

Experimental Vaccine Protection against Homologous and Heterologous Strains of Feline Immunodeficiency Virus

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Received 20 July 1992/Accepted 15 October 1992

More than 90% of cats immunized with inactivated whole infected-cell or cell-free feline immunodeficiency virus (FIV) vaccines were protected against intraperitoneal infection with 10 50% animal infectious doses of either homologous FIV Petaluma (28 of 30 cats) or heterologous FIV Dixon strain (27 of 28 cats). All 15 control cats were readily infected with either strain of FIV. Protection appears to correlate with antiviral envelope antibody levels by a mechanism yet to be determined.

Infection of domestic house cats with feline immunodeficiency virus (FIV) is an established cause of fatal immunosuppressive disease under both natural and experimental conditions and is becoming a very useful small animal model for AIDS antiviral therapy and vaccine testing (4, 14). We previously showed that immunity against low-dose intraperitoneal (i.p.) infection with homologous FIV could be achieved in about 75% (11 of 15) of cats immunized with inactivated whole FIV vaccines made up of either fixed infected allogenic T cells or cell-free virus harvested from these cells (20). Protection appeared to correlate in general with vaccine induction of a strong antiviral immune response, including virus-neutralizing (VN) antibodies. However, the relative titer of VN antibodies did not completely correlate with protection. In contrast to the simian immunodeficiency virus (SIV) macaque model (17), immunization of cats with the uninfected T cells used to grow the virus for vaccine production and challenge did not protect any cats against challenge infection with FIV (20). Thus, in the FIV model, although the allogeneic T-cell antigens could be shown to contribute an adjuvant effect to the vaccines (20), they did not appear to be the critical correlate of immune protection. In the present study, we expanded upon this FIV vaccine model by immunizing a larger number of cats with a slightly modified protocol and by challenging cats protected against the homologous FIV with a heterologous strain of FIV, differing by 11% in outer envelope amino acid sequences.

Preparation of the FIV vaccines using the interleukin-2-independent feline T-cell line (FL-4) has been described previously (19, 20). The formulation of the adenylyl-muramyl dipeptide (A-MDP) adjuvant has been reported (2). Both fixed infected-cell and inactivated whole-virus vaccines were shown to be free of infectious virus and viable cells for more than 2 years by *in vitro* virus isolation and lymphocyte

proliferation assays and by monitoring of vaccinated unchallenged cats for infectious virus using previously described (20) virus isolation and polymerase chain reaction (PCR) methods (data not shown).

Forty specific-pathogen-free (SPF) cats, 4 to 6 months of age, were divided into three groups. Group A (15 cats) animals were vaccinated subcutaneously with 2.5×10^7 fixed infected FL-4 cells per dose plus A-MDP adjuvant, group B (15 cats) received 250 μ g of inactivated whole virus per dose plus A-MDP adjuvant, and group C (10 cats) received adjuvant alone. All cats were given the first booster injection 2 weeks after the primary immunization, and the second booster injection was given 3 weeks later. Because of apparent A-MDP toxicity, i.e., diarrhea and vomiting, after the primary immunization, the dose of A-MDP was reduced to 125 and 10 μ g for the first and second booster injections, respectively.

After vaccination with either vaccine but before challenge (day zero; Fig. 1 to 4), all cats were completely negative for infectious virus in their peripheral blood lymphocytes by both virus isolation and PCR (Table 1), further evidence that the vaccines were completely inactivated *in vitro*. By 3 weeks after the second booster injection, at the time of homologous virus challenge (day zero), substantial levels of antibodies binding to FIV core (p25) and envelope (SU and TM) antigens were detected. Enzyme-linked immunosorbent assay (ELISA) titers were 1:1,000 to 1:50,000 for antibodies to purified core p25 and 1:500 to 1:50,000 for SU and TM peptides, with higher titers to the TM peptide (Fig. 1 to 3). By immunoblot, antibody titers of 1:50 to 1:5,000 were detected against purified SU and TM glycoproteins (data not shown). In general, epitope-specific envelope antibodies detected by ELISAs using SU(595-615) and TM(694-705) peptides were higher in titer (thus more sensitive) than the overall anti-envelope antibody titer detected by ELISA or immunoblot assays with purified SU and TM glycoproteins. Using a previously described microassay (20), significant titers of VN antibody (mean titers of 100 and 800) were also achieved, generally higher in those cats immunized with the fixed infected-cell vaccine (Fig. 4). Unimmunized adjuvant

