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NOTES

Protection against Homologous but Not Heterologous Challenge Induced by Inactivated Feline Immunodeficiency Virus Vaccines

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Whole inactivated virus vaccines from the FL4 cell line protected against challenge with homologous feline immunodeficiency virus (Petaluma strain) but not against a heterologous FIV isolate (GL-8) which is distinct from the Petaluma strain in virus neutralization. Protection was associated with a type-specific neutralizing antibody response and was retained when the challenge virus was propagated in an unrelated cell line.

Given the many biological similarities between feline immunodeficiency virus (FIV) and human immunodeficiency virus (HIV) (8, 18), there is much interest in FIV as an animal model in which to develop vaccines against HIV. FIV infection of domestic cats occurs worldwide, and sequence variation among FIV strains reveals a pattern similar to that of HIV-1 (4, 5, 7, 9, 10). By using criteria similar to those used to group HIV-1 strains, FIV isolates from domestic cats have been classified into three subtypes (A, B, and C) (12). There appears to be antigenic diversity within FIV subtypes (6), and escape from neutralization by infected cat sera can occur (11). These features show FIV to be an attractive model with which to examine the relevance of natural antigenic diversity to vaccine protection against lentivirus infection.

Protection against challenge with FIV was achieved by Yamamoto and coworkers (15, 17) following vaccination of cats with immunogens prepared from FL4 cells, an interleukin-2-independent cell line infected with the Petaluma strain of FIV (FIV/PET) which constitutively releases large numbers of virus particles (16). Immunization with either inactivated FL4 cells or inactivated whole virus prepared from FL4 cells protected cats from subsequent challenge with either the homologous FIV/PET isolate (17) or the heterologous FIV/Dixon isolate (15).

The purpose of this present study was to investigate the breadth of protection afforded by inactivated whole FIV vaccines derived from FL4 cells, by testing whether protection extended to the FIV/Glasgow-8 (FIV/GL-8) isolate. Although a subtype A isolate like FIV/PET, FIV/GL-8 is antigenically distinct since it is neutralized to only a limited extent by sera from FIV/PET-infected cats (6).

In the first experiment, we immunized cats with an inactivated FIV/PET vaccine, prepared as described by Yamamoto et al. (15, 17). Four groups of specific-pathogen-free cats aged between 18 and 20 weeks were immunized as shown in Table 1. As detailed in Table 1, the cats received six doses and were challenged on week 20 by the intraperitoneal route with either FIV/PET or FIV/GL-8. Following challenge, the cats were monitored for infection by virus isolation as described elsewhere (2). Samples of culture supernatant were tested weekly by enzyme-linked immunosorbent assay (FIV antigen detection kit; IDEXX, Portland, Maine) for the production of FIV p24. Cultures which did not produce FIV p24 were maintained for a minimum of 18 days before being scored as negative.

As shown in Table 1, all of the unvaccinated control cats but only one of six vaccinated cats became virus-positive following challenge with FIV/PET, confirming previous results (15, 17). These five protected cats remained negative by virus isolation until the termination of the experiment, 38 weeks after challenge. In contrast, all of the vaccinated or unvaccinated cats challenged with FIV/GL-8 became virus positive. Thus, we demonstrated that the FL4 vaccine failed to prevent infection with the heterologous virus.

Protection induced by inactivated simian immunodeficiency virus vaccines grown in human T cells has been shown to be associated with immune responses to human antigens and restricted to simian immunodeficiency virus grown on human cells (1, 13). Although our FIV vaccine experiments involved no xenogenic antigenic challenge, the possibility remained that polymorphic cellular antigens might be targets for immune responses contributing to protection. This question was prompted further by our observation in this study that FL4 vaccines did not protect against FIV/GL-8 grown in Q201 cells, feline interleukin-2-dependent T cells (14), while previously protection was observed against FIV/PET or FIV/Dixon grown in FeT-1 cells (16), which were derived from the same culture of peripheral blood cells as FL4 cells (14a). It was therefore possible that FeT-1 and FL4 cells had common polymorphic cell surface protein antigens which were not shared by Q201 cells. Although immunization with uninfected FeT-1 cells did not confer protection against challenge with FeT-1-derived FIV/PET (17), the possibility remained that protective host cell components might be up-regulated by FIV infection and/or concentrated on virions. Therefore, we considered it important to test the effect of cellular origin of the challenge virus on vaccine protection.

