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Review of the first feline leukemia virus vaccine

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As soon as a virus was proven to be one cause of leukemia in cats, the potential for immunoprophylaxis was recognized, and a number of attempts were made to immunize cats against the FeLV. Early immunization efforts failed, however, and in some cases immunization of kittens with inactivated whole virus vaccines actually led to heightened susceptibility to infection, rather than resistance.¹ The immunosuppressive effects of FeLV were believed to be associated with the envelope protein, p15e, which in its native form substantially inhibits lymphocyte blastogenesis.²

Nevertheless, immunity to FeLV clearly existed in nature. Cats that had been exposed to the virus, but did not develop persistent viremia, were immune to rechallenge exposure.³ Low doses of live virus conferred immunity in some cats, but the risk of FeLV-induced disease was too great to consider such vaccines for general application. Likewise, live attenuated strains were considered impractical because of the risk of oncogenesis or genetic recombination. The FeLV is a retrovirus; replication proceeds only after a DNA copy of the viral genome is integrated into the host cell. Therefore, research concentrated on efforts to develop an effective inactivated immunogen.

Discovery of Subunit Vaccines

The foundation of the first commercial FeLV vaccine was the observation by Wolff et al⁴ that the FL-74 cell line, infected with the Kawakami-Theilen strain of FeLV, released soluble tumor and virus antigens when propagated in serum-free medium. Olsen and Lewis⁵ inoculated cats with the resulting tissue culture fluids and discovered that the cats responded not only to feline oncornavirus-associated cell membrane antigen (FOCMA), a cell-

surface protein on FeLV-transformed cells, but also to the viral proteins gp70 and p27, suggesting that the system might be useful to immunize cats against FeLV infection.

Mastro et al⁶ provided direct evidence that a vaccine based on these soluble viral subunits immunized cats against experimental FeLV challenge exposure, and Olsen et al⁷ provided further evidence of protection of naturally exposed cats in multicat households. In contrast to earlier studies of inactivated whole virus vaccines, immunosuppressive effects were not observed. Olsen and Lewis⁵ speculated that the p15e in the soluble subunit vaccine existed in a precursor form that lacked the immunosuppressive properties of whole virus p15e, although direct evidence to support this hypothesis was not presented.

Development of the First FeLV Vaccine

Commercial development of a subunit FeLV vaccine based on these observations required process improvements to increase yields and to "scale-up" to commercial production quantities. Likewise, a safe effective adjuvant system was needed; the original experimental vaccine had incorporated Freund incomplete adjuvant, which is too reactive for use in privately owned cats. The resulting vaccine^a was federally licenced in 1985, and a full report was published by Sharpee et al.⁸

The vaccine consisted of chemically inactivated culture filtrate from FL-74 feline lymphoid cells infected with all 3 subtypes (A, B, and C) of the Kawakami-Theilen strain of FeLV. Viral gp70 as well as FOCMA were detected by ELISA and the immunofluorescent antibody (IFA) test, respectively. A dual adjuvant, incorporating aluminum hydroxide and partially purified saponin was cho-

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^aLeukocell, SmithKline Beecham Animal Health, West Chester, Pa.

sen as the most effective additive on the basis of in-house comparative studies.

Published Studies

In the studies reported by Sharpee et al,⁸ cats were vaccinated 3 times and challenge exposed intranasally with $10^{5.7}$ focus-forming units of the Rickard strain of FeLV on 2 successive days. To increase the likelihood of infection, controls and vaccinates were immunosuppressed with 5 mg of methylprednisolone/kg of body weight at the time of challenge exposure.

Persistent viremia was observed in 5 of 25 vaccinates (20%), as opposed to 7 of 10 (70%) control cats. Lymphoid tumors developed in 2 of 25 (8%) vaccinated cats and 6 of 10 (60%) control cats. In agreement with previous studies of FeLV infection, tumor development and mortality during the 26-month observation period were strongly associated with persistent viremia. Indeed, tumors were observed only in persistently viremic cats, and all but 3 of the deaths occurred in persistently viremic cats.

Twelve of the surviving vaccinates were observed for 4 years, then tested for latent infection by methylprednisolone immunosuppression and bone marrow culture.⁹ Although 8 of the cats tested had transient viremia after challenge exposure, latent infection could not be detected, indicating that vaccination may also reduce the probability of developing latent infection.

Serologic responses were similar between cats given either 2 or 3 doses of the vaccine, but challenge-exposure data indicated possible superior protection after 3 inoculations; therefore, 3 doses of the original product were required. Pedersen et al¹⁰ failed to confirm evidence of protection in an experimental trial of the vaccine. A number of methodologic problems made the data difficult to interpret, however, including the fact that 1 experimental group was given only 2 doses of the 3-dose product. Serologic evidence is available that the cats may have been exposed to FeLV prior to completion of immunization.¹¹

Development of Vaccine 2

In practice, the 3-dose vaccination schedule proved costly, and veterinarians experienced difficulty obtaining owner cooperation in completing all vaccinations on the recommended schedule. Therefore, efforts were made to develop a 2-dose product that afforded the same protection as the original 3-dose vaccine. These development efforts led to introduction of an improved potency vaccine,^b as reported by Haffer et al.¹²

Vaccine 2 differs from the original FeLV vaccine in that it contains a greater concentration of antigen, so that it induces serologic responses and

immunity to challenge-exposure after 2 inoculations rather than 3.¹² In the study reported by Haffer et al,¹² cats were vaccinated twice, immunosuppressed, and challenge exposed as described.⁸ Of 25 vaccinated cats, 7 (28%) became persistently viremic after challenge exposure, compared with 6 of 10 (60%) controls.