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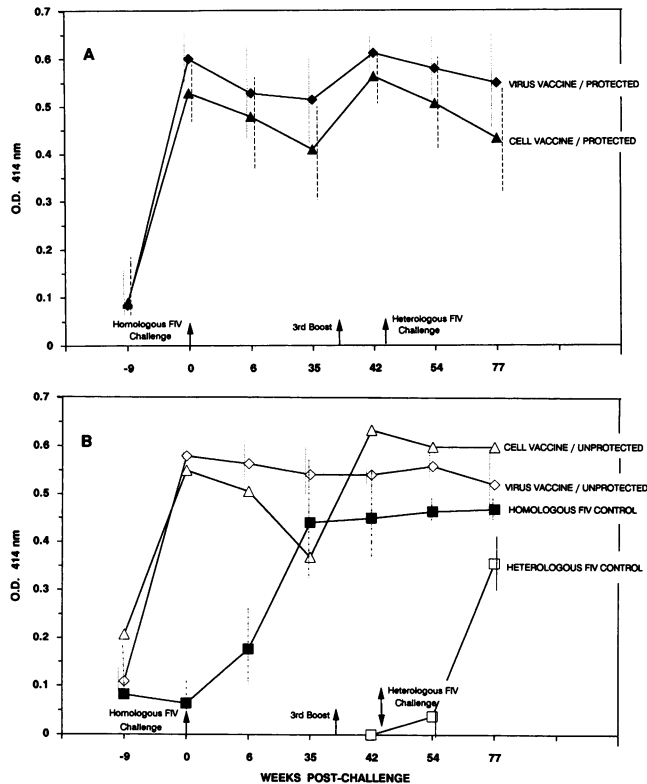


FIG. 1. Relative levels of anti-p25 antibodies in sera from vaccinated and adjuvant-control cats were determined by ELISA (20), using electrophoretically purified p25 as substrate antigen (50 μ g per well). Cats were immunized three times with fixed infected-cell vaccine (\blacktriangle , \triangle), inactivated whole-virus vaccine (\blacklozenge , \lozenge), or placebo prior to homologous FIV Petaluma challenge at zero weeks. ELISA was performed at a serum dilution of 1:100, and the results are expressed in optical density (O.D.) values (20). The dilution titer of vaccinated cats after the second booster injection ranged from 1:1,000 to 1:50,000 by immunoblot analysis. Panels A and B represent results from vaccine-protected (\blacklozenge , \blacktriangle) and unprotected (\lozenge , \triangle) cats, respectively, compared with the results from placebo-administered cats infected with homologous (\blacksquare) and heterologous (\square) FIV. Cats A40 and 249 (panel B; \lozenge) became virus isolation positive at 3 weeks postchallenge with homologous virus. All vaccine-protected cats (panel A; \blacklozenge , \blacktriangle) and five naive SPF cats (panels A and B; \square) were given booster injections with their respective vaccine or placebo, respectively, at 38 weeks postchallenge and challenged 7 weeks later with heterologous FIV Dixon. Cat 346 (panel B; \triangle) became positive by virus isolation and PCR at 16 weeks after heterologous FIV challenge (or 61 weeks postchallenge with homologous FIV). Five cats from each vaccinated and unvaccinated placebo group were randomly selected to be tested for their anti-p25 antibody titers, and the results are expressed as mean plus or minus standard deviation (vertical bars). Only those immunized cats which were virus isolation and/or PCR positive were specifically tested for the antibody titers.

