

NOTES

Passive Antibody Protection of Cats against Feline Immunodeficiency Virus Infection

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All six cats passively immunized with sera from either feline immunodeficiency virus (FIV)-vaccinated cats or cats infected with FIV (Petaluma strain) were protected from homologous FIV infection at a challenge dose that infected all six control cats. Passive immunization with sera from cats vaccinated with uninfected allogeneic T cells used to grow the vaccine virus did not protect either of two cats against the same FIV challenge. These results suggest that antiviral humoral immunity, perhaps in synergy with anticellular antibodies, may be responsible for previously reported vaccine protection.

Feline immunodeficiency virus (FIV)-induced immunodeficiency disease in domestic cats represents an important veterinary health problem and serves also as a very useful small-animal model for AIDS antiviral therapy and vaccine development (2, 4, 7). The virus is transmitted by blood and saliva; about 1 to 6% of cats worldwide are infected (5, 10, 18). We reported that over 90% of cats immunized with either inactivated infected-cell or inactivated cell-free whole-FIV vaccines were protected against experimental infection with homologous and heterologous strains of FIV (19, 20). Protection appeared to correlate with vaccine induction of strong antiviral immune responses including high-titered neutralizing antibodies. In contrast to the observed protection against simian immunodeficiency virus (SIV) by the infected human T cells used to grow the virus (14), immunization with analogous uninfected feline T cells did not protect cats against challenge infection with FIV grown in the same cells (20). However, anticellular antibodies did add a nonspecific adjuvant effect to the vaccines (20). To further determine the mechanism of FIV vaccine protection, we evaluated the role of antiviral and anticellular antibodies by passive immunization against experimental FIV infection.

Eighteen specific-pathogen-free (SPF) cats, 3 to 6 months of age, were divided into three study groups as shown in Table 1. In the first study, three cats were passively immunized with pooled sera from vaccinated cats and three control cats received phosphate-buffered saline (PBS) or pooled control cat sera. The vaccine serum pool was from nine unchallenged SPF cats that had been immunized three times at 3- to 4-week intervals with inactivated FIV vaccines, consisting of either fixed infected allogeneic T cells (FL-4 cells) or semi-purified cell-free virus harvested from the same cells; both vaccines were mixed in threonyl muramyl dipeptide (SAF-1) adjuvant (250 µg per dose), as described previously (19). This vaccination protocol previ-

ously protected cat against intraperitoneal (i.p.) infection with a low dose of homologous cell-free FIV (Petaluma strain) (19, 20). The FIV-neutralizing (VN) titer of this pooled serum was 1,280. The control serum pool, from nine SPF cats which were either unimmunized or immunized with adjuvant only, had no VN titer.

In the second study, two groups of three cats each were passively immunized with pooled sera from either six experimentally FIV-infected or six control SPF cats. Any live virus in the pooled sera, collected from cats at 9 to 10 months postinfection with 10 50% animal infectious doses (10 ID₅₀) of FIV Petaluma, was inactivated by a combination of heat (56°C for 40 min) and HCl (pH 4 for 2 h) treatment, a method previously reported to completely inactivate human immunodeficiency virus type 1 (HIV-1) in infected human sera (9, 16). The pooled control serum was similarly treated. The VN titer of the pretreated pooled serum from the infected cats was about 2,560, and that of control sera was <8 (below the detectable level). The heat and acid pretreatment did not lower the VN titer.

In the third study, two SPF cats each were passively immunized with either pooled sera from two SPF cats vaccinated with fixed uninfected FeT1 cells or from two SPF cats vaccinated with fixed uninfected 3201 cells. FeT1 cells are an interleukin 2-dependent feline T-cell line from which interleukin-2-independent FL-4 cells used to grow the FIV vaccine virus were derived (17). 3201 cells are another allogeneic feline T-cell line susceptible to infection with FIV-Petaluma. One cat received only PBS.

