Evaluation of a killed, whole virion feline leukemia virus vaccine

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There are now 5 licensed FeLV vaccines available to veterinarians in the United States. Three of these contain killed whole virions and 2 contain viral subunits. One vaccine[a] contains viral subcomponents released by a stressed, infected, feline cell line. The mixture of proteins secreted by this cell line is complex, but it is rich in gp70, the major immunogen of FeLV. The other subunit vaccine[b], contains a recombinant product consisting of a major portion of the gp70 molecule secreted by a recombinant strain of *Escherichia coli*.

Although gp70 has been recognized for many years as the major immunogenic component of FeLV, the commercial development of a vaccine had been delayed by fears that the other envelope glycoprotein p15e, was potently immunosuppressive. It was assumed that the presence of p15e would doom any attempt to generate an effective whole virion vaccine.[1] This opinion persisted despite evidence that healthy adult cats may develop strong immunity to FeLV infection after natural exposure to the virus, thus indicating that field strains of FeLV are immunogenic.[2,3] It is also clear, however, that natural immunity cannot be relied on to protect exposed cats.

Despite reports that documented whole virions to be effective immunogens, vaccine companies sought to develop methods of producing a gp70-containing vaccine in the absence of p15e. Investigators at a commercial laboratory[c] found that a persistently infected feline cell line appeared to produce p70, with low amounts of p15e. An investigator at another company[c] believed that perhaps p15e was not as important as initially assumed. He reasoned that a killed whole virion vaccine rich in gp70 could provide sufficient stimulus to confer protective immunity, provided that the antigen concentration was sufficiently high and that an effective adjuvant was used. Thus, 4 criteria had to be met to produce an effective, killed, whole virion vaccine: a strain of FeLV had to be found that had high concentration of gp70; the strain should grow to high titer in cell culture; an effective adjuvant should be available; and the virus must be killed in a manner that retains maximal immunogenicity.

The second laboratory[d] released its FeLV vaccine[e] in 1988. There has never been any doubt about the vaccine's efficacy as an immunogen. But problems in manufacturing led to low incidence of allergic reactions, and the vaccine was voluntarily withdrawn. The vaccine has been reformulated and is again available to veterinarians.

Vaccine Efficacy

Specific-pathogen-free 10-week–old kittens were used in all vaccine efficacy studies. Prior to vaccination, these kittens were negative for p27 antigenemia as measured by ELISA[f] and the immunofluorescent antibody (IFA) test,[4] as well as for antibodies to FeLV, feline parvovirus (FPV) feline calicivirus (FCV), feline rhinotracheitis virus (FRV), and rabies virus.

The aforementioned vaccine[e] (vaccine A), was used in all experiments. This vaccine contains a unique isolate of FeLV selected for optimal growth characteristics and maximal immunogenicity. A specific isolate,[g] was used to establish a persistently infected nontransformed feline diploid cell line as a source of vaccine virus. The virus suspension was chemically inactivated and contained whole virions. The vaccine contains an adjuvant.

The efficacy of the vaccine was documented by challenge-exposure of vaccinated kittens with virulent FeLV. The vaccine was administered to the kittens at 10 and at 13 weeks of age, and all vaccinated and control kittens were challenge-exposed 2 weeks later at 15 weeks of age. Challenge exposure consisted of 2 oronasal administrations of approximately 10^6 focus-forming units of virulent FeLV-A, Rickard Strain given on 2 successive days.

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[a]Leukocell, SmithKline Beecham, Lincoln, Neb.
[b]Genetivac FeLV, Pitman-Moore, Mundelein, Ill.
[c]SmithKline Beecham, Lincoln, Neb.
[d]Diamond Scientific, Des Moines, Iowa.
[e]Covenant, Haver/Diamond Scientific, Shawnee, Kan.
[f]Leukassay, Pitman-Moore Co, Mundelein, Ill.
[g]LUNA Strain, Haver/Diamond Scientific, Shawnee, Kan.

Table 1—Efficacy study of an FeLV killed whole virion vaccine

Trial No.	Vaccinates: No. of cats	Vaccinates: No. viremic	Vaccinates: Proportion viremic (%)	Controls: No. of cats	Controls: No. viremic	Controls: Proportion viremic (%)
1	6	0	0	6	5	83
2	7	0	0	19	12	63
3	29	6	21	...	...	...
4	6	0	0	4	3	75
6	16	2	12	9	5	55
7	19	1	5	10	4	40
8	20	1	5	9	4	44
9	10	1	10	14	13	93
Totals	113	11	9.7	71	46	64.7

Table 2—Comparison between vaccines A and B, and C and D5

	Vaccinates: No. of cats	Vaccinates: No. viremic	Vaccinates: Percent protection (%)	Controls: No. of cats	Controls: No. viremic	Controls: Percent protection (%)
Vaccine B	10	9	10	8	7	87.5
Vaccine A	30	11	63	20	17	85
Vaccine C	10	4	60	18	13	72
Vaccine D	10	1	90	18	13	72

Challenged-exposed cats were simultaneously immunosuppressed by IM administration of methylprednisolone acetate (10 mg/kg of body weight) on the day of first challenge. The challenge dose was standardized to induce persistent viremia and/or FeLV syndromes and death in 50 to 85% of control cats.