controls had no detectable FIV antibody. Three weeks after the second booster injection, all cats were challenged i.p. with 10 50% animal infectious doses (ID_{50}) of the homologous Petaluma strain of FIV, for which the titer had previously been determined in vivo (20). For the next 9 months the cats were monitored, as previously described (20), for FIV infection, for FIV antibody response, and for clinical status. Virus isolation and PCR results are summarized in Table 1. All 15 cats immunized with the fixed infected-cell

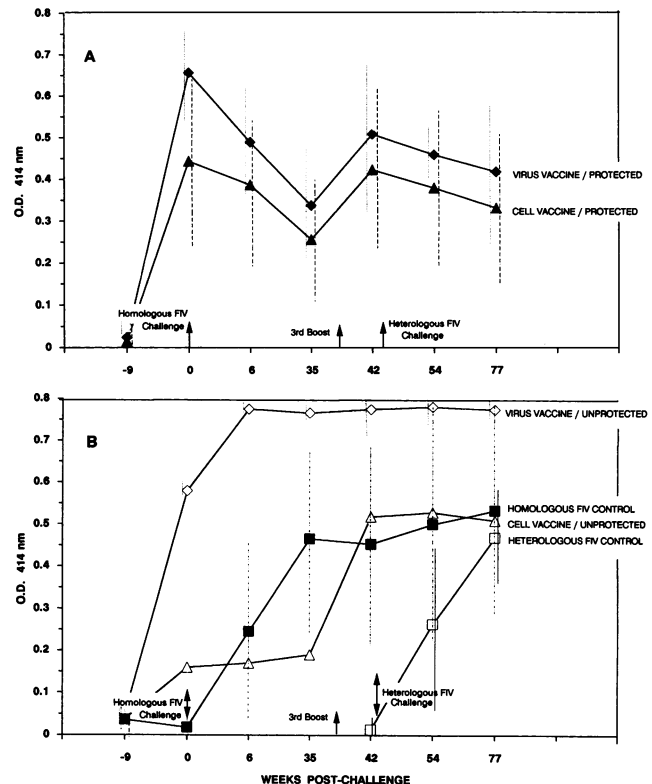


FIG. 2. Vaccinated and adjuvant-administered control cats were tested by ELISA for serum antibodies to transmembrane peptide TM(694-705), at a serum dilution of 1:500. The amino acid sequence of this TM peptide is QELGCNQNQFFC. (A) The means of all vaccine-protected cats are shown (\blacklozenge , virus vaccine; \blacktriangle , infected-cell vaccine). (B) Cats A40 and 249 (\lozenge , mean) from the inactivated whole-virus group and cat 346 (\triangle) from the fixed infected-cell group were unprotected from FIV challenge. The means of the two adjuvant-administered control cats infected with either homologous FIV Petaluma (\blacksquare) or heterologous FIV Dixon (\square) are shown.

vaccine (group A) remained uninfected and PCR negative, and their FIV antibody levels gradually declined over the 9-month interval after challenge (Fig. 1A, 2A, and 3A). Thirteen of 15 cats immunized with the inactivated whole-virus vaccine (group B) were also completely protected against infection and showed a similar, progressive decline in FIV antibody levels (Fig. 1A, 2A, and 3A). The two cats (249 and A40) in group B that were not protected became persistently infected and PCR positive by 3 weeks after challenge, and their FIV antibody levels increased and/or remained elevated for the next 9 months (Fig. 1B, 2B, and 3B). All 10 adjuvant control cats seroconverted and became persistently infected within several weeks after challenge.

