Feline Leukemia Virus Vaccine: New Developments

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INTRODUCTION

A safe and effective vaccine for feline leukemia has been a primary goal of researchers studying feline leukemia virus (FeLV) since the early 1970s. This emphasis was generated following the identification of feline leukemia as a virally induced disease (Jarrett et al., 1964) and with the isolation of viral strains by Kawakami et al. (1967) and Rickard et al. (1969) in the late 1960s. A need existed for an FeLV vaccine since the virus, with its attendant associated syndromes, represents a leading cause of cat mortality worldwide. In January 1985, a commercial vaccine ("Leukocell", Norden Laboratories, Lincoln, NE) was licensed for use in the U.S.A. using as its basis a prototype vaccine developed at the Ohio State University (Lewis et al., 1981). The prototype contained FeLV antigens released from lymphoid cells persistently infected with the Kawakami isolate of FeLV. These FeLV-associated proteins were found to be non-infectious and capable of producing an immune response without the immunosuppressive pathology characteristic of live attenuated (Salerno et al., 1979) and killed FeLV (Olsen et al., 1977).

In order to obtain a commercial license, studies on the vaccine's efficacy were performed and reported (Sharpee et al., 1986). Continued research and improvements on the vaccine have generated new data that explores various secondary issues, including: (a) possible immunosuppression following vaccination; (b) protection against the establishment of latent FeLV infections upon virus exposure; (c) immunogenicity of an alternate route of administration; (d) clinical performance in high-risk environments; (e) development of cytotoxic antibodies following vaccination. A review of this new information is presented here.

EFFECTS OF IMMUNOSUPPRESSIVE ELEMENTS PRESENT IN THE VACCINE

It is well established and has been demonstrated in our laboratory (Olsen et al., 1977; Mathes et al., 1978, 1979) that FeLV p15E (an envelope protein which is common to all retroviruses) profoundly suppresses feline cellular immunity. We have shown, for example, that purified p15E inhibits a number of normal immune functions and cell types both in vivo and in vitro (Hebebrand et al., 1977; Olsen et al., 1977; Mathes et al., 1978, 1979) while other FeLV proteins have no such effect.

The immunosuppressive envelope protein, p15E, is present in some form in the feline leukemia vaccine. This was established by Lewis et al. (1981) showing that cats respond immunologically to p15E when they are vaccinated. Studies have been performed to determine if the presence of p15E has any detrimental effects upon the immune system of the host. However, 2 avenues of inquiry have reliably shown that the vaccine produces no immunosuppressive effect upon lymphocyte function. First, vaccination does not impair lymphocyte blastogenesis. Second, when "Leukocell" was given concurrently with other feline vaccines, cats responded immunologically to all the immunizing agents administered. This indicates that, although p15E is present, it must be in an alternate or precursor form that does not induce the immunosuppressive pathology associated with it in its processed form.

Studies were conducted to determine if "Leukocell" had similar effects on lymophocyte blastogenes and showed that the vaccine has no effect on lymphocyte mitogenesis. In one study, 1:2 or 1:20 dilutions of the unadjuvanted, soluble vaccine proteins were combined in vitro with concanavalin A-stimulated lymphocytes. These vaccine proteins had no significant effect on simulated growth of cat lymphocytes when compared with control samples (Table 1). Similar tests using whole virus or p15E cause a profound suppression of T-cell mitogenesis (Hebebrand et al., 1977; Mathes et al., 1977). In a second test,

TABLE 1

Effect of "Leukocell" preparation on lymphocyte mitogenesis

Cat. No.	Disintegrations per minute and vaccine dilution[a]		
	1:2	1:20	Control
OA-I	51 222	62 828	59 908
OD-4	117 236	128 058	126 998
OC-3	116 849	102 340	85 945
NK-2	91 896	99 329	85 346

[a]Pre-adjuvanted vaccine added to medium containing concanavalin A and purified cat peripheral blood lymphocytes.

cats were vaccinated twice with a 10-fold concentration of adjuvanted vaccine. As shown in Table 2, lymphocytes from these cats showed no change in LBT (lymphocyte blast transformation) response when comparing pre- and post-vaccination values, nor did their LBT values differ appreciably from those in non-vaccinated cats. These studies suggest that although p15E antigenic sites exist within the "Leukocell" preparation, the protein is not in an active suppressive form. Previous studies have found a level of p15E as low as 5 μg ml^{-1} (Mathes et al., 1978) to be suppressive, so that if the protein is present at all, it is at levels of <5 μg ml^{-1}.

