Experimental Oncornavirus Vaccines in the Cat


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Summary

An experimental approach to the immunoprophylactic control of feline oncornavirus-mediated diseases has included induction of antiviral immunity and antibodies to the feline oncornavirus-associated membrane (tumor) antigens. A suitable model for exploring the effectiveness of killed oncornavirus vaccines in the cat has been provided by the use of feline sarcoma virus. Immunization of seven pregnant queens over a 6-week period with ultraviolet light-inactivated Gardner-Arnstein feline sarcoma virus resulted in significant protection among 12 kittens challenged with a tumor-forming Dose 90 at 7 days of age. This immunity was not present in kittens challenged at 35 days of age. Among 12 kittens born of queens immunized during pregnancy with ultraviolet light-inactivated Kawakami-Theilen feline leukemia virus and challenged with the same live virus at 4 days of age, significant protection was noted, ranging from prolongation of survival time to complete protection in 3 kittens. In general, the higher the antibody titer in the mother, the more effective the protection afforded the kittens.

Immunization of 43 kittens during their first 5 weeks of life with the same vaccines used in adult cats did not immunize sufficiently to protect against feline sarcoma virus challenge at 5 weeks of age. Neutralizing antibody responses in these kittens were significantly lower than in pregnant queens. That kittens of this age are immunologically responsive was established, since complete protection of 9 kittens to feline sarcoma virus was obtained by immunization with a crude tumor extract inactivated with 5 to 7 megarads of γ-irradiation. All these kittens developed feline oncornavirus-associated membrane antibodies while 3 developed demonstrable levels of virus-neutralizing antibodies.

The results of these studies are believed indicative that killed virus vaccines and tumor vaccines can be effective immunoprophylactic measures in the control of RNA tumor virus oncogenesis in the cat. Developments in this model system should be relevant to any consideration given similar vaccines in humans.

Introduction

The feline leukemia-sarcoma virus-induced diseases provide excellent models in which the role of immunity to mammalian oncornaviruses can be evaluated, since these diseases occur naturally (17), can be produced experimentally in outbred animals, and are transmitted horizontally in nature. Accordingly, experimental studies with postnatal viral inoculation simulate the natural pathogenesis of these diseases. Thus it is reasonable to develop and evaluate RNA tumor virus vaccines in the cat as a means of preventing experimental and naturally occurring oncornavirus-induced disease.

There is evidence that both active (12, 15) and passive (5) immunity can be induced to feline oncornaviruses and that each protects against oncornaviral disease. Precedent for the feasibility of using immunoprophylaxis to prevent leukemia has been shown in the murine oncornavirus systems (1, 7, 13).

The objectives of the studies reported here were to characterize the immune response of the cat to feline oncornavirus and to determine whether oncogenesis by feline oncornaviruses could be prevented by immunoprophylaxis. The eventual objective of this work is to provide a nucleus of data that may be applied to future development of vaccines against human oncornaviruses.

Our ultimate goal will be to define the various viral vaccine and host factors that lead to optimum production of antiviral neutralizing antibody, anti-tumor antigen antibody, and protection from oncogenesis in vivo following viral challenge.

Materials and Methods

Cats. All cats used in this study were from a closed breeding colony maintained at The Ohio State University. These animals are of hysterectomy-derived ancestry and are maintained in isolation (Shope units) from conventional cats and other laboratory animals. The cats are similar in most respects to conventional cats but are uniformly free of antibody to the feline oncornaviruses and other feline viruses.

Viruses. The GA-FeSV1 (10) used in vaccination studies was obtained from Electronucleonics Inc. (Bethesda, Md.) as tissue culture-grown and sucrose gradient-purified virus. Virus for in vitro serum neutralization studies was the ST-FeSV (19) prepared as cell-free 10% tumor homogenates. Homogenized tumor suspensions were clarified at 2,300 ×

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1 Presented at the symposium "Immunological Control of Virus-associated Tumors in Man: Prospects and Problems," April 7 to 9, 1975, Bethesda, Md. Supported in part by NIH Contract CP-43217 and American Cancer Society Grant IM 27.

