Investigation of the induction of antibodies against Crandell-Rees feline kidney cell lysates and feline renal cell lysates after parenteral administration of vaccines against feline viral rhinotracheitis, calicivirus, and panleukopenia in cats

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Objective—To determine whether administration of Crandell-Rees feline kidney (CRFK) cell lysates or vaccines against feline viral rhinotracheitis, calicivirus, and panleukopenia (FVRCP vaccines) that likely contain CRFK cell proteins induces antibodies against CRFK cell or feline renal cell (FRC) lysates in cats.

Animals—14 eight-week-old cats.

Procedure—Before and after the study, renal biopsy specimens were obtained from each cat for histologic evaluation. Each of 4 FVRCP vaccines was administered to 2 cats at weeks 0, 3, 6, and 50. Between weeks 0 and 50, another 3 pairs of cats received 11 CRFK cell lysate inoculations SC (10, 50, or 50 µg mixed with alum). Clinicopathologic evaluations and ELISAs to detect serum antibodies against CRFK cell or FRC lysates were performed at intervals.

Results—Cats had no antibodies against CRFK cell or FRC lysates initially. All cats administered CRFK cell lysate had detectable antibodies against CRFK cell or FRC lysates on multiple occasions. Of 6 cats vaccinated parenterally, 5 had detectable antibodies against CRFK cell lysate at least once, but all 6 had detectable antibodies against FRC lysate on multiple occasions. Cats administered an intranasal-intraocular vaccine did not develop detectable antibodies against either lysate. Important clinicopathologic or histologic abnormalities were not detected during the study.

Conclusions and Clinical Relevance—Parenteral administration of vaccines containing viruses likely grown on CRFK cells induced antibodies against CRFK cell and FRC lysates in cats. Hypersensitization with CRFK cell proteins did not result in renal disease in cats during the 56-week study. (*Am J Vet Res* 2005;66:506–511)

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Renal disease commonly develops in cats and is believed to be one of the most common causes of death.[1,2] For example, at Colorado State University, 18,938 cats were evaluated in the period from 1992 through 2001 and chronic renal failure was diagnosed in 676 (3.6%) of those cats. Pyelonephritis, nephrolithiasis, systemic arterial hypertension, toxins, and hypokalemia are some of the known or suspected causes of chronic renal failure in cats.[1-4] However, in most instances, the cause is undetermined. In cats with chronic renal failure, lymphocytic-plasmacytic interstitial nephritis is the most common histopathologic finding, suggesting that immune-mediated reactions may play a role in the disease.[5]

In the 1970s, it was shown that the **Crandell-Rees feline kidney (CRFK)** cell line could be used to propagate feline viruses such as **feline herpesvirus 1 (FHV-1)**, calicivirus, and panleukopenia virus.[6-11] During virus purification for production of vaccines against FHV-1, calicivirus, and panleukopenia (ie, FVRCP vaccines) or immunoassay development, it is impossible to remove all CRFK cell proteins or other cell constituents. Thus, it is likely that some CRFK cell proteins contaminate the viral preparations and, if so, it is also likely that commercially available FVRCP vaccines contain CRFK cell proteins. As a consequence, during the course of routine vaccination, cats may be exposed to CRFK cell proteins and may develop an immune response against those proteins. Because the CRFK cell line is derived from a feline kidney tissue preparation, administration of FVRCP vaccines to cats could induce antibodies that also bind to feline renal tissues.

The objectives of the study reported here were to determine whether administration of CRFK cell line lysates or FVRCP vaccines that contain CRFK cell lysates to cats induces antibodies against CRFK cell proteins or antibodies that react with **feline renal cell (FRC)** lysates. In addition, we intended to determine whether FVRCP vaccination or hypersensitization with CRFK cell lysate induces clinical pathologic or histologic abnormalities in treated cats over a 56-week period.

Materials and Methods

Vaccines—Three of the market-leading FVRCP vaccines[a-c] that are administered parenterally and the only triva-

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lent, modified-live FVRCP vaccine[d] available commercially for intranasal and intraocular administration were chosen for the study.