Lymphocyte blastogenesis assays were conducted after vaccination to evaluate immunosuppressive effects of the vaccine. The mean proliferative response to concanavalin A was not statistically different between control and vaccinated cats, confirming the earlier observation that the subunit vaccine is not immunosuppressive.

Basis of Immunity

The basis of immunity to FeLV infection after vaccination is incompletely understood. Among the general population, only about a third of cats become persistently viremic after natural exposure, with another third recovering after a period of transient viremia.¹³ In studies of the original FeLV vaccine and its successor, positive correlation was noted between anti-gp70 titer and resistance to viremia.^{8,12} Cats with optical density reading > 0.8 by ELISA for anti-gp70 rarely became infected. Cats that became viremic were either seronegative or tended to have low gp70 titer.

Virus neutralizing (VN) titer is directly correlated with ELISA titer to gp70 in tests performed by the manufacturer (data not shown). However, VN titer to FeLV is generally low and, as such, does not necessarily correlate with resistance to persistent viremia. After challenge exposure, many cats with low titer were resistant to infection¹²; thus, VN antibody titer alone appears to be a poor predictor of resistance to FeLV infection. It is probable that immunity to FeLV involves cell-mediated immunity and other factors, possibly genetically determined, in addition to humoral immunity.

Additional Studies

Nine additional challenge-of-immunity studies were conducted by the manufacturer in the 3 years after licensing of vaccine 2. In each trial, specific-pathogen-free cats were vaccinated with vaccine 2 according to label instructions. Commercial production lots of vaccine were used. In all trials except 89B, cats were immunosuppressed with corticosteroids, challenge exposed intranasally as previously described, and evaluated by biweekly virus isolation attempts. Any cat that remained ELISA-positive for more than 8 weeks was considered persistently viremic. In trial 89B, cats were challenge exposed SC with the NCE strain of FeLV.^c In all 9 studies (Table 1), cats inoculated with vaccine 2 were significantly less likely to develop persistent viremia. However, in 8 of 9 studies, one or

^bLeukocell-2, SmithKline Beecham Animal Health, West Chester, Pa.

^cHaffer K. 1987-1989 efficacy studies of Leukocell and Leukocell-2 (abstr). *Proceedings. 8th ACVIM Forum* 1990;415-418.

Table 1—Results of challenge-of-immunity studies of vaccine 2

Study No.	No. of cats		Proportion persistently viremic (%)		Preventable fraction (%)
	Vaccinated	Control	Vaccinated	Control	
87A	32	10	16	50	68
87B	26	12	8	33	76
88A	10	10	28	70	60
88B	10	10	0	60	100
89A	39	10	10	50	80
89B	14	5	7	60	88
89C	11	9	36	89	60
90A	10	10	30	90	67
90B	10	10	20	60	67

more of the vaccinated cats became persistently viremic. This indicates that the immunity engendered by FeLV vaccine administration is not absolute; vaccination must be used in concert with test-and-removal programs and other control measures designed to reduce exposure.

Safety

In the pre-licensing field trial of vaccine 1 involving almost 7,000 vaccinations, some type of reaction was observed in 12.6% of vaccinates.¹⁴ Approximately three-fourths of these were local reactions—discomfort or pain on injection—but 3.1% involved systemic signs—most often lethargy or inappetence—and 0.6% involved hypersensitivity or miscellaneous other reactions. An independent study of vaccine reactions¹⁵ conducted after the commercial introduction of vaccine-1 reported higher rates, involving more than a quarter of all cats. Again, most reactions were local, injection-site reactions or mild systemic signs of fever, listlessness, inappetence, and the like.

A second independent study was undertaken to assess the effect of manufacturing changes and SC administration.¹⁵ Disappointingly, the reaction rate appeared to increase. The authors examined a number of confounding variables and found that simultaneous administration of another vaccine significantly increased the apparent rate of reactions to the FeLV vaccine. Differences among clinics also were significant.

Unfortunately, a comparison group was not included in either of these studies so that it is difficult to know how these results compare with those of other commonly used feline vaccines or to what extent the results were affected by the study design. Heightening owners' awareness of the potential for reactions, and calling them the next day to check for any untoward effects, introduces bias. The high rates reported almost certainly reflect a degree of over-reporting. Meaningful assessment of reaction rates will require a simultaneous blind comparison of different vaccines, ideally including a placebo.