Nine major trials were conducted, using vaccine A (Table 1). In total, 113 kittens were vaccinated in all these trials, and 71 kittens were used as nonvaccinated controls. Collectively, these trials resulted in 11 of 113 vaccinated cats developing viremia (90.3% protection). In contrast, 46 of 71 of the nonvaccinated control kittens developed viremia.

Comparative Vaccine Studies

Each veterinary vaccine company tests its own vaccine. The great diversity of challenge-exposure procedures and methods of evaluation makes comparisons difficult. For this reason, it was decided to test an FeLV vaccine that did not contain whole virions. Vaccine B[a] was used according to manufacturer's instructions. Kittens were challenge-exposed according to the standard protocol. Of 10 kittens given vaccine B in 2 doses, 3 remained nonviremic. Of 8 nonvaccinated controls, 7 (88%) became persistently viremic and 2 of them died of FeLV-associated syndrome (Table 2). A group of 30 kittens given a reduced dose of vaccine A (50% dose) had 69% protection (11 developed viremia) after identical challenge exposure. Thus, in this single experiment, vaccine B-inoculated kittens did not have greater protection than did controls ($\chi^2 = 0.787$) whereas those vaccinated with vaccine A had greater protection than did controls ($\chi^2 = 6.54$).

Table 3—Interference study of a combination vaccine containing FeLV, calicivirus, rhinotracheitis, and parvovirus

Vaccinates: No. of cats	Vaccinates: No. viremic	Vaccinates: Percent protection (%)	Controls: No. of cats	Controls: No. viremic	Controls: Percent protection (%)
7	0	100	19	12	63

In a similar experiment, a second-generation vaccine[h] (vaccine C) was compared with vaccine A in a challenge-exposure study. The FeLV component of vaccine A was incorporated in a vaccine[i] containing FeLV, FRV, FCV, and FPL (vaccine D). Of 10 kittens inoculated with vaccine C, 4 became persistently viremic, whereas only 1 of 10 inoculated with vaccine D developed viremia. Of 18 control, nonvaccinated kittens, 13 also developed viremia (Table 2; χ^2 for vaccine C = 2.79; χ^2 for vaccine D = 9.95).

Studies on Multiple Component Vaccines

Two experiments were conducted to determine efficacy of vaccine A when used in conjunction with other viral vaccines. One experiment was designed to determine the effect of using vaccine D (FeLV, FPV, FRV, and FCV) on the efficacy of the FeLV component of the vaccine.

Vaccine D was administered to 7 kittens at 10 and 13 weeks of age. Twenty nonvaccinated kittens served as controls. After FeLV challenge exposure at 15 weeks, all vaccinates were tested for evidence of protection as indicated by absence of FeLV viremia (Table 3).

The second experiment was designed to determine whether the FeLV component interfered with the response of kittens to other vaccine components. Serologic responses were used to identify possible interference. Kittens were allotted to 3 groups: group A—7 kittens were inoculated with vaccine D; group B—6 kittens were inoculated with vaccine containing the 3 viruses other than FeLV (vaccine E) and used as negative controls; group C—5 nonvaccinated kittens were also used as controls.

[h]Leukocell II, SmithKline Beecham, Lincoln, Neb.
[i]Confirm, Diamond Scientific, Des Moines, Iowa.

Table 4—Serologic response to vaccines D and E in an antigen interference study

		Antibody titer*					
		Rhinotracheitis		Calicivirus		Parvovirus	
Group/Vaccine	N	Day 0	Day 35	Day 0	Day 35	Day 0	Day 35
1 D	4	< 2†	59	< 2	94	< 2	> 128
2 E	6	< 2	78	< 2	144	< 2	≥ 128
3 Control	5	< 2	< 2	< 2	< 2	< 2	< 2

*Virus neutralization titer; †geometric mean titer.

Of the 7 kittens given vaccine D, all remained free of viremia after challenge exposure, whereas, 12 of 20 controls (60%) became persistently viremic and 8 of 20 controls died of FeLV-associated diseases (Table 3). Therefore, simultaneous vaccination with FeLV, FCV, FRV, and FPV did not interfere with the protective response to the FeLV component of the vaccine.

Response to Vaccine D

All kittens used in the second interference experiment were seronegative to all viruses prior to vaccination. Samples obtained 2 weeks after administration of vaccine D indicated seroconversion and development of protective antibody titer. All nonvaccinated control kittens remained negative to all components of the vaccine (Table 4). Statistical analysis revealed no significant difference between antibody titers in group-A and -B kittens on the basis of a 2-tailed analysis of variance.

Discussion

Evidence indicates that vaccine A, an inactivated whole virion vaccine, is highly efficacious when administered to cats.

