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Serum Neutralization of Feline Immunodeficiency Virus Is Markedly Dependent on Passage History of the Virus and Host System

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Sera from feline immunodeficiency virus (FIV)-infected cats exhibited extremely low levels of neutralizing antibodies against virus passaged a few times in vitro (low passage), when residual infectivity was assayed in the $CD3^+$ $CD4^-$ $CD8^-$ MBM lymphoid cell line or mitogen-activated peripheral blood mononuclear cells. By sharp contrast, elevated titers of highly efficient neutralizing activity against FIV were measured, by use of high-passage virus, in assays on either the fibroblastoid CrFK or MBM cell line. However, high-passage virus behaved the same as low-passage virus after one in vivo passage in a specific-pathogen-free cat and reisolation. Subneutralizing concentrations of infected cat sera enhanced the production of low-passage virus by MBM cells, an effect not seen with high-passage virus in CrFK cells. These qualitative and quantitative discrepancies could not be attributed to differences in the amount of immunoreactive viral material, to the amount of infectious virus present in the viral stocks, or to the presence of anti-cell antibodies. The observed effects were most likely due to the different passage history of the viral preparations used. The observation that neutralizing antibodies detected with high-passage virus were broadly cross-reactive in assays with CrFK cells but isolate specific in MBM cells suggests also that the cell substrate can influence the result of FIV neutralization assays. This possibility could not be tested directly because FIV adapted to grow in CrFK cells had little infectivity for lymphoid cells and vice versa. In vitro exposure to infected cat sera had little or no effect on the ability of in vivo-passaged FIV to infect cats. These data reveal no obvious relationship between titers against high-passage virus and ability to block infectivity of FIV in cats and suggest caution in the use of such assays to measure vaccine efficacy. In conclusion, by contrast with what has been previously reported for the use of CrFK cells and high-passage virus, both natural and experimental infections of cats with FIV generate poor neutralizing antibody responses with regard to in vivo protection.

The importance of humoral antibody responses in protection against lentiviruses is unclear. For example, human immunodeficiency virus (HIV)-infected patients and simian immunodeficiency virus-infected macaques that developed antibodies capable of neutralizing these viruses in vitro were reported to survive longer than hosts who did not (9, 16, 21), and in certain vaccine experiments protection of primates against HIV type 1 (HIV-1) or simian immunodeficiency virus correlated with induction of neutralizing antibodies (NA) (3, 15, 30). Other studies, in contrast, failed to demonstrate a positive correlation between protection and presence of serum NA (5, 10, 18, 25, 31, 36). More importantly, reports of the 6th Annual Conference on Advances in AIDS Vaccine Development suggest that HIV vaccines that generated NA in humans against laboratory strains of HIV all failed to neutralize fresh HIV isolates (7). Factors which might contribute to blurring the picture are numerous: selection of highly neutralizable variants of the virus with repeated in vitro propagation, presence in the sera of anti-cell antibody and infection-enhancing antibodies which can interfere with neutralization, emergence of escape mutants during the neutralization assay, use of improper methods for measuring NA, etc. (for reviews, see references 28, 29, and 32).

The feline immunodeficiency lentivirus (FIV) produces clinical symptoms in domestic cats similar to those of human AIDS. As already demonstrated for other aspects of AIDS pathogenesis (35), studies of FIV may help our understanding of the role and properties of NA especially in regard to their protective action in vivo. This laboratory previously described a neutralization assay for FIV which exploits the ability of the virus to induce syncytia in the Crandell feline kidney fibroblastoid cell line (CrFK) (43). The NA detected by that method develop within weeks of infection and plateau at high titers in 4 to 6 months. Interestingly, such NA are broadly cross-reactive, as shown by their presence in field cats of different geographical origin and in specific-pathogen-free (SPF) cats infected with FIV isolates from different geographical regions (26, 44). Similar conclusions were reached by Fevereiro et al. (13, 14) using a slightly different method. Such broad cross-reactivity is at variance with those of HIV and simian immunodeficiency virus, whose sensitivity to antibody-mediated neutralization is largely strain specific (28, 29, 32). As the effectiveness of NA to the latter viruses is generally evaluated in cultures of T lymphocytes (45), we reasoned that divergences between neutralization of FIV and neutralization of primate lentiviruses might stem from the different cellular substrates used to measure the residual infectivity of the viruses following exposure to putative neutralizing sera. This study compared the ability of sera of infected cats to inhibit FIV infectivity for CrFK cells, the recently established T-null cell line MBM (27), primary blasts (PB) obtained by polyclonal stimulation of peripheral blood mononuclear cells (PBMC), and cats. The results demonstrate that serum neutralization of FIV is dependent upon (i) the cells on which the assay is

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TABLE 1. Passage history and other characteristics of the stocks of FIV used