2 Presenter.

3 The abbreviations used are: GA-FeSV, Gardner-Arnstein feline sarcoma virus; ST-FeSV, Snyder-Theilen feline sarcoma virus; R-FeLV, Rickard feline leukemia virus; KT-FeLV, Kawakami-Theilen feline leukemia virus; FeSV, feline sarcoma virus; FeLV, feline leukemia virus; FOCMA, feline oncornavirus membrane antigen; SN, serum-neutralizing antibody; IFA, immunofluorescence.
g for 20 min, followed by an 18,000 × g clarification for 1 min. Supernatants were aliquoted and stored at −70°C. The R-FeLV (18) was prepared from 20% tumor homogenates of thymus, spleen, and bone marrow in our 2nd and 4th in vivo passages. These homogenates were clarified at 2,300 × g for 20 min, aliquoted, and stored at −70°C and −170°C. The KT-FeLV (16) was prepared from tissue culture supernatants of feline lymphoblastoid line FL-74 (20); these preparations were concentrated by ultracentrifugation and twice banded in sucrose gradients.

**Vaccine Preparation.** UV inactivation of purified GA-FeSV was accomplished at a surface dose rate of 150 ergs/sq mm/sec for an accumulated total dose of 35,000 ergs/sq mm. The D_{10} for the ST-FeSV was approximately 3,500 ergs and was close to that obtained for the murine sarcoma viruses (21). Formalin inactivation of GA-FeSV was performed by the method of Fink and Rauscher (6). Immediately prior to use, inactivated virus vaccines were emulsified in an equal volume of Adjuvant 65 or Freund’s complete adjuvant.

Inactivation of virus in tumor antigen vaccines was by γ-irradiation. Aliquots of a 10% homogenate of ST-FeSV-induced tumor cells were treated with various doses of γ-irradiation by Dr. Darwood B. Rowley, at the United States Army Nadiq Laboratories. Vaccination experiments used tumor cell vaccines irradiated with 5, 6, and 7 megarads, respectively.

**In Vitro Virus Assay Methods.** FeSV’s were assayed for focus-forming activity in early passages (3 to 15) of feline embryo cells. Actively growing cells were seeded in plastic Petri dishes (2 × 10^5/dish) in 4.5 ml of Medium L-15 and 15% fetal calf serum. After 24 hr, the growth medium was removed and the cells were treated with 1 ml DEAE-dextran (40 μg/ml) in Medium L-15 without fetal calf serum. After 20 min, this medium was removed and the plates were rinsed with 5 ml of growth medium. Monolayers were then overlaid with 0.2 ml of appropriate virus dilutions for 2 hr. Plates were rocked at 10- to 15-min intervals to maintain an even distribution of the inoculum. The latter was removed and replaced with 5 ml of growth medium. Monolayers were fed with fresh medium on the 7th day after infection and fixed with buffered formalin and stained with Giemsa. 3 to 4 days later. Foci were counted at ×25 or ×40 magnification with a dissecting microscope.

R-FeLV was assayed for helper focus-inducing activity on a sarcoma-positive, leukemia-negative feline cell line kindly supplied by Fischinger et al. (8). This cell line is designated subclone 81 of a Moloney murine sarcoma virus transformed CCC cat cell line 6c (9). The focus-induction assay procedure was similar to that described above for ST-FeSV on feline embryo cells except that McCoy Medium 5A with 15% fetal calf serum was used as growth medium (8).