Additional information can be seen in studies showing that "Leukocell", given concurrently with modified live virus (MLV) or inactivated rabies vaccine or with a combination MLV feline panleukopenia–feline calicivirus–feline rhinotracheitis vaccine, caused no loss of amnestic response to any of the immunizing agents (Sharpee et al., 1986). For example, cats with an immune history to FeLV and rabies virus were inoculated concurrently with "Leukocell" and MLV rabies vaccine. Within 3 weeks, test cats developed a 10-fold increase in their mean gp70 antibody level and a 25-fold increase in their mean FOCMA (feline oncornavirus membrane antigen) antibody titer. A similar pattern of serologic response occurred following concurrent administration of "Leukocell" and other routinely used feline vaccines. We have previously shown that p15E interferes with both antiviral and anti-tumor responses (Olsen et al., 1977) and that such immunosuppression occurs rapidly when p15E is introduced to the host (Mathes et al., 1979). Therefore, a post-vaccination sero-

TABLE 2

In vivo effect of 10× "Leukocell" concentration on lymphocyte

Test group and cat No.	Disintegrations per minute and test interval[a]		
	Day 0	1st vaccine dose	2nd vaccine dose
Vaccinates			
SK-2	26 527	34 706	23 049
SJ-3	15 008	38 096	ND[b]
SJ-4	20 945	34 132	44 385
SK-1	47 553	41 865	39 363
Controls			
OC-3	58 852	38 459	20 518
NK-2	45 482	39 012	28 828

[a]Concanavalin A-stimulated cat peripheral blood lymphocytes tested 1–14 days post-vaccination.
[b]ND = not done.

logic response to FeLV and heterologous vaccines is a significant indicator of the absence of immune interference following administration of "Leukocell".

In a recent study by Henderson et al. (1984), new light was shed on how the p15E protein may acquire its immunosuppressive properties. Retrovirus envelope protein (in precursor form) is processed before virion assembly and maturation. One of the structural changes that occurs is cleavage by cellular enzymes of a 2000-dalton peptide from the precursor molecule. This takes place after the viral protein is produced, while it is being transported to the outer cellular membrane. Thus, prior to virus assembly, p15E undergoes proteolytic processing. This event may prove to be necessary for p15E to become immunosuppressive. The p15E moiety present within the vaccine may be in an unprocessed form, thus allowing antibody development without immunosuppressive effects. Mastro et al. (1986) have shown that vaccinated cats respond to different viral antigens in the vaccine preparation than those found with whole virus. They saw that the envelope gp70 of FeLV is associated with protection, but the gp70 present on the virion is not responded to strongly until after challenge. This suggests that the gp70 moiety in the vaccine is similar, but different to that found on mature virions. The vaccine may induce a primary immunization for gp70 and the challenge then produces a strong amnestic response. A similar mechanism may occur with p15E, with an altered or immature form present in the vaccine which can induce antibody against virion p15E.

PROTECTION FROM THE DEVELOPMENT OF LATENT INFECTIONS

In cases of latent (non-productive) FeLV infection, virus is not actively produced in bone marrow or peripheral blood lymphocytes and is not detectable by conventional means. However, the FeLV genome is present in target cells and is capable of viral reactivation under certain conditions, most notably administration of corticosteroids (Rojko et al., 1982). In addition, recent reports by Lewis et al. (1986) and Lafrado and Olsen (1986) indicate that a neutrophil defect occurs rapidly upon FeLV infection and that this defect persists even in a latent host. This defect is observed in neutrophils isolated from FeLV-negative cats previously exposed to FeLV (Table 3). The neutrophils lose their ability to form oxygen-free radicals, thus losing their ability to kill invading organisms. Latency thus became an intriguing test of "Leukocell" efficacy. If the vaccine could reliably protect cats from latent infection, its value as a prophylactic agent would be enhanced considerably.

