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Evaluation of subunit vaccines against feline immunodeficiency virus infection

Ernst J. Verschoor*, Marja J. Willemse†, Jeanette G. Stam, Arno L.W. van Vliet*, Henk Pouwels†, Stuart K. Chalmers†, Marian C. Horzinek*, Paul J.A. Sondermeijer†, Wim Hesselink† and Anthony de Ronde*‡§

Subunit vaccines prepared against feline immunodeficiency virus (FIV) infection were evaluated in two trials. First, cats were immunized with bacterial expression products of an envelope fragment that contained the V3 neutralization domain of the FIV surface protein fused to either galactokinase (K-SU3) or glutathione-S-transferase (G-SU3). Quantitative and qualitative differences in the humoral immune response were observed with three adjuvants of which Quil A was the best in terms of total and virus neutralizing antibody. Notwithstanding the responses induced, 19 of 20 immunized cats did not resist challenge and became infected. To determine whether priming with a live viral vector would confer protection, cats were inoculated oronasally and subcutaneously with a feline herpesvirus (FHV) mutant expressing the FIV env *gene; two booster immunizations followed using the K-SU3 product in either Quil A or a mineral oil/Al(OH)$_3$ adjuvant. FIV-specific antibody responses were only weak, and the vaccinates did not withstand challenge with a low dose of homologous virus. Copyright © 1996 Elsevier Science Ltd.*

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Since the onset of the AIDS epidemic and the following identification of a lentivirus, the human immunodeficiency virus type 1 (HIV-1) as its cause[1,2], development of an HIV vaccine has become an important objective. A convenient small animal model has been found in feline immunodeficiency virus (FIV) infection of cats that can be exploited for the study of lentivirus pathogenesis and vaccine development[3–5]. Like HIV-1, FIV causes an immunodeficiency syndrome in its host. The virus is more prevalent in the cat population than HIV is in man, and development of a vaccine is therefore of obvious veterinary importance.

Lentiviral vaccine studies have met with varying degrees of success[6–13]. Cats have been protected against homologous and heterologous FIV challenge using whole-virus and fixed-infected cell vaccines[14,15]. Passive immunization has demonstrated the protective potential of antibodies against FIV infection[16]. Their nature and the epitopes to which they are directed are as yet unknown. High-titered virus neutralizing antibody (VNA) responses can be elicited in cats by immunization with the V3 region of the FIV envelope surface (SU) protein[17,18]. Moreover, the V3 region acts as an immunodominant neutralization domain in FIV-infected cats[18] and determines cell tropism *in vitro*[19]; it has these properties in common with its HIV-1 counterpart[20].

We have used bacterial expression products containing the FIV V3 region in the vaccination experiments. The polypeptides proteins were presented in combination with three different adjuvants: a mineral oil/Al(OH)$_3$-based adjuvant, Quil A, and a combination of Quil A and Al(OH)$_3$ were used.

In the second part of this study we primed the immune system with a live-attenuated feline herpesvirus-1 (FHV-1) vector expressing the FIV *env* gene, combined with booster immunizations with the V3-containing protein. All vaccination protocols were tested for their ability to protect against FIV by challenging the cats with a low dose of the homologous strain FIV-UT113.

MATERIALS AND METHODS

Bacterial fusion proteins

Two *Escherichia coli* expression products were used which consisted of the amino acid residues 361–445 of the FIV SU protein fused to galactokinase (K-SU3) or glutathione-*S*-transferase (G-SU3). The SU moiety encodes an immunodominant epitope that was shown to induce VNA in cats[18]. Expression and purification of the fusion proteins has been described previously[18].

