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Passive Immunity to Feline Leukemia: Evaluation of Immunity from Dams Naturally Infected and Experimentally Vaccinated

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Antibodies against feline leukemia virus (FeLV) and the feline oncornavirus-associated cell membrane antigen (FOCMA) were transferred from pregnant cats to their suckling kittens. All of these kittens were protected against infection and oncogenesis by virulent FeLV when challenged at 2 weeks of age. Suckling kittens acquired 25 to 100% of maternal virus-neutralizing and FOCMA titers by 3 days of age, and titers underwent linear decay to undetectable levels by 2 to 3 months of age. FOCMA antibody in dams and kittens was identified as immunoglobulin G (IgG) by use of goat anti-human IgG serum, which cross-reacts with feline IgG in the indirect membrane immunofluorescence test for FOCMA antibody. In an attempt to induce protective maternal antibody by vaccination, 10 pregnant cats were immunized by three to five weekly intramuscular injections with purified FeLV inactivated by ultraviolet irradiation. After the course of immunization, neither virus-neutralizing nor FOCMA antibody was detectable in the dams or in 19 kittens born to these cats. When these kittens were challenged with FeLV at 2 weeks of age, 18 of 19 developed persistent viremia and FeLV-related disease.

Two approaches currently being evaluated for control of feline leukemia virus (FeLV) infection are (i) elimination of infected cats and (ii) immunoprophylaxis of susceptible cats. Because FeLV is transmitted horizontally among outbred cats (10, 14), both approaches are feasible. Hardy et al. (9) have shown that testing and removal of actively infected (viremic) cats is effective in reducing or eliminating FeLV infection and disease in test populations of cats. Although a limitation of this approach is the need for constant monitoring and control of the population to insure continual exclusion of FeLV-infected cats, the data have established conclusively that feline leukemia has epidemiological characteristics of a contagious disease. Vaccination of cats, therefore, is a logical alternative, particularly since humoral immunity appears to be important in resistance to feline oncornaviruses (1-5, 21).

Active humoral immunity against the feline oncornavirus-associated cell membrane antigen (FOCMA) (2) develops in cats that resist natural or experimental infection with virulent FeLV (1-5, 21). It has been possible to induce active immunity to FOCMA by immunizing cats with killed FOCMA-containing tumor cells (15, 18, 19); however, comparable neutralizing antibody against FeLV or feline sarcoma virus (FeSV) has been difficult to induce in kittens with inactivated virus (19, 27). The latter is especially relevant, since pre-existing antibody to FOCMA does not necessarily protect cats from FeLV viremia (18, 19). Passively acquired FOCMA and virus-neutralizing (VN) antibody also protects against challenge with virulent FeSV, as shown by Essex et al. (2) for kittens suckling dams previously exposed horizontally to feline oncornaviruses and by Olsen et al. (19) for kittens suckling dams immunized with inactivated FeSV. One implication of these data is that since natural susceptibility to feline oncornaviruses is highest in the first 2 months of life (12, 25), vaccination of mother cats would confer passive immunity to suckling kittens during the period of maximal susceptibility. Subsequently, active immunization of weanling kittens could be initiated.

The objectives of this study were (i) to evaluate an inactivated FeLV vaccine for its ability to induce humoral immunity in pregnant cats and thereby protect kittens born to these cats from challenge with virulent FeLV and (ii) to compare this immunity with that possessed by

pregnant cats immunized by previous exposure to virulent FeLV.

MATERIALS AND METHODS

Cats. All cats were from a closed breeding colony of the Department of Veterinary Pathobiology, The Ohio State University. The cats are of hysterectomy-derived ancestry and are free of ectropic FeLV infection and FeLV-related diseases. Pregnant cats used for immunization were estimated by abdominal palpation to be between 28 and 35 days of gestation.