**In Vivo Virus Assay Methods.** Vaccinated and nonvaccinated kittens received injections in the flank area with infectious FeSV at 5 to 6 weeks of age after active immunization, or during the 1st week of life in passive immunization studies (pregnant queen immunization). Inoculation sites were palpated at frequent intervals, and tumor sizes were measured with calipers to determine mean tumor induction times and to estimate tumor growth rates. Serum samples were collected at weekly intervals during the 1st 6 to 8 weeks, and biweekly thereafter. Cats inoculated with FeSV were observed for 35 to 40 weeks, while those given FeLV were observed 12 to 18 months. All cats that expired or were euthanized at terminus were necropsied, and the extent of metastases was determined.

**Serological Tests.** Indirect immunofluorescent antibody titers to FOCE were determined according to procedures described by Essex et al. (4).

SN’s were determined by a modified microtest procedure originally described by Jarrett et al. (14), using Falcon Microtest II plastic plates. Twofold serum dilutions (inactivated at 56°C for 30 min) were prepared in Leibowitz Medium L-15 with 10% γ-globulin-free fetal calf serum in plastic Falcon microtest plates (0.1 ml/well). Five to 10 focus-forming units of FeSV (ST tumor homogenate) in a volume of 0.025 ml were added to all the serum and the cell control wells. Plates were shaken briefly and incubated for 60 min at room temperature or at 4°C. Ten thousand feline embryonic indicator cells were added in a volume of 0.025 ml. After shaking, the plates were incubated for 18 hr at 37°C, and the supernatant medium was then replaced. Medium was again replaced after 3 to 4 days before being fixed on the 11th or 12th day in 10% buffered formalin and stained with Giemsa. The total number of foci in 3 wells/dilution was counted. Virus survival was plotted as a function of serum dilution, and survival curves were constructed for 3 or more points. Serum-neutralizing titers represented that serum dilution that neutralized 80% of the viral infectivity (2). Hyperimmune goat anti-KT-FeLV served as positive antibody control for all SN assays.

**Results**

Owing primarily to the greater rapidity of detectable clinical responses, most studies were performed with FeSV rather than FeLV.

**Serum Neutralization Responses in Cats Immunized with Killed Feline Oncornavirus Vaccines.** In order to evaluate the immunogenicity of inactivated Ga-FeSV, 43 kittens and 7 pregnant queens were immunized (Table 1). No significant levels of serum-neutralizing antibodies were observed in kittens during the 1st 4 weeks (4 injections of 3 × 10^6 virus particles). In contrast, 6 of 7 pregnant queens given 4 injections of the same vaccines responded with a mean SN titer of approximately 1:8. By 7 weeks, the mean SN titer of the queens was 1:14, whereas only 1 group of kittens, those given FeSV inactivated with UV and emulsified in Adjuvant 65, indicated some response. By 2 to 5 weeks postchallenge (Table 1, Columns 7 to 10), this group showed a mean SN titer of approximately 1:5. No other significant SN antibody titers developed in the kittens. None of the queens or kittens produced antibodies to FOCE.

**Effects of Immunization of Kittens with UV or FormalinInactivated GA-FeSV on Tumor-Growth Patterns and Survival Times.** Two groups of 14 kittens given killed GA-FeSV vaccines and challenged with a 90% tumor dose of FeSV at 5 to 6 weeks of age (Table 2) showed no significant differences in tumor latent periods and in mean survival times.
than did a nonvaccinated group. Similarly, no differences were observed in tumor-growth patterns among nonimmunized and immunized kittens. Approximately 50% of kittens in both categories died from their tumors or were sacrificed during terminus. These results indicate that the low levels of antibody induced in the kittens (Table 1) were not protective against a 90% tumor dose of GA-FeSV.