Experimental cats—Fourteen 8-week-old unvaccinated kittens of both sexes were purchased from commercial vendors for inclusion in the study. The kittens were gang housed, and food was available ad libitum; they were observed daily throughout the study. In addition, 2 other kittens were included in the study to provide FRC lysates. The experimental design was reviewed and approved by the Colorado State University Animal Care and Use Committee in compliance with federal guidelines.

Experimental design—After acclimation, the kittens were anesthetized and ovariohysterectomized or castrated; a wedge of renal tissue (3 mm wide × 4 mm deep × 7 mm long) was surgically obtained from each kitten and placed in neutral-buffered 10% formalin. Two weeks later (week 0), the kittens were randomly allocated into groups of 2. To 2 kittens each, 1 of the 4 FVRCP vaccines was administered on weeks 0, 3, and 6. To 2 kittens each, 10 µg of CRFK cell lysate, 50 µg of CRFK cell lysate, or 50 µg of CRFK cell lysate mixed thoroughly with an equal volume of alum (1 mg)[e] was administered SC at weeks 0, 2, 4, 6, 8, 12, 16, 20, 24, 32, and 40. At week 50, the appropriate booster vaccine or dose of CRFK cell lysate was administered to each cat. At week 56, renal biopsies were performed as described. At the beginning and end of the study, concentrations of antibodies against FHV-1, calicivirus, and panleukopenia virus were measured in serum samples obtained from the cats by use of a commercially available ELISA.[9,f]

At weeks 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 50, 54, and 56, 3 mL of blood was collected via jugular venipuncture and 6 mL of urine was collected via cystocentesis. Blood was immediately placed into a 1.5-mL tube containing EDTA and a sterile tube without anticoagulant. After the blood clotted in the latter, it was centrifuged and the serum was removed and divided into 2 aliquots. The blood sample in the tube containing EDTA, 1 of the 2 serum aliquots, and 4 mL of urine were submitted for a CBC, serum biochemical analyses, and urinalysis, respectively. The other serum aliquot and 2 mL of urine were stored at –70°C. At weeks 12, 28, and 56 of the study, a urine sample was collected from each cat and evaluated for microalbuminuria by use of a commercially available kit.[g]

Histologic evaluation—The 28 renal biopsy specimens were sectioned, stained with H&E, and examined by 2 pathologists (RJB and CAB) who independently applied a predetermined scoring system for the presence of inflammation or glomerular lesions. The pathologists did not know whether the samples had been obtained before or after inoculation nor the identity of the cat from which each of the renal tissue samples had been obtained.

Preparation of CRFK cell and FRC lysates—Frozen CRFK cells (purchased from a commercial vendor[h]) were thawed quickly at 37°C. The cell suspension was diluted in 50 mL of minimal essential medium (with nonessential amino acids and Earle salts medium[i]) containing 5% fetal bovine serum and pelleted via centrifugation at 220 × *g* for 5 minutes. The pellet was removed, suspended in fresh medium, and plated in a cell culture flask.[j] Cells were then amplified in similar flasks and harvested by washing the cell layers with 1× trypsin-EDTA solution.[i] The cells collected from 3 flasks[j] were washed 3 times in PBS solution (pH, 7.2), resuspended in 3 mL of PBS solution in a 15-mL centrifuge tube, and lysed by use of a sonicator (at 30% power with 1 minute of lysis time cycled with 1 minute of cooling time) while in an ice bath (20 cycles). The lysate was then centrifuged at 18,000 × *g* for 20 minutes at 4°C. Results of an assay indicated that the protein content of the supernatant was 440 mg/dL; aliquots were stored at –70°C until used.

