INFECTION AND IMMUNITY, June 1971, p. 833-837

Vol. 3, No. 6
Printed in U.S.A.

Pathogenesis of Feline Panleukopenia Virus in Susceptible Newborn Kittens

I. Clinical Signs, Hematology, Serology, and Virology[1]

C. K. CSIZA,[2] F. W. SCOTT, A. DE LAHUNTA, AND J. H. GILLESPIE

Departments of Microbiology and Anatomy, New York State Veterinary College, Cornell University, Ithaca, New York 14850

Received for publication 8 January 1971

Inoculation of susceptible newborn kittens with a large dose of panleukopenia virus caused subclinical infection in 19 of 23 cases. All infected kittens developed severe and prolonged leukopenia. Cell-free virus was present in the blood from 1 to 7 postinoculation days. The virus spread to all organs, regardless of the route of inoculation. The thymus, spleen, mesenteric lymph nodes, and the cerebellum were the most severely infected organs. Kittens responded to virus infection by production of specific antibodies, first detectable in the circulatory system 6 to 8 days after infection. Antibody production preceded recovery from leukopenia by 3 days.

Feline panleukopenia (FPL; feline enteritis) is a highly contagious disease of Felidae (1, 16), raccoons (18), and mink (12, 19). The transmission of gastroenteritis by bacteria-free filtrate from a Siamese cat to another cat was demonstrated in 1928 by Verge and Christoforoni (17). Extensive studies in the 1930's (4, 5, 10) confirmed the viral etiology of the disease and described the clinical signs and pathological changes in the post-weaned cats. In vivo tests for virus and antibody assay were not developed until intranuclear inclusions were recognized in cell cultures (7, 11) of actively dividing cells (6, 9) similar to those observed in the intestinal epithelium and lymphoid tissues of infected cats by earlier investigators (4, 10). Another important development in the history of the disease was the demonstration that a transmissible agent of feline ataxia (9) was identical to FPL virus (8).

This investigation was undertaken to define the pathogenesis of FPL virus infection in newborn kittens following the natural routes of exposure (oral or intranasal, or both). Special attention was paid to the action of the virus on the central nervous system and the development of the cerebellar disease.

[1] Based upon a thesis presented by C. K. Csiza, to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the Ph.D. degree.

[2] Present address: Veterinary Science Laboratories, Division of Laboratories and Research, New York State Department of Health, Albany, N.Y. 12201.

MATERIALS AND METHODS

Cell cultures. Preparation of secondary feline kidney (FK) cell cultures used for virus isolation and serum neutralization (SN) tests have been described in detail (14). The growth medium for the secondary FK cells was medium 199, supplemented with 0.5% lactalbumin hydrolysate and 10% fetal calf serum.

Virus. Panleukopenia virus used for this study was the "ataxia" isolate supplied by L. Kilham as a 10% ferret cerebellar suspension and passaged in secondary FK cell cultures in our laboratory. The second passage virus pool was kept at −60 to −70 C and was used for animal inoculations. The second and third passages of virus pools were used for SN tests in cell cultures and for preparation of antisera.

Quantification of virus was accomplished by inoculation of 0.1 ml of a series of twofold dilutions into secondary FK cells in Leighton tubes (LT) within a few hours after seeding. The cell cultures were incubated at 37 C for 4 days. Cover-slip cultures were removed and stained with May-Grünwald-Giemsa (MGG) (14) and examined for presence of typical intranuclear inclusions. Fifty per cent end points ($TCID_{50}$), a dose producing cytopathic effect (CPE) in 50% of the inoculated cell cultures, were calculated by the Spearman-Karber method (3).

Animal inoculations. Pregnant cats with no history of FPL vaccination or clinical illness resembling panleukopenia were tested for presence of neutralizing antibodies. Five susceptible pregnant cats were located and placed in Horsfall isolation units. A total of 23 kittens were born. They were inoculated by the intracerebral, oral, or intranasal routes, or by oral and intranasal routes, with 9,000 to 18,000 $TCID_{50}$ of FPL virus on the day of bith (day zero). The kittens were

sacrificed 1 to 43 days postinoculation (DPI). The queens were held as contact controls.