In tests conducted at Norden Laboratories, 17 cats that were vaccinated and challenged were evaluated for viral latency 2–3 years after virus challenge. Test cats had received the recommended 3 vaccine doses and were challenged with the Rickard strain of FeLV (FeLV-R) using procedures previously described (Sharpee et al., 1986). Annual booster vaccinations with a single dose were

TABLE 3

Effect of latent FeLV on neutrophil oxidative burst

	Days after exposure to FeLV-R						
	0	7	28	96	168	351	768
Latent FeLV							
3125	23726[a]	706	34	33[b]	32	126	254
3133	3021	19	26[b]	12	21	223	262
3136	5260	284	346[b]	158	158	62	ND[c]
Controls							
3130	7466	9035	10546	9684	1935	8752	9692
3129	5699	ND	9015	ND	9175	11564	11564
3132	8752	9576	ND	10751	ND	19872	10872

[a]Light release index (peak cpm/control).
[b]Day tested FeLV negative in serum and bone marrow.
[c]ND = not done.

also administered. In an attempt to activate latent FeLV 24–36 months after challenge, methylprednisolone was administered to all test cats once a week for 4 consecutive weeks at the rate of 7.5 mg kg^{-1}. This immunosuppressive treatment induced a mean 60% reduction in lymphocyte count, indicating that immunocompetence of the test cat was, in fact, compromised (15 of the 17 cats experienced a reduction in lymphocyte count). Latency was assessed by culturing bone marrow aspirates obtained from femoral shafts prior to and 1 week after the last of the 4 immunosuppressive treatments. Cultures were maintained for 21 days. Culture media contained hydrocortisone phosphate which has been shown to considerably enhance FeLV reactivation (Rojko et al., 1982). The presence of the FeLV group-specific protein in culture fluids was determined by ELISA methods and co-cultivation plaque assays.

The 15 test cats had a diverse viremic status after challenge, creating a varied group for evaluating virus reactivation. Four cats were aviremic and 11 were transiently viremic. Latent post-challenge infections were not observed in the 15 potentially latent cats (Table 4), even when repeated immunosuppressive treatment was administered. This result takes on added significance in view of: (1) the large viral challenge burden (5×10^6 infectious units), which increases the likelihood of latent infection; (2) the use of FeLV-R as a challenge agent, a strain which has a predisposition towards viral latency (Pedersen et al., 1984); (3) enhancement of culture media with hydrocortisone acetate in order to promote viral reactivation; (4) demonstrated immunosuppression of test cats; (5) transient FeLV infection in 11 of 15 cats after challenge, indicating that genome integration occurred, but was subsequently eliminated as a result of the host immune response.

TABLE 4

Activation of latent FeLV-R in vaccinated cats tested 24 and 36 months post-challenge

	Isolation after steroid treatment in vivo and in vitro			
	Serum		Bone marrow	
	Pre	Post	Pre	Post
15 cats[a]	−	−	−	−
ED-3	+	+	+	+
KC-3	−	−	+	+

[a]Eight with transient GSA+ during first 12 weeks after challenge.

SUBCUTANEOUS ADMINISTRATION

Approval has been obtained in the U.S.A. for "Leukocell" to be administered subcutaneously as well as intramuscularly. This route was granted on the basis of data showing that subcutaneous (SC) immunization elicits gp70 (Table 5) and FOCMA responses (Table 6) equivalent to or greater than intramuscular (IM) vaccination. This means that the vaccine can be given with a greater degree of convenience and reduced patient discomfort.