**Live Virus Challenge of Kittens Born of Queens Immunized with Inactivated FeSV or FeLV.** Evidence of passively transferred immunity to FeSV or FeLV was sought by challenging kittens born of queens immunized during their pregnancy. In 1 part of this study, 12 kittens of GA-FeSV(UV-65)-vaccinated queens and 12 kittens from nonvaccinated queens were challenged in the 1st week of life with GA-FeSV (Table 3). All 12 kittens from nonimmunized queens developed tumors, and 11 of 12 died from their tumors within 21 weeks of age. In contrast, only 6 of the 12 kittens from immunized queens developed tumors, while tumors regressed in 3, and 3 failed to develop tumor during the 36-week observation period. In general, kittens born of queens with the highest neutralizing antibody titer (~1:16) at time of delivery were afforded the greatest protection. No serological tests were made on colostrum; however, it seems likely that the protection was provided by maternal antibody.

In the 2nd part of this study, all 7 FeLV-inoculated kittens from nonimmunized queens died within 19 weeks after R-FeLV challenge, whereas a significant proportion (25%) of kittens from R-FeLV(UV-65)-immunized queens failed to develop disease after more than 70 weeks of age. Of the 12 challenged kittens from FeLV-immunized queens, 7 died from thymic lymphoma and 2 died from FeLV-related diseases (17). Their mean survival was 32 weeks, a significantly prolonged survival time, compared with kittens from nonimmunized queens.

**FeSV Challenge of Kittens Immunized with Irradiated Tumor Cell Vaccine.** Table 4 summarizes the effects of various doses of γ-radiation on infectivity of a ST-FeSV tumor cell homogenate. The untreated homogenate contained nearly $8 \times 10^7$ and $1.4 \times 10^6$ focus-forming units per ml of FeLV and FeSV, respectively. Irradiation with 3 megarads reduced titers of both viruses by nearly 103 while 4 megarads eliminated all infectivity detectable in vitro. In vivo sarcomogenic activity for newborn kittens was still

<table>
<thead>
<tr>
<th>Vaccinea</th>
<th>Recipients</th>
<th>Geometric highest SN titers achieved/time interval</th>
<th>0–2 wk</th>
<th>2–5 wk</th>
<th>5–7 wk</th>
<th>7–10 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA-FeSV(UV-65)</td>
<td>20 kittens b</td>
<td>$2.0 \pm 0.0$ (0/3)</td>
<td>$2.6 \pm 0.2$ (5/20)</td>
<td>$3.2 \pm 0.2$ (7/16)</td>
<td>$4.6 \pm 0.4$ (7/11)</td>
<td></td>
</tr>
<tr>
<td>GA-FeSV(For-65)</td>
<td>13 kittens</td>
<td>$2.0 \pm 0.0$ (0/9)</td>
<td>$3.0 \pm 0.3$ (4/13)</td>
<td>$2.7 \pm 0.2$ (4/13)</td>
<td>$2.1 \pm 0.1$ (1/14)</td>
<td></td>
</tr>
<tr>
<td>GA-FeSV(UV-FCA)</td>
<td>10 kittens</td>
<td>$2.0 \pm 0.0$ (0/6)</td>
<td>$2.7 \pm 0.2$ (0/14)</td>
<td>$2.4 \pm 0.1$ (0/14)</td>
<td>$3.7 \pm 0.3$ (3/9)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>17 kittens</td>
<td>$2.0 \pm 0.0$ (0/5)</td>
<td>$2.5 \pm 0.3$ (4/17)</td>
<td>$2.4 \pm 0.2$ (2/14)</td>
<td>$3.7 \pm 2.4$ (4/14)</td>
<td></td>
</tr>
<tr>
<td>GA-FeSV(UV-65)</td>
<td>7 queens c</td>
<td>$2.4 \pm 0.2$ (1/7)</td>
<td>$7.4 \pm 0.2$ (6/7)</td>
<td>$14.0 \pm 0.3$ (7/7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations and numbers in parentheses in Column 1: UV, UV inactivation; For, formalin inactivation; 65, Adjuvant 65; FCA, Freund’s complete adjuvant.

Kittens received 4 weekly immunizations of $3 \times 10^{11}$ inactivated virus particles beginning at 4 to 6 days of age prior to challenge with a 90% tumor dose of GA-FeSV at 35 to 40 days of age.