Collection of the specimens. All kittens were bled from the jugular vein two to three times per week. Blood samples for SN test were allowed to clot, and the separated serum was stored at −20 C until used. Blood for total leukocyte counts was collected in Unopette (Becton, Dickinson and Co., Rutherford, N.J.) blood-diluting pipettes, and counts were made with Fusch-Rosenthal counting chambers. To demonstrate lack of influence of bleeding on total leukocyte counts, 12 kittens born to immune queens were inoculated on the day of birth with FPL virus and were bled by the scheme used on kittens in pathogenesis study.

Upon euthanasia, 4 central nervous system tissues and 12 visceral organs were collected in triplicate for virus isolation, histopathology, and fluorescent-antibody (FA) technique. Tissues for FA were stored at −60 to −70 C. Cerebrospinal fluid, urine, feces, and tissues for virus isolation were stored at −20 C.

Virus isolation. One-tenth milliliter of body fluids and 10% tissue suspensions (2) was inoculated into each of two roller tissue culture tubes shortly after the tubes were seeded with secondary FK cells and incubated at 37 C. The tubes were examined for CPE (a nonspecific type of cell degeneration) at 4 DPI. If no cell degeneration was present, cell culture and fluid were frozen and thawed once. The contents of two tubes were pooled and centrifuged at 1,500 rev/min for 10 min, and 0.5 ml of the supernatant fluid was reinoculated into two tubes. If no CPE was observed, this procedure was repeated two more times. The fourth passage was carried out by infecting two LT cultures, and cover slips were stained with MGG at 4 DPI.

If CPE was detected on the first or second blind passage, it was confirmed by inoculation of LT cultures and examination of the stained cover slip for the presence of the typical Cowdry type A intranuclear inclusions, produced by the FPL virus. At a later date, the original sample was titrated and the virus titer was expressed as $\log_{10}$ per 0.01 g of tissue (cultures inoculated with 0.1 ml of 10% tissue suspension) or 0.1 ml of body fluid. Specimens from which virus was recovered on the third or fourth passage contained a small amount of virus and were not titrated.

SN test. The technique used in SN test has been described elsewhere (14). The tests were carried out on groups of 4 to 8 heat-inactivated (56 C for 30 min) serum samples from one animal on the same day. To a serial twofold dilution, an equal volume of virus containing 900 $TCID_{50}$ units per 0.1 ml was added, and the mixture was incubated at room temperature for 1 hr. A 0.2-ml amount was inoculated into each of two LT culture tubes with freshly seeded, secondary FK cells. Inoculated and control cultures were stained with MGG stain at 4 DPI and were examined for CPE.

RESULTS

Clinical signs. The kittens appeared healthy and nursed well during the course of the disease as indicated by the high lipid content of the sera. Clinical signs of infection were absent, except in one kitten which was found moribund in lateral recumbency at 14 DPI and was sacrificed. Three other kittens were found dead at 6, 7, and 10 DPI without any indication of illness the previous day.

Clinical pathology. Total white blood cell counts in the newborn kittens varied from 5,300 to 15,000 per mm^3. The mean leukocyte count was 10,160 with standard deviation at 2,750 per mm^3. The mean total white blood cell count dropped below 5,000 per mm^3 by the 3rd day after inoculation and remained at a low level for 7 days. Counts were as low as 200 to 600 per mm^3 in three kittens at 8 to 10 DPI. One of these three animals was found dead at 10 days of age, but the other two remained clinically normal until sacrificed at 22 and 29 days after infection, respectively. Many of the blood samples collected had a rusty brown color, concurrent with severe leukopenia. Recovery from the prolonged leukopenia was followed in some kittens by leukocytosis 13 to 15 DPI, and the cell counts gradually returned to a normal range a few days later. In contrast, the kitten in lateral recumbency at 14 days after infection had developed severe leukopenia a second time. This was concurrent with suppurative infection of the central nervous system. Bacteriological examination of kittens that died 6 and 7 DPI revealed enteric bacteria of the genus *Escherichia* from a number of visceral and thoracic organs.