The prototype vaccine was tested as an intramuscular regimen. While the route of administration was conventional, some patient discomfort had been associated with this route. An alternate site of injection was suggested as a solution to this problem. The commercial preparation was tested for its effectiveness when given via a SC route in 2- and 3-dose regimens. The serologic

TABLE 5

Comparison of anti-gp70 response following subcutaneous or intramuscular vaccination with "Leukocell"

Test group	Number of cats	Test interval and anti-gp70 response[d]			
		Pre-vaccination	Post 1st vaccination	Post 2nd vaccination	Post 3rd vaccination
2-dose SC[a]	27	0.041	0.025	0.412	NA[c]
3-dose SC	27	0.050	0.012	0.542	1.218
3-dose IM[b]	12	0.051	0.034	0.164	0.563
Controls	12	0.048	0.030	0.019	0.007

[a]SC = subcutaneous vaccination.
[b]IM = intramuscular vaccination.
[c]NA = not applicable.
[d]Geometric mean values expressed as ELISA optical density readings at 405 nm (0.2 is positive).

TABLE 6

Comparison of anti-FOCMA response following subcutaneous or intramuscular vaccination with "Leukocell"

Test group	Number of cats	Test interval and anti-FOCMA response[d]			
		Pre-vaccination	Post 1st vaccination	Post 2nd vaccination	Post 3rd vaccination
2-dose SC[a]	27	2	3	52	NA[c]
3-dose SC	27	2	4	60	212
3-dose IM[b]	12	3	3	9	68
Controls	12	3	1	1	3

[a]SC = subcutaneous vaccination.
[b]IM = intramuscular vaccination.
[c]NA = not applicable.
[d]Geometric mean values expressed as reciprocal or indirect immunofluorescence antibody titer (8.0 is positive).

responses generated were compared with that for IM vaccinates. The geometric mean anti-gp70 and anti-FOCMA values were considerably greater following SC vaccination than after IM vaccination. Only 2 SC doses were needed to produce strong gp70 and FOCMA antibody values equal to those observed following the 3-dose IM regimen. Thus, the SC route elicited an antibody response that was both stronger and more rapid than the IM response. It appears that route of administration, indeed, affects the immune response to antigens and that a 2-dose regimen may be sufficient for efficacy, although the manufacturers still suggest 3 doses for maximal effect. This experience suggests that investigating alternate routes of administration should probably be an obligatory part of vaccine development for other vaccines, especially for adjuvanted preparations.

CLINICAL PERFORMANCE IN A HIGH-RISK ENVIRONMENT

Within 18 months after "Leukocell" had been licensed in the U.S.A. a noteworthy report appeared attesting to the vaccine's clinical performance in a high-risk environment (Henby et al., 1986). The report confirmed the previous efficacy studies used in the laboratory (Lewis et al., 1981; Sharpee et al., 1986). In addition, the study was performed by independent practitioners administering feline leukemia vaccination under everyday field conditions and was not associated with the previous researchers.

The most convincing aspect of their report was the description of vaccination in a colony of 46 cats maintained in a private home. Sanitation, nutrition and routine immunizations (other than for feline leukemia) had been observed

by its owners. The colony was essentially a closed population in good health, but had occasional contact with stray cats. In August 1984, 2 of the colony's cats experienced a generalized malaise and subsequently died with what was suspected to be feline leukemia. The entire colony was subsequently tested for FeLV and 10 of the 46 cats were found to be FeLV positive.

All of the cats in the colony, both FeLV-positive and FeLV-negative animals, received 3 doses of "Leukocell" at the recommended intervals. Two subsequent tests for FeLV (8 and 50 weeks after the initial test) found that all 36 FeLV-negative cats were still negative. The FeLV-positive cats all remained positive for the duration of the 1-year observation period, confirming that the colony had experienced a feline leukemia outbreak and that the negative cats had remained in contact with FeLV-infected cats. Four of the 10 FeLV-positive cats died during the test period.

Multiple diagnostic tests confirmed that 35 vaccinated cats remained FeLV negative despite nearly a year of continuous and unrestricted physical contact (including common eating and sleeping facilities) with FeLV-infected cats. Performance of the vaccine in this colony takes on added significance in that some of the FeLV-negative vaccinates were geriatric animals as old as 19 years, thus falling into one of the population groups most susceptible to FeLV infection (Hardy, 1981).