Virus preparation and inactivation. The Kawakami-Theilen strain of FeLV (16) used for immunization of pregnant cats was harvested from a feline lymphoblast cell line (FL-74) (26). The FeLV-KT contains A-, B-, and C-subgroup specificities. Ten-liter batches of FL-74 culture fluid were concentrated 10- to 20-fold using a Pellicon cassette system (Millipore Corp., Bedford, Mass.) with a 5-square-foot (0.46-m^2) cassette (10^6 nm/μl). Retentate fluids containing virus were then centrifuged at 56,700 × g over 50% ribonuclease-free sucrose (Schwarz/Mann, Div. of Becton, Dickinson & Co., Orangeburg, N.Y.) cushion prepared in TE buffer [0.1 ml of NaCl, 0.01 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.2) and 0.001 M ethylenediaminetetraacetate]. Virus harvested from the cushion was then purified by two cycles of isopycnic banding in 0 to 50% sucrose (centrifugation for 18 h at 57,600 × g in a Beckman SW27 rotor; Beckman Instruments, Inc., Fullerton, Calif.). Virus bands were collected, dialyzed against TE buffer to remove sucrose, and stored in 2-ml portions at −70°C. Inactivation of purified virus was accomplished by ultraviolet (UV) irradiation at 150 ergs/mm^2 per s for a total accumulated dose of 35,000 ergs/mm^2 (27). The purified FeLV-KT contained between 5 × 10^{10} and 10^{11} viral particles per ml, as determined by electron microscopic enumeration.

The Rickard strain of FeLV (FeLV-R; subgroup A) (20) was used for oncogenic challenge of kittens. The inoculum consisted of a clarified 20% (wt/vol) homogenate of thymic lymphosarcoma tissue and contained 10^5 focus-forming units/ml as assayed in the 81 cell culture system originated by Fischinger et al. (6). The biological response of cats to the FeLV-R inoculum has been described (12).

Immunization with UV-inactivated FeLV. Eight pregnant adult female cats were immunized with three to five weekly intramuscular injections of 1 ml of concentrated UV-inactivated FeLV-KT emulsified in an equal volume of Freund complete adjuvant. Injections were begun as soon as pregnancy was diagnosed (28 to 35 days) and continued until parturition occurred. Two nonpregnant adult female cats were given eight weekly immunizations with the same vaccine. Serum samples and blood smears were collected from each cat before each immunization.

Immunization by natural exposure to virulent FeLV. Two female cars were exposed to virulent FeLV-R by contact with FeLV shed by suckling litters of kittens that had been inoculated with FeLV-R. Thus, the means of natural FeLV exposure duplicated that described by Essex et al. (2) for FeSV in adult female cats.

Challenge of kittens. Kittens born to vaccinated or naturally immunized dams were challenged at 2 weeks of age by intraperitoneal injection of an established oncogenic dose of FeLV-R of tissue origin. The inoculum had been shown to produce an incidence of persistent viremia and FeLV-related disease (notably, thymic lymphosarcoma) in 85 to 100% of cats inoculated at less than 8 weeks of age (12). Serum samples and blood smears were collected from all kittens 2 days after birth and at biweekly intervals thereafter. All kittens were observed for evidence of FeLV-related disease for up to 6 months postchallenge.

Serological tests. The presence of feline leukemia group-specific antigen (gsa) in circulating leukocytes was determined by the indirect immunofluorescence test described by Hardy et al. (8). The FeLV antiserum was of goat, rather than rabbit, origin (18).

FOCMA antibody was assayed by the indirect membrane immunofluorescence (IMI) test described by Essex et al. (2). The immunoglobulin class of FOCMA antibody was determined by a tertiary (IMI) test in which either goat anti-human immunoglobulin G (IgG) or goat anti-human IgM serum (Hyland, Div. of Travenol Laboratories, Inc., Costa Mesa, Calif.) was substituted for the rabbit anti-feline globulin serum (Sylvana, Millburn, N.J.) usually used as the secondary reagent in the IMI FOCMA test (2). The cross-reactivity and specificity of the anti-human IgG and IgM reagent with putative feline IgG and IgM were determined by immunoelectrophoresis against whole feline serum. A single line of precipitation formed in the region and shape corresponding to the migration of feline IgG and IgM (23) (Fig. 1). The tertiary reagent was fluorescein isothiocyanate-conjugated rabbit anti-goat immunoglobulin (Hyland Laboratories).

VN antibody against FeLV subgroup A was determined as described by Schaller and Olsen (22).

RESULTS

Adult female cats vaccinated with UV-inactivated FeLV. Of 10 pregnant cats vaccinated from three to five times over a period of 5 to 6 weeks, 9 failed to develop demonstrable VN antibody titers (i.e., < 1:2). One cat developed a titer of 1:16 after four immunizations but died of suppurative pyelonephritis prior to parturition. The VN titers of two nonpregnant cats also remained at <1:2 after eight weekly immunizations with UV-inactivated FeLV-KT (Table 1).