The doses, routes, and schedule for both passive immunization and the live virus challenge were identical for all three studies. Cats were given a 20-ml intravenous transfusion of stored (-20°C), pooled sera or PBS 18 h prior to i.p. challenge with 5 ID₅₀ of FIV Petaluma. An additional 15 ml of the same sera or PBS was given i.p. at 24 and 48 h postchallenge (p.c.). All cats were routinely monitored for FIV antibodies by immunoblotting (20), enzyme-linked immunosorbent assay (ELISA) (19), and virus-neutralizing

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TABLE 1. Isolation of FIV from passively immunized cats before and after challenge

Study and cat	Source of sera	Virus isolation at wk:												
		2	3	4	5	6	7	8	9	10	11	13	16	19
1														
328	Vaccinated cats ^a	-	-	-	-	-	-	-	-	-	-	-	-	-
334	Vaccinated cats ^a	-	-	-	-	-	-	-	-	-	-	-	-	-
338	Vaccinated cats ^a	-	-	-	-	-	-	-	-	-	-	-	-	-
336	Nonvaccinated cats ^b	-	+	+	+	+	+	+	+	+	+	+	+	+
337	Nonvaccinated cats ^b	-	+	+	+	+	+	+	+	+	+	+	+	+
339	PBS-immunized cats ^c	-	+	+	+	+	+	+	+	+	+	+	+	+
2														
X1 ^d	Infected cats ^e	-	-	-	-	-	-	-	-	-	-	-	-	-
X3	Infected cats ^e	-	-	-	-	-	-	-	-	-	-	-	-	-
X5	Infected cats ^e	-	-	-	-	-	-	-	-	-	-	-	-	-
32C	Infected cats ^e	-	-	-	-	-	-	-	-	-	-	-	-	-
X2	Uninfected cats ^f	-	-	+	+	+	+	+	+	+	+	+	+	+
X4	Uninfected cats ^f	-	+	+	+	+	+	+	+	+	+	+	+	+
32A	Uninfected cats ^f	-	-	+	+	+	+	+	+	+	+	+	+	+
3														
400	Immunized cats ^g	-	-	+	+	+	+	+	+	+	+	+	+	+
402	Immunized cats ^g	-	-	+	+	+	+	+	+	+	+	+	+	+
401	Immunized cats ^h	-	-	+	+	+	+	+	+	+	+	+	+	+
403	Immunized cats ^h	-	-	+	+	+	+	+	+	+	+	+	+	+
32D	PBS-immunized cats ^c	-	-	+	+	+	+	+	+	+	+	+	+	+

^a Pooled vaccine sera consisted of sera from nine cats immunized with either inactivated whole-virus or fixed infected-cell vaccines.
^b Pooled nonvaccine sera consisted of sera from nine SPF cats which were unimmunized or immunized with adjuvant.
^c PBS was used directly for immunization in place of pooled sera.
^d Cat X1 was the unchallenged control used to monitor the level of the immunized antibodies throughout the study.
^e Inactivated infected sera consisted of a pooled serum preparation from six experimentally FIV-infected cats which was inactivated by a combination of heat and acid treatment.
^f Pooled sera from six unvaccinated unchallenged SPF cats.
^g Pooled vaccine sera from cats immunized with uninfected feline T cells (FeT1 line).
^h Pooled vaccine sera from cats immunized with uninfected feline T cells (3201 line).

assay (19) and for infectious virus by virus isolation (19, 20). One cat was passively immunized with inactivated sera from infected cats and monitored for FIV infection as a control to determine whether any residual viable virus remained after the heat and acid treatment.

Passive immunization with vaccine sera (study 1). High titers of anti-transmembrane peptide (anti-TM) (Fig. 1A), (titer, VN 64 to 128) (Fig. 1B), and anti-p25 (titer, 1:10,000 to 1:50,000) (data not shown) antibodies were detected at 1 week after challenge, 8 days after the first passive immunization, in cats passively immunized with vaccine sera. In all three cats, titers of viral antibody derived from passive immunization gradually decreased and were undetectable by 16 to 19 weeks p.c. (Fig. 1). At 19 weeks p.c., FIV infection was undetectable in the cats by virus isolation (Table 1). The lack of an antibody response to virus challenge (Fig. 1) and the lack of isolatable virus indicated that all three cats passively immunized with vaccine sera were protected from homologous FIV challenge. In contrast, the three control cats developed anti-p25 antibody titers (from 1:100 at 6 weeks p.c to 1:10,000 at 19 weeks p.c.; data not shown), anti-TM antibody titers (Fig. 1A), and VN antibody titers (Fig. 1B) starting 4 to 10 weeks p.c. and became persistently positive for FIV isolation as early as 4 weeks p.c. (Table 1).

