INFECTION AND IMMUNITY, Apr. 1973, p. 547-549

Vol. 7, No. 4
Printed in U.S.A.

Absence of an Immune Response after Oral Administration of Attenuated Feline Panleukopenia Virus

RONALD D. SCHULTZ AND FREDRIC W. SCOTT

Department of Veterinary Microbiology, New York State Veterinary College at Cornell University, Ithaca, New York 14850

Received for publication 24 November 1972

Cats were orally vaccinated with attenuated feline panleukopenia virus to compare this route with parenteral immunization. Cats receiving vaccine virus by mouth did not produce a systemic or local antibody response to the virus. Intranasal and subcutaneous vaccination produced high levels of neutralizing antibodies and provided protection from challenge with virulent virus. The results suggest that virus does not initially infect the tissue of the oral pharynx or gastrointestinal tract as previously suspected.

Feline panleukopenia is an important viral disease of cats. It is presumed that the virus first established itself in the oral pharynx, although the sites of initial virus replication are not known (J. H. Gillespie and F. W. Scott, Advan. Vet. Sci. Comp. Med., in press). Microscopic lesions are principally found in the mucosa of the small intestine, most often the jejunum and ileum and the lymphoreticular system (F. W. Scott and J. H. Gillespie, in press; reference 4).

Recommended prevention and control of the disease are by vaccination of susceptible cats with tissue culture origin, inactivated or modified live virus vaccines (1). There is a direct relationship between serum-neutralizing antibody titers and protection from feline panleukopenia infection (5).

Panleukopenia virus, a member of the parvovirus group, is extremely stable and is resistant to many chemicals, low pH, and high temperatures (30 min, 56 C). It may remain infectious after standing at room temperature for as long as 1 year.

With knowledge of the resistant characteristics of panleukopenia virus and the awareness that during natural or experimental disease, virus multiplies and produces pathological lesions in the intestinal tract, an attempt was made to vaccinate cats by the oral route. It was anticipated that the following questions would be answered by this study. (i) Could the efficacy of the live attenuated vaccine virus be increased by oral inoculation? (ii) Would locally synthesized antibody in the intestinal tract protect cats from oral challenge with virulent virus?

MATERIALS AND METHODS

The vaccine virus used throughout this study was Leukogen-TC, a modified live virus vaccine (Bio-Ceutic Lab., Inc., div. of Philips Roxane, Inc., St. Joseph, Mo., 64502). The mean tissue culture infective dose, calculated at the time of or shortly after vaccination for each 1-ml vial of vaccine virus, was approximately 10^4.

Serum neutralization tests were performed in microchamber slides (Lab-Tek Products, div. of Miles Lab., Westmont, Ill. 60559) by the procedure described by Scott et al. (5).

Fecal samples were collected at 7, 14, and 21 days postvaccination. Dry fecal material (20 g) was homogenized with 15 ml of phosphate-buffered saline and centrifuged at 3,500 × *g* for 20 min, and the supernatant fluid was removed and concentrated with polyvinylpyrilodone to 1 ml. Viral neutralization activity of the fecal supernatant was determined by the method described for serum neutralization.

Cats were challenged orally with 10^4 mean tissue culture infective doses of a virulent feline ataxia virus strain of panleukopenia virus per ml, as described (3). Tissues collected from euthanatized cats were processed for viral isolation (3). Small and large intestines were thoroughly rinsed with sterile cold saline to prevent viral isolation from intestinal contents.

Animals. Four adult cats, seven adolescent cats, and four newborn kittens were inoculated by the oral route, three adolescent and four newborn kittens were inoculated by the intranasal or oral-intranasal route, and three adolescent and two newborn kittens were inoculated subcutaneously with panleukopenia vaccine virus.

Oral inoculations were performed by opening the cat's mouth and placing the 1 ml of virus sus-

pension on the tongue at a rate such that the cat could swallow the liquid. If liquid was observed to bubble from the nostril, the inoculation was considered oral-intranasal. This occurred most often when oral inoculations were attempted in newborn kittens. Intranasal inoculations were accomplished by placing 1 ml of virus suspension into the nostrils with a syringe.

RESULTS

Serum neutralization titers of cats inoculated orally, intranasally, and subcutaneously are presented in Fig. 1. Only 1 of the 15 cats inoculated orally produced serum-neutralizing antibody, whereas all of the cats inoculated intranasally or subcutaneously produced antibody. A difference in serum neutralization titers with regard to the age of the cat was not observed. Fecal samples obtained from the cats inoculated orally were negative for viral-neutralizing antibodies at a 1:5 dilution. Three orally vaccinated cats without detectable serum antibody when challenged with virulent viruses developed clinical signs of panleukopenia. Two orally vaccinated cats, inoculated intranasally 6 weeks after oral inoculation, developed serum-neutralizing antibodies in a manner similar to cats inoculated by the intranasal route alone.