At 38 weeks after challenge, the 15 protected (uninfected) cats in vaccine group A and the 13 protected cats in vaccine group B were given a third booster injection with their respective FIV vaccines. To avoid confusion, the cat groups given booster injections were called groups 1A and 1B in study 2 (Table 2). In both groups given booster injections, a marked anamnestic antibody response to FIV core and envelope antigens was elicited (Fig. 1 to 3). Anti-envelope (SU) peptide and VN antibody titers peaked higher than the levels reached after the second booster injection (Fig. 3 and 4). Seven weeks after the third booster injection the 28

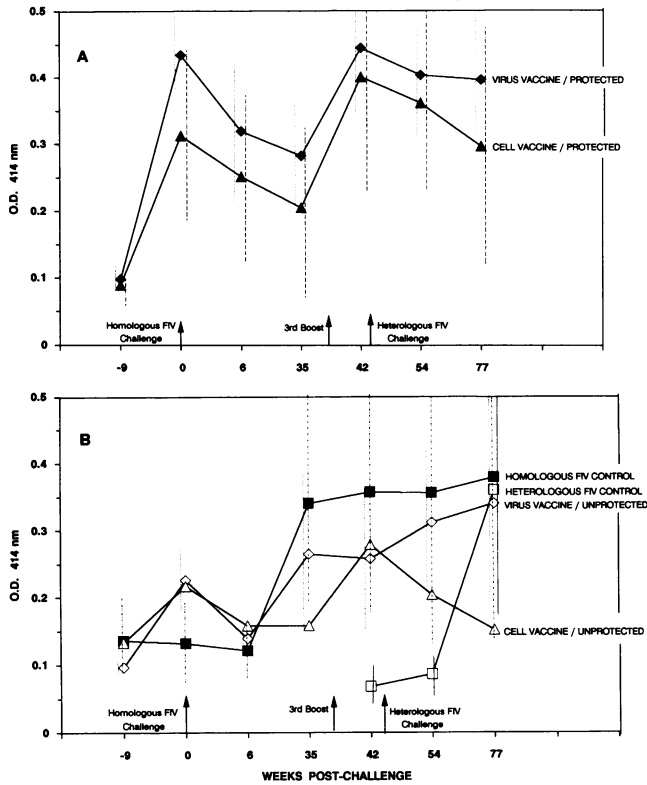


FIG. 3. Vaccinated and adjuvant-administered control cats were tested by ELISA for serum antibodies to viral surface envelope peptide SU(595-615), at a serum dilution of 1:100. This envelope sequence has been shown to be immunogenic in cats infected with the Petaluma isolate (1). By immunoblot analysis, the dilution titers of vaccinated cats after the second booster injection ranged from 1:50 to 1:5,000. The description of the animals is as described in the legends to Fig. 1 and 2.

vaccine-protected animals were challenged i.p. with 10 ID₅₀ of the heterologous Dixon strain of FIV. This strain differs by 11 and 4%, respectively, in the outer envelope and transmembrane envelope amino acid sequences from the FIV Petaluma strain (12) (both GenBank no. L00607 and L00608 for FIV Dixon). In vivo titration of this FIV strain in eight SPF cats (two per dose from 10⁻¹ to 10⁻⁴) established that a 10⁻³ dilution of the stock represented 10 ID₅₀. Five of five SPF controls were readily infected with this dose of FIV

TABLE 1. FIV vaccine protection against homologous FIV challenge

Study 1 group (n) ^a	Virus isolated at indicated wk postchallenge								No. protected/ total no. (%)	
	-1		3	6	9	16		37		
	V ^b	PCR ^b	V	V	V	V	PCR	V		PCR
A (15) ^c										15/15 (100)
B (15) ^d			249, A40 ^e	249, A40	249, A40	249, A40	249, A40	249, A40	249, A40	13/15 (87)
Control (10)			55U, 55X, 354	55U, 55X, 354, 250, 9410, A42, JK1, 7K2, DK2	All	All	All	All	All	0/10 (0)

^a Vaccinated and adjuvant control cats were challenged i.p. with homologous FIV.
^b Virus isolation (V) and PCR were performed as previously reported (20).
^c Group A cats were given fixed infected-cell vaccine.
^d Group B cats were given inactivated virus vaccine.
^e Identification numbers of cats that became positive by virus isolation or PCR.