Passive immunization with infected sera (study 2). Significant titers of anti-TM (Fig. 2A), VN (128 to 256) (Fig. 2B), and anti-p25 (1:5,000 to 1:20,000) (data not shown) antibodies were detected at 1 week p.c. in all four cats passively immunized with inactivated sera from infected cats. In both challenged and unchallenged cats, the FIV antibody titers

steadily declined and after 9 weeks p.c., the antibodies were no longer detectable (Fig. 2). In contrast, starting 7 weeks p.c., the three cats passively immunized with pretreated control sera developed significant titers of anti-p25 (data not shown) and anti-TM (Fig. 2A) antibodies. As of 19 weeks p.c., only cats immunized with control sera were positive for FIV by isolation (Table 1). These results strongly suggest that antiviral antibodies were responsible for immune protection.

Passive immunization with anticellular sera (study 3). To determine the possible role of anticellular antibodies in the passive antibody protection observed, cats were passively immunized with pooled sera from cats previously vaccinated with uninfected allogeneic feline T-cell lines (FeT1 or 3201 cells); one SPF cat received placebo (PBS). The pooled sera contained high levels of anticellular antibodies but no detectable VN antibodies (<8) (Table 2). None of these cats developed anti-p25, anti-TM, or VN antibody titers until 5 to 10 weeks p.c. (data not shown), at which time all cats were positive for FIV by virus isolation (Table 1). No significant differences in the rates of FIV isolation (Table 1) and viral antibody induction (data not shown for study 3) between the cats receiving anti-FeT1 and anti-3201 sera (study 3) and the cats receiving PBS (studies 1 and 3) or control sera (studies 1 and 2) were observed. Thus, anticellular antibodies alone could not confer protection against virus infection despite the presence of levels of anticellular antibodies comparable to those induced by the FIV vaccines in study 1 (Table 2).

In vitro cell absorption. Vaccine sera (from study 1) and infected sera (from study 2) were absorbed with fixed

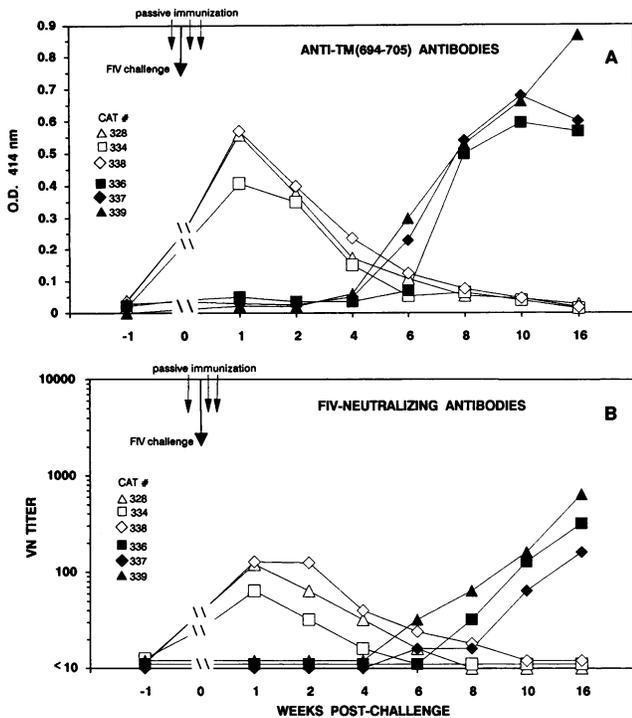


FIG. 1. Three SPF cats (cats 328, 334, and 338) were passively immunized with pooled sera from vaccinated SPF cats, and an additional three SPF cats (cats 336, 337, and 339) were passively immunized with PBS or pooled sera from unimmunized SPF cats or from SPF cats immunized with adjuvant only. All cats were monitored for anti-TM (amino acids 694 to 705) (A) and VN (B) antibodies. All six cats were given 20-ml intravenous transfusion (first small arrow) of pooled vaccine sera, nonimmune control sera, or PBS 18 h prior to i.p. challenge with 5 ID_{50} of FIV Petaluma (large arrow). An additional 15 ml of vaccine sera, control sera, or PBS was given i.p. at 24 and 48 h p.c. (second and third small arrows). O.D. 414 nm, optical density at 414 nm.