Accordingly, we performed a further experiment using a

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TABLE 1. Virus-specific protection induced by FL4 inactivated virus vaccines

Expt no.	Vaccine prepn[a]	Immunization schedule (wk)	Challenge virus (cell origin)	Dose (CID_{50})[b]	Challenge (wk)	No. infected/ no. challenged
1	Pelleted virus	0, 2, 4, 7, 10, and 17	PET (FeT-1)	10	20	1/6
	Unvaccinated					6/6
	Pelleted virus	0, 2, 4, 7, 10, and 17	GL-8 (Q201)	5	20	5/5
	Unvaccinated					5/5
2	Gradient-purified virus	0, 3, and 6	PET (FeT-1)	10	9	0/5
	Unvaccinated					5/5
3	Gradient-purified virus		PET (Q201)	10	9	1/5
	Adjuvant alone	0, 3, and 6				5/5
	Gradient-purified virus		GL-8 (Q201)	10	9	4/5
	Adjuvant alone					5/5

[a] Each vaccine dose contained 250 μg of antigen in 250 μg of threonyl muramyl dipeptide (tMDP) in SAF-M emulsion (provided by Chiron Corporation).
[b] CID_{50}, 50% cat infectious dose.

challenge stock of FEV/PET produced in Q201 cells, which are unrelated to FL4 cells. Two groups of specific-pathogen-free cats aged between 10 and 12 weeks were immunized with inactivated FIV/PET, and two groups of age-matched control cats were immunized with adjuvant alone as shown in Table 1. The vaccine virus was produced by a modified protocol in which the virus was inactivated with 0.5% (vol/vol) paraformaldehyde prior to partial purification by two cycles of sucrose gradient centrifugation. This process yielded immunogens containing a significantly greater proportion of virus-specific proteins compared with the original vaccine (data not shown). A preliminary experiment with this vaccine (experiment 2) indicated that only three immunizations were required for protection against challenge with FIV/PET (Table 1).

Following vaccination, the cats were challenged with either FIV/PET or FIV/GL-8 grown in Q201 cells (Table 1). At 3, 6, 9, and 12 weeks after challenge, FIV was isolated from all of the control cats and from four of five vaccinated cats challenged with FIV/GL-8 but from only one of five vaccinated cats challenged with FIV/PET (Table 1). The five protected cats remained virus negative for 31 weeks postchallenge. Thus, significant protection was achieved against Q201-grown FIV/PET but not against FIV/GL-8. These results demonstrate that protection by FL4 vaccination is virus specific.

Given the antigenic difference between FIV/PET and FIV/GL-8 (6, 10), sera taken on the days of challenge from the vaccinated cats which were not protected from the FIV/GL-8 challenge were analyzed for virus neutralizing antibody (VNA) against either virus. In both experiments, the mean $\log_{10}$ VNA titers were significantly higher against FIV/PET than against FIV/GL-8 (Table 2), suggesting that the lack of protection against FIV/GL-8 may have been the result of its antigenic divergence from FIV/PET. The relatively weak neutralization of FIV/GL-8 by sera from FL4-immunized or FIV/PET-infected cats does not reflect an intrinsically higher resistance of FIV/GL-8 to neutralization, since sera from many naturally infected pet cats show the reciprocal pattern, neutralizing FIV/GL-8 more potently than FIV/PET (6).

TABLE 2. Type specificity of VNA response in FL4-vaccinated cats at day of challenge

Expt no.	No. of cats	$\log_{10}$ VNA titer (mean ± SD)	
		FIV/PET	FIV/GL-8
1	11	3.93 ± 0.19	3.23 ± 0.14[a]
3	10	3.76 ± 0.23	2.56 ± 0.36[a]

[a] $P < 0.01$.

Our results indicate that the homologous protection afforded by the FL4 virus vaccine is indeed virus specific and contrast with the findings in the simian immunodeficiency virus system, in which protection was not achieved when the challenge virus was propagated in simian cells unrelated to the vaccine source (3). In addition, this study demonstrates that the resistance induced by the inactivated FIV vaccines is type specific and does not extend to the antigenically distinct heterologous FIV/GL-8 isolate. This result confirms the antigenic heterogeneity among FIV isolates, even within subtype A (10, 12), revealed by virus neutralization (6). Since the immunized cats had markedly lower titers of VNA against FIV/GL-8 than against FIV/PET, VNA may have a role in protection. However, whether neutralizing antibodies are sufficient for protection remains to be established. Since it appears from this study that antigenic diversity can confound vaccine protection, a framework is provided for future endeavors to characterise and broaden the protection afforded by FIV vaccines.

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