To provide FRC lysates for use in this study, the kidneys were collected from 2 additional unvaccinated kittens after euthanasia or death. One kitten was obtained from a commercial vendor and was included in another research project until euthanasia was performed (via IV administration of pentobarbital sodium) because of behavioral problems. The other kitten was relinquished from a local humane society to a foster parent and died as a result of unknown causes. A piece of renal cortex (2 mm deep × 2 mm wide × 2 mm long) was removed from the relinquished kitten, placed in 0.5 mL of sterile PBS solution, and cultured for viruses.[k] The remaining renal tissues from both kittens were stored at –70°C until the results of culture were known to be negative for viruses. Following removal of the renal capsule, a kidney from each kitten was macerated separately in 3 mL of sterile PBS by use of sterile scissors; the tissues were then lysed and processed as described for the CRFK cells. The protein concentration of FRC lysates obtained from the euthanatized kitten and the kitten that died was 1.3 and 2.5 g/dL, respectively.

Development of ELISAs—Indirect ELISAs were developed to measure the concentration of antibodies against CRFK cell lysates (designated the CRFK-ELISA) or FRC lysates (designated the FRC-ELISA). Sera from 5 kittens obtained prior to inoculation and after administration of 4 inoculations of CRFK cell lysate (week 8) were randomly selected for use in titration of the ELISAs. Multiple antigen concentrations, micro-ELISA plates, buffers, blocks, and secondary antibody concentrations were assessed during optimization of the ELISAs.

In the optimized ELISAs, 100 µL of a 1:400 dilution of CRFK cell lysate or 100 µL of a 1:4,000 dilution of FRC lysate in 0.06M carbonate buffer (pH, 9.6) was incubated in micro-ELISA plates[l] for approximately 16 hours at 4°C. Plates were washed 3 times with **PBS solution containing 0.05% Tween 20[k] (PBS-Tween solution)** and blocked with 300 µL of PBS-Tween solution containing 1% casein acid hydrolysate[m] for 1 hour at approximately 20°C. One serum sample obtained prior to inoculation and 1 sample obtained at week 8 (from a cat inoculated with CRFK lysate and alum) were randomly selected for use as the negative and positive controls, respectively, for all experiments. A positive control sample, a negative control sample, and the sera to be tested were diluted 1:50 in PBS-Tween solution, after which 100 µL was pipetted into triplicate wells of a micro-ELISA plate; the plate was incubated for 30 minutes at 37°C. Plates were then washed 3 times with 200 µL of PBS-Tween solution. One hundred microliters of a 1:1,000 dilution of peroxidase-conjugated goat anti-cat IgG (heavy-chain specific)[n] in PBS-Tween solution was pipetted into appropriate wells. After a 30-minute incubation period at 37°C, the plates were washed as described and 100 µL of substrate was added to each well.[o] The enzyme reaction was stopped after 10 minutes at approximately 20°C by pipetting 100 µL of 0.18M H_2SO_4 into each well. The optical density of each well (compared with a substrate control blank) was read at 450 nm with an automated micro-ELISA reader.[p]

The mean absorbance value for the positive control sample, the negative control sample, and each test sample was calculated. The mean absorbance values were converted to %ELISA units by use of the following formula: (test sample mean absorbance minus the negative control sample mean absorbance)/(positive control sample mean absorbance minus the negative control sample mean absorbance) multiplied by 100. In the CRFK-ELISA and FRC-ELISA, an individual cat was considered positive for antibodies against the cell lysates if the %ELISA value was greater than the mean

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%ELISA value plus 2 SD of the preinoculation samples for that group of cats.

Data analyses—To determine the interassay variation of the CRFK-ELISA and FRC-ELISA, the mean of the positive control wells was divided by the mean of the negative control wells for 14 CRFK-ELISAs and 14 FRC-ELISAs performed on different days and the coefficient of variation of these ratios was calculated. Because there were only 2 cats/group, most results of the study are presented descriptively. Mean CRFK-ELISA results and FRC-ELISA results were grouped for the 2 cats administered the intranasal-intraocular vaccine and the 6 cats administered the parenteral vaccines. The results were entered into a spreadsheet and analyzed by use of ANOVA software appropriate for a repeated-measures experiment.[q] The statistical model included vaccine group, week of the study (time), and interaction between week (time) and group. The FRC-ELISA results were log transformed because they were not normally distributed. Because there were numerical differences in week 0 results between groups, these results were included as a covariate. A value of $P < 0.05$ was considered significant.