uninfected cells (combination of FeT1 and 3201), fixed FIV-infected cells (FL-4 line), or fixed feline leukemia virus (FeLV)-infected feline T cells (FL-74 line) (15). The cells were fixed at 5×10^6 /ml with 1.25% paraformaldehyde in PBS for 24 h at 5°C, washed three times with sterile PBS, resuspended in sterile PBS, and stored at 5°C. Absorption was performed with 5×10^7 cells per 0.3 ml of serum sample for 1.5 h at 37°C, and the serum sample was clarified (made free of cells) by microcentrifugation. Uninfected-cell absorption consisted of two absorptions with FeT1 cells followed by one absorption with 3201 cells. Infected-cell absorption consisted of two absorptions with the infected cells (FL-4 or FL-74). The control sera were similarly incubated but without adding cells. Antibodies to uninfected FeT1 cells were measured by fixed-cell ELISA. ELISA plates were coated with fixed uninfected FeT1 cells (2×10^5 per well) with bicarbonate buffer (pH 9.6), incubated overnight at 37°C, and washed once with washing buffer before being used (19).

Absorption of pooled vaccine sera (study 1) with fixed uninfected cells (FeT1 and 3201) did not significantly lower the VN titer (1,280 to 640), whereas absorption with an identical number of fixed FIV-infected FL-4 cells completely removed the VN titer (<8) (Table 2). The titer of anti-FeT1 antibody remaining in the FL-4-absorbed sera was similar to the titer of antibody remaining in sera absorbed with unin-

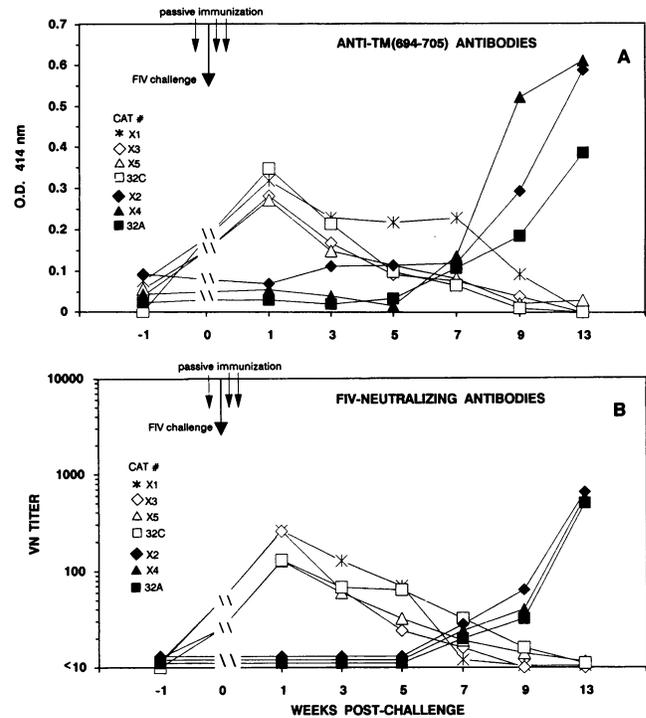


FIG. 2. Four SPF cats (cats X1, X3, X5, and 32C) were passively immunized three times (small arrows) with heat- and acid-pretreated, pooled sera from cats experimentally infected with FIV Petaluma. Three SPF cats (cats X2, X4, and 32A) received immunizations with heat- and acid-pretreated control sera. All cats except for cat X1 were challenged at 0 weeks p.c. with homologous FIV Petaluma (large arrow) and were monitored for anti-TM (amino acids 694 to 705) (A) and VN (B) antibodies.

fected cells. A similar nonspecific twofold decrease in VN titer (2,560 to 1,280) in infected sera (from study 2) was observed upon absorption with uninfected cells. This decrease was probably due to nonspecific attachment and to dilution from the traces of PBS still remaining in the pelleted fixed cells used for absorption. Thus, the VN titer of the sera from cats immunized with either fixed infected-cell or inactivated whole-virus vaccine was unaffected by absorption with the uninfected cells used to grow the virus. Similarly, absorption with FeLV-infected feline T cells (FL-74 line) did not affect the VN titers.