*Virology Unit, Institute of Infectious Diseases and Immunology, Veterinary Faculty, University Utrecht, Yalelaan 1, 3584 CL, Utrecht, The Netherlands. †Intervet International BV, PO Box 31, 5830 AA, Boxmeer, The Netherlands. ‡Present address: Human Retrovirus Laboratory, Academic Medical Center, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands. §To whom correspondence should be addressed. (Received 16 May 1995; revised 7 September 1995; accepted 12 September 1995)

Table 1 Vaccine formulations and vaccination schedules

Trial		Vaccination					
			Antigen				
Group	Animal	Adjuvant	Week 0	Week 4	Week 6	Week 8	Week 10
Trial 1							
I	1–5	Mineral oil/$Al(OH)_3$	K-SU3	K-SU3	K-SU3	K-SU3	K-SU3
II	5–10	Mineral oil/$Al(OH)_3$	K-SU3	K-SU3	K-SU3	K-SU3	G-SU3
III	11–15	Quil A	K-SU3	PBS	K-SU3	PBS	K-SU3
IV	16–20	Quil A/$Al(OH)_3$	K-SU3		K-SU3		G-SU3
V	21–25	Controls	PBS		PBS		PBS
Trial 2							
I	1–5	Mineral oil/$Al(OH)_3$	FHV-FIV-env				
II	6–10	Quil A	FHV-FIV-env				
III	11–15	Controls	FHV/β-gal				

Vaccine preparation

Antigen was mixed with adjuvant to a concentration of 100 μg ml^{-1} of vaccine; the respective adjuvants are listed in *Table 1*. The oil/$Al(OH)_3$-based vaccines were water-in-mineral oil emulsions with the antigen absorbed to alum in the water phase. Saponin Quil A was used in two adjuvant formulations: either antigen was mixed with Quil A directly (100 μg ml^{-1}; Superfos, Vedbaek, Denmark) or after pre-absorption to 0.3% (final concentration) of $Al(OH)_3$ gel (Superfos, Vedbaek, Denmark). Phosphate-buffered saline (PBS) was used as placebo vaccine. Each adjuvant formulation contained 0.01% thiomersal for preservation and was stored at 4°C until use.

Construction of recombinant FHV virus vectors

Recombinant FHV viruses were developed that express either the *E. coli* β-galactosidase gene or the envelope gene of FIV-UT113 (EMBL accession no. X60725)[21]. The development of the FHV/β-gal vector, strain 25B3B11, has been described before[22]. The construction of FHV/FIV-env vector was essentially as described for FHV/β-gal, using the same insertion site in the U_L region. However, to drive the expression of the FIV gene a strong promotor was selected that had been derived from the long terminal repeat (LTR) of Rous sarcoma virus[23]. The resultant transfer plasmid pFHV37 was co-transfected with FHV DNA into CRFK cells and recombinant viruses were generated by homologous recombination. Recombinant viruses were detected by immunofluorescence and stocks were prepared by plaque-purification.

Design of the trials

Trial 1. All cats were vaccinated at 0, 6, and 10 weeks with 1 ml of vaccine; challenge was by subcutaneous (s.c.) injection of 10–20 50% cat infectious doses (CID_{50}) of FIV-UT113 at week 14 (*Table 1*). Four groups of 5 cats each were vaccinated with the fusion proteins while one group of 5 cats was immunized with the placebo vaccine. Mineral oil/$Al(OH)_3$-based vaccines were given intramuscularly (i.m.) while the other vaccines were applied subcutaneously (s.c.). Group I was vaccinated three times with 100 μg of K-SU3. Groups II, III, and IV were immunized twice (t=0 and 6 weeks) with 100 μg of K-SU3 and received a final booster immunization with 100 μg of G-SU3 at week 10. Cats in group V received three immunizations with PBS.

Trial 2. Three groups of 5 cats were used in this study. At t=0 weeks cats from groups I and II were inoculated oronasally and s.c. with 10^5 plaque-forming units (p.f.u.) of the FHV/FIV-env recombinant while group III was inoculated via both routes with 10^5 p.f.u. of the FHV/β-gal vector. At 4 and 8 weeks after priming with the FHV recombinants all animals received booster injections. Group I was immunized i.m. with 100 μg K-SU3/dose in mineral oil/$Al(OH)_3$ adjuvant. Group II received the same antigen s.c., formulated in Quil A adjuvant, while group III was s.c. immunized twice with PBS (*Table 1*). At t=12 weeks the animals were challenged s.c. with 10–20 CID_{50} of FIV-UT113.