Virus stock	Passage history	Source[a]	Titer (ml^{-1})	p24 content[b]
FIV-M2^{CAT}	10 times in SPF cats	PL	10^3 CID_{50}	<3
FIV-M2^{MBM}	Isolated from 8th passage in SPF cats and passaged 4 times in MBM cells	CF	$10^{2.5}$ $TCID_{50}$	4 ± 0.4
FIV-M2$^{MBM/CrFK}$	As FIV-M2^{MBM} and passaged 17 times in low-serum CrFK cells	CF	$10^{3.3}$ SFU	42 ± 7
FIV-Pet^{FL4}	181st subculture of persistently infected FL4 cells	CF	$10^{4.5}$ $TCID_{50}$	39 ± 4
FIV-Pet$^{FL4/MBM}$	As FIV-Pet^{FL4} and passaged 3 times in MBM cells	CF	$10^{4.0}$ $TCID_{50}$	9 ± 2
FIV-Pet$^{FL4/CAT}$	As FIV-Pet^{FL4} and passaged 1 time in SPF cats	WB	$>10^{2.0}$ CID_{50}	ND[c]
FIV-Pet$^{FL4/CAT/MBM}$	Isolated from an SPF cat infected with FIV-Pet^{FL4} and passaged 3 times in MBM cells	CF	$10^{2.50}$ $TCID_{50}$	ND
FIV-Pet$^{FL4/CrFK}$	As FIV-Pet^{FL4} and passaged 23 times in low-serum CrFK cells	CF	$10^{3.6}$ SFU	55 ± 1
FIV-Pet^{CrFK}	76th subculture of persistently infected CrFK cells	CF	$10^{2.0}$ CID_{50}	ND

[a] PL, plasma; CF, tissue culture fluid; WB, whole blood.
[b] p24 antigen content in virus suspensions concentrated 10 times compared with those used in neutralization assays and expressed as nanograms per milliliter ± standard deviation.
[c] ND, not determined; used only for infecting cats as long-term-infected donors of serum.

performed, (ii) the strain of the virus used to infect serum donors, (iii) the challenge strain of virus, and (iv) whether the challenge virus has been propagated in cats or cell culture.

MATERIALS AND METHODS

Cats and sera. SPF cats were purchased from Iffa Credo (L'Asbrege, France), caged individually in pathogen-free quarters, and infected when 5 to 7 month old. Immune sera were obtained from SPF animals inoculated intravenously with various stocks of FIV 1 to 28 months earlier and, when indicated, from random-source naturally infected field cats. They contained high titers of FIV-specific antibody according to a commercial enzyme-linked immunosorbent assay (ELISA; IDEXX, Portland, Maine), an in-house ELISA (24), and Western immunoblotting. Normal sera were obtained from noninfected SPF cats and were nonreactive in the above assays. All cats were negative for feline leukemia virus according to ELISA (IDEXX). Prior to neutralization assays, the sera were treated at 56°C for 30 min and checked for infectious FIV by standard culture and, in the case of the in vivo assay, also by inoculation into SPF cats.

Cell cultures. CrFK cells were grown in Eagle's minimal essential medium supplemented with 0.5% fetal bovine serum and additional supplements (43). The interleukin-2- and concanavalin A-dependent feline cell line MBM has been established from the PBMC of an FIV- and feline leukemia virus-negative SPF cat, has been phenotyped as $CD3^+$ $CD4^-$ $CD8^-$ by use of appropriate anti-feline sera (27), and is grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 5 μg of concanavalin A (Sigma, St. Louis, Mo.) per ml, and 20 U of recombinant human interleukin-2 (Boehringer, Mannheim, Germany) per ml. Feline PBMC were obtained by Ficoll (1,077 mg/ml) density gradient separation and extensively washed. PB were obtained by prestimulating PBMC of SPF cats with 5 μg of concanavalin A (Sigma) per ml in RPMI 1640 supplemented as described above and infected at day 3 of culture (26).

Viruses. The local Pisa-M2 isolate of FIV (FIV-M2), obtained from an asymptomatic FIV-seropositive feline leukemia virus-negative field cat, has been freed of the initially present feline syncytium-forming spumavirus by two consecutive passages of cell-free plasma in cats (26). The Petaluma strain (FIV-Pet) isolated in California was obtained from N. C. Pedersen as persistently infected CrFK cells and from J. Yamamoto as persistently infected FL4 cells. The passage histories, sources, titers, and p24 antigen contents of the virus stocks used are given in Table 1. All virus preparations were filtered and stored at −80°C in 1-ml aliquots until use. Titers in MBM and PB were determined by inoculating 100 μl of 10-fold dilutions of the virus stocks into quadruplicate wells of 96-well microplates containing 5×10^4 cells in 100 μl of RPMI 1640 medium. The cultures were monitored by measuring Mg^{2+}-dependent reverse transcriptase (RT) and FIV p24 antigen in the supernatants after 8 days of incubation at 37°C in 5% CO_2, and virus titers were expressed as 50% tissue culture infective doses ($TCID_{50}$) calculated by the Reed and Muench method (39). Titers in CrFK cells were determined as described elsewhere (43) and expressed in syncytium-forming units (SFU). Titers in vivo were determined by inoculating 1 ml of 10-fold dilutions of virus intravenously into groups of four SPF cats, which were then monitored for seroconversion and virus isolation for 6 months, and expressed as 50% cat infectious doses (CID_{50}).