Cats passively immunized the sera from either FIV-vaccinated or FIV-infected cats were protected from FIV infection at an i.p. challenge dose (5 ID_{50}) which infected all control cats. High titers of anticellular antibodies were detected in the vaccine sera, but no such antibodies were present in the infected sera used for immunization. Moreover, these antibodies could not be the critical correlate for vaccine protection because passive immunization of cats with sera containing only high titers of anticellular antibodies did not protect these animals from FIV challenge. Furthermore, we previously showed that cats immunized with the uninfected allogeneic T cells used to grow the vaccine virus were not protected against i.p. challenge infection (20). Thus, in contrast to immune protection elicited in macaques by inactivated SIV-infected-cell or cell-free virus vaccines (1, 14), anticellular antibodies do not appear to be critical to the passive antibody protection observed in the current studies. However, our previous results with inactivated

TABLE 2. Titers of VN and anticellular antibodies in sera before and after absorption with FIV-infected and uninfected cells

Source of sera	Type of cell absorption (infection status)	VN titer	Anti-FeT1 antibody ELISA (OD) ^a	
Vaccinated cats	Whole-virus + infected-cell vaccine ^b	None	1,280	0.498 ± 0.008
		FeT1 + 3201 (uninfected)	640	0.180 ± 0.002
		FL-74 (FeLV infected)	1,280	0.188 ± 0.006
		FL-4 (FIV infected)	<8	0.162 ± 0.001
	Whole-virus vaccine ^c	None	1,280	0.602 ± 0.004
		FeT1 + 3201 (uninfected)	1,280	0.157 ± 0.011
		FL-74 (FeLV infected)	640	0.096 ± 0.003
		FL-4 (FIV infected)	<8	0.055 ± 0.001
	Infected-cell vaccine ^d	None	2,560	0.514 ± 0.021
		FeT1 + 3201 (uninfected)	2,560	0.105 ± 0.003
		FL-74 (FeLV infected)	2,560	0.073 ± 0.005
		FL-4 (FIV infected)	<8	0.046 ± 0.008
	Infected cats ^e	None	2,560	0.074 ± 0.031
FeT1 + 3201 (uninfected)		1,280	0.081 ± 0.011	
FL-74 (FeLV infected)		2,560	0.058 ± 0.006	
FL-4 (FIV infected)		8	0.058 ± 0.001	
Cats immunized with: Uninfected FeT1 cells	None	<8	0.490 ± 0.001	
	FeT1 + 3201 (uninfected)	<8	0.035 ± 0.011	
Uninfected 3201 cells	None	<8	0.662 ± 0.002	
	FeT1 + 3201 (uninfected)	<8	0.118 ± 0.022	

^a OD, optical density.

^b Pooled vaccine sera consisting of both whole-virus and fixed infected-cell vaccinated cat sera used in study 1.

^c Pooled vaccine sera from cats immunized with inactivated whole-virus vaccine.

^d Pooled vaccine sera from cats immunized with fixed infected-cell vaccine.

^e Pretreated (heat- and pH 4-inactivated) infected sera used in study 2.

^f Pooled vaccine sera from cats immunized with uninfected feline T cells (FeT1 and 3201 cells). These are the anti-FeT1 and anti-3201 cell sera used in study 3.

whole-virus vaccines suggest that anticellular antibodies exert an adjuvant effect to the vaccine immunogenicity (20).

In Sweden, passive protection with sera from SIV-infected and HIV-2-vaccinated monkeys has also been reported. Three of four monkeys passively immunized (9 ml/kg of body weight) with inactivated pooled sera with high VN titers from monkeys infected with SIV_{sm} were protected against 10- to 100-ID₅₀ homologous challenges given 5 h after immunization (12). Protection of two of three cynomolgus monkeys was obtained with sera (9 ml/kg) (VN titer, 2,560) from a monkey given an HIV-2 vaccine 6 h before intravenous challenge with 10 ID₅₀ of HIV-2 (12). The virus for the vaccine was grown in human cells, while the virus for the challenge was passaged twice in human peripheral blood lymphocytes and subsequently twice in monkey peripheral blood lymphocytes. Both of these studies suggested that high titers of virus-specific antibodies were required for protection (12). However, passive immunization results at the University of California, Davis, have indicated a stronger correlation of anticellular antibodies than of antiviral antibodies to protection against infection with SIV_{mac} (13). Therefore, in the SIV macaque model, the relative importance of anticellular antibodies compared with antiviral antibodies in the protective immunity elicited by the inactivated whole-virus vaccine remains unclear.