Virus isolation and serology

Blood samples were taken for serology and virus isolation at regular intervals. Isolation of FIV from peripheral blood mononuclear cells was described earlier[24]. FHV was isolated from oropharyngeal swabs as described by Willemse *et al.*[22]

Antibodies directed to the FIV core and envelope proteins were detected by an ELISA based on *gag* and *env* expression products[25]. VNA titers were determined in CRFK cells[18]. VNA titers were defined as the reciprocal of the highest dilution at which the virus was completely neutralized.

RESULTS

Vaccine trial 1

Development of antibody after immunization with the fusion proteins was measured in an ELISA using baculovirus-expressed gp150 envelope protein. Differences in kinetics and strength of the anti-envelope response between the immunization groups could be distinguished (*Figure 1*). A rapid antibody response was observed in the Quil A (group III) vaccinees; after two immunizations this group had a mean response twice as high as the others. At challenge (week 14) cats in the oil/$Al(OH)_3$ (group II) and Quil A (group III) sets had generated the highest FIV-specific antibody response. All SU3-vaccinated groups had a mean anti-envelope

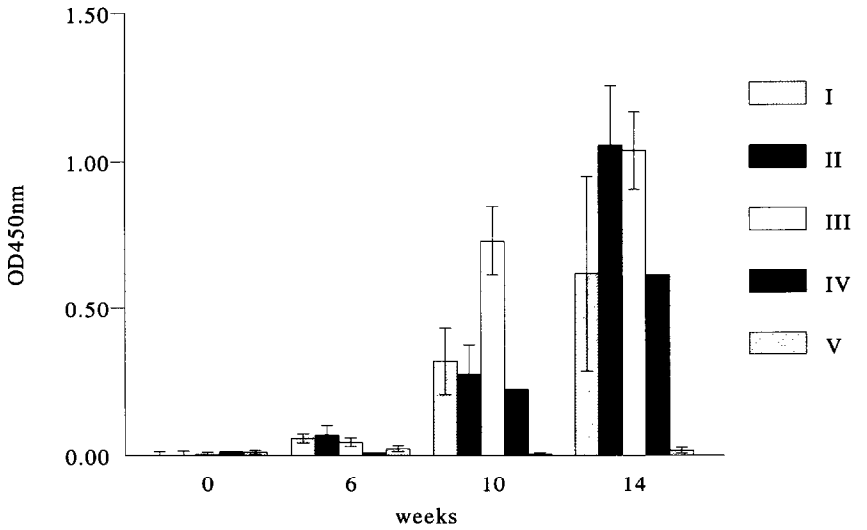

Figure 1 Envelope antibody responses of cats immunized with different adjuvant formulations. Mean responses for each vaccine group are represented by bars

Table 2 Antibody titers and virus isolation (trial 1)

Group	Cat	AB[a]	VNA[b]		VI[c]	Seroresp[d]
			Week 10	Week 14		
	1	400	16	16	+	+/+
	2	800	<16	256	+	+/+
I	3	800	64	256	-	+/+
	4	800	16	16	-	+/+
	5	800	16	64	+	+/+
	6	1600	16	256	+	-/±
	7	400	64	64	+	+/+
II	8	800	64	16	+	+/+
	9	1600	64	64	+	+/+
	10	1600	256	256	+	+/+
	11	1600	64	256	-	±/-
	12	3200	256	256	+	+/+
III	13	3200	256	256	+	+/+
	14	1600–3200	64	16	+	+/-
	15	3200	1024	256	-	-/±
	16	200	64	16	+	+/+
	17	200–400	64	64	-	-/+
IV	18	800	64	256	+	+/+
	19	400	<16	64	+	+/+
	20	800	64	256	+	+/+
	21	<100	<16	<16	+	+/+
	22	<100	<16	<16	-	-/-
V	23	<100	<16	<16	+	+/+
	24	<100	<16	<16	+	+/+
	25	<100	<16	<16	+	±/-

[a]Antibody titer to the FIV envelope protein at moment of challenge (t=14 weeks). [b]Virus neutralizing antibody titer at t=10 and 14 weeks after first immunization. [c]Virus isolation from PBMC cultures; (-) negative at each time point; (+), positive at one or more time points. [d]Seroresponse after challenge (VNA/ELISA); (-) no increase in antibody response; (+) increase in antibody titer; (±) indefinite

response exceeding that of our control serum taken from a FIV-infected cat (0.583 ± 0.033). VNA titers were tested before challenge at week 10 and 14 (*Table 2*). A correlation was discernible between the total anti-FIV response and the VNA titer though an exception could be found (cat no. 14).