Neutralization assays. The assay on CrFK cells was performed as described elsewhere (43) against 100 SFU of virus. Briefly, equal volumes of twofold dilutions of sera and FIV-M2$^{MBM/CrFK}$ or FIV-Pet$^{FL4/CrFK}$ diluted to contain 1,000 SFU/ml were mixed, incubated at room temperature for 1 h, and then inoculated into duplicate wells (200 μl per well) of 24-well plates which where seeded with 10^4 CrFK cells in 500 μl of RPMI 1640 medium. Six days later, the plates were stained with 0.2% crystal violet in 30% methanol and examined microscopically for syncytia. NA titers are expressed as the reciprocal of the highest dilution of serum that completely prevented the formation of syncytia.

For the assays on MBM cells and PB, FIV-M2^{MBM} or the indicated stock of FIV-Pet diluted to 200 $TCID_{50}$/ml was incubated with an equal volume of twofold dilutions of sera at 37°C for 1 h and then inoculated (100 μl per well) into quadruplicate wells of 96-well flat-bottomed microplates containing 5×10^4 cells in 100 μl. NA titers are expressed as the reciprocal of the highest dilution of serum which reduced by 50% the levels of RT activity or FIV p24 antigen detected in the supernatant of the test cultures at day 8 of incubation, as compared with control cultures receiving FIV incubated with medium. All experiments were repeated at least twice.

For the neutralization assay in cats, pooled sera diluted 1:8 were mixed with an equal volume of FIV-M2^{CAT} (final concentration, 10 CID_{50}/ml of mixture) and incubated at 37°C for 1 h. The mixtures were then injected intravenously into groups of 4 SPF cats (1 ml per cat), which were monitored as described above for 7 months. Each neutralization assay

TABLE 2. Neutralization of FIV-M2$^{MBM/CrFK}$ and FIV-Pet$^{FL4/CrFK}$ by sera of experimentally infected SPF cats, with CrFK cells as the host system

Virus infecting serum donors	Cat no.	Neutralizing titer	
		FIV-M2$^{MBM/CrFK}$	FIV-Pet$^{FL4/CrFK}$
FIV-M2^{CAT}	258	1,024	1,024
	708	256	256
	1374	4,096	4,096
	4216	1,024	1,024
	4270	1,024	1,024
	4282	1,024	1,024
FIV-Pet^{FL4}	338	4,096	4,096
	867	512	512
	871	512	512
	2906	4,096	4,096
	3368	4,096	4,096
	3522	512	256
	3524	512	1,024
FIV-Pet^{CrFK}	1696	1,024	1,024
FIV-Pet$^{FL4/CAT}$	3735	32	32
	3788	512	256

included the appropriate controls for cells, virus, immune serum, and normal serum.

FIV isolation and quantitation of viral burden in infected cats. These assays were performed by coculturing freshly harvested PBMC with MBM cells (27). Each batch of cocultures included sham-infected cells as negative controls and was monitored for FIV growth by measuring RT and p24 antigen in the supernatants once a week. Negative cultures were terminated 5 or more weeks after initiation. Results are expressed as number of infected cells per 10^6 PBMC.

RT and p24 antigen assays. RT activity was measured as previously described (2). The p24 antigen was measured with an in-house capture ELISA based on two monoclonal antibodies which recognize different epitopes (24). The cultures were considered positive for FIV when RT counts per minute and p24 antigen optical density readings were fivefold and twofold higher, respectively, than the corresponding values given by supernatants of mock-infected cultures.

RESULTS

FIV neutralization with CrFK cells as the host system. Previous studies had shown that sera of FIV-infected cats inhibit the production of syncytia by FIV in CrFK cells and that the effect is due to true neutralization of virus infectivity, since it correlates with inhibition of virus production (13, 14, 26, 43, 44). In such studies, the infectivities for CrFK cells of FIV-Pet and the Dutch isolate Amsterdam 6 (FIV-A6) were neutralized with similarly high efficiencies (44). Initially, FIV-M2 did not grow on CrFK, but after several passages in MBM cells it could be propagated also in CrFK, where it produced syncytia at least as effectively as did FIV-Pet (42). This property made it possible to measure the neutralizing activity of sera against FIV-M2 with CrFK cells as a host system. Table 2 shows titers obtained by simultaneously titrating a panel of representative immune sera against FIV-M2$^{MBM/CrFK}$ and FIV-Pet$^{FL4/CrFK}$. All of the immune sera tested consistently neutralized the two viral strains with similar efficiencies. Also, no differences were seen in the kinetics of neutralization over incubation periods ranging from 1 to 30 min (42). These findings confirmed that the NA measured in the CrFK assay are broadly cross-reactive. Sera of noninfected cats were devoid of neutralizing activity for both viral strains (data not shown).

