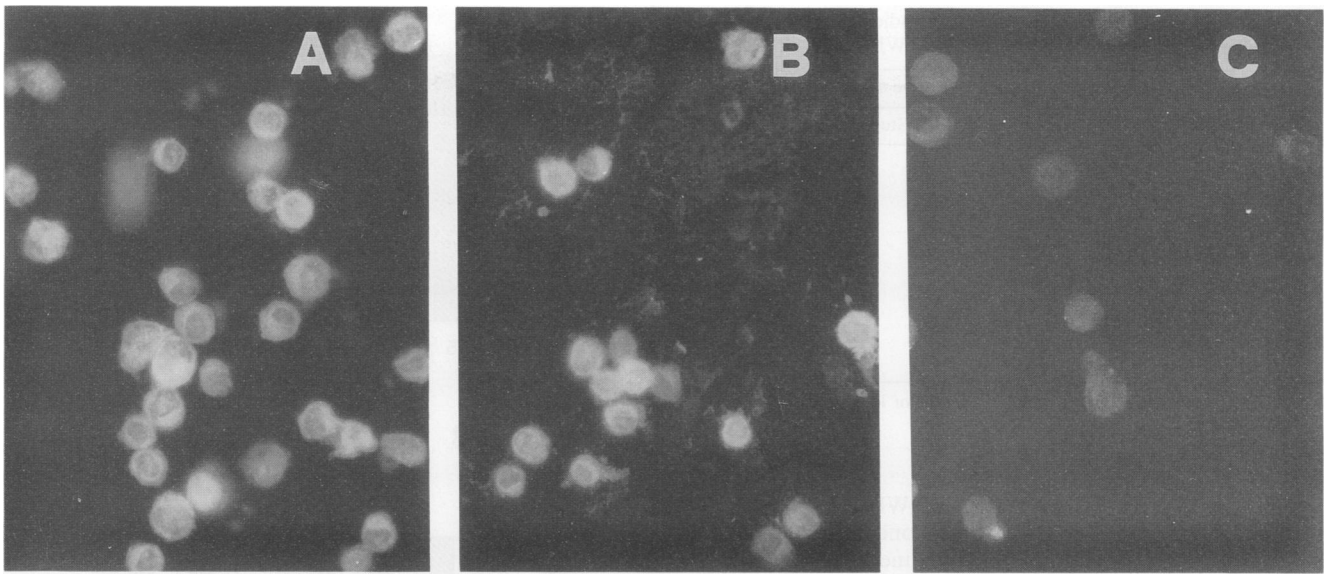

FIG. 1. FIV-infected CrFK cells reacted with serum (A) or fresh saliva (B) from a seropositive cat or with saliva from a seronegative cat (C). The photograph in panel C was overexposed to allow visualization of the cells.

(WB-IgA) sera (Bethyl Laboratories, Montgomery, Tex.) for 1 h, washed again, and developed with 3',3'-diaminobenzidine tetrahydrochloride (Sigma). The anti-IgA serum used was alpha chain specific and produced a single precipitation arc against cat serum by immunoelectrophoresis. Controls included standard FIV-positive and FIV-negative cat sera as well as strips prepared with extracts of uninfected CrFK cells. The strips were read both visually and by densitometric scanning (Ultrascan; LKB, Bromma, Sweden), immediately after the reaction was stopped with distilled water. The molecular weights of the reactive peptides were established by comparison with prestained low-molecular-weight markers (Bio-Rad, Richmond, Calif.). A sample was scored as positive when it produced at least any two of p24, gp41, and gp120 FIV-specific bands.

RESULTS

Total Ig levels in saliva. Seropositive cats showed enhanced levels of salivary IgG. This change may at least partly reflect the enhanced prevalence of oral lesions observed in seropositive cats, as suggested by the higher ratios of salivary IgG to serum IgG found in seronegative and seropositive cats with oral lesions compared with those found in seronegative cats without oral lesions. It is important to emphasize, however, that salivary IgG was also enhanced in the few seropositive cats with no detectable oral lesions (Table 1). Salivary IgG is considered a transudate of plasma IgG (19); this may be due to the hypergammaglobulinemia of seropositive cats because, consistent with previous findings (1), IgG levels in serum were higher in seropositive cats than they were in control cats. In contrast, no differences were found in the serum IgM content between infected and uninfected cats; mean ± standard deviation levels were 0.9 ± 0.2 and 0.9 ± 0.1 mg/ml, respectively.

