Use of modified live feline panleukopenia virus vaccine to immunize dogs against canine parvovirus

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SUMMARY

Modified live feline panleukopenia virus (FPLV) vaccine immunized dogs against canine parvovirus (CPV) infection. Unlike the long-lived (≥ 20-month) immunity engendered by CPV infection, the response of dogs to living CPV was variable. Doses of FPLV (snow leopard strain) of 10^5.7 TCID_50 were necessary for uniform immunization; smaller inocula resulted in decreased success. The duration of immunity, as measured by the persistence of hemagglutination-inhibiting antibody, was related to the magnitude of the initial response to vaccination; dogs with vigorous initial responses resisted CPV challenge exposure 6 months after vaccination, and hemagglutination-inhibiting antibodies persisted in such dogs for > 1 year. Replication of FPLV in dogs was demonstrated, unlike CPV, the feline virus did not spread to contact cats. Adverse reactions were not associated with FPLV vaccination, and FPLV did not interfere with an immune response to attenuated canine distemper virus.

Canine parvovirus (CPV) is an important canine pathogen. A variety of vaccines have been used for immunoprophylaxis against CPV infection, including inactivated and living feline panleukopenia virus (FPLV), mink enteritis virus, and CPV. Detailed studies of inactivated vaccines have been reported previously; there also have been preliminary reports concerning the safety and efficacy of modified live (ML) FPLV vaccines in dogs.

The objectives of the present study were to examine the immune response of dogs to ML-FPLV vaccines, to identify factors that influence successful immunization, and to compare the immunity engendered by living FPLV with that which occurs after CPV infection.

Materials and Methods

Experimental animals—Beagles (8 to 20 weeks old) from the Baker Institute’s specific-pathogen-free (SPF) colony were used for duration of immunity studies, except in one experiment in which 6 young adult Beagles were purchased from a seronegative research colony (Cornell Dog Farm). Dogs were housed in isolation facilities in groups of 3 to 6 animals. Seronegative sentinel animal in each group served as an indicator of unintentional exposure to CPV. Studies of the duration of immunity after CPV infection were done in dogs maintained in isolation facilities after experimental CPV infection and in naturally infected dogs maintained in a closed colony. In all experiments, seronegative sentinel animals were monitored to ensure that unplanned exposure to CPV had not occurred.
Field trials—A total of 114 seronegative dogs were vaccinated. They comprised 3 populations: group A—a research breeding colony (Cornell Dog Farm, n = 24); group B—a closed research colony (Mary Imogene Bassett Hospital, Cooperstown, NY, n = 46); and group C—privately owned working dogs from several kennels in southern Georgia (n = 44). All dogs were inoculated IM with a single 1-ml dose of the commercial ML-FPLV vaccine. Serum samples were collected and evaluated by HI tests before vaccination and 2 to 3 weeks thereafter. Thirty of the 114 dogs were reinoculated 3 weeks (n = 18) or 7 weeks (n = 12) after the initial vaccination. Serum samples were again tested for HI antibody 2 to 3 weeks after the 2nd inoculation. Nonvaccinated contact dogs were monitored to control for inadvertent CPV exposure during the course of the experiment.

Viral isolation and assay—Viral isolations and assays were performed as described.

Response to ML-FPLV vaccine—Except where otherwise noted, a single serial of a commercial ML-FPLV vaccine (snow leopard strain) produced for use in cats was used. All dogs were inoculated IM with a single 1-ml dose. The vaccine contained 10⁷ TCID₅₀/dose.

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Dose-response relationship—The snow leopard strain was added to freshly passed CCL64 cells (mink lung cell line) at a multiplicity of infection of approximately 1. After 3 days of incubation, the cells were split 1:3 and were incubated for an additional 3 days. Cells were then disrupted by 3 cycles of freezing and thawing. Tissue culture fluids were pooled, clarified by low-speed centrifugation (300 × g), and concentrated by trituration to approximately 1/100th of the original volume. The final suspension contained 10⁷ TCID₅₀/ml.