Mean ± S.E.

Numbers in parentheses in Columns 3 to 6: number of cats with SN titers >1:4/total number of cats tested/time period.

Queens received $3 \times 10^{11}$ inactivated virus particles within the 1st 4 days after copulation and again at 1, 2, 3, 5, and 7 weeks during pregnancy (6 injections, total).

**Table 2**

The effect of immunization with inactivated GA-FeSV on tumor growth and survival times following inoculation with live GA-FeSV

<table>
<thead>
<tr>
<th>Immunization category a</th>
<th>No. of cats</th>
<th>Rapid progressor</th>
<th>Slow progressor and/or temporary regressor</th>
<th>Complete regressor and/or no tumor</th>
<th>Mean latent period b</th>
<th>Survival time (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA-FeSV(UV-65)</td>
<td>14</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>3.5 ± 0.8 c</td>
<td>28.9 ± 9.9</td>
</tr>
<tr>
<td>GA-FeSV(For-65)</td>
<td>14</td>
<td>6</td>
<td>7</td>
<td>1</td>
<td>4.7 ± 1.4</td>
<td>29.0 ± 6.7</td>
</tr>
<tr>
<td>Nonimmunized</td>
<td>15</td>
<td>2</td>
<td>6</td>
<td>7</td>
<td>4.1 ± 1.7</td>
<td>34.4 ± 5.5</td>
</tr>
</tbody>
</table>

a Regimen given in Table 1, Footnote a.

b The number of animals showing the designated tumor growth pattern.

c Time in weeks between challenge with live virus and appearance of tumor, excluding animals that did not develop a detectable tumor.

Mean ± S.D.
Experimental Oncornavirus Vaccines in the Cat

Table 3
Influence of immunization of pregnant queens with inactivated FeSV and FeLV vaccines on susceptibility of kittens to FeSV or FeLV

<table>
<thead>
<tr>
<th>Immunization of queens</th>
<th>Challenge of kittens</th>
<th>Disease response</th>
<th>Tumor growth response</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA-FeSV(UV-65)</td>
<td>GA-FeSV</td>
<td>9/12</td>
<td>6 (20)</td>
</tr>
<tr>
<td>None</td>
<td>GA-FeSV</td>
<td>12/12</td>
<td>11 (21)</td>
</tr>
<tr>
<td>R-FeLV(UV-65)</td>
<td>R-FeLV</td>
<td>9/12</td>
<td>9 (32)</td>
</tr>
<tr>
<td>None</td>
<td>R-FeLV</td>
<td>7/7</td>
<td>7 (19)</td>
</tr>
</tbody>
</table>

- Regimen given in Table 1, Footnote a.
- Number of kittens responding/number receiving injections.
- Kittens were challenged between 2 and 4 days of age.
- Numbers in parentheses, mean survival time in weeks.
- Kittens were challenged between 5 and 8 days of age.
- p = 0.05 ($\chi^2$).

Table 4
Effect of $\gamma$-radiation on inactivation of FeLV and FeSV in a 10% tumor cell homogenate of a ST-FeSV-induced tumor

<table>
<thead>
<tr>
<th>Radiation dose (megarads)</th>
<th>In vitro assay (foci-forming units/ml)</th>
<th>Sarcomagenesis in vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FeLV</td>
<td>FeSV</td>
</tr>
<tr>
<td>None</td>
<td>$7.9 \times 10^7$</td>
<td>$1.4 \times 10^6$</td>
</tr>
<tr>
<td>3</td>
<td>$2.2 \times 10^7$</td>
<td>$5.7 \times 10^4$</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- Titrated on CCC cells.
- Titrated on feline embryo cells.
- NT, Not tested.