In the FIV cat system, the picture appears to be clearer in that passive antibody protection may be mediated primarily

by VN antibodies. The vaccine serum pool used for passive immunization had a VN titer of 1,280, and the recipient cats had VN titers of 64 to 128 at 8 days after the first transfer. A slightly higher VN titer of 2,560 was detected in the infected-cat sera used for passive immunization, and the recipients of these sera had VN titers of 128 to 256 at 8 days after the first immunization. In contrast, cats immunized with uninfected-cell or control sera which had no VN titer were unprotected against FIV infection. Whereas protected cats immunized with inactivated whole-virus vaccine had an average VN titer of 100 at the time of FIV challenge (19), immunized cats with lower VN titers were unprotected from low-dose virus challenge (10 to 20 ID₅₀) (7). It seems unlikely that virus-infected FL-4 cells and virus derived from them induced a more vigorous or qualitatively different anticell response than the uninfected FeT1 counterpart because the titers of anticellular antibodies were similar in cats immunized with infected fixed feline T cells and in those immunized with uninfected T cells (Table 2).

These passive protection results demonstrate that protection observed in cats with active immunization (19, 20) could be mediated by a threshold level of antiviral antibodies, including VN antibodies. Antibody-mediated complement lysis of virions or virus-infected cells, antibody-dependent cellular cytotoxicity, or cytotoxic T lymphocytes may also contribute to protection. Our preliminary results suggest that passive immunity with cat antisera to FIV Petaluma also

protects against heterologous FIV Shizuoka strain. These results for positive passive protection against FIV complement the positive results with passive immunization obtained in the HIV-1 chimpanzee and HIV-2 macaque models (3, 11, 12) and support the use of passive immunization against HIV-1 with plasma from healthy carriers (6, 8) or V3 loop-specific human monoclonal antibodies (3). However, results from the SIV macaque model indicate the need for caution in using this approach. The feline model should therefore be helpful for further evaluating the use of passive immunization for prophylaxis against experimental FIV transmission in certain clinical settings such as during pregnancy or following needle stick accidents.