Adult female cats exposed to virulent FeLV. Two cats that had suckled litters of FeLV-inoculated kittens developed VN titers of 128 and 64 (geometric mean = 90) and FOCMA antibody titers of 32 and 16 (geometric mean = 21), respectively (Table 2). Neither cat became FeLV gsa positive.

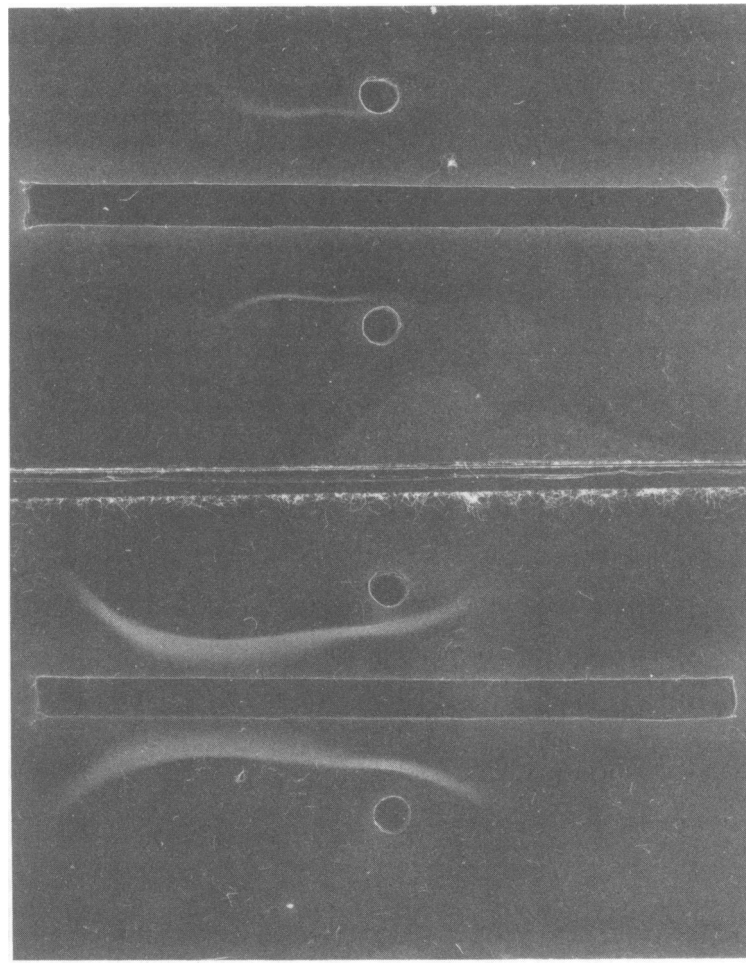

FIG. 1. *Immunoelectrophoresis patterns with goat anti-human IgM (top trough) of anti-human IgG (bottom trough) versus whole feline serum. A single line of precipitation overlies the expected position of the arcs for feline IgM and IgG, respectively. Cathode at left.*

Kittens born to cats immunized with UV-inactivated FeLV. Eighteen of 19 kittens born to six dams immunized with UV-inactivated FeLV became persistently FeLV gsa positive (viremic) after challenge with virulent FeLV. All of the 18 FeLV gsa-positive kittens developed FeLV-related disease in 6 to 35 weeks (Table 1). Twelve of the 18 cats developed thymic lymphosarcoma, and 6 died either of early undetermined causes (no necropsy possible) or of bacterial sepsis. The one cat to remain FeLV gsa negative after challenge developed persistent FOCMA and VN antibody titers and remained free of disease after 40 weeks of observation.

Kittens born to cats immunized by virulent FeLV. By 3 days of age, all kittens had acquired VN and FOCMA antibody titers ranging from 25 to 100% of maternal titers. In all kittens, VN and FOCMA antibody titers decayed to <1:2 by 2 months of age (Table 2, Fig. 2). A secondary rise in both titers occurred between 4 and 5 months of age in all four kittens of one litter but in neither of two kittens in the second litter. At the time of FeLV challenge at 2 weeks of age, kittens had a geometric mean VN titer of 13 (range, 8 to 16), and the mean FOCMA titer was 8 (range, 4 to 16) (Table 2, Fig. 2). After FeLV inoculation, none of the six kittens became FeLV gsa positive and none developed disease after 7 months of observation (Table 2).