Table 5
Evaluation of killed ST-FeSV tumor cell vaccine as determined by in vivo protection and FOCMA antibody production

<table>
<thead>
<tr>
<th>Tumor or vaccine (megarads)</th>
<th>Cats tested</th>
<th>Responsiveness to ST-FeSV challenge</th>
<th>FOCMA antibody titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FL-74</td>
<td>C thy C</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>509-3</td>
<td>No tumor</td>
<td>1:4</td>
</tr>
<tr>
<td>5</td>
<td>509-4</td>
<td>No tumor</td>
<td>1:32</td>
</tr>
<tr>
<td>5</td>
<td>509-5</td>
<td>No tumor</td>
<td>1:8</td>
</tr>
<tr>
<td>5</td>
<td>509-6</td>
<td>No tumor</td>
<td>&lt;1:2</td>
</tr>
<tr>
<td>6</td>
<td>620-2</td>
<td>No tumor</td>
<td>1:8</td>
</tr>
<tr>
<td>6</td>
<td>620-3</td>
<td>No tumor</td>
<td>1:2</td>
</tr>
<tr>
<td>6</td>
<td>620-4</td>
<td>No tumor</td>
<td>1:2</td>
</tr>
<tr>
<td>7</td>
<td>295-2</td>
<td>Regressor</td>
<td>1:2</td>
</tr>
<tr>
<td>7</td>
<td>295-3</td>
<td>Nonprogressor</td>
<td>1:16</td>
</tr>
<tr>
<td>7</td>
<td>295-4</td>
<td>Nonprogressor</td>
<td>1:2</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>All died</td>
<td>NT</td>
</tr>
</tbody>
</table>

- Immunized 3 times prior to challenge at 35 days of age.
- FOCMA antibody after the 3rd immunization as tested on FL-74 cells and canine thymus cells (C thy C) infected with KT-FeLV virus.
- NT, Not tested.
- Three nonimmunized cats challenged at 35 days of age.

The immune response of 9 cats immunized with ST-FeSV tumor cell vaccines irradiated with 5, 6, or 7 megarads, respectively, is summarized in Table 5. Virus-neutralizing antibodies were induced in only 3 animals.

Target cells used in the indirect IFA assay for FOCMA were FL-74 cells (feline hemopoietic cell line shedding KT-FeLV) and a KT-FeLV-infected dog thymus cell. All kittens were negative for antibodies to FOCMA prior to immunization. Significant IFA titers to FOCMA developed after 3 weeks of immunization. The specificity of the FOCMA response was supported by the evidence that (a) IFA to FOCMA in FL-74 cells was not removed by absorption with cat tissues, and (b) similar IFA patterns to FOCMA were obtained with the KT-FeLV-infected dog thymus cells. Moreover, noninfected thymus dog cells were not reactive with FOCMA-positive cat sera.

An important aspect of this study (Table 5) was that immunized cats were resistant to ST-FeSV challenge. Only cats immunized with the 7-megarad-treated tumor cells developed tumors. However, these tumors regressed or failed to progress. It seems likely that the 7-megarad dose of $\gamma$-irradiation destroyed some antigenicity of the homogenate.

Age-related Factors in the Response to FeLV. With the exception of 1 experiment wherein kittens born of queens immunized with UV-inactivated R-FeLV were challenged at 4 days of age with R-FeLV (Table 3), all other studies were performed with FeSV. In order to assess the efficacy of FeLV vaccines by means other than disease induction and/or survival, a working knowledge of susceptibility to infection and immune responses to infection at various ages is necessary.

Table 6 summarizes the serological, virological, and biological response of newborn, weanling, and young adult cats to R-FeLV and KT-FeLV infection.

Cats inoculated when newborn developed persistent FeLV viremia but little or no neutralizing or FOCMA antibody. The converse generally was true of cats inoculated in the postweaning or young adult period of life. Lymphosarcoma, aplastic anemia, or lymphoid depletion disease has occurred in many of the cats in the group inoculated as newborns, whereas only 1 of the 20 inoculated older cats has developed aplastic anemia after 20 weeks. This cat was present after 3 megarads; however, doses of 5 megarads or more abolished in vivo oncogenicity.