Twenty-four adult dogs in a seronegative research colony at Cornell Dog Farm were allocated into 3 groups of 8 dogs each. Dogs in group E were inoculated IM with 1 ml of the vaccine containing 10⁷ TCID₅₀/ml.
Replication of vaccinal FPLV in dogs—To determine whether vaccinal FPLV replication occurred in dogs, six 8-week-old SPF pups (group H) were inoculated in the gastrocnemius muscle with 104.5 TCID50 of ML-FPLV. A pup was killed with an overdose of sodium pentobarbital at PVW 1, 2, 3, 4, 5, and 7. The popliteal and sublumbar lymph nodes on the injected side, and samples of kidney, jejunum, ileum, mesenteric lymph nodes, liver, spleen, heart, thymus, and serum were collected aseptically and stored at −70 °C until assayed.

Frozen sections were prepared from each tissue and were stained with a fluorescent antibody conjugate for CPV. Sections were examined by ultraviolet microscopy for specific nuclear fluorescence. Tissue samples were weighed and triturated in minimal essential medium to a 10% (w/v) suspension. Tenfold dilutions were made and 0.2-ml amounts were added to freshly passed NLK cells in 24-well plates that contained 1.2-cm diameter round glass coverslips. Cultures were incubated 4 days, after which the coverslips were removed, stained by fluorescent antibody, and examined for specific nuclear fluorescence. End points (50%) were calculated from 2 or 3 replicate cultures at each dilution. Virus titers were expressed as TCID50/g of tissue.

For comparison, an 8-week-old SPF pup was inoculated IM with a similar dose (104.5 TCID50) of virulent CPV and was killed on postinoculation day (PID) 4. Tissues were collected and evaluated as described previously.

Onset of immunity—Eight littermate SPF Beagle pups (group I) were used. Dogs were vaccinated IM with the commercial ML-FPLV vaccine. Two dogs each were challenge exposed on PID 1, 3, 5, and 7. Challenge-inoculum virus and the evaluation of response to challenge exposure have been described. Serum samples were collected daily and antibody titers were determined by the HI test. Dogs were observed daily for clinical signs of illness, and CPV isolation was attempted from fecal samples collected before challenge exposure and 1, 3, 5, 7, 9, and 11 days after challenge exposure.

Duration of humoral immune responses—The persistence of antibody after vaccination was studied in 22 dogs (group J) maintained in contact with seronegative sentinel animals. Serum
TABLE 5—Recovery of virus from tissues of dogs inoculated IM with ML-FPLV vaccine or virulent CPV (group H)

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>PVD</th>
<th>Sublumbar lymph node</th>
<th>Kidney</th>
<th>Jejunum</th>
<th>Mesenteric lymph node</th>
<th>Liver</th>
<th>Spleen</th>
<th>Heart</th>
<th>Thymus</th>
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<tr>
<td>ML-FPLV inoculation</td>
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Data expressed as TCID_{50}/g of tissue. - = No virus recovered; parentheses contain results of FA examination of tissues, relative score = to ++ + + + + + + + + + .

Fig 3—Serologic responses of SPF Beagle pups (group I) to IM vaccination with $10^{6.7}$ TCID_{50} of ML-FPLV.

Fig 4—Persistence of antibody in dogs (group K) after experimental infection. Range (I) and mean HI titers. Numbers in parentheses indicate the number of dogs at each sampling time. Dogs were maintained with seronegative (noninfected) sentinels to preclude unrecognized exposure.

Results

Response to ML-FPLV vaccine—Of 114 dogs (groups B, C, and D), 105 (92.1%) developed detectable > 1:80 HI titers to CPV after a single inoculation of the multivalent vaccine for dogs (group D) that contained 10^{5.7} TCID_{50} FPLV (Table 1). Titers ranged from < 10 to 5,120; the distribution was bimodal (Fig 1). Vaccinated dogs could be classified into 2 groups, those with titers ≤ 80 (47.5%, median = 70) and those with titers > 80 (52.5%, median = 320).

Thirty dogs were revaccinated 3 or 7 weeks after initial inoculation. Twelve of 17 dogs with HI titers ≤ 80 had 4-fold or greater increases in titer after revaccination, but none of 13 dogs with HI titers > 160 had detectable HI titer in the reverse passive hemagglutination test or after revaccination (Table 2).

Similar results were observed after vaccination with a multivalent vaccine for dogs (group D) that contained FPLV as the parvovirus component (Table 3). There were no differences in the persistence of HI titters between vaccinated and nonvaccinated dogs. Immunity was measured by the failure of dogs to shed virulent CPV after challenge exposure and absence of serologic responses to challenge exposure.