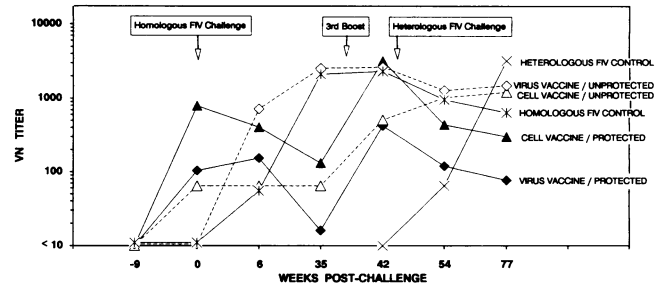


FIG. 4. VN antibody titers of vaccinated and adjuvant-administered control cats were tested at -9, 0, 6, 35, 42, 54, and 77 weeks after homologous FIV Petaluma challenge. The mean VN titers of six or seven randomly selected cats from the fixed infected-cell and the inactivated whole-virus groups were averaged. Two unprotected cats (A40 and 249) (mean VN titer; ◇) from homologous FIV challenge and the single unprotected cat (346) (△) from heterologous FIV challenge were specifically tested for VN titer. Mean VN titers of seven adjuvant-administered control cats challenged with homologous FIV Petaluma (×) and two adjuvant-administered control cats challenged with heterologous FIV Dixon (×) are also shown. Only vaccine-protected cats were given the third booster vaccine at 38 weeks postchallenge, and they were challenged 7 weeks later with heterologous FIV Dixon. The viral status of the different groups is presented next to the corresponding mean VN titers. The VN titer was defined as the reciprocal of the highest final dilution which gave ≥50% reduction in reverse transcriptase or ELISA p25 optical density (O.D.) values of the infected control culture which had no serum exposure (20).

Dixon strain. At 16 weeks after the heterologous FIV challenge, all but 1 of the 28 vaccinated cats in groups 1A and 1B remained uninfected (Table 2) and showed a steady decline in FIV antibody titers. The single infected cat in vaccine group 1A became positive for infectious FIV by 16 weeks postchallenge and showed an increase in FIV antibodies. The time of virus isolation and positive PCR detection suggests that this cat was infected with FIV Dixon instead of FIV Petaluma; however, further studies (i.e., sequencing isolated virus from cat 346) will be necessary to identify the FIV strain.

These results confirm and extend our previous findings (20) in showing that positive immune protection against homologous FIV challenge infection can be achieved in cats by either inactivated infected-cell or cell-free virus vaccines. The increased efficacy noted in the present study (>90% versus 75% in the previous study) can probably be attributed to the use of a stronger immunogen, i.e., increased number

TABLE 2. FIV vaccine protection against heterologous FIV challenge

Study 2 group (n) ^a	Virus isolated at indicated wk postchallenge ^b								No. protected/ total no. (%)
	3 (48)		5 (50)		8 (53)		16 (61)		
	V ^c	PCR ^c	V	PCR	V	PCR	V	PCR	
1A (15) ^d							346 ^e	346	14/15 (93)
1B (13) ^f									13/13 (100)
Control (5) ^g	2MK, IK2, IK3	2MK	2MK2, IK2, IK3	2MK2, IK2, IK3	2MK2, IK2, IK3	2MK2, IK2, IK3	2MK2, IK2, IK3, IK4	2MK2, IK2, IK3, 2MK6	0/5 (0)

^a For study 2, all protected cats from study 1 were given booster injections at 38 weeks after the homologous FIV challenge and then were challenged 7 weeks later with heterologous FIV.

^b Weeks postchallenge with heterologous virus (weeks postchallenge with homologous FIV).

^c Virus isolation (V) and PCR were performed as previously reported (20).

^d Cats in group 1A consisted of the 15 cats from study 1, group A, that were protected from homologous FIV challenge.

^e Identification number of cat that became positive by virus isolation or PCR.

^f Cats in group 1B consisted of the 13 cats from study 1, group B, that were protected from homologous FIV challenge.