FIV neutralization with the lymphoid T cell line MBM as the host system. Sera that had exhibited high NA titers in the CrFK assay were examined for NA against FIV-M2^{MBM} with MBM cells as substrate. Table 3 shows representative results obtained with sera from SPF cats infected with FIV-M2^{CAT} or

TABLE 3. Neutralization of FIV-M2^{MBM} by sera of SPF cats infected for various periods with different preparations of FIV-M2, with CrFK or MBM cells as the host system

Virus infecting serum donors	Virus dose used for infection	Cat no.	Time postinfection (mo)	Neutralizing titer on:		
				CrFK	MBM	
					RT	p24
FIV-M2^{CAT}	1,000 CID_{50}	4282	1	1,024	<8	<8
			7	1,024	16	16
		708	7	1,024	8	8
			12	256	8	32
		258	13	1,024	8	16
			18	1,024	<8	128
		1694	19	>2,048	8	8
		2986	25	1,024	<8	<8
			27	>2,048	<8	8
		2576	28	>2,048	<8	<8
	20 CID_{50}	4216	9	1,024	<8	32
		4270	9	1,024	8	32
	10 CID_{50}	3575	7	>2,048	<8	32
		3581	7	>2,048	<8	16
	1 CID_{50}	3590	7	>1,024	<8	8
		3591	7	>1,024	<8	<8
FIV-M2^{MBM}	100 $TCID_{50}$	2863	5	>2,048	<8	<8

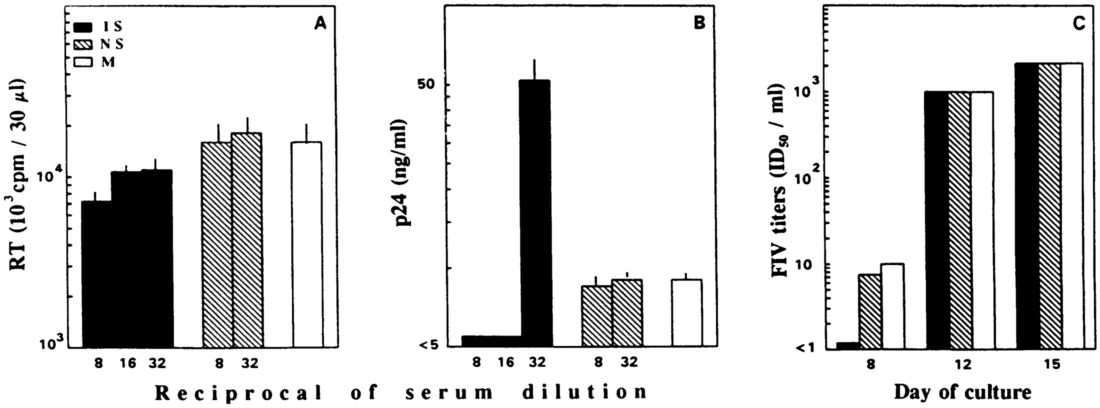

FIG. 1. Neutralization of FIV-M2^{MBM} by the serum of a cat infected with FIV-M2^{CAT} with MBM cells as the host system. Virus (10 $TCID_{50}$) was incubated for 1 h at 37°C with the indicated dilutions of serum from a long-term-infected (IS) or a normal cat (NS) or with medium alone (M) and then inoculated into MBM cultures. (A and B) Means ± standard deviations of RT and p24 antigen content in the supernatant of the test cultures, respectively. (C) Residual virus content at the end of the 1-h incubation period, on the basis of the RT readings done at day 8, 12, or 15 of virus titration on MBM cells. The immune serum completely neutralized FIV infectivity for CrFK cells up to a dilution of 1:1,024, whereas the normal serum diluted 1:8 did not. ID_{50}, 50% infectious dose.

FIV-M2^{MBM}, including the animal from which FIV-M2 was isolated in culture (cat 258). With the 50% inhibition of RT production as an end point to monitor virus neutralization, the titers ranged between <8 and 16. With the 50% inhibition of p24 antigen production as an end point, the titers were slightly higher but were again much lower than those found in CrFK cells. That the presence of anti-p24 antibody in sera may sometimes lead to an overestimate of the NA when evaluated with the latter end point readout is intuitively plausible and also has been documented by others (4). In subsequent experiments, the NA titers obtained with both methods are reported for the sake of comparison; however, interpretation of neutralization is mainly based on the titers obtained with RT levels.