Fourteen weeks after the first immunization all cats were challenged with 10–20 CID_{50} of homologous FIV-UT113. Virus isolation from PBMC and antibody titers were used to assess vaccine efficacy. The post-challenge data are listed in *Table 2*. Virus isolation was possible from PBMC cultures of 19 cats. However, 22 of 25 cats

Table 3 Antibody response and virus isolation (trial 2)

Group	Cat	VNA titer		VR[a]	Seroresp[b]
		Week 8	Week 12		
	I-31	<16	16	+	+
	I-39	<16	16	+	+
I	I-43	16	16	+	+
	I-46	<16	16	+	+
	I-65	<16	16	+	+
	I-42	<16	16	+	+
	I-50	<16	16	+	+
II	I-51	<16	256	+	+
	I-57	16	ND	+	+
	I-63	16	64	+	+
	I-27	<16	16	+	+
	I-37	<16	<16	+	+
III	I-44	<16	<16	+	+
	I-52	<16	<16	+	+
	I-62	<16	<16	+	+

Pre-challenge VNA titers were determined 8 and 12 weeks after the start of the trial. [a]Virus isolation after challenge. [b]Seroresponse after challenge (VNA); (+), positive in virus isolation from PBMC cultures at one or more time points or increase in VNA titer after challenge; (-) negative in virus isolation from PBMC cultures at all time points or lack of increase VNA titer after challenge

were considered as virus-infected when these data were analyzed in combination with the seroresponses after challenge. One PBS control cat (cat no. 22) remained protected against FIV challenge. Interestingly, this animal withstood a second challenge with 10–20 CID_{50} of FIV-UT113 at t=22 weeks, a phenomenon we also have encountered in other vaccine trials[24].

Vaccine trial 2

Four weeks after oronasal inoculation with the FHV recombinant sera were screened for FIV-specific antibodies. No humoral response was detectable in an immunoprecipitation assay using radiolabelled FIV[21] (data not shown). Subsequent booster immunizations with the bacterial fusion protein K-SU3 induced low levels of FIV-specific antibodies (*Figure 2*), as well as low VNA titers (*Table 3*). Quantitative differences in total anti-envelope antibodies and VNA responses between groups I and II were noticeable, similar to those

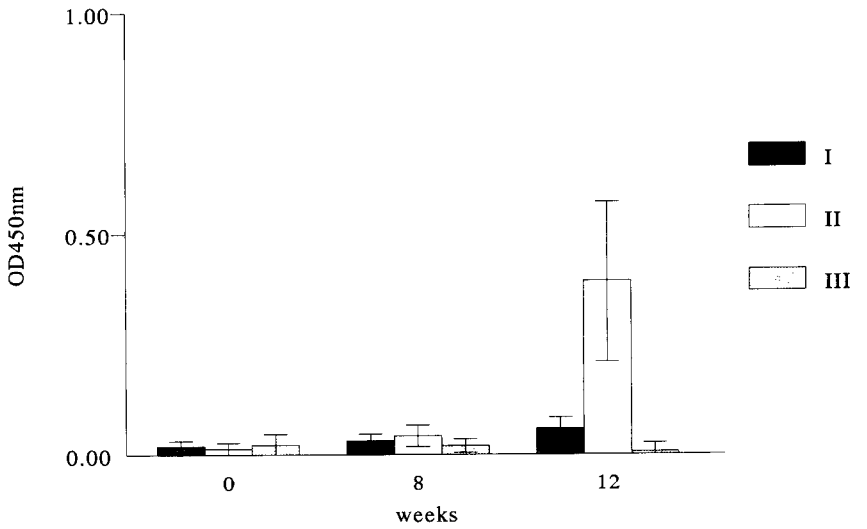

Figure 2 Development of FIV-specific antibody response after immunization of cats with a live virus vector (FHV/FIV-env or FHV/β-gal) in combination with subunit booster immunizations at *t*=4 and 8 weeks. Mean responses for each vaccine group are represented by bars

in trial 1. Upon challenge all cats became infected as evidenced by virus isolation from PBMC cultures and by a further rise in antibody titers (*Table 3*).