Dogs that had recovered from experimental CPV infection 12 months previously (n = 2) and 20 months after infection (n = 2) were challenged exposed and monitored for clinical signs of illness and fecal viral shedding. Two additional dogs were challenged exposed 6 and 20 months after infection, respectively, and killed 4 days later. Response to challenge exposure was determined by fluorescent antibody and by viral isolation from the feces, small intestine, mesenteric lymph nodes, thymus, and tonsils.

Duration of immunity—Persistence of immunity after vaccination (group L; n = 21) or infection (group M; n = 6) was determined by oronasal challenge with virulent CPV. Dogs were maintained in isolation units during the period between vaccination or initial infection and challenge exposure. Seronegative sentinel animals were included in each group to detect exposure to CPV.

Dogs were considered immune only if they resisted infection. Immunity was measured by the failure of dogs to shed virulent CPV in the feces after challenge exposure and absence of serologic responses. Fecal viral shedding was evaluated by (i) spread of infection to a susceptible nontreated dog introduced 3 days after challenge exposure and/or (ii) isolation of CPV in tissue cultures from fecal specimens.

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Persistence of antibody in dogs vaccinated with ML-FPLV

<table>
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<th>PW 3</th>
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<td>5,120</td>
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Replication of ML-FPLV in dogs—There was evidence of limited replication of vaccinal FPLV in dogs. Feline panleukopenia virus was recovered primarily from lymphatic tissues of dogs (group H) for up to PW 5 (Table 5). Viremia was demonstrated in 2 of 5 dogs on PW 2 and in 1 of 5 dogs on PW 3. On PW 4, the amount of virus in the tissues of the dog given FPLV was less than that of the dog given a similar dose of CPV; the distribution of FPLV was also restricted, compared with that of CPV (Table 5).

Examination of frozen sections stained with fluorescent antibody conjugate for CPV confirmed the viral isolation results. Specific nuclear fluorescence was observed only in tissues from which FPLV was also isolated. Cells infected with FPLV were few in number and widely scattered. In contrast, diffuse areas of specific fluorescence were prominent in the tissues of the dog infected with CPV; more than 50% of the thymic cells were judged infected.

In dog 1139 (group H, Table 5), FPLV was isolated from the jejunum. Fluorescent antibody examination, however, revealed that infected cells were restricted to the lamina propria. Specific fluorescence was not observed in the intestinal epithelium in any vaccinated dog, but was prominent in the small intestine of the CPV-infected dog.

Onset of protection—Antibody was first detected in dogs (group J) by the HI test on PW 3 (Fig 3). Titers rapidly increased to maximal amounts by PW 7. Dogs challenged oronasally with CPV on PW 1 and 3 became infected; they shed CPV in their feces on the 3rd to 5th day after challenge exposure. In contrast, dogs challenged on PW 5 or 7 did not become infected with CPV, and virus was not demonstrated in their feces.

Clinical illness was not observed in any of the immunized dogs. However, as reported previously,10 signs were not pronounced in the nonvaccinated controls either, and consisted of 1 or 2 days of increased body temperature, depression, and a loose mucoid feces. Canine parvovirus was recovered from the feces of nonvaccinated controls on postchallenge-exposure days 3 to 7.

Antibody persistence—Antibody titers in dogs (group K) that had recovered from CPV infection decreased 2- to 4-fold during the first 3 months. Thereafter, titers decreased only slowly (Fig 4). Dogs held in isolation for 10 months (n = 5) or 20 months (n = 3) after infection had titers ≥ 320.

Antibody titers in dogs (group J) that had responded initially to ML-FPLV with titers ≥ 80 persisted in a manner similar to those of dogs given CPV. Ten of 11 such dogs had titers ≥ 80 at PW 18; 4 of 5 dogs had titers ≥ 80 at PW 56 (Table 6).

Duration of immunity—Recovery from CPV infection conferred sustained immunity in dogs (group M). Two dogs challenged oronasally 1 year after inoculation and 2 dogs challenged 20 months after inoculation were immune; they had no clinical signs of illness, their antibody titers to CPV did not increase, and CPV could not be recovered from fecal samples collected between postchallenge exposure days 1 and 13.