In the experiment depicted in Fig. 1, the neutralizing activity of one immune serum (NA titer on CrFK cells, 1,024) was determined as above and also by titrating the residual viral infectivity for MBM cells found in the virus-serum mixtures at the end of the 1-h incubation period. The serum inhibited RT and p24 production in the test cultures at the expected dilutions (Fig. 1A and B). A dilution of 1:8 also reduced the residual viral infectivity, but the effect was visible only when the virus titers were calculated from the readings of day 8 of titration. The residual infectivities determined on later readings were identical, regardless of whether the virus had been incubated with immune serum, normal serum, or medium (Fig. 1C). Subneutralizing dilutions of sera obtained from cats infected with the homologous virus often led to increased production of FIV-M2 (Fig. 1B, serum at 1:32; Fig. 2). The effect was occasionally seen also at the lowest dilutions of immune sera tested but was not observed with normal sera (Fig. 2) or with sera of cats infected with other FIV isolates (not shown).

Additional studies showed that the neutralizing effect disappeared completely when the virus-antibody inocula were removed from the host cultures after 24 h of incubation. Prolonging the time of exposure of FIV to immune sera or incubating the virus-serum mixtures with a polyclonal anti-cat immunoglobulin serum or with cat complement for an additional 1 h prior to inoculation into the test cultures failed to augment the neutralizing activity (data not shown). Interestingly, sera obtained from SPF cats infected with FIV-Pet or from naturally infected field cats behaved like normal sera, in

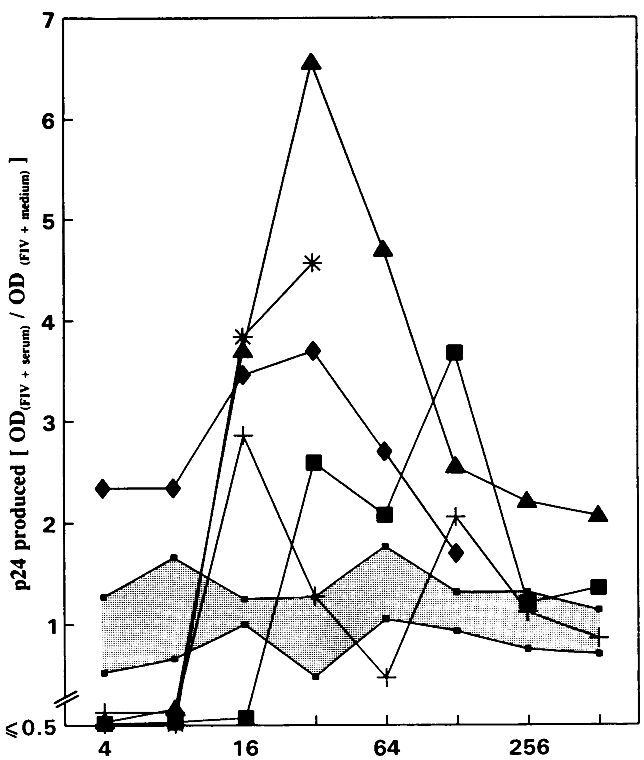

FIG. 2. Enhancement of FIV-M2 production by MBM cells in the presence of subneutralizing concentrations of sera from cats infected with FIV-M2^{CAT}. Virus (10 $TCID_{50}$) was incubated for 1 h at 37°C with the indicated dilutions of sera from five long-term-infected cats (▲, *, ◆, ■, and +) or from five normal cats (shaded area) and then inoculated into MBM cells. The levels of p24 in the supernatants of the cultures were measured 12 days later. The results are expressed as the ratio of the optical density (OD) given by the supernatants of the cultures inoculated with virus preincubated with the sera to that given by the supernatants of the cultures inoculated with virus preincubated with medium alone. The infected cat sera used completely neutralized FIV infectivity for CrFK cells up to dilutions of 1:1,024 or higher, whereas normal cat sera diluted 1:8 did not.

TABLE 4. Neutralization of FIV-M2^{MBM} by sera of SPF cats infected with heterologous virus strains, with CrFK or MBM cells as the host system

Virus infecting serum donors	Cat no.	Neutralizing titer on: CrFK	MBM RT	MBM p24
FIV-Pet^{FL4}	338	4,096	<8	<8
	867	512	ND[a]	<8
	871	512	<8	8
	2906	4,096	<8	<8
	3522	512	<8	<8
	3368	4,096	<8	<8
	3524	512	<8	<8
FIV-Pet^{CrFK}	1696	1,024	ND	<8
FIV-Pet$^{FL4/CAT}$	3788	512	<8	<8
	3735	32	<8	<8
Field[b]	1	1,024	<8	<8
	2	1,024	<8	8
	3	1,024	<8	<8
	4	1,024	<8	<8
	5	32	<8	8
	6	512	<8	<8
	7	>128	<8	8
	8	512	<8	<8
	9	128	<8	<8
	10	<32	<8	<8

[a] ND, not determined.
[b] Random-source naturally infected field cats.