Results

The coefficients of variation for the CRFK-ELISA and FRC-ELISA were 9.8% and 13.4%, respectively. Each of the vaccinated cats developed antibodies against FHV-1, calicivirus, and panleukopenia virus after vaccination. Prior to inoculation with CRFK cell lysates or vaccines, antibodies against CRFK cell lysates were detected in none of the cats via the CRFK-ELISA. Antibody concentration in sera obtained from all 6 cats administered CRFK cell lysates exceeded the positive cutoff value of the CRFK-ELISA on multiple sample dates after inoculation **(Figure 1)**. During the course of the study, the concentration of antibodies against CRFK cell lysates in sera obtained from either of the cats administered the intranasal-intraocular vaccine did not exceed the positive cutoff value in the CRFK-ELISA **(Figure 2)**. However, in sera obtained from 5 of the 6 cats administered parenteral vaccines, antibody concentration exceeded the positive cutoff value of the CRFK-ELISA at least once during the study. When the results for the 2 cats administered the intranasal-intraocular vaccine were grouped and compared with results for the 6 cats administered vaccines parenterally, there was a significant ($P < 0.05$) difference between groups regardless of time **(Figure 3)**. None of the cats had antibodies against FRC lysate proteins prior to inoculation. On multiple sample dates, all 6 cats administered

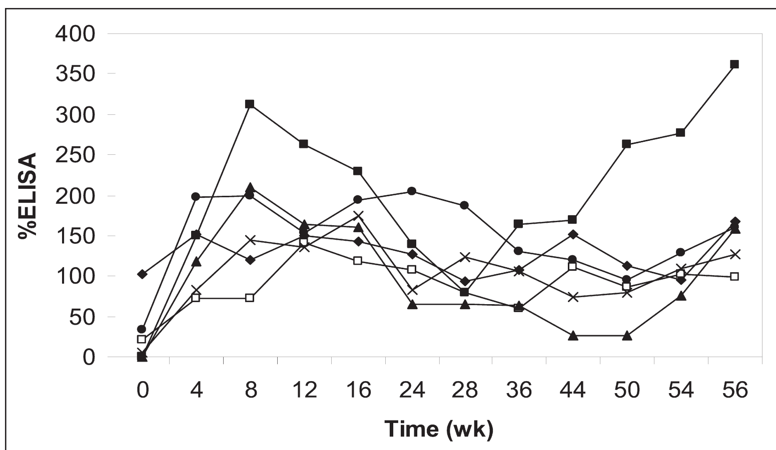

Figure 1—Results of an ELISA (%ELISA units*) to detect antibodies against Crandall-Reese feline kidney (CRFK) cell lysates in cats inoculated SC with 10 μg of CRFK cell lysate (n = 2; diamonds and open squares), 50 g of CRFK cell lysate (2; triangles and crosses), or 50 μg of CRFK cell lysate mixed with alum (2; closed squares and circles) at weeks 0, 2, 4, 6, 8, 12, 16, 20, 24, 32, 36, 40, and 50. The positive cutoff %ELISA value for these samples was 105. *Mean absorbance values were converted to %ELISA units by use of the following formula: (test sample mean absorbance minus the negative control sample mean absorbance)/(positive control sample mean absorbance minus the negative control sample mean absorbance) multiplied by 100.