Immunoglobulin class of FOCMA antibody in dams and kittens. Table 3 illustrates the IgG and IgM profiles of kittens with passively and actively acquired FOCMA antibody. In both the dams' and kittens' sera, the major FOCMA

antibody was IgG. In most cases, IMI FOCMA titers were within one twofold dilution of each other, whether rabbit antiserum against feline globulin (the usual secondary reagent in the IMI FOCMA test) or goat anti-human IgG was used as the secondary reagent. In two of the four kittens in which secondary FOCMA titers developed, both initial (passive) and secondary

TABLE 1. *Response of pregnant cats to immunization with UV-inactivated FeLV and susceptibility of their kittens to challenge with virulent FeLV*

Immunized pregnant cats					Kittens born to immunized pregnant cats							
Animal no.	No. of immunizations	Serological data postimmunization (time of parturition)			Animal no.	Serological data pre-FeLV challenge[a] (3 days to 2 weeks of age)			Serological data post-FeLV challenge (6 to 30 weeks of age)			
		VN antibody	FOCMA antibody	FeLV gsa		VN antibody	FOCMA antibody	FeLV gsa	VN antibody	FOCMA antibody	FeLV gsa	FeLV-related disease
83R	5	<2	2	–	83R-1	<2	<2	–	<2	<2	+	+
					-2	<2	<2	–	NT[b]	<2	+	+
					-3	NT	<2	–	NT	<2	+	+
					-4	NT	<2	–	NT	<2	+	+
88R	5	<2	<2	–	88R-1	<2	<2	–	<2	<2	+	+
					-2	<2	<2	–	NT	<2	+	+
					-3	NT	<2	–	NT	<2	+	+
					-4	NT	<2	–	NT	<2	+	+
152R	3	<2	<2	–	152R-2	<2	<2	–	<2	<2	+	+
					-4	NT	<2	–	NT	<2	+	+
					-5	NT	<2	–	NT	<2	+	+
					-6	NT	<2	–	NT	<2	+	+
895	5	<2	<2	–	895-2	<2	<2	–	<2	<2	+	+
921	5	<2	<2	–	921-1	<2	<2	–	<2	<2	+	+
964	5	<2	2	–	964-1	<2	<2	–	<2	<2	+	+
					-2	<2	<2	–	NT	<2	+	+
					-3	<2	<2	–	NT	<2	+	+
					-4	<2	<2	–	<8	<8	–	–
822[c]	4	<2	<2	–	None							
890[d]	4	16	4	–	None							
81B[e]	8	<2	<2	–	None							
59B[e]	8	<2	<2	–	None							

[a] Challenge by intraperitoneal injection of a known oncogenic dose of FeLV-R at 2 weeks of age.
[b] NT, Not tested.
[c] Kittens stillborn.
[d] Dam died of suppurative pyelonephritis before parturition.
[e] Nonpregnant cat.

TABLE 2. *Passive transfer of VN and FOCMA antibody from mother cats immunized by exposure to virulent FeLV and susceptibility of their kittens to FeLV challenge*

Animal group (no.)	Time after parturition	Geometric mean titer		FeLV gsa status	FeLV-related disease
		VN	FOCMA		
Dams (2)	0	90	22	neg[c]	neg
	2 weeks[a]	NT[b]	NT	neg	neg
	1 mo	90	22	neg	neg
	3 mo	90	32	neg	neg
Kittens (6)	0	27.9	13.9	neg	neg
	2 weeks[a]	13.0	8.0	neg	neg
	1 mo	4.6	3.2	neg	neg
	3 mo	0.0	1.2	neg	neg
	6 mo	2.5	7.0	neg	neg
	7 mo	6.5	3.5	neg	neg

[a] FeLV challenge of kittens at 2 weeks of age.
[b] NT, Not tested.
[c] neg, Negative.