Virus isolation by direct cell culture or in feline kidney tissue culture from three cats inoculated orally with virulent virus and euthanatized at 12, 18, and 24 h postinoculation are listed in Table 1.

DISCUSSION

The unexpected and somewhat contradictory results suggested that oral administration of vaccine virus did not elicit an immune response in the majority of the cats. To determine if the one cat that produced serum-neutralizing antibody did so because of inapparent intranasal inoculation, cats were intentionally inoculated by that route. As shown in Fig. 1, cats vaccinated by the intranasal route produced high levels of antibody to the virus. It is concluded, therefore, that the one cat that responded after oral vaccination may have received an intranasal inoculation. The immune response to intranasal immunization with vaccine virus has not been reported previously. The possibility of local protective immunity in the absence of serum antibody was tested to determine if local intestinal antibody was produced after oral vaccination. The negative fecal titers and susceptibility to oral challenge with virulent virus suggested that oral immunization with modified live panleukopenia

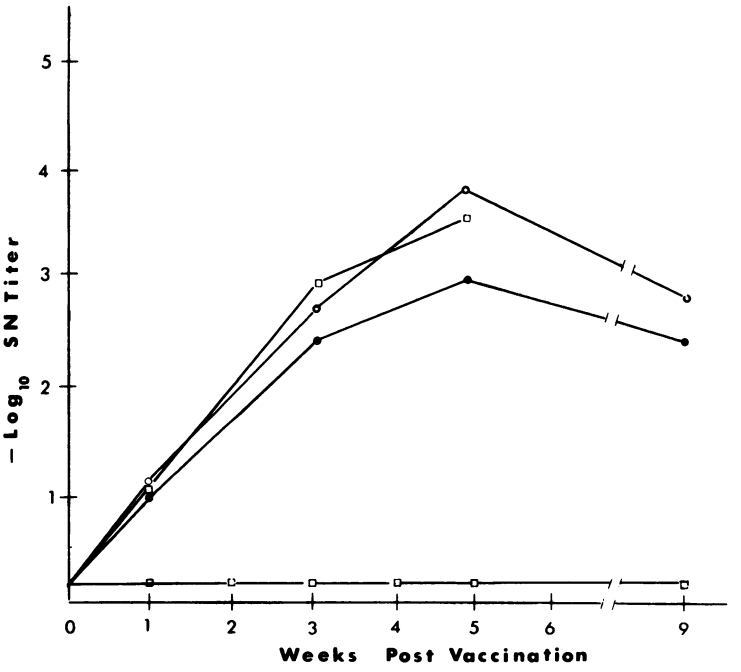

FIG. 1. *Average serum-neutralizing antibody titers to attenuated panleukopenia virus after oral, intranasal, and subcutaneous vaccination. Symbols:* ○, *intranasal or oral-intranasal vaccination, average of 7 cats;* ●, *subcutaneous vaccination, average of five cats;* □, *oral vaccination, average of 14 cats. (The titer of the one cat that responded was not included in the average and is graphed separately.)*

TABLE 1. *Virus isolation after oral infection with virulent virus (feline ataxia strain)*

Time after infection (h)	Spleen	Heart	Thymus[a]	Lung[a]	Mesenteric[a] lymph node	Small intestine[b]	Colon	Feces
12				FP[c]				ND[d]
18				FP				+[e]
24	+			FP			+	+

[a] Direct cell culture and isolation in feline kidney cells.
[b] One section from duodenum and jejunum and two sections from ileum.
[c] FP, Feline picornavirus (calicivirus).
[d] ND, Not done.
[e] +, Feline panleukopenia virus isolated.

virus was totally ineffective in producing a local immune response. Cats initially vaccinated orally that did not produce an immune response to virus, when vaccinated intranasally at a later time, produced a response which was similar to a primary intranasal immunization. This is further evidence suggesting that there was no immune response after oral vaccination.

Csiza et al. reported that virus was isolated from the small intestine 18 h after an oral-intranasal inoculation; however, it was not detected by immunofluorescence (2, 3). In the present study, the procedure of flushing the intestine with saline to prevent the intestinal contents from giving false positive results of viral infection suggested that the small intestine did not become infected by the oral route during the first 24 h. In agreement with the results reported by Csiza et al., virus was present systemically in 24 h (3).

We conclude from this study that infection of the small intestine does not occur in a majority of animals after oral vaccination with modified live panleukopenia virus. This route of vaccination, therefore, should not be used for immunization. We also conclude that intranasal vaccination is effective in producing a systemic response to the vaccine virus. Studies are in progress to determine if this route is effective in overcoming the immunosuppressive effect of maternal antibody.

ACKNOWLEDGMENTS

We are most grateful to Eleanor Tompkins, Carol Hoff, James Buck, and Nancy Aagaard for their technical assistance.

This study was supported by Public Health Service research grant AI-10042 from the National Institute of Allergy and Infectious Diseases.

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