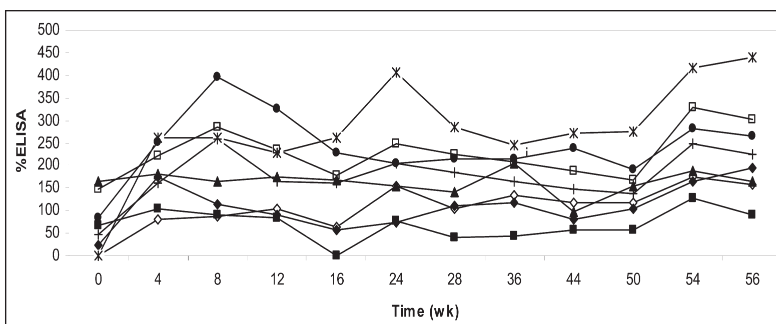

Figure 2—Results of an ELISA to detect antibodies against CRFK cell lysates in 8 cats each vaccinated with 1 of 4 commercially available vaccines against feline viral rhinotracheitis, calicivirus, and panleukopenia (ie, FVRCP vaccines) at weeks 0, 3, 6, and 50. Two modified-live vaccines were each administered parenterally to 2 cats (triangles and closed diamonds; and crosses and circles) and a killed virus vaccine was administered parenterally to 2 cats (vertical line and open squares). An intranasal-intraocular vaccine was administered to 2 cats (open diamonds and closed squares). The positive cutoff %ELISA value for these samples was 193.

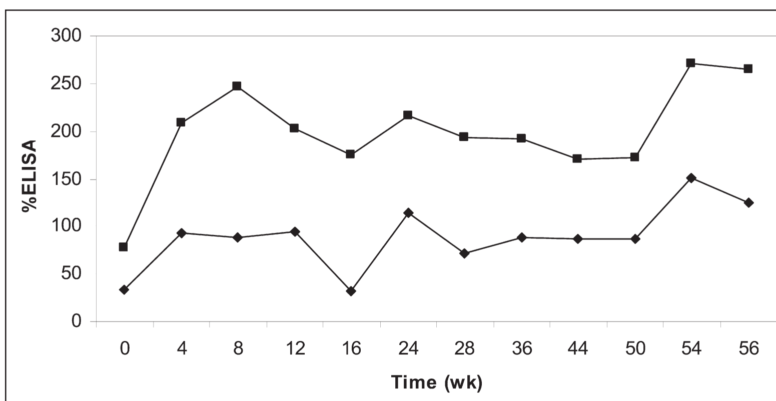

Figure 3—Comparison of mean values obtained by use of an ELISA to detect antibodies against CRFK cell lysates in 2 cats administered an intranasal-intraocular FVRCP vaccine (diamonds) and 6 cats administered an FVRCP vaccine parenterally (squares). The positive cutoff %ELISA for these samples was 193. Values at each time point are significantly ($P < 0.05$) different between groups after week 0.

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CRFK cell lysate had serum antibody concentrations that exceeded the positive cutoff value of the FRC-ELISA (**Figure 4**). During the course of the study, neither of the cats administered the intranasal-intraocular vaccine had serum antibody concentrations that exceeded the positive cutoff value of the FRC ELISA (**Figure 5**). However, at various times during the study, sera obtained from all 6 cats administered vaccines parenterally had concentrations of antibodies against FRC lysate proteins that exceeded the positive cutoff value of the FRC-ELISA. When results from the 2 cats administered the intranasal-intraocular vaccine were grouped and compared with results of the 6 cats administered vaccines parenterally, there was a significant (P < 0.05) difference between groups regardless of time (**Figure 6**). There were no clinically important changes detected between findings of CBCs, serum biochemical analyses, or urinalyses in any cat at any time point and the baseline data. Of the 42 urine samples that were evaluated for microalbuminuria, a total of 3 samples from 3 different cats that received vaccines parenterally were positive for microalbuminuria. However, each of those samples had considerable hematuria attributed to the collection procedure (cystocentesis), which likely explained the positive test result. On histologic evaluation, glomerular lesions that might be attributed to vaccination or administration of CRFK cell lysate were not detected. In 4 cats, mild interstitial inflammatory infiltrates comprised of lymphocytes, plasma cells, macrophages, and rare eosinophils were observed; 2 of these cats had been inoculated with CRFK cell lysate, and 2 had received vaccination parenterally. In 1 parenterally vaccinated cat and 1 CRFK cell lysate-inoculated cat, these inflammatory infiltrates were detected histologically in renal biopsy samples obtained before and after vaccination or inoculation; therefore, the inflammation was probably not related to vaccination or inoculation with CRFK cell lysate in these cats.