FIV antibody in saliva. Attempts to detect FIV-specific antibody in saliva by using commercial or laboratory-made ELISAs that performed well with serum gave large numbers of false-positive and false-negative results. We therefore decided to use the IIF and WB assays, which were also considered to provide more informative results for the purposes of this study.

After preliminary tests, saliva was assayed at a dilution of 1:10 and serum was assayed at a dilution of 1:50 for both IIF and WB. Testing of fresh saliva by IIF (Fig. 1B) and WB-IgG detected antibodies in all but 1 of the 16 seropositive cats examined, while WB-IgA on fresh saliva missed 3 seropositive subjects. When WB-IgA was performed on serum samples, it was also found to perform worse than the other assays. Saliva samples kept at −20°C for 4 weeks or more gave substantial numbers of false-negative results by both IIF and WB. No false-positive saliva samples were detected by IIF or WB among the 16 seronegative cats (Table 2). The presence of oral lesions did not appear to influence the sensitivity of saliva testing. Eleven of the 16 infected cats examined had evident signs of gingivitis and/or periodontitis; antibodies were detected in 10 such animals, whereas antibodies were detected in 4 of 5 cats with no oral lesions. This is in keeping with the results obtained with experimentally infected specific-pathogen-free cats, showing that saliva was

TABLE 1. IgG concentrations in fresh saliva and serum of FIV-seropositive and control cats with or without oral lesions

Cat (no. examined)	IgG concn (mg/ml [mean ± SD]) in:		Ratio saliva/serum
	Saliva	Serum	
Controls			
With OL[a] (3)	1.2 ± 0.6	29.1 ± 4.9	1:24
Without OL (13)	0.3 ± 0.2	20.2 ± 4.3	1:66
Total (16)	0.5 ± 0.4	21.9 ± 5.5	1:44
FIV seropositive			
With OL (11)	2.4 ± 1.2	35.8 ± 5.1	1:15
Without OL (5)	1.4 ± 0.7	26.2 ± 5.2	1:19
Total (16)	2.1 ± 1.1[b]	32.8 ± 6.8[b]	1:16

[a] OL, oral lesions ranging from periodontitis to severe ulceroproliferative stomatitis.
[b] Significantly different from controls ($P < 0.001$; Student's t test).

TABLE 2. Detection of anti-FIV antibodies in the saliva of FIV-seropositive and control cats by IIF, WB-IgG, and WB-IgA

Cat group (no. examined) and test	No. of cats whose samples were reactive		
	Fresh saliva	Stored saliva[a]	Serum
Controls (16)			
IIF	0	0	0
WB-IgG	0	0	0
WB-IgA	0	0	0
FIV-seropositive (16)			
IIF	15	10	16
WB-IgG	15	8	16
WB-IgA	13	ND[b]	15

[a] Saliva was maintained at −20°C for 4 weeks or longer.
[b] ND, not done.

already positive by IIF, WB-IgG, and WB-IgA as early as 6 to 8 weeks after infection, that is, concomitantly or very soon after seroconversion, and remained positive for the entire period of observation (24 months) in the complete absence of oral lesions. In these animals, WB-IgM gave negative or inconsistent results when it was performed on saliva, although IgM was detected in serum by WB throughout the first 6 months of infection (data not shown).

The bands developed by positive saliva samples in WB-IgG were similar to those given by positive sera in this study (Fig. 2A) and previous studies (14), but there was a lower prevalence of bands to gp41 (Fig. 3). WB-IgA of most positive saliva and serum samples was negative for p50 and gp120 and generally gave weaker bands than WB-IgG (Fig. 2B and 3).