This report also described vaccination of 400 cats in a second colony at a public cat welfare shelter with a history of feline leukemia. Prior to vaccination, the entire colony of 272 cats was ELISA tested for FeLV and all FeLV-positive cats were removed. A total of 39 cats (14.3%) were FeLV positive. The remaining 233 cats were vaccinated with 2 or 3 doses of "Leukocell". Thereafter, incoming cats were tested for FeLV status prior to entry into the colony. Cats found to be negative were vaccinated and added to the colony. The purpose of this study was primarily to remove any FeLV cats from the colony and then to be able to maintain it as FeLV free.

Although the screening program in this colony eliminated the majority of positive cats, infected cats may have still entered the shelter due to false-negative ELISA tests, due to latent infection or during the initial pre-test holding period. Despite the possibility of FeLV exposure in an open shelter with a large transient population, only 6 of 400 vaccinates during the ensuing 12 months tested FeLV positive. Mitigating circumstances, such as possible FeLV exposure prior to vaccination, existed in 5 of these 6 cats. The experience of this colony is significant in that it involved a large number of vaccinates over an extended period with a significant number having been exposed to FeLV-infected cats; secondly, it demonstrated that a program of diagnostic screening and vaccination can effectively eliminate feline leukemia, even in a facility with a history of the disease and a transient population involving high risk of exposure.

SIGNIFICANCE OF ANTIBODY RESPONSE TO VACCINATION

The ultimate test of vaccine efficacy is protection against virulent challenge that affects non-vaccinated controls. In challenge-of-immunity studies, the licensed vaccine protected 80% of cats from persistent viremia and 92% from tumor development for > 2 years after challenge, even though vaccinates were artificially immunosuppressed with corticosteroids (Lewis et al., 1981; Sharpee et al., 1986). This qualification is an important one that is often overlooked when pointing out simply that "Leukocell" has 80% efficacy. The vaccine has 80% efficacy in artificially immunosuppressed cats subjected to a massive challenge dose with a highly virulent agent on 2 successive days (Lewis et al., 1981). This challenge regime was able to infect 100% of the control population regardless of their ages. We believe that 80% efficacy under these circumstances would be equal to 100% efficacy under typical field conditions.

Although serologic response to vaccination is secondary to protection, vaccinated cats in these studies exhibited antibody responses to whole FeLV, FOCMA or gp70. The licensed vaccine elicited mean post-vaccination anti-FeLV and anti-FOCMA values in cats that exceeded mean post-challenge titers of non-vaccinated controls (Sharpee et al., 1986) and the prototype vaccine elicited a gp70 antibody response in all test cats (Lewis et al., 1981), although significant virus neutralizing antibody was not detected prior to challenge. A later study by independent investigators showed mean FOCMA and gp70 antibody values in 70 seronegative cats exceeded protective levels (Stallman and Legendre, 1986). However, with a seroconversion rate in this study of 64% to either FOCMA or gp70, leaves open to question the importance of these conventional measures of FeLV immunity.

A strong and consistent serologic response is desirable, but certainly not the sole or possibly even the most critical indicator of protection, particularly protection resulting from cellular immunity. Although antibody production is stimulated by the vaccine and is easily measured, it is not the only immune response generated. Therefore, is a vaccinated cat that does not express virus-neutralizing antibody resistant to disease? Previous studies indicate that the answer is "yes", as was shown by Lewis et al. (1981). A protective response was generated, even without significant virus-neutralizing antibody. In all probability, the marked amnestic response that was observed in all the vaccinated cats following challenge (Lewis et al., 1981; Sharpee et al., 1986) allowed for a protective response. Initial serologic response in some animals was modest, but challenge was followed by a much more pronounced antibody response and protection.

In addition to the development of a conventional serologic antibody response, a strong cytotoxic response is also needed. Studies have shown that feline complement-dependent cytotoxic (CDC) antibodies in the presence of cat complement will lyse homologous FeLV-infected tumor cells (Grant et al., 1977). This may be particularly important in the case of FeLV since, unlike

herpes- or coronavirus, FeLV is a non-lytic virus. Thus, an infected cell will produce virus for an extended period. Lysis will interrupt cell transformation and viral replication. Virus-neutralizing antibody will neutralize cell-free FeLV, but has no effect on FeLV transformation of cells or viral replication within those cells. Removal of FeLV-infected and/or transformed cells is essential for total protection from future development of FeLV-associated disease. The development of CDV antibody is one way for this to occur.

