Abrogation of Resistance to Feline Oncornavirus Disease by Immunization with Killed Feline Leukemia Virus

Richard G. Olsen, Edward A. Hoover, Joseph P. Schaller, Larry E. Mathes, and Linda H. Wolff

Department of Veterinary Pathobiology, The Ohio State University, Columbus, Ohio 43210

SUMMARY

Four-week-old specific-pathogen-free cats were immunized with a combined vaccine composed of killed feline leukemia virus and killed feline oncornavirus-associated cell membrane antigen-containing tumor cells. Immunization induced feline oncornavirus-associated cell membrane antigen antibody titers ranging from 1:32 to 1:256 but did not elicit detectable virus-neutralizing antibody titers. Kittens immunized with tumor cells alone developed higher feline oncornavirus-associated cell membrane antigen antibody titers (ranging from 1:512 to 1:2048) than those given the combined vaccine.

All kittens were challenged with virulent Snyder-Theilen feline sarcoma virus at 12 weeks of age. Seventy-five % of the kittens vaccinated with combined vaccine and 67% of the combined vaccine.

INTRODUCTION

FeLV\(^2\) disease includes an active virus infection and the formation and growth of malignant transformed cells. Subclinical FeLV infection of cats induces antibodies to virus envelope subgroup-specific antigens (1) that prevent viremia. In addition, these animals produce active immunity to FOCMA (4). The presence of FOCMA antibody in cats correlates with resistance to oncogenicity by FeLV or FeSV (3). Thus resistance to and recovery from feline oncornavirus disease depends upon development of at least 2 separable host immunological responses: 1 toward the infecting virus and 1 toward surface antigen of neoplastic cells.

Vaccination against FeSV with a killed virus vaccine alone does not appear to protect actively immunized kittens (15) against homologous virulent virus challenge. Kittens that have suckled similarly vaccinated queens, however, were protected from the same oncogenic virus challenge (15).

Kittens actively vaccinated with FOCMA-containing killed tumor cells were resistant to FeSV-induced fibrosarcomas (13). However, kittens that completely regressed fibrosarcomas did not produce VN antibody to FeSV and became persistently viremic.

The objective of the study reported here was to evaluate immunization of cats against combined killed FeLV and an inactivated feline oncornavirus tumor cell vaccine on: (a) oncornavirus infection, as determined by the prevention of FeLV viremia; and (b) protection from malignant tumor formation after the challenge of immunized cats and control cats with virulent feline oncornavirus.

MATERIALS AND METHODS

Cats. All cats used in this study were from a closed breeding colony maintained at The Ohio State University (17). These animals were of hysterectomy-derived ancestry and were maintained in isolation from conventional cats and other laboratory animals. The cats were similar in most respects to conventional cats, but they were uniformly free of antibody to the feline oncornaviruses and other feline viruses. All kittens were 4 weeks old at the time of 1st vaccination and then were vaccinated at 6 and 8 weeks thereafter. The animals were subsequently challenged with oncogenic virus at 12 weeks at age.

Feline Oncornavirus Tumor Cell Vaccine. Tumor cell vaccines were prepared from an established cell line of FL-74, originally established by Thelen et al. (20) from a FeLV-induced lymphoma.

FL-74 cells were grown (14) in 1000-ml roller bottles (Falcon Plastics, Oxnard, Calif.) with McCoy's 5A growth medium (Grand Island Biological Co., Grand Island, N. Y.) and were titrated with 2% NaHCO\(_3\) to pH 7.0. Antibiotics, 50 IU of penicillin and 50 µg of streptomycin per ml, were added to the culture medium.

The method of thermal inactivation of FL-74-associated infectious FeLV and inactivation of tumor cell viability has been described (7). FL-74 cells were harvested, washed 3 times in Hanks' balanced salt solution, supplemented with 5% calf serum, and resuspended at a concentration of 3.6 x 10\(^6\) cells/ml in the same buffered salt solution. The cells were dispensed in 3-ml quantities and were incubated in a 56° water bath for 3 min with constant agitation. To stop the reaction, the cells were transferred immediately to a 4° ice bath. This heat treatment inactivated all cell-bound infectious FeLV and did not significantly reduce the antigenicity of the FOCMA on the cell membrane (7). All FL-74 cell
vaccine preparations were prepared on the day that they were to be used.