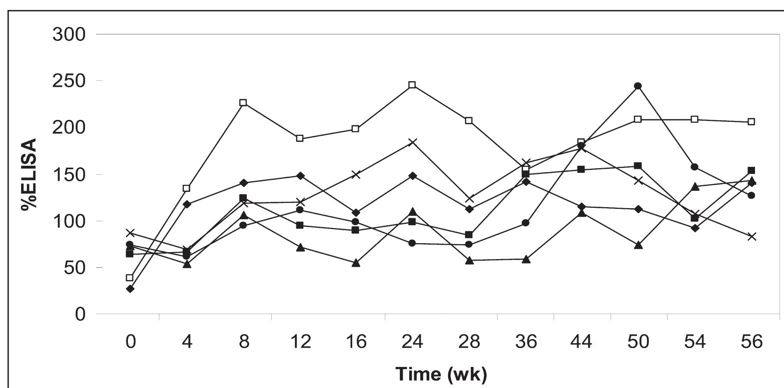

Figure 4—Results of an ELISA to detect antibodies against feline renal cell (FRC) lysates in cats inoculated SC with 10 µg of CRFK cell lysate (n = 2; diamonds and open squares), 50 mg of CRFK cell lysate (2; triangles and crosses), or 50 mg of CRFK cell lysate mixed with alum (2; closed squares and circles) CRFK lysates on weeks 0, 2, 4, 6, 8, 12, 16, 20, 24, 32, 40, and 50. The positive cutoff %ELISA value for these samples was 106.

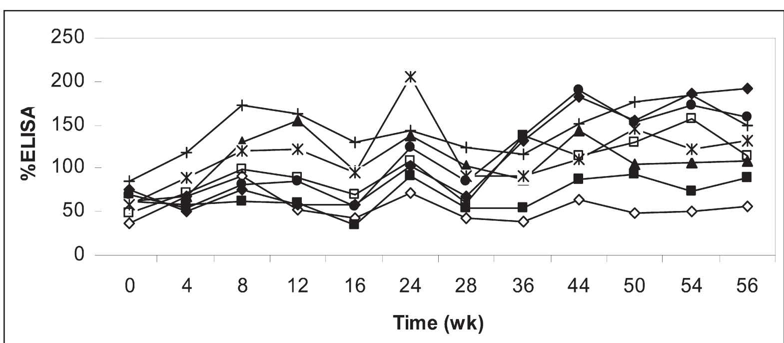

Figure 5—Results of an ELISA to detect antibodies against FRC lysates in 8 cats each vaccinated with 1 of 4 FVRCP vaccines at weeks 0, 3, 6, and 50. Two modified-live vaccines were each administered parenterally to 2 cats (triangles and closed diamonds; and crosses and circles) and a killed virus vaccine was administered parenterally to 2 cats (vertical line and open squares). An intranasal-intraocular vaccine was administered to 2 cats (open diamonds and closed squares). The positive cutoff %ELISA value for these samples was 93.