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Table 6

<table>
<thead>
<tr>
<th>Virus</th>
<th>Age at inoculation</th>
<th>FOCMA antibodies</th>
<th>Virus neutralizing antibodies</th>
<th>FeLV gs antigen*</th>
<th>FeLV-related disease</th>
<th>Observation period (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-FeLVb</td>
<td>Newborn</td>
<td>3/21</td>
<td>0/7</td>
<td>20/21</td>
<td>14/21</td>
<td>8-30</td>
</tr>
<tr>
<td>KT-FeLVb</td>
<td>Newborn</td>
<td>0/4</td>
<td>0/4</td>
<td>4/4</td>
<td>4/4</td>
<td>8</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>3/25</td>
<td>0/11</td>
<td>24/25</td>
<td>18/25</td>
<td></td>
</tr>
<tr>
<td>R-FeLVa</td>
<td>3-4 mo.</td>
<td>8/10</td>
<td>7/10</td>
<td>1/10</td>
<td>0/10</td>
<td>30</td>
</tr>
<tr>
<td>R-FeLVa</td>
<td>1 yr</td>
<td>5/6</td>
<td>6/6</td>
<td>1/6</td>
<td>0/6</td>
<td>30</td>
</tr>
<tr>
<td>KT-FeLVa</td>
<td>3 mo.</td>
<td>4/4</td>
<td>NT1</td>
<td>1/4</td>
<td>1/4</td>
<td>20</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>17/20</td>
<td>13/16</td>
<td>3/20</td>
<td>1/20</td>
<td></td>
</tr>
</tbody>
</table>

* Presence of feline group-specific antigen in peripheral leukocytes as determined by the Hardy test.

1 One ml of a 20% tissue homogenate containing 10⁵ infectious units of virus (by focus induction on CCC cells).

2 One ml containing 10⁷ infectious units.

NT, not tested.

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Discussion

These studies were initiated to evaluate the feasibility of immunizing an outbred mammal, the cat, against horizontally transmissible oncogenic RNA tumor viruses with killed virus vaccines. The initial vaccine studies were performed with FeSV, owing primarily to the relative rapidity of evaluation of protective efficacy, compared with similar studies with FeLV.

To date, these studies have established that kittens can be effectively protected against FeSV by active immunization with crude irradiated tumor cell vaccines. This observation is important since it argues against the possibility that kittens are not responsive to immunogens responsible for inducing immunity to oncornaviruses. Such a possibility was raised by the observation that 4 injections of UV-irradiated or formalin-inactivated FeSV failed to protect kittens and to induce detectable levels of serum-neutralizing antibodies by the time kittens were 5 to 6 weeks of age, the time of challenge with a 90% tumor-inducing dose of virus, whereas the same number of injections induced detectable levels of neutralizing antibodies in 6 of 7 pregnant queens.

Further immunization of queens with 2 additional doses of vaccine resulted in serum-neutralizing antibody titers that ranged from 1:8 to 1:32 (mean, 1:14). A significant number of kittens, 6 of 12, born of these queens survived a normally lethal dose of FeSV.

These data suggest that our apparent inability to effectively protect 5- to 6-week-old kittens by direct immunization with killed virus vaccines may be attributable to an insufficient number of immunizations, to an insufficient amount of immunogen in the vaccine, or to an inappropriate vaccine regimen.

Another factor may be that the virus challenge was too great. However the fact that a significant proportion of 1-week-old kittens (6 of 12) born of immunized queens survived a normally lethal dose of FeSV argues against this possibility. Furthermore, the solid protection afforded 5-week-old kittens to FeSV by immunization with irradiated crude tumor cell vaccines indicates that sufficient protection against the viral dose used can be achieved in 5- to 6-week-old kittens.