TABLE 5. Neutralization of FIV-Pet^{FL4} by sera of infected SPF and field cats, with MBM cells as the host system

Virus infecting serum donors	Cat no.	Neutralizing titer RT	Neutralizing titer p24
FIV-Pet^{FL4}	338	>512	>512
	867	>2,048	128
	871	>2,048	256
	2906	>512	>512
	3368	>512	>512
	3522	32	32
	3524	>2,048	>2,048
FIV-Pet^{CrFK}	1696	>64	64
FIV-Pet$^{FL4/CAT}$	3735	<8	<8
	3788	<8	<8
FIV-M2^{CAT}	258	<8	ND[a]
	708	<8	ND
	4216	<8	ND
	4270	<8	ND
	4282	<8	ND
Field[b]	1	<8	8
	2	<8	<8
	3	<8	<8
	4	<8	<8
	5	<8	16
	6	<8	<8
	7	<8	16
	8	<8	<8
	9	<8	<8
	10	<8	<8

[a] ND, not determined.
[b] Random-source naturally infected field cats.

that they completely failed to neutralize FIV-M2 or had extremely low titers although this isolate was effectively neutralized by the same immune sera in CrFK cells (Table 4). Thus, the FIV neutralizing activity demonstrable in the MBM cells not only was weak but also was virus strain specific.

Because the results obtained with FIV-M2 were in conflict with published data obtained with FIV-Pet (47), we decided to examine our immune sera for NA against FIV-Pet, using MBM cells as the host system. As shown in Table 5, sera of cats infected with FIV grown in FL4 or CrFK cells neutralized FIV-Pet^{FL4} effectively, with titers that resembled those reported in previous studies (17, 47). Such high NA titers remained unchanged when the virus-serum inocula were washed off the test cultures (data not shown). In contrast, two serum samples obtained from cats infected with FIV-Pet passaged once in vivo (FIV-Pet$^{FL4/CAT}$) showed no neutralizing activity. Sera from cats infected with FIV-M2^{CAT} or from field cats also had little neutralizing activity if any. Thus, FIV-Pet appeared to exhibit a higher sensitivity to neutralization than FIV-M2; however, this occurred only with sera taken from cats infected with the homologous virus that had been grown in tissue culture. As shown in Table 6, FIV-Pet passaged once in cats and then reisolated in MBM cells (FIV-Pet$^{FL4/CAT/MBM}$) was not sensitive to neutralization, similar to FIV-M2^{MBM} (Table 3). That the reversal to low susceptibility to neutralization was due to the passage in vivo and not to growth in MBM was shown by the fact that FIV-Pet passaged in MBM cells but not in vivo (FIV-Pet$^{FL4/MBM}$) was as neutralizable as FIV-Pet^{FL4}.

FIV neutralization with PB as the host system. To exclude the possibility that the lower NA titers in MBM cells were due to some unique property of these cells, we titrated a number of immune sera against FIV-M2 with fresh PB as the host system. Figure 3 depicts one experiment similar to that in Fig. 1, except that PB were used instead of MBM cells. The results were superimposable on those obtained with MBM cells; the same was true for all other sera tested (data not shown). Thus, the NA titers obtained with MBM cells accurately reflect those obtained with fresh PB.

FIV neutralization with cats as the host system. To determine whether NA in CrFK or in MBM cells more accurately reflect the situation in vivo, we exposed in vivo-grown FIV-M2 to pooled sera from cats experimentally infected with the homologous virus, pooled sera from naturally infected cats, or

TABLE 6. Neutralization of FIV-Pet^{FL4}, FIV-Pet$^{FL4/MBM}$, and FIV-Pet$^{FL4/CAT/MBM}$ by sera of SPF cats infected with FIV-Pet^{FL4} or FIV-Pet^{CrFK}

Virus infecting serum donors	Cat no.	Neutralizing titer on MBM cells: FIV-Pet^{FL4} RT	FIV-Pet^{FL4} p24	FIV-Pet$^{FL4/MBM}$ RT	FIV-Pet$^{FL4/MBM}$ p24	FIV-Pet$^{FL4/CAT/MBM}$ RT	FIV-Pet$^{FL4/CAT/MBM}$ p24
FIV-Pet^{FL4}	338	>512	>512	>256	>256	<8	8
	871	>2,048	256	>256	>256	<8	<8
	2906	>512	>512	>256	>256	<8	<8
	3368	>512	>512	>256	>256	<8	<8
	3522	32	32	>256	>256	<8	<8
	3524	>2,048	>2,048	>256	>256	<8	<8
FIV-Pet^{CrFK}	1696	>64	64	ND	ND	<8	<8