Two additional dogs were challenged oronasally: one 6 months after inoculation and one at 20 months...
after inoculation. Both were killed 4 days after challenge exposure. Canine parvovirus was not isolated from the feces, serum, thymus, tonsil, small intestine, mesenteric lymph nodes, or spleen of either dog. In contrast, viral titers of $\geq 10^4.0$ TCID$_{50}$/g were observed on postchallenge exposure day 4 in tissues from nonvaccinated dogs. Recovered dogs had serum titers $\geq 320$ at the time of challenge exposure.

The duration of immunity to CPV in dogs (group L) after ML-FPLV vaccination was related to the magnitude of the initial antibody response. Dogs that initially developed titers $\geq 80$ were found to be immune to challenge exposure at PVW 3 (n = 3), 10 (n = 8), and 24 (n = 2; Table 7). The dogs that had initially developed HI titers $\leq 80$ after vaccination responded to CPV challenge exposure in a manner similar to that observed in dogs given inactivated virus vaccines. All became infected when challenge exposed at PVW 3 (n = 3), 10 (n = 1), or 24 (n = 4). However, the only clinical signs were mild pyrexia (39.1°C) in 1 dog and mucoid feces in 2 others.

**Discussion**

This study demonstrated that recovery from CPV infection resulted in high antibody titers to CPV that persisted. Although response to ML-FPLV vaccines was variable, those dogs that developed vigorous initial responses to vaccination (HI titers $\geq 90$) tended to maintain high titers of circulating anti-CPV antibody. Antibody that persists can be interpreted as sustained immunity because a direct correlation between antibody response and resistance to infection has been demonstrated for CPV in dogs and FPLV in cats.

Dogs that had recovered from CPV infection maintained high ($\geq 320$) antibody titers in the absence of reinfection and were immune to reinfestation for at least 20 months. This suggests that the CPV genome may persist for periods in dogs, although such a chronic carrier state has not been demonstrated. The persistence of rodent parvoviruses in their respective hosts is apparently important to the maintenance of high antibody titers over a long period of time. A similar mechanism may account for the sustained immunity after CPV infection or ML-FPLV vaccination. Dogs vaccinated with ML-FPLV were almost equally into 2 groups: (i) those that developed relatively low antibody titers which decreased rapidly over time and (ii) those that had initially high titers which persisted. The antibody response and the resistance to CPV challenge exposure of dogs in the latter group were similar to those of dogs vaccinated with inactivated virus. Dogs in the latter group were immune in the manner of dogs that had recovered from CPV infection.

Replication of FPLV in dogs was demonstrated in reports$^{15,16}$ that FPLV did not infect dogs but failed to produce disease after experimental inoculation, rather than on virologic or serologic examination. Nevertheless, the growth of FPLV in dogs was recorded as compared with the growth of CPV in dogs$^3$ or cats.$^{17}$ Only lymphoid tissues appeared to support FPLV replication in the dog. Serologic results indicated, however, that infection does not occur in all dogs.

The reasons for the variable response to vaccination are not fully understood. A hypothesis consistent with these observations is that high antibody titers are developed only in dogs in which FPLV replication occurs. Maximal antibody responses to ML-FPLV are higher and more sustained than those in dogs given inactivated or CPV,$^3$ but they are lower than the typical responses...
The lower antibody responses to ML-FPLV probably reflect the relatively restricted growth of canine parvovirus (CPV) in the dog. Also, when CPV is used as the test antigen in HI tests (as in this study), anti-FPLV sera generally have 2-fold lower titers than when FPLV is used as antigen. Nevertheless, immunity to CPV infection was demonstrated 6 months after vaccination, and antibody responses at high titers in some vaccinated dogs for more than 1 year, in which FPLV replication apparently did not occur.

Such animals did not resist CPV infection, in that intestinal replication of challenge inoculum virus was not observed in fecal samples (as in this study), anti-FPLV sera generated was proportional to the amount of living virus excreted, however, did not infect contact animals. This is consistent with the observation that ML-FPLV is not infectious by the oral route for cats or dogs.

Dogs vaccinated with ML-FPLV, therefore, do not appear to be a source of infection for other dogs or cats. These studies, then, revealed that ML-FPLV safely immunizes dogs against CPV, but that its efficacy depends on the viral dose administered. Responses to vaccination were variable at all viral doses tested, but more dogs developed vigorous responses (HI titers ≥ 80) when larger viral inocula were used. The duration of immunity appeared correlated with the intensity of the initial immune response; HI titers persisted in some, but not in all, dogs for more than a year.

References