DISCUSSION

In this study we examined whether V3-fusion proteins could protect cats against an FIV challenge. Both peptides were previously shown to be immunogenic in cats and able to induce VNA[18]. Our protocol consisted of three immunizations within a 3-month period; variations were made in the antigen used for final booster immunization and in the adjuvant formulation. G-SU3 was incorporated in final immunization to specifically boost the SU3-directed immune response and not that to the galactokinase (K-) part of the immunogen. Use of G-SU3 in the final immunization considerably increased the FIV-specific antibody levels. The adjuvants used are all applied in veterinary practice and were selected on the basis of their potential to induce divergent immune responses. Quil A stimulates both humoral and cell-mediated immunity[26,27]. Oil/Al(OH)$_3$ induces mainly the humoral immunity[28] while Al(OH)$_3$ can strongly potentiate the adjuvanticity of Quil A[29,30].

The Quil A vaccine had induced the strongest humoral response after two immunizations. Strong and rapid immunoresponses after use of Quil A-containing vaccines are also reported by others[31–33]. At challenge the mineral oil/Al(OH)$_3$ vaccination group had a mean antibody titer comparable to that of the Quil A group. Al(OH)$_3$ in combination with Quil A (group IV) was successfully employed in a feline leukaemia virus subunit vaccine with *E. coli*-expressed gp70 as antigen[34], but in our experiments the combination negatively influenced the level and quality (VNA) of the response. Such a phenomenon has been described by Bomford *et al.*[30] who reported a relation between efficacy of the combination-adjuvant and the immunogenicity of the antigen.

Priming with a FHV/FIV-env recombinant in combination with two booster immunizations was also examined for its protective capacity. The rationale for such a protocol was that both cell-mediated and humoral immunity would be induced. Similar protocols have been successful in protecting macaques and chimpanzees against SIV and HIV-1 infection, respectively[7,8]. The reason for the failure to induce protection in our experiments remains as yet to be elucidated. The FHV/FIV-env recombinant replicated well as it could be routinely reisolated until 3 weeks after inoculation (data not shown). In addition, it was capable of eliciting protection to a virulent FHV strain in an earlier study (M.W., unpublished observations). Local nature of the immune responses induced by FHV, which primarily replicates in the oronasal tract, may have contributed to the lack of protection against subcutaneous FIV challenge whereas systemic immunity is preferred. We attempted to overcome this limitation by s.c. inoculation of the FHV recombinants in combination with intramuscular boostering of the animals with the V3-containing protein. A similar protocol successfully protected cats against i.m. inoculation with the feline retrovirus FeLV[35]. The antibody response elicited after the two booster immunizations with the SU3 protein was weaker than after immunization with only the protein and may suggest interference by the priming with the FHV/FIV-env vector.

Our efforts to induce immunity to FIV infection have failed. Successful protection against FIV has been obtained by a vaccine consisting of fixed-FIV-infected T-cells (FL-4 cells)[14,15]. Passive transfer of serum from immunized animals can prevent cats from FIV infection, indicating that antibodies can protect[16]. The nature of these protective antibodies and the epitopes to which they are directed are unknown. Failure to elicit protective antibodies in our study, despite induction of high anti-envelope antibody responses, may indicate that epitopes other than within the V3 region may be involved or that *in vitro* (VNA) titers cannot be directly translated to the *in vivo* situation. Enhancement of infection after the immunization with FIV subunit vaccines has been observed too[36–38]. We have not encountered the latter event as post-challenge titers and virus isolations from vaccinated animals were comparable to those of the controls. However, the design of our study, particularly the infrequent sampling immediately following challenge, may have prevented detection of enhancement. The observation of enhancement illustrates that

FIV vaccination may not always be beneficial and once again emphasizes the many obstacles which may be encountered during the development of an efficacious lentivirus vaccine.

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