Virus isolation. The serum contained cell-free virus 2 to 7 days after infection. Cerebrospinal fluid had a higher quantity of virus than serum when paired titrations were performed. Virus was recovered from urine 2 to 22 days after infection. Feces also contained panleukopenia virus at 22 DPI.

Panleukopenia virus was isolated in secondary FK cell cultures from 6 of 15 samples tested (including cerebellum) at 18 hr after a combined oral and intranasal inoculation. The concentration of virus was highest from 4 to 7 DPI (3 to 7 logs of virus per g of tissue) in all organs tested.

Recovery of virus from the kitten sacrificed at 22 DPI was successful only from the central nervous system tissues, kidneys, urine, and feces on the third or fourth blind passage in cell cultures. Virus isolation was unsuccessful by this method from tissues of kittens sacrificed at 29 to 43 DPI.

Based on virus isolation, the bladder, gonads, and spinal cord were the least susceptible organs for virus multiplication. Virus titers were high and comparable in thymus, spleen, mesenteric lymph nodes, and cerebellum. Among these four organs the thymus was the first to be infected (18 hr postinoculation), and infectivity was maintained the longest in the cerebellum (22 DPI). These results (Table 1) are comparable to the immunofluorescent-antibody studies (part II).

TABLE 1. *Virus isolation in secondary feline kidney tissue culture from 23 serologically susceptible kittens after inoculation with ataxia strain of panleukopenia virus at birth*

Cat	Route of inoculation[a]	Euthanasia PID[b]	Virus titers of tissue or body fluid[c]																			
			Thymus	Lung	Heart	Liver	Spleen	Mesenteric lymph node	Pancreas	Kidney	Bladder	Ovary	Testes	Small intestine	Large intestine	Olfactory bulb	Cerebrum	Cerebellum	Spinal cord	Cerebrospinal fluid	Serum	Urine	Feces
590	IN + OR	¾	+4	—[d]	+3	—	—	+2	—	+4	—	—		+2	ND[e]	—	—	+4	—	ND	ND	—	ND
589	IN + OR	2	1.3	0.35	+3	0.35	1.15	2.2	—	+3	ND	—		1.75	1.15	+3	+3	+3	+3	+3	+3	+4	ND
591	IN + OR	3	2.9	1.3	0.85	1.45	2.05	2.95	0.85	0.7	+3		ND	1.15	1.45	0.7	1.00	+3	+2	2.05	+3	+3	ND
518	IC	4	4.15	3.25	ND	3.55	3.55	3.25	2.05	2.95	2.20	2.20		3.10	ND	2.35	3.10	3.55	2.20	ND	2.65	2.20	ND
519	IN	4	4.15	3.40	ND	3.55	3.85	4.15	1.45	2.35	2.05		2.35	2.95	ND	2.35	3.85	3.55	2.20	+1	3.55	+3	ND
521	OR	4	4.15	3.25	ND	3.85	3.85	3.85	2.95	3.25	2.05		2.05	3.55	ND	2.35	3.55	3.25	ND	ND	3.25	+3	ND
592	IN + OR	5	4.15	3.25	2.95	3.55	3.55	2.95	3.25	2.35	+2	+2		3.25	3.25	1.75	2.95	3.25	1.75	2.20	+2	+1	ND
593	IN + OR	6	3.55	2.65	3.25	3.25	2.95	3.25	2.05	2.50	1.15		1.00	3.85	3.85	2.20	2.95	2.80	2.50	1.90	+4	ND	ND
451	IC	6	4.55	4.85	ND	4.85	4.85	4.25	4.10	4.55	4.25	3.35		4.45	ND	3.95	4.85	5.15	ND	ND	ND	ND	ND
452	IC	7	3.40	3.10	ND	3.10	3.25	2.35	3.85	3.40	3.25	ND		3.55	4.90	3.25	3.55	4.15	ND	ND	ND	ND	ND
520	IN	7	3.10	4.30	ND	4.15	ND	3.55	3.55	3.55	2.05	1.60		4.00	ND	2.05	3.70	4.45	1.75	+1	3.25	+3	ND
522	OR	7	3.10	3.25	ND	3.55	2.95	2.95	2.80	2.95	2.05	1.75		4.15	ND	2.05	3.10	3.85	2.50	+1	+2	+2	ND
662	IN + OR	9	+4	+4	3.55	+4	2.50	+3	0.35	+4	+4		—	+4	4.85	+3	1.00	4.45	+3	ND	ND	+2	ND
663	IN + OR	10	—	4.15	+4	+4	+3	+3	+3	+3	+4	—		2.35	4.55	1.75	2.05	4.45	+4	ND	ND	+4	ND
683	IN + OR	10	—	2.50	2.20	—	+4	—	—	+4	—	—		2.20	1.60	1.45	0.85	3.55	+4	ND	ND	+4	ND
664	IN + OR	11	—	—	—	—	—	+4	—	—	—		—	—	3.85	+4	+4	3.55	—	—	ND	—	ND
665	IN + OR	12	+4	4.15	0.85	+4	3.25	2.65	+4	+4	+3	+4		3.40	4.70	2.35	3.55	3.85	+3	ND	ND	—	ND
666	IN + OR	13	—	—	—	—	—	—	—	+4	—		—	—	2.95	+4	+4	3.85	—	—	ND	—	ND
517	IC	14	—	+4	ND	—	+3	+4	+4	+3	+3		—	—	ND	+3	1.45	1.45	+4	ND	—	+3	ND
686	IN + OR	22	ND	—	—	—	—	—	—	+4	ND		—	—	—	+4	—	+3	+4	—	—	+4	+4
685	IN + OR	29	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	ND	—	—	—
684	IN + OR	36	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	ND	—	—	—
687	IN + OR	43	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—	ND	—	—	—