In a limited number of cats tested at Norden Laboratories, "Leukocell" consistently elicited marked levels of CDV antibodies, as shown in Fig. 1. Four specific pathogen-free (SPF) cats exhibited a CDC antibody response after each of 3 vaccine doses. Interestingly, CDV antibody responses were significant in all cats following 2 doses (well above the 5.0 index considered positive), while gp70 values at the same test interval were still modest and below the 0.2 optical density value considered positive. Thus, the vaccine's immunizing properties should not be assessed on the basis of anti-gp70 or anti-FOCMA
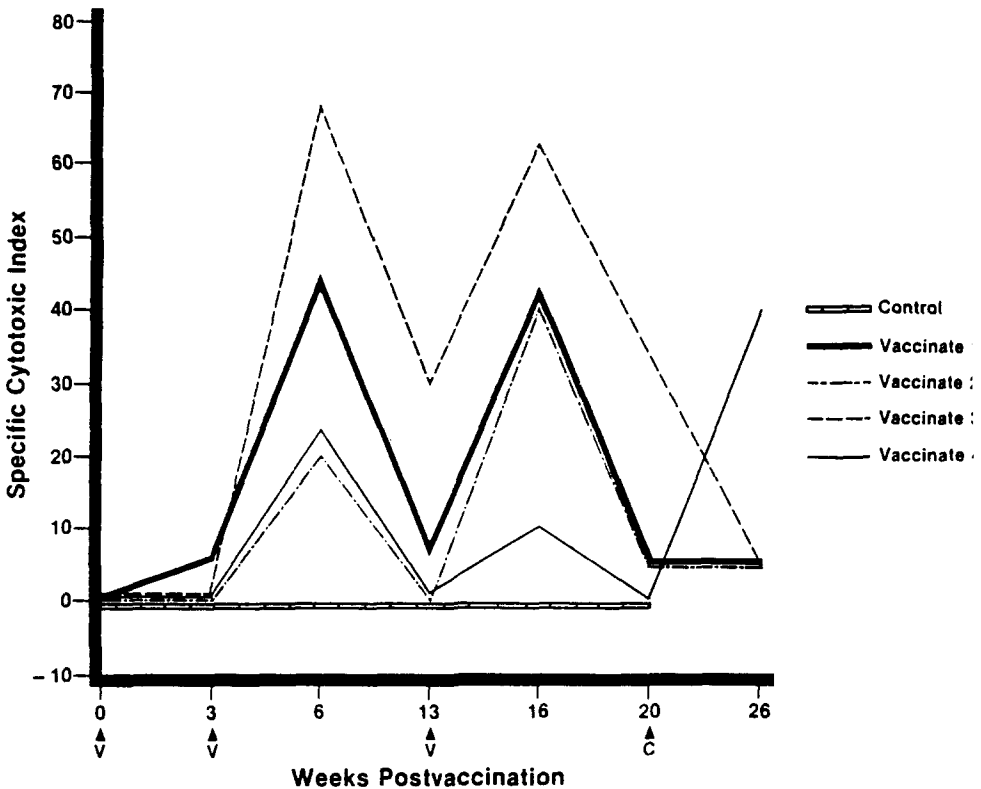

Fig. 1. Complement-dependent cytotoxic antibody indexes in SPF cats following 3 doses of "Leukocell" (V) plus FeLV challenge (C).

values alone, and the development of a strong cytotoxic response should also be considered as a potentially protective response.

CONCLUSION

This first year for commercial use of the FeLV vaccine has been very successful. Research has continued to develop a safer and more effective vaccine and also to determine possible side effects. The actual effectiveness of the vaccine in the general cat population would be hard to access due to the limited time of availability, but preliminary results suggest that a reduction of FeLV disease and associated syndromes should be expected. In addition, with the success of a retrovirus vaccine in the cat population, a model now exists which gives better access to the potential of a vaccine for retroviral diseases in other animals, including man.

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