Effects of Cobra Venom Factor Treatment on Latent Feline Leukemia Virus Infection

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The role of the complement system in containment of feline leukemia virus infection was studied by cobra venom factor treatment of feline leukemia virus-immune cats. One to three weeks after cobra venom factor treatment, an increase in viral antigen in marrow myelomonocytic cells and circulating immune complexes was noted. Prevention of reactivation of feline leukemia virus infection may in part depend on an intact complement system.

The majority of adult cats exposed to feline leukemia virus (FeLV) develop a transient viremia, and clearance of the virus is associated with the development of virus-neutralizing antibody and antibody to the feline oncovirus-associated cell membrane antigen (FOCMA) (3, 7, 12, 28).

These cats are designated as regressors, and many of them have latent infection present in the bone marrow and lymph nodes as demonstrated by the presence of viral antigen in cultured myelomonocytic and lymphoid cells (29). Prevention of reactivation of FeLV infection in vivo may depend on an intact host immune system, since administration of high-dose steroids to regressor cats results in recurrence of viremia (29).

Although the natural history of FeLV infection and the importance of the immune response in controlling this infection have been intensively studied (4, 8, 13, 14), all the factors important in containing latent infection have not been established. We hypothesized that the complement system might play a role in containment of latent infection because of the following observations. (i) Addition of FeLV to normal human or cat serum activates the complement system with virolysis secondary to the generation of the C5 through C9 membrane attack complex (16). (ii) Antibody to FOCMA present on FeLV-transformed cells can only induce cell lysis in vitro in the presence of complement (6). (iii) Hypocomplementemia, possibly secondary to circulating immune complexes, is found in cats with persistent viremia or feline lymphosarcoma or both (2, 15). (iv) Antibody production against certain antigens and antibody-mediated cytotoxicity may require normal complement activity (17, 20, 21). (v) An intact complement system has been shown to be important in clearance of other viral infections (10).

In this study, we evaluated the role of complement in preventing reactivation of FeLV infection by examining the effects of complement depletion on regressor cats with latent infection.

Self-limiting FeLV infection was established in five adult specific-pathogen-free cats by intraperitoneal injection of the Rickard strain of FeLV in an inoculum of a thymic lymphosarcoma homogenate (5, 24). The regressor status of the cats was confirmed by demonstration of persistent FOCMA antibody titers by a standard membrane immunofluorescence assay (3) and absence of FeLV P27 in peripheral blood

neutrophils by a modification of the Hardy test (9, 14, 26). At 16 weeks postinfection, cats were evaluated for reactivatable productive infection. Femoral and humeral bone marrow was collected from cats by a standard technique, and the freshly isolated marrow cells (5×10^6) were then cultured for 6 to 14 days. Reactivable productive infection was demonstrated by cell-associated P27 in cytocentrifuge preparations (25, 26, 29).

Total hemolytic complement in these cats was determined by a modification of the method of Mayer (22) with sheep erythrocytes sensitized with rabbit immunoglobulin M (IgM) antibodies (Cordis, Miami, Fla.) as the target cells.

Immune complex levels were measured by using a modification of an enzyme-linked immunosorbent assay as described by Singh and Tingle (32). In this assay (34), human complement C1q was used as the basic coat, and the staphylococcus protein A horseradish peroxidase (Boehringer Mannheim Chemicals, Indianapolis, Ind.), was used as the enzyme conjugate. Values were expressed as aggregated cat IgG equivalents per milliliter of serum.

Complement depletion was achieved by intraperitoneal injection of cobra venom factor (CVF) derived from *Naja Haje* species (lot no. 74160; Cordis, Miami, Fla.); 200 U/kg were given in four divided doses over 24 h (1).

Before complement depletion, all cats had negative smears for FeLV P27 on blood and bone marrow exams but had evidence of latent viral infection as demonstrated by myelomonocytic cells that were positive for P27 after in vitro culture (29).

TABLE 1. Effect of CVF on latent FeLV infections in vivo

Weeks post-treatment	No. of cats positive for P27 in ^a :		FOCMA-L ^b	Total hemolytic complement ^c
	Marrow	Blood		
0	0	0	5.2 ± 0.6	52.8 ± 4.4
1	3	0	4.2 ± 1.3	0
2				50 ± 8
3	2	1	6.6 ± 0.7	ND ^d

^a The test group contained five cats.

^b FOCMA antibody titers are expressed as the base 2 logs of the titers and represent the geometric mean ± standard error of the mean.

^c Total hemolytic complement units are expressed as the mean ± standard error of the mean.

^d ND, Not done.

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FIG. 1. Immune complex levels in latent stage of FeLV infection. Cats were separated into two groups according to the peak level of immune complexes: cats with high levels (●) and cats with low levels (△) after CVF treatment. Level of immune complex is expressed as aggregated cat IgG (ACG) equivalents per milliliter.

One week after CVF treatment, when complement depletion was present, three of the five cats were positive for P27 in either blood or bone marrow myelomonocytic cells (Table 1). Two of the five cats were studied 3 months later when there was no evidence of virus expression in the peripheral blood or bone marrow. Treatment with saline alone did not induce viral replication in blood or bone marrow in either cat.

Since an increase in immune complex levels has been seen in cats with persistent viremia (2, 34), we next measured immune complex levels after CVF treatment. Three of the five cats showed a significant increase in C1q-precipitable immune complexes within 1 to 3 weeks after treatment. In two of the cats, this correlated with an increase in P27 in the bone marrow or blood. However, in the third cat, immune complexes were elevated without alteration of virus expression (Fig. 1).

Previous studies have demonstrated that immunosuppressive therapy such as corticosteroids and nitrosoureas significantly impaired host resistance to experimental infection with FeLV (29, 31) and allowed for the reactivation of latent infection in FeLV-immune cats (29). The host immune mechanisms which appeared to play a significant role in clearing FeLV and preventing reactivation included complement-dependent antibodies to FeLV-infected cells and antibody to virus alone (4, 12, 18, 19, 25).

In this study, we found that regressor cats made hypocomplementemic by CVF treatment had evidence of increased viral replication in bone marrow myelomonocytic cells and elevated levels of circulating immune complexes, suggesting a role for complement in preventing viral reactivation. Although these cats did not develop the persistent viremia seen when regressor cats were treated with pharmacological doses of steroids, this may be due to the difference in degree and duration of immunosuppression achieved (1, 20, 21, 27).

The rise in circulating immune complexes which followed CVF treatment is consistent with either an increase in circulating antigen due to viral reactivation or alteration in tissue binding and release of the complexes (23). It is not possible to differentiate between these two mechanisms.

We conclude that FeLV may persist as a latent infection in marrow myelomonocytic cells. Alteration in either the cel-

lular or humoral immune system which can occur due to infection with other viruses or bacteria may lead to viral recrudescence.

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