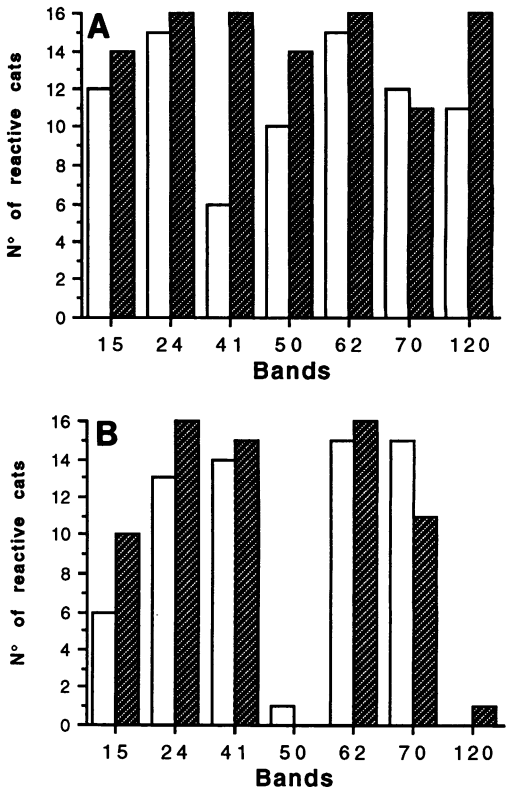

FIG. 3. Number of fresh saliva (□) and serum (▨) samples from the 16 FIV-seropositive cats that were reactive to individual FIV gene products by WB-IgG (A) and WB-IgA (B).

DISCUSSION

IgG antibodies to HIV proteins have been detected in 80 to 100% of saliva samples obtained from HIV-seropositive individuals, while the search for HIV-specific salivary IgA has provided contradictory results (2, 4, 8, 9, 15, 22). The data presented here show that the saliva of FIV-infected cats also contains FIV-specific IgG and IgA that can be consistently detected by IIF or WB from the early stages of infection. In HIV-infected individuals, impaired gingival health increased the titers of HIV antibody found in saliva but not the rate of HIV-positive saliva samples (8). Similarly, in the present study of FIV-seropositive cats, the presence of oral inflammatory lesions enhanced the levels of total IgG in saliva, possibly as a result of augmented gingival vascular transudation and bleeding, but this was not a prerequisite for antiviral antibody detection in saliva.

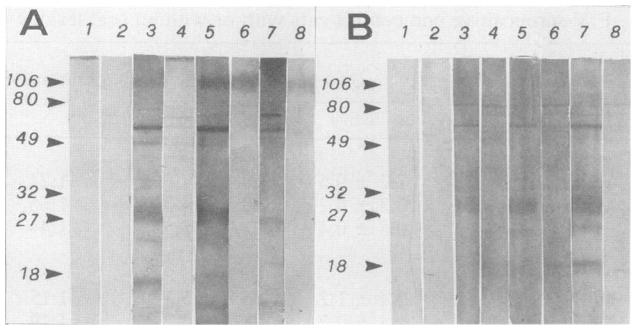

FIG. 2. WB analysis of serum (lanes 1, 3, 5, and 7) or fresh saliva (lanes 2, 4, 6, and 8) specimens from one seronegative cat (lanes 1 and 2) and three seropositive cats (lanes 3 to 8) developed with anti-cat IgG (A) or IgA (B) sera.

In cats, it is much simpler to collect saliva than it is to collect blood. Because there was a good correlation between the results obtained with saliva and serum, saliva might be exploited for FIV diagnostic and epidemiological purposes. To detect FIV antibody, in the present study we used IIF and WB, which are labor-intensive and costly, because initial attempts with ELISAs gave inconsistent results. Similar problems have been encountered in the testing of saliva for HIV antibody by ELISAs designed for use with serum (4, 9). Further investigations aimed at developing an ELISA suitable for cat saliva are warranted, because such an assay would open the way for an FIV test on a noninvasive sample for use in the home. It may be of practical value that saliva testing has also been found to be valuable in the diagnosis of feline leukemia virus infection (12, 13). The observation that stored saliva was inferior to fresh saliva for FIV antibody detection may be partly explained by the presence of proteolytic enzymes (20). The addition of protease inhibitors has increased the reliability of testing of human saliva for HIV antibody (8) and should be tried for use in the detection of FIV in feline saliva as well.

Bites represent the major means of FIV transmission. The presence in cat saliva of antiviral antibody, and possibly of other virus-inhibitory factors similar to those described for human saliva (6, 7, 18), may contribute to an explanation of why the rate of FIV transmission is generally low (16). It should be mentioned, however, that in preliminary studies,

the saliva of FIV-infected cats was seen to contain much lower FIV-neutralizing activity than serum, if any (21).

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