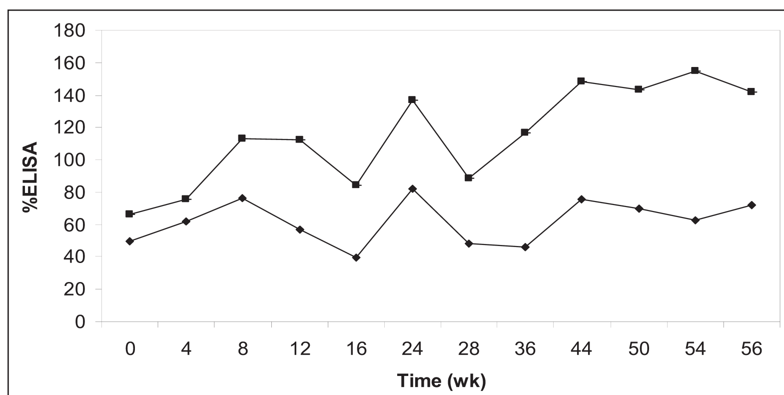

Figure 6—Comparison of mean values obtained by use of an ELISA to detect antibodies against FRC lysates in 2 cats administered an intranasal-intraocular FVRCP vaccine (diamonds) and 6 cats administered an FVRCP vaccine parenterally (squares). The positive %ELISA cutoff for these samples was 93. Values at each time point are significantly (P < 0.05) different between groups after week 0.

Discussion

Healthy cats that are inoculated parenterally with a sufficient quantity of antigen on multiple occasions should mount a detectable antibody response against the inoculum. In the present study, as expected, each cat that was inoculated 12 times with CRFK cell lysate without vaccine viruses had serum concentrations of antibodies against CRFK cell lysate greater than the positive cutoff value of the CRFK-ELISA on multiple sample dates. The vaccines used in the study of this report are suspected[a-c] or known[d] to be grown on the CRFK cell line, but only 4 doses were administered to the study cats over the 56-week period. Subsequent to vaccination, 5 of the 6 cats that received FVRCP vaccines parenterally had serum concentrations of antibodies

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against CRFK cell lysate that exceeded the positive cutoff value of the CRFK-ELISA; 4 of those 6 cats had serum antibody concentrations that exceeded the positive cutoff value of the CRFK-ELISA at most sample points after vaccination. The parenterally vaccinated cat in which the serum concentration of antibodies against CRFK cell lysates did not exceed the CRFK-ELISA positive cutoff value did develop detectable antibodies against FHV-1, calicivirus, and panleukopenia virus, indicating that its humoral immune responses were intact. These data suggest that the vaccines used in the study of this report contain CRFK antigens. However, the CRFK cells used in the experiment were grown with media containing **bovine serum albumin (BSA)**, and it is possible that the cats injected with CRFK cell lysate (and the vaccinated cats if the vaccine viruses were grown with BSA) may also have developed an antibody response against BSA or other cell culture proteins. Immune responses against constituents of vaccines other than the primary antigens have been suspected or proven previously in dogs and humans.[12-14] The FVRCP vaccine that requires intranasal-intraocular administration is grown on the CRFK cell line, but in the present study, the serum concentrations of antibodies against CRFK cell lysate in the 2 cats administered this product never exceeded the positive cutoff value of the CRFK-ELISA over time. When the serum antibody concentration data obtained by use of the CRFK-ELISA for the 2 cats administered the intranasal-intraocular FVRCP vaccine and the 6 cats administered vaccines parenterally were grouped and compared over time, the results were significantly higher in the cats administered the vaccines parenterally. However, because only 2 cats/group were used in our study, additional experiments with larger numbers of cats per group would be required to further confirm these initial findings. In the study of this report, an FRC-ELISA was used to assay sera from FVRCP-vaccinated cats; therefore, it would be possible to obtain false-positive test results if FHV-1, calicivirus, or panleukopenia antigens were present in the FRC lysate used in our study. However, the kitten from which tissues were obtained for use in the FRC-ELISA (for which results are reported here) was young and unvaccinated, and the renal tissues did not yield virus growth on culture. In addition, results were similar between the 2 FRC lysates prepared from each of 2 kittens and the FRC lysate was made without BSA or other known immunogenic substances. Therefore, we believe that the antibodies against FRC lysates detected in the sera of vaccinated cats and CRFK cell lysate-hypersensitized cats had developed against feline antigens. In the present study, cats that had been administered CRFK cell lysates on multiple occasions and those that had received FVRCP vaccines that contained CRFK cell protein parenterally consistently developed detectable antibodies against FRC lysates, whereas cats that had been administered the intranasal-intraocular FVRCP vaccine did not. In addition, when the data obtained from the cats that received vaccines parenterally were grouped, mean FRC-ELISA results over time were significantly greater than the grouped data obtained from the cats that received the intranasal-intraocular vaccine. These results indicate that administration of vaccines containing CRFK cell proteins to cats will induce antibody responses that react with FRC lysates; these data also suggest that antibody response rates will be greater in cats administered vaccines with high concentrations of CRFK cell proteins that are given parenterally, compared with CRFK-containing vaccines given intranasally. Additional studies involving larger numbers of cats would be required to further confirm these findings. Immune responses to vaccine constituents have been associated with autoimmunity previously. In 1 study,[13] recent vaccination of dogs with some products suspected to contain bovine thyroglobulin resulted in the development of anti-canine or anti-bovine thyroglobulin antibodies. In the present study, clinical, hematologic, serum biochemical, or urine abnormalities were not detected in any of the cats. In addition, histologic abnormalities potentially attributable to inoculation with CRFK cell lysate or parenteral administration of a FVRCP vaccine were mild and only detected in 2 cats. We chose to give the cats 12 injections of CRFK cell lysate to approximate half the amount of CRFK cell protein that a cat would receive over its lifetime if the cat were vaccinated yearly with FRVCP vaccines that contained CRFK cell proteins. On the basis of these data, it appears that even hypersensitization with CRFK cell lysate was not associated with development of notable renal inflammation, glomerular disease, or urine abnormalities in cats during the study period. Because evaluation of BUN and serum creatinine concentrations and urinalysis performed by use of a dipstick can provide false-negative results, we applied the microalbuminuria assay to select urine samples. This assay is more sensitive than other techniques for detecting early renal damage.[15,16,q-s] Our data did not indicate that there is any risk of renal damage associated with parenteral administration of FVRCP vaccines containing CRFK cell proteins to cats, at least during the study period. However, small groups of cats were evaluated over a 56-week period, and there may be individual susceptibilities to renal damage associated with production of antibodies against CRFK cell lysate or FRC lysate.CRFK cells have some characteristics of fibroblasts and so may be in multiple tissues. There are many diseases of cats, including uveitis, cholangiohepatitis, inflammatory bowel disease, pancreatitis, hyperthyroidism, type I diabetes mellitus, hypoadrenocorticism, and some forms of stomatitis, that have been proposed to have an immune-mediated component. The potential association between antibodies against CRFK cell proteins and these syndromes remains to be elucidated.