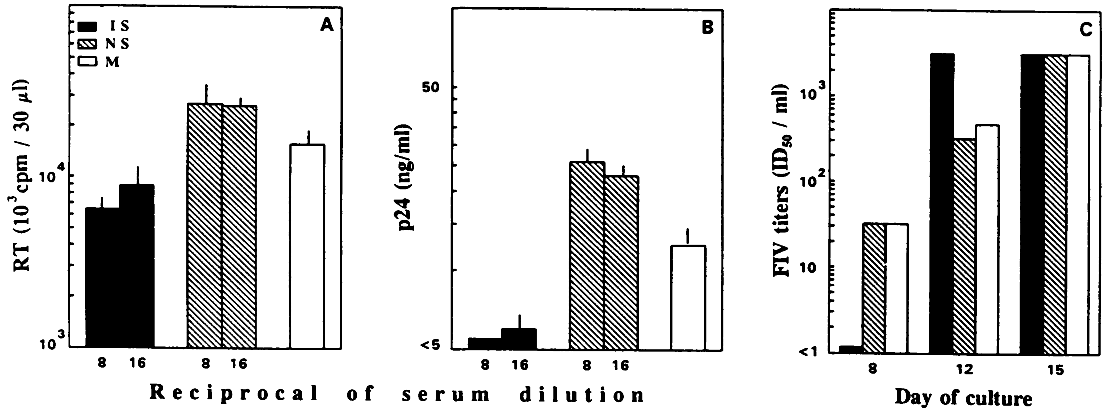

FIG. 3. Neutralization of FIV-M2 with PB cells as the host system by the serum of a cat infected with FIV-M2^{CAT}. The experiment was performed exactly as in Fig. 1, except that the host system was PB rather than MBM cells. ID_{50}, 50% infectious dose.

pooled sera from noninfected cats. The stock of virus used (FIV-M2^{CAT}) in the assay was plasma from SPF cats infected with FIV-M2 passaged only in vivo and harvested at 2 weeks of infection when the levels of actively produced anti-FIV antibody in serum are only beginning to appear (44). The immune sera chosen were among those with the highest NA titers, both in the CrFK assay and in the MBM assay. As shown in Table 7, the neutralizing effect of pooled homologous immune sera was low if there was any (2 of 4 cats became infected), while there was no indication that heterologous sera obtained from naturally infected field cats, living in the area where FIV-M2 had been isolated, reduced infectivity (3 of 4 cats became infected, as in the group inoculated with FIV preincubated with pooled normal serum). In the three groups, all infected cats seroconverted at approximately the same time and exhibited similar virus burdens.

DISCUSSION

When assayed in the fibroblastoid cell line CrFK, sera from infected cats exhibited elevated titers of highly efficient, widely cross-reactive neutralizing activity against FIV. They neutralized effectively and at similarly high titers the Californian isolate FIV-Pet, the Dutch isolate FIV-A6, and the Italian isolate FIV-M2, which differ substantially in the amino acid sequence of the surface glycoprotein (40). Neutralization was not affected by washing off the input virus-serum mixtures after adsorption to the cells (this study and references 13, 14, 26, 43, and 44). The presence of a linear epitope involved in this broadly reactive neutralizing effect in the third variable region of the surface glycoprotein of FIV has been described previously (23).

Neutralization with the T-null lymphoid cell line MBM or PB as substrate yielded strikingly different results in that (i) the titers of NA were surprisingly low, (ii) neutralization was evident only with sera derived from cats infected with the homologous virus isolate, (iii) neutralization was lost if cells treated with virus-serum mixtures were incubated for 12 to 15 days instead of the standard 8-day assay, and (iv) neutralization was readily reversed by removing the virus-serum mixtures after adsorption to the test cultures.

The reasons why antibody-mediated neutralizations of FIV infectivity are quantitatively and qualitatively different depending on whether residual FIV infectivity is measured on lymphoid or on CrFK cells can be several. The differences were not artifacts due to variations in the ratio of defective virus and/or immunoreactive viral material to infectious virus present in the FIV preparations used in the various assays, as the extent of neutralization did not correlate with their content in p24 antigen (Table 1) and RT (data not shown). Cellular molecules incorporated into the viral envelope may contribute to neutralization of HIV and simian immunodeficiency virus (1, 33), and anti-cell antibodies have been implicated in the protection conferred by certain experimental vaccines against such viruses (8, 22). That anti-cell antibody might contribute also to FIV neutralization is suggested by the results obtained when sera derived from cats infected with FIV-Pet grown in tissue cultures were tested on MBM cells for NA to FIV-Pet^{FL4} (Table 5). In these cases, the neutralizing activity reached titers of 2,048 or more, but one passage in vivo of FIV-Pet^{FL4} before its use for infecting the serum donor lowered the NA titers detected to the levels found with FIV-M2. However, a major role for anti-cell antibodies in FIV

TABLE 7. Neutralization of FIV-M2^{CAT} by sera of infected cats, with SPF cats as the host system

Virus infecting serum donors	No. of cats/group	No. of cats seronverted	Wk postinfection at seroconversion	No. of FIV-positive cats[a]	Viral burden[b]
None[c]	4	3	4, 4, 8	3	50, 50, 500
FIV-M2^{CAT}[d,e]	4	2	6, 8	2	500, 500
Field[e,f]	4	3	4, 6, 6	3	50, 50, 5,000