The tentative conclusion is that the vaccine regimen used in the kittens was insufficient and a regimen that will induce significant levels of viral neutralizing antibodies must be sought. It is not implied that induction of neutralizing antibodies will necessarily be protective; however, the results of the queen immunization indicated a strong correlation between induction of virus-neutralizing antibodies and protection to virus challenge. In order to establish this correlation conclusively, the FeSV system should provide this information more rapidly than the FeLV system.

Ultimately, it is desired that the efficacy of a viral vaccine be evaluated in the FeLV system under simulated natural conditions, i.e., an evaluation of protection against horizontally transmitted virus by virus shedders to contacts. For these studies, markers of infection (other than disease) in the contacts may be required. These may include detection of viremia (11), of antiviral antibodies by neutralization or complement-fixation inhibition, development of FOCMA antibodies, and alterations in immunological responses attributable to FeLV infection or leukemogenesis.

We are currently examining kittens from newborn to 4 months of age for an age group of cats that responds immunologically to FeLV and FeLV-associated antigen (FOCMA) but are still moderately to highly susceptible to FeLV infection. Among this age group, evaluation of an FeLV vaccine for production of active immunity to FeLV should be experimentally feasible. The results to date (Table 6) indicate that infection of 3-month-old kittens with R-FeLV or KT-FeLV results in humoral antibody responses to viral envelope antigens and to FOCMA. The development of FOCMA antibodies may prove to be a valuable marker, since immunization with killed virus vaccines has not induced FOCMA antibodies.
antibodies. Accordingly, it should be possible to immunize with killed virus and induce neutralizing antibodies, challenge at 3 months or younger with R-FeLV or KT-FeLV, and monitor protection by the absence of FOCMA antibody formation. Infection of 3-month-old kittens with R-FeLV or KT-FeLV, as demonstrated by neutralizing and FOCMA antibody formation, did not result in clinical disease or consistent viremia (Hardy test), thereby negating these as reliable indices of FeLV infection in this age group. Yet to be determined is whether younger kittens, 6 to 8 weeks of age, develop persistent viremia following infection with R-FeLV or KT-FeLV.

Jarret et al. (15) have obtained persistent viremia in cats infected at 6 months of age with 10^9.5 FeLV-infected feline embryo fibroblasts. This infection led to high antibody titers to virus subgroup glycoprotein antigens common to the infected cells and virion envelopes. These antibodies are reported to have the same specificity as those responsible for virus neutralization and cell cytotoxicity but are measured by an indirect immunofluorescence test on the infected cells. Their relationship to FOCMA antibodies was not clarified. In this study, we were able to induce virus-neutralizing antibodies in the absence of FOCMA and cytotoxic antibodies, and vice versa.

The studies by Essex et al. (3) have established a correlation between presence of antibody to the FOCMA and the capability of the cat to resist feline oncornavirus-induced neoplasia. On the basis of these studies, it is conceivable that a vaccination regimen that included immunization with FOCMA will be necessary for efficient immunological protection from FeLV- and FeSV-induced disease. Results described herein revealed that immunization of kittens with irradiated ST-FeSV tumor homogenates (free of replicating virus) (a) induced specific anti-FOCMA responses and (b) protected the kittens from subsequent ST-FeSV challenge, even though virus-neutralizing antibody titers were very low or undetectable. Further studies are needed to define a nucleic acid-free tumor immunogen that induces anti-FOCMA response and provides active immunity to oncogenic virus challenge. The experimental studies reported here with SPF cats may be interpreted to support the concept (11) that both anti-FeLV and anti-FOCMA may be required for solid immunity to virus infection and tumor formation. The ultimate combining of vaccination with both viral envelope and FOCMA immunogens may provide ultimate prophylactic control of feline oncornavirus-induced diseases and hopefully serve as a useful model for control of similar diseases in other outbred animal systems.

References

Experimental Oncornavirus Vaccines in the Cat


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