^g Five naive SPF cats were immunized with adjuvant alone and then were challenged 7 weeks later with heterologous FIV.

of infected cells and amount of cell-free virus antigen per dose and/or stronger adjuvant preparation. The duration of protection has not yet been tested, and the antibodies induced tended to decline rather rapidly, approaching baseline levels by 6 to 8 months after the last booster injection. However, the strong anamnestic VN antibody response observed when the animals were given a booster injection 10 months after their second booster injection indicates that both vaccines have induced strong B-cell memory. It is not known, however, whether such a prompt memory cell response would protect against rechallenge infection in the absence of a recent booster immunization. It does not seem likely that this anamnestic response can be attributed to exposure of these animals to the homologous live-virus challenge given 38 weeks previously, because there was no sign of transient or latent infection at that time or thereafter and the antibody levels progressively declined. Not only do the current vaccines exhibit enhanced efficacy against the homologous strain of FIV (Petaluma strain) but they also protect very well against i.p. challenge infection with a heterologous (Dixon strain) FIV that differs by 11% in outer envelope amino acid sequences. This difference is similar to that between the San Diego (FIV PPR), Glasgow, and Petaluma strains of FIV (12, 15, 16). However, this difference is considerably less than the 21 and 22% differences at the external envelope sequences observed among the FIV Petaluma and the Japanese (FIV TM2) (11) and Maryland (FIV MD) (13) isolates, respectively. Preliminary results showing cross-neutralizing antibody activity between different European isolates and FIV Petaluma (18) suggest that conserved immunodominant regions in the viral envelope may exist in the present vaccines produced from FIV Petaluma.

The type of immunity (i.e., without any sign of transient infection) achieved and the parameters of immune protection, including protection against heterologous virus strains, are remarkably similar to those observed with SIV vaccines (4, 5). In both FIV cat and SIV macaque models, the mechanism of immune protection achieved by the inactivated whole-virus vaccines remains to be elucidated. Although antibodies to the viral envelope appear most important, a protective effect of VN antibodies in the FIV and SIV models remains uncertain. In the SIV model, nonviral cellular antigens (i.e., CD4 and HLA classes I and II) picked up by the virions from the uninfected human T cells that were used to grow the virus for vaccination and challenge also appear to contribute significantly to the immune protection

(3, 10, 17). By contrast, in the FIV model, the picture appears somewhat clearer in that immunization with the uninfected feline T cells used to grow the vaccine virus does not protect against challenge infection with FIV grown in the same cells and protection correlates at least equally well with antiviral as with anticellular antibodies. The few vaccinated cats that failed to be protected against infection had lower antiviral (anti-SU and VN) antibody titers prior to challenge than the protected cats, but some vaccinated cats with extremely low levels of VN antibodies were still protected from challenge. The threshold of antiviral antibody response required for protection may vary between individual animals and thus obscure the correlation between humoral antibody response and protection. The results from this study clarify and support the importance of vaccine-induced antibodies in protection. Passive immunization with pooled sera with high VN antibodies but no cellular antibody from healthy FIV-infected cats protected all recipients, and passive transfer of pooled sera from cats hyperimmunized with the uninfected cats' cells used to grow the vaccine virus failed to protect against challenge infection (7). The role of vaccine-induced antibody-dependent cellular cytotoxicity and antibody complement lysis of virions remains to be determined.

Initial efforts in Glasgow, United Kingdom, to prepare similarly efficacious inactivated whole-virus vaccines, using the FIV Glasgow strain, probably failed because little intact envelope was present in the vaccine and little or no VN antibodies were induced (9). Instead of protection, enhancement of infection was elicited by the vaccine (8). Using a technique for detecting enhancing antibodies to feline infectious peritonitis virus (6), we have been unable to show the presence of enhancing antibodies in our vaccine sera with or without added complement (5a). Using the FIV Petaluma strain grown in FL-4 cells, investigators from the United Kingdom have now confirmed our positive vaccine results (7a).

This work was supported by NIH grants CA-39016, AI30904, and AI27732 and by a research contract from Synbiotics Corporation.

We thank Renee Nakamura, Violet Martinez, and Sherry Trujillo for technical assistance.

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