**Killed FeLV Vaccine.** Killed Kawakami-Theilen FeLV which contains a-, b-, and c-type specificities was used as source of vaccine virus.

Spent culture media from FL-74 were concentrated by continuous-flow filtration (Millipore Corp., Bedford, Mass.) and were purified twice by sucrose gradient density centrifugation as described by Mathes *et al.* (12). Virus preparations of 1.0 × 10^{11} virus particles/ml were routinely obtained and subsequently inactivated by UV radiation (22).

**Feline Oncornavirus Challenge.** ST-FeSV, prepared by a modified procedure of Moloney (10), was used as the oncogenic challenge in vaccinated and control cats. This virus contains a- and b-type specificities. Ten % homogenates of ST-FeSV-induced fibrosarcomas were prepared in a Sorvall Omnimixer in L15 medium. Homogenized tumor suspensions were clarified at 2,300 × g for 20 min followed by a 18,800 × g treatment for 1 min. Cell-free supernatants were prepared in 1.0-ml aliquots and were stored at −70°.

One ml of challenge ST-FeSV inoculated s.c. in the shoulder area produced a greater than 75% incidence of tumors in 12-week-old kittens. Vaccinated and nonvaccinated kittens of comparable ages were inoculated similarly with the same pool of infectious ST-FeSV.

**Determination of FeLV Viremia.** The presence of FeLV group-specific antigen in circulating leukocytes was determined by the indirect immunofluorescence test described by Hardy *et al.* (6). The primary reagent, goat anti-Kawakami-Theilen FeLV serum (19), was adsorbed *in vivo* according to the procedure described by Hardy *et al.* (6). In brief, the goat serum (35 ml) was injected i.p. in a 2.7-kg, 4-month-old, specific-pathogen-free cat. After 18 h, the cat was exsanguinated. The serum was recovered, aliquoted, frozen, and stored at −70°.

**Serological Tests.** Indirect immunofluorescent antibody titers to FOCMA were determined according to procedures described by Essex *et al.* (3). VN antibody was determined by a modified microtest procedure previously described (19). The test determined the capacity of cat sera to neutralize focus-forming activity of Subgroup A FeLV on 81C sarcoma-positive leukemia-negative cells (5).

**RESULTS**

**Incidence and Growth of Tumor in Vaccinated Cats.** Results of immunization of 12 kittens with a combined vaccine of 1.0 × 10^{6} heat-killed FL-74 cells and 1.0 × 10^{11} killed Kawakami-Theilen FeLV particles are summarized in Table 1. Control kittens were of 2 groups: (a) kittens immunized with complete Freund’s adjuvant alone; and (b) kittens immunized by the same vaccine protocol, with heated, killed FL-74 cells alone.

All kittens were monitored weekly throughout the experiments for group-specific antigen in the peripheral leukocytes and for FOCMA antibody and VN antibody. Palpation for tumors was performed daily.

After ST-FeSV challenge, 9 of 12 (75%) kittens immunized with combined vaccine developed progressive fibrosarcoma. The incidence of tumors in the nonvaccinated control kittens was similar (67%). By contrast, kittens vaccinated with FL-74 cells alone developed no progressive tumors. The tumor responses in the control cats and cats given only killed FL-74 cells were very similar to responses that we reported earlier (13).

After challenge, 83, 83, and 75% of control, FeLV/FL-74 cell-vaccinated, and FL-74 cell-vaccinated cats, respectively, became viremic after ST-FeSV challenge. None of the vaccinated cats developed VN antibody before challenge.

Chart 1 shows the comparison of the mean tumor diameter of each group of cats after infection with ST-FeSV. By 2 weeks postinfection, the mean tumor size of the control cats was larger than either vaccinated group. However, the cats that received the combined vaccine had a similar incidence of progressive fibrosarcoma. By 3 to 5 weeks postchallenge, the mean size of tumors in cats vaccinated with FL-74 cells alone was significantly smaller (*p* = 0.001) as compared to either of the other 2 groups.

**FOCMA Antibody Responses in Vaccinated Cats.** One hundred % of all kittens immunized with either the combined vaccine or the FL-74 cell vaccine alone developed FOCMA antibodies (Table 1).