[a] Based on repeated virus isolations performed up to 7 months postinfection.
[b] Infected cells per 10^6 PBMC at 6 months postinfection.
[c] Pool of five normal SPF cat serum samples. NA titers on CrFK and MBM cells were <8.
[d] Pool of sera of five SPF cats infected 8 to 13 months earlier. NA titers ranged from 8,192 to 16,384 on CrFK cells and from 8 to 32 on MBM cells.
[e] When injected into naive cats, these serum pools proved noninfectious.
[f] Pool of sera of infected field cats. NA titers ranged from 1,024 to 8,192 on CrFK cells and were <8 in MBM cells.

neutralization on CrFK cells is ruled out by the observation that high titers of NA were present also in random-source-infected cats, most of which were never exposed to tissue-culture-grown vaccines.

We must conclude that the discrepancies observed between neutralization on CrFK and on lymphoid cells are due to differences in the viral preparations or in the cells used. These possibilities could not be tested directly because FIV adapted to produce syncytia in CrFK cells proved very poorly infectious for lymphoid cells and vice versa (data not shown). It is clear, however, that the high-passage FIV used in the CrFK assay might be more neutralizable than the low-passage FIV used for the MBM and PB assays. This scenario is supported by the observation that the high-culture-passage FIV^{FL4}, which was effectively neutralized by homologous immune sera in MBM cells, lost its sensitivity to neutralization almost completely once reisolated from an infected cat (Table 6). Evidence suggests that FIV evolution is driven by the selective pressure of host immune response (34, 41). As it is likely that the opposite process may also take place, repeated passages in culture in the absence of immunological pressure might progressively narrow the antigenic polymorphism of the virus (46).

Abundant evidence shows that the host is an integral part of virus neutralization reactions and may markedly influence their outcome (for a review, see reference 11). That the host cell can influence the result of FIV neutralization is suggested by the observation that NA detected by use of high-passage virus were broadly reactive in CrFK cells and isolate specific in MBM cells. Generally, the influence of target cells on neutralization has been attributed to the fact that the virus uses different receptors to infect the cells (11). Our present understanding of FIV receptor(s) is limited; nonetheless, a recently described monoclonal antibody which is thought to recognize the FIV receptor (20) prevented FIV infection of MBM cells as effectively as infection of CrFK cells (data not shown), suggesting that FIV receptors on these two cell types are similar. It should be noted, however, that in a previous study antibodies that neutralized HIV-1 in $CD4^+$ lymphocytes were inactive on CD4-expressing HeLa cells, implying that the environment of the receptor is also important (6). Alternatively, since sera of hosts infected with lentiviruses are known to contain infection-enhancing as well as infection-inhibiting antibodies (29), it is possible that CrFK cells do not permit the action of the former kind of antibody because of the absence of appropriate Fc receptors or for other reasons. In this study, subneutralizing concentrations of heated immune sera exerted a substantial enhancement of FIV infectivity for lymphoid cells, an effect not seen with CrFK cells (13, 14, 43, 44). As enhancement of infection has been noted in cats treated with putative FIV vaccines (19), the FIV system might be ideal for understanding the role of enhancing antibody in lentiviral pathogenesis.

This study also shows that exposure to anti-FIV antibody has little effect on the ability of FIV to infect cats, at least when the virus used has been propagated in vivo. Virtually no protection was observed in the cats injected with 10 CID_{50} of FIV preincubated with a pool of homologous immune sera that neutralized FIV at high titers in CrFK cells and also had some neutralizing activity on MBM cells. Thus, neutralization assays performed on lymphoid cells seem to reflect the situation in vivo more closely than assays on CrFK cells. This has practical implications because cell systems which reflect the situation in vivo should preferably be used in neutralization assays (11). The CrFK assay conserves, however, a considerable value as a confirmatory test for infection.

The in vivo experiment reported here represents the first attempt to evaluate the biological significance of antibody-mediated in vitro neutralization of FIV. The poor neutralization observed contrasts with the results of a passive immunization study in which adoptive transfer of sera obtained from FIV-infected or -vaccinated animals effectively protected cats against subsequent challenge with the homologous strain of FIV (17). Protection has been reported in most (12, 37, 38) but not all (36) the published attempts to neutralize the infectivity of primate lentiviruses in vivo by preincubating the viruses in vitro or pretreating animals with immune sera. However, to our knowledge the present study is the first of its kind to make use of virus derived directly from infected animals. The relevance of this study is suggested by reports from the 6th Annual Conference on Advances in AIDS Vaccine Development that NA measured with culture-adapted HIV do not reflect the ability of sera to neutralize real-world HIV (7). We feel, therefore, that our results may be representative of what occurs under natural circumstances of infection, thus casting further doubts on the feasibility of protecting hosts against lentiviral infections with antisera that contain NA or vaccines that elicit NA.

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