a. Felocell III, Pfizer Animal Health, NY.
b. PureVax, Merial, Athens, Ga.
c. Fel-O-Vax, Fort Dodge Animal Health, Overland Park, Kan.
d. Trivalent intranasal/intraocular vaccine, Heska Corp, Fort Collins, Colo.
e. Imject Alum, Pierce Biotechnology Inc, Rockford, Ill.
f. HESKA Veterinary Diagnostic Laboratories, Fort Collins, Colo.
g. ERD-Healthscreen feline urine test, Heska Corp, Fort Collins, Colo.
h. CCL-94, ATCC, Manassas, Va.
i. Irvine Scientific, Santa Ana, Calif.
j. *T-175* flasks, Nalge Nunc International, Rochester, NY.
k. Veterinary Diagnostic Laboratory, Colorado State University, Fort Collins, Colo.

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l. Immulon 2, 96-well microtiter plates, Dynatech Laboratories, Alexandria, Va.
m. Sigma Chemical Co, St Louis, Mo.
n. Peroxidase-labeled, anti-feline IgG (heavy-chain specific), Kirkgaard & Perry Laboratories Inc, Gaithersburg, Md.
o. TMB microwell peroxidase system, Kirkgaard & Perry Laboratories Inc, Gaithersburg, Md.
p. Multiskan Ascent plate reader, ThermoElectron Corp, Beverly, Mass.
q. MIXED procedure in SAS, SAS Institute Inc, Cary, NC.
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s. Vaden SL, Jensen WA, Longhofer S, et al. Longitudinal study of microalbuminuria in soft-coated wheaten terriers (abstr). *J Vet Intern Med* 2002;16:300.
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