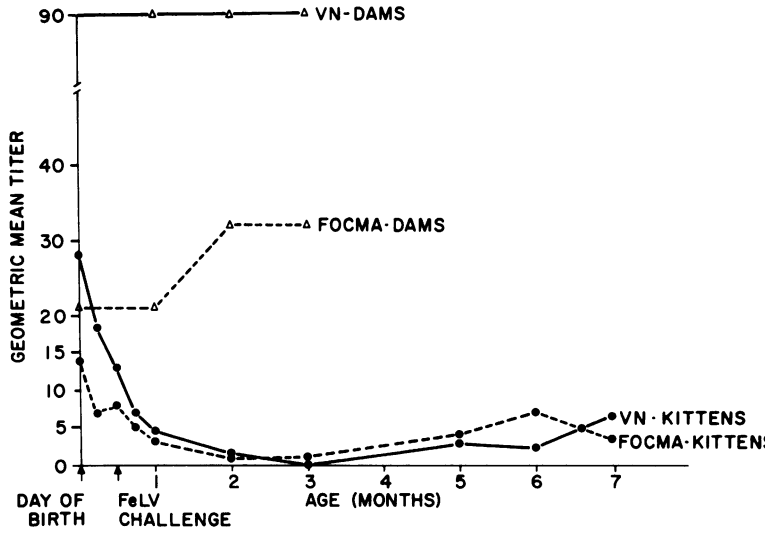

FIG. 2. *Passive transfer of VN and FOCMA antibody from mother cats immunized by exposure to virulent FeLV to their suckling kittens.*

TABLE 3. *Immunoglobulin class of FOCMA antibody in serial serum samples from a kitten (956-1) born to a dam with FOCMA antibody acquired by prior exposure to virulent FeLV*

Time after birth (mo)	Secondary reagent used to determine IMI FOCMA titer		
	Anti-feline globulin[a]	Anti-IgG	Anti-IgM
0	32	NT[b]	NT
1	4	8	2
2	2	2	16
3	2	4	2
5	16	32	2
7	32	32	2

[a] Reciprocal of highest positive dilution.
[b] NT, Not tested; serum at this interval exhausted.

titers were composed of IgG with trace amounts of IgM. In the remaining two kittens, putative IgM IMI FOCMA antibody was detected in the absence of IgG activity at 2 months of age (Table 3) when little or no FOCMA titer was demonstrable using the usual anti-feline immunoglobulin antiserum (which is directed almost exclusively toward feline IgG).

DISCUSSION

This report verifies that maternal transfer of protective levels of VN and FOCMA antibody occurs. The presence or absence of humoral antibody in dams correlated directly with resistance or susceptibility of their kittens to FeLV. One hundred percent of kittens with VN titers of at least 8 were protected against a known oncogenic FeLV challenge that proved fatal in 95% of kittens from dams without detectable VN titers. Our data concerning passive transfer of FOCMA antibody, resistance to oncogenic challenge, and the IgG nature of FOCMA antibody required by suckling kittens are similar to those obtained by Essex et al. (2) with FeSV. In addition, we found that decay of VN and FOCMA antibody in suckling kittens was virtually linear over a 2-month period, as expected for passively acquired antibody. Nomographs for maternal antibody to feline panleukopenia virus in kittens are similar, although feline panleukopenia virus titers are usually higher initially and thereby persist longer (24).

Our data also document that neutralizing antibody to FeLV is difficult to induce with inactivated virus. Little or no VN antibody was demonstrable after as many as eight weekly injections of concentrated UV-inactivated FeLV-KT. These observations agree with those of Olsen et al. (19) and Yohn et al. (27) concerning inactivated FeSV vaccines and with those of Hardy et al. (11) concerning FeLV preparations inactivated with formalin. Some possible explanations for the cats' minimal immune response to inactivated FeLV include the following. (i) Amplification of antigenic mass or the direct exposure of lymphoreticular organs that are associated with FeLV replication in vivo are critical to development of an effective immune response; (ii) immunogenicity of the virus is compromised during purification or inactivation; and (iii) FeLV glycoproteins must be presented in association with a cell membrane complex for appropriate recognition and trig-

gering of the VN antibody response. The inefficacy of inactivated FeLV as an immunogen is in contrast to the response of mice to inactivated Gross (13), Friend (7), and Rauscher (17) murine leukemia virus. Furthermore, mice suckling mothers vaccinated with formalized Gross or Friend murine leukemia virus were protected from homologous oncogenic challenge by passive maternal antibody (13, 17).

In conclusion, the presence of maternal humoral immunity to FeLV is transferred to suckling kittens and is effective in protecting these kittens from FeLV infection and disease. A major limitation to immunization procedures involving inactivated FeLV was the difficulty in obtaining an immune response to nonreplicating virus. Other protocols aimed at improving the immune response of cats to killed FeLV are currently being evaluated.

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