Chart 2 compares the FOCMA antibody responses of the 2 vaccinated groups. Five weeks after the initial vaccination, the geometric mean antibody titer of the combined vaccine group was approximately 1:16. By contrast, the group of cats that received FL-74 cells alone developed a geometric mean of 1:1024. This significantly different FOCMA response (*p* = 0.001) was continually observed through 2 weeks after challenge.

None of the nonvaccinated cats developed FOCMA antibody before ST-FeSV challenge.

**DISCUSSION**

FOCMA is antigenically distinct from FeLV structural components (2), and immunity to FOCMA correlates with the cat’s capability to regress tumors (4). This was supported by studies that included the immunization of cats with killed FL-74 cells (13) and living FL-74 cells (11). In these studies, cats produced specific FOCMA antibody and were 100% resistant to progressive FeSV-induced fibrosarcomas. However, immunization did not induce VN antibody or prevent viremia after oncogenic virus challenge.

In the present study cats immunized with FL-74 cells were again resistant to progressive fibrosarcomas but were not resistant to induction of persistent FeLV viremia after challenge with oncogenic FeSV.

The same ST-FeSV challenge in cats immunized with the combined inactivated FL-74 cell and killed FeLV preparation induced an incidence of 75% progressive fibrosarcomas. This was similar to the incidence in the unvaccinated control cats (67%). Likewise, cats immunized with combined FeLV-tumor cell vaccine exhibited a strikingly lower FOCMA antibody response in comparison to cats immunized with cells alone. Thus, it appears that the protective immunity to feline oncornavirus disease was hindered rather than enhanced by inclusion of inactivated, purified FeLV as a vaccine component.

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Table 1
Evaluation of the responses of cats immunized with a combined vaccine composed of inactivated FL-74 cells and inactivated FeLV and then challenged with feline sarcoma virus

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% FOCMA antibody
% malignant tumor development
% viremia

* BC, before challenge; -, negative reaction; +, positive reaction; +/-, transient viremia; AC, after challenge; P, progressively growing fibrosarcoma; R, regression of fibrosarcoma; NT, no tumor developed.

Induction of immune serum factors that block cytotoxic immune factors (8) may account for the loss of protective immunity in the feline system. However, cats given the combined FeLV/FL-74 cell vaccine did not develop detectable levels of VN antibody. Wood (21) recently reported that immunization with attenuated Moloney sarcoma virus abrogates protective immunity in adult BALB/c mice and that the attenuated virus was apparently immunosuppressive. In contrast to the murine system reported by Wood (21), it has been reported that the adult cats respond to killed FeLV by producing high-titer VN antibodies (15). Moreover, kittens that suckle FeLV-immunized queens exhibited a high degree of resistance to feline oncornaivirus disease (15, 22).

By contrast to the adult cat, the young kitten (less than 4 months old) produces little or no VN antibody to either killed FeLV (15) or infectious FeLV (9). Schaller et al.3 have presented evidence that the killed FeLV vaccination of young kittens, in fact, causes tumor enhancement.

We have recently demonstrated (unpublished observations) that killed FeLV inhibits the cat lymphocyte blast transformation to concanavalin A. Dr. A. Hellman and Dr. A. Fowler (personal communication) demonstrated the same type of inhibition of mouse lymphocytes by murine leukemia virus proteins.

Immunosuppression is associated with persistent FeLV infection, and it precedes oncogenesis (16). The present study suggests to us that either FeLV itself or the cat's
Resistance to Feline Oncornavirus Disease

Chart 1. Fibrosarcoma growth s.c. at sites of inoculation in vaccinated and control cats. All cats were 12 weeks old at challenge with a single dose of Snyder-Thilen FeSV. The difference in mean tumor diameter of cats vaccinated with combined vaccine as opposed to cats immunized with FL-74 cells alone was significant at 0.01 and 0.005 at 4 and 5 weeks after challenge, respectively.

Chart 2. Geometric mean antibody titer s of cats immunized with the various feline oncornavirus vaccines. The differences in FOCMA antibody response of cats given combined vaccine as opposed to cats immunized with FL-74 cells alone were significant (p = 0.001) at all time periods from 4 weeks postvaccination through 2 weeks after FeSV challenge.

encounter with FeLV results in an immunosuppressive or enhancing factor that favors development of FeLV-related neoplasia.

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REFERENCES

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