In developing a vaccine against FeLV infection, it is critical to ensure that the vaccine contains adequate amounts of the major protective antigen gp70. It is probably also important that other, less well defined, viral antigens be present. Whole FeLV virions may not only provoke antiviral antibodies, but also stimulate cell-mediated immunity.[5] An adequate antiviral response should prevent persistent viremia, immunosuppression, and other FeLV-associated syndromes. It is desirable, although perhaps not essential, that an anti-FeLV vaccine also contain feline oncorna virus-associated cell membrane antigen (FOCMA) because antibodies to FOCMA have been claimed to prevent development of FeLV-induced tumors.[6]

Immunity to FeLV infection and its associated diseases can be ascertained with confidence by documenting protection against viremia. Serologic response alone, after vaccination, may not be sufficient to prevent viremia and cannot, therefore, be used as assurance of protection. Isolates of FeLV differ widely in virulence; so when establishing appropriate FeLV challenge exposure, it is essential that a virus strain of consistent, high virulence be selected. It is also desirable that the challenge strain be free of feline sarcoma virus or other agents that might complicate interpretation of results. The third prerequisite for reproducible challenge exposure is the need to transiently immunosuppress challenged-exposed animals through use of steroids to permit establishment of infection. Use of appropriate standardized challenge exposure will result in establishment of virus infection in susceptible cats, as manifested by development of persistent viremia. Infected, viremic cats will eventually develop leukemia or other FeLV-associated diseases and die. Viremia may be detected by direct virus isolation from blood or other body fluids[7]; by detection of viral antigen in blood smears, using the immunofluorescent antibody (IFA) test[4]; or by detection of p27 antigen in serum using ELISA. Some investigators have regarded viremia of up to 10 weeks' duration as transient and of no pathogenic relevance. It is our opinion that viremia lasting for > 3 weeks after challenge exposure is relevant and has the potential to establish latent infection. Such latent infection may re-express itself in stressed cats. For this reason, we regarded viremia > 3 weeks' duration in vaccinated cats to be evidence of lack of protection.

Simultaneous vaccination with FeLV, FRV, FCV, and FPV (vaccine D) did not interfere with the protective response to the FeLV component of the vaccine (Table 3). In addition, and possibly more importantly, the FeLV component of the vaccine did not influence antibody responses to FRV, FCV, and FPV (vaccine E).

Cats are known to be most susceptible to FeLV when they are young.[5] However, there is ample evidence to indicate that passive immunity can be conferred on kittens by nursing from immune mothers.[5,8,9] It is essential, therefore, to protect kittens as early as possible. We have evaluated the efficacy of this vaccine in kittens initially inoculated at 10 weeks of age. A second dose of vaccine was administered 3 weeks later, and protection was documented 2 weeks later—only 5 weeks after initiation of vaccination. The advantages of a vaccine that confers effective immunity in 2 doses are many. The most obvious is that protective immunity is conferred early, so that kittens are protected by 15 weeks of age.

Conclusion

Vaccine A was administered to 10-week-old kittens in 2 doses given 3 weeks apart. Protection against challenge exposure, as documented by

prevention of persistent viremia and other FeLV disease syndromes, was observed.

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Feline leukemia virus vaccine development

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Over the years, a great deal of controversy has surrounded the value of vaccination for the prevention of diseases associated with FeLV.[1] As a result, several research workers have studied the pathogenesis of the disease and the immunologic response of cats to the disease and to vaccination.[2,3] Testing of commercial and experimental vaccines has resulted in mixed conclusions with regard to prevention of infection and the associated diseases.[4-7] Those reports often describe differing challenge exposure and evaluation protocols, making data comparison difficult. During the development of an FeLV vaccine, studies on many vaccine prototypes were performed in our laboratory. Once successful prototypes were identified, comparative studies were performed to determine vaccine efficacy.

The efficacy tests reported herein were executed by vaccination of cats followed by either intraperitoneal or natural challenge exposure. Nonvaccinated cats were challenge exposed with the same material. In the earlier tests, cats were immunosuppressed, using a corticosteroid, then challenge exposed by intraperitoneal inoculation with a virulent FeLV, in accordance with a USDA-approved procedure. This challenge-exposure procedure consistently induced persistent FeLV viremia in most of the control cats. The nonvaccinated control cats were commingled with the vaccinated cats during the entire study to provide continuous, natural challenge exposure. In later tests, cats were naturally challenge exposed by housing them with infected carrier cats that had been challenge exposed by use of the intraperitoneal method and had developed a peristent viremia.[8]

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Initial Formulations

The purpose of our studies was to determine how to produce a vaccine that would provide the desired degree of protection. The first test included use of a live vaccinia virus recombinant vaccine, 3 killed virus recombinant vaccines (containing the same antigen but 3 different adjuvant systems), and a whole killed FeLV vaccine (vaccine A), (which was an experimental vaccine that contained the same whole killed virus in all its variants but 4 different adjuvant systems). The recombinant vaccines were designed to enhance expression of gp70, which was implicated as the important antigen involved in eliciting a protective immune response in an earlier study.[9] However, other studies indicated that whole killed FeLV vaccine preparations protected cats against FeLV challenge exposure, whereas gp70 subunit vaccines did not.[10]