Another source of data on vaccine safety is the adverse effect reports filed with the biologics producers. All complaints reported to the technical

services department of the manufacturer of vaccines 1 and 2 since 1989 have been tabulated. Complaints are categorized by type of complaint, product involved, and clinical signs of disease. Analysis of these records indicated that in the period January 1, 1989 to June 10, 1991, the manufacturer received 5.4 complaints of alleged reactions/100,000 doses of vaccine 2 sold.^d Of these, 28% involved local reactions (eg, stinging reaction upon injection, and 72% or 3.9/100,000 doses involved systemic signs, such as lethargy, fever, inappetence). This analysis undoubtedly underestimates the true number of reactions, particularly mild reactions, owing to under-reporting. It nevertheless underscores the overall safety of the product in routine practice, because all alleged reactions were included; no attempt was made to exclude cases of intercurrent disease or simultaneous administration of multiple vaccines.

Field Studies

As with most veterinary biologics, the number of controlled field trials of FeLV vaccines is limited. Anecdotal evidence indicates that use of the vaccine may have greatly decreased the prevalence of FeLV infection in clinical practice.¹⁶ Likewise, a report of efforts to control FeLV infection in 2 high-risk shelters indicated that inoculation with vaccine 1 had a key role in reducing the incidence of new cases.¹⁷ Because test-and-removal programs were instituted simultaneously, however, it was not possible to ascribe the success solely to the FeLV vaccine.

The largest controlled field trial of any FeLV vaccine to date was reported by Pollock and Scarlett.¹⁸ Seventy-eight cats were randomly assigned to vaccine or placebo groups. Six weeks after the third vaccination, the cats were placed in a single room in contact with 44 FeLV-positive cats. All cats were housed together, sharing food bowls, litter, and perches, for 2 years. Blood samples were collected at 4-month intervals, coded, and tested for viremia by ELISA and IFA methods in 2 laboratories. After 12 months of exposure, 5 of the 34 (14.7%) vaccinated cats with sufficient data for analysis were persistently viremic, compared with 15 of 36 (41.6%) placebo-inoculated cats.

In analyzing these data, Pollock and Scarlett argued that studies designed to evaluate diseases like FeLV infection, in which < 100% of the controls become ill, must take into account the proportion of cats that are "naturally resistant" to infection. The true measure of vaccine efficacy for such diseases is the preventable fraction (PF), the proportion of expected cases that were prevented by vaccination. If a vaccine had no efficacy at all, the proportion of cases among the vaccinates would be the same as the proportion among the

^dStarr R, Technical Services, SmithKline Beecham Animal Health, Exton, PA: Unpublished data, 1991.

placebo-inoculated cats. Comparing the proportion of cases among the vaccinates with that among the placebo group yields the PF, the diminution in cases attributable to the vaccine. The general formula is:

$$PF = \frac{I(c) - I(v)}{I(c)} \quad \text{in which:}$$

$I(c)$ = incidence in controls
 $I(v)$ = incidence in vaccinates

For the study reported by Pollock and Scarlett, the PF was 62%, based on crude incidence, and 68%, based on incidence density (new infections per cat-month of exposure). These results were similar to the original data reported by Sharpee et al,⁸ which yielded PF of 72%.

Direct comparison of these results with claims for other FeLV vaccines is potentially misleading. Data for other FeLV vaccines are limited and largely unpublished; most studies suggest PF in the 60 to 90% range, although they are seldom presented as such. The number of cats in any one trial is usually too small to provide precise estimates of efficacy, and individual trials cannot be legitimately combined when they differ in schedule, challenge exposure, or other variables. Likewise, differences in challenge virus strain and titer, route of exposure, sample collection procedures, and outcome measures (eg, virus isolation vs the less sensitive IFA test) make direct comparisons among different products impossible.

In general, the efficacy of FeLV vaccines is less than that expected for other feline vaccines. This probably reflects the complex nature of the disease and its portal of entry and initial site of replication.¹⁹ Legendre et al²⁰ reported a field trial of vaccine 1 that failed to find efficacy. Kittens were given 2 inoculations of vaccine 1. Challenge exposure consisted of housing the cats in contact with naturally infected cats at a ratio of 1:5 for 24 weeks. Difference was not seen in the rate of infection as determined by ELISA between the vaccinated and control cats. The authors suggested that one possible explanation might be virus strain differences. If this were true, it would affect all FeLV vaccines. However, independent evidence does not exist that indicates strains circulating in the general population fail to cross-immunize within a given subgroup. An alternative hypothesis is that the apparent lack of efficacy reflects general immunosuppression of the kittens in the trial. Almost 50% of the deaths among vaccinated and control cats were attributable to feline infectious peritonitis. That the trial kittens failed to respond normally to standard vaccines for upper respiratory tract disease supports the view that they may have been immunocompromised.

Conclusion

The first commercially available vaccine

against FeLV was introduced in 1985. Repeated experimental studies have supported the efficacy of the vaccine and its successor. A large, independent and well controlled field trial documented the vaccine's effectiveness under conditions of chronic natural exposure. Because no vaccine provides 100% protection, however, test-and-removal programs and other measures designed to reduce exposure should continue to be part of the overall control strategy.

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