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REFERENCES

1. Cranage, M. P., L. A. E. Ashworth, P. J. Greenaway, M. Murphey-Corb, and R. C. Desrosiers. 1992. AIDS vaccine developments. *Nature (London)* 335:685-686.
2. Egberink, H. F., K. Hartman, and M. C. Horzinek. 1991. Chemotherapy of feline immunodeficiency virus infection. *J. Am. Vet. Med. Assoc.* 199:1485-1487.
3. Emini, E. A., W. A. Schleif, J. H. Nunberg, A. J. Conley, Y. Eda, S. Tokiyoshi, S. D. Putney, S. Matsushita, K. E. Cobb, C. M. Jett, J. W. Eichberg, and K. K. Murthy. 1992. Prevention of HIV-1 infection in chimpanzees by gp120 V3 domain-specific monoclonal antibody. *Nature (London)* 355:728-730.
4. Gardner, M. B. 1991. Simian and feline immunodeficiency viruses: animal lentivirus models for evaluation of AIDS vaccines and antiviral agents. *Antiviral Res.* 15:267-286. (Minireview.)
5. Hosie, M. J., C. Robertson, and O. Jarrett. 1989. Prevalence of feline leukaemia virus and antibodies to feline immunodeficiency virus in cats in the United Kingdom. *Vet. Rec.* 128:293-297.
6. Jackson, G. G., J. T. Perkins, M. Rubenis, D. A. Paul, M. Knigge, J. C. Despostes, and P. Spencer. 1988. Passive immunoneutralisation of human immunodeficiency virus in patients with advanced AIDS. *Lancet* ii:647-652.
7. Jarrett, O., J. K. Yamamoto, and J. C. Neil. 1991. Feline immunodeficiency virus as a model for AIDS vaccination. *AIDS* 5(Suppl. 1):S163-S165.
8. Karpas, A., I. K. Hewlett, F. Hill, J. Gray, N. Byron, D. Gilgen, V. Bally, J. K. Oates, B. Gazzard, and J. E. Epstein. 1990. Polymerase chain reaction evidence for human immunodeficiency virus 1 neutralization by passive immunization in patients with AIDS and AIDS-related complex. *Proc. Natl. Acad. Sci. USA* 87:7613-7617.
9. Kempf, C., F. Barre-Sinoussi, J.-J. Morgenthaler, and D. Germann. 1991. Inactivation of human immunodeficiency virus (HIV) by low pH and pepsin. *J. Acquired Immune. Defic. Syndr.* 4:828-830.
10. Povey, R. C., and G. J. Hawkins. 1989. Feline immunodeficiency virus: a commentary. *Can. Vet. J.* 30:559-562.
11. Prince, A. M., H. Reesink, D. Pascual, B. Horowitz, I. Hewlett, K. K. Murthy, K. E. Cobb, and J. W. Eichberg. 1991. Prevention of HIV infection by passive immunization with HIV immunoglobulin. *AIDS Res. Hum. Retroviruses* 7:971-973.
12. Putkonen, P., R. Thorstensson, L. Ghavamzadeh, J. Albert, K. Hild, G. Biberfeld, and E. Norrby. 1991. Prevention of HIV-2 and SIV_{sm} infection by passive immunization in cynomolgus monkeys. *Nature (London)* 352:436-438.
13. Rosenthal, A., M. B. Jennings, R. Cotterman, S. Schmerl, J. L. Yee, S. Joye, and M. B. Gardner. 1992. Passive immunization and challenge of rhesus macaques with SIV_{mac}. abstr. 126. 10th Annual Symposium on Nonhuman Primate Models for AIDS.
14. Stott, E. J. 1991. Anti-cell antibody in macaques. *Nature (London)* 353:393.
15. Theilen, G. H., T. G. Kawakami, J. D. Rush, and R. J. Munn. 1969. Replication of cat leukemia virus in cell suspension cultures. *Nature (London)* 272:589-590.
16. Vittecoq, D., B. Mattinger, F. Barre-Sinoussi, A. M. Courouce, C. Rouzioux, C. Doinel, M. Bary, J. P. Viard, J. F. Bach, P. Rouger, and J. J. Lefrere. 1992. Passive immunotherapy in AIDS: a randomized trial of serial human immunodeficiency virus-positive transfusions of plasma rich in p24 antibodies versus transfusions of seronegative plasma. *J. Infect. Dis.* 165:364-368.
17. Yamamoto, J. K., C. D. Ackley, H. Zochlinski, H. Louie, E. Pembroke, M. Torten, H. Hansen, R. Munn, and T. Okuda. 1991. Development of IL 2 independent feline lymphoid cell lines chronically infected with feline immunodeficiency virus: importance for diagnostic reagents and vaccines. *Intervirolgy* 32:361-375.
18. Yamamoto, J. K., H. Hansen, E. W. Ho, T. Y. Morishita, T. Okuda, T. R. Sawa, R. M. Nakamura, and N. C. Pedersen. 1989. Epidemiologic and clinical aspects of feline immunodeficiency virus (FIV) infection in cats from the continental United States and Canada and possible mode of transmission. *J. Am. Vet. Med. Assoc.* 194:213-220.
19. Yamamoto, J. K., T. Hohdatsu, R. A. Olmsted, R. Pu, H. Louie, H. A. Zochlinski, V. Acevedo, H. M. Johnson, G. A. Soules, and M. B. Gardner. 1993. Experimental vaccine protection against homologous and heterologous strains of feline immunodeficiency virus. *J. Virol.* 67:601-605.
20. Yamamoto, J. K., T. Okuda, C. D. Ackley, H. Louie, H. Zochlinski, E. Pembroke, and M. B. Gardner. 1991. Experimental vaccine protection against feline immunodeficiency virus. *AIDS Res. Hum. Retroviruses* 7:911-922.