[a] Abbreviations: IN, intranasal; OR, oral; IC, intracerebral.
[b] Postinoculation day.
[c] Titers expressed as $\log_{10}$/0.01 g of tissue or 0.1 ml of body fluid.
[d] No virus isolated through four blind passages +1, etc. FPL virus isolation in the first, etc., blind passage.
[e] Not determined.

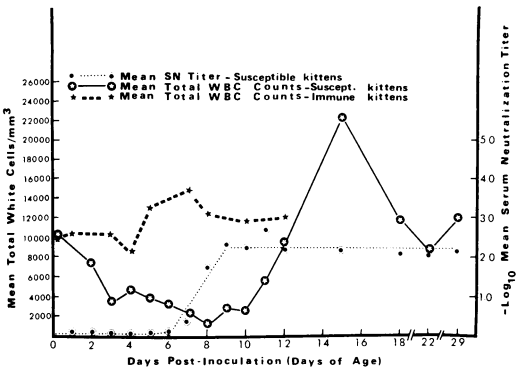

FIG. 1. *Comparison of total white blood cell (WBC) counts in 12 immune and 21 susceptible newborn kittens after panleukopenia virus infection on the day of birth. Correlation of WBC and immune response in the susceptible newborn kittens.*

The route of inoculation (intracerebral, intranasal, or oral) made no difference on the spread of virus or viral concentration in different organs that were compared at 4 DPI (cats 518, 519, 521) and at 7 DPI (cats 452, 520, 522). Animals identified with numbers 518 to 522 were littermates and were sacrificed, whereas 452 was from another litter and died.

Serology. Measurable neutralizing antibody titer was first detected 6 days after infection (1 of 6 kittens bled, titer 1:2.5) and at 7 DPI (one of four kittens bled, titer 1:28). From 8 DPI on, all kittens had neutralizing antibody levels. SN-antibody titers (Fig. 1) reached maximum by 10 to 12 DPI and persisted at those levels until the animals were sacrificed (up to 43 DPI). Seven of nine cats had titers ranging from 1:56 to 1:220, whereas the remaining two had titers of 1:900 and 1:1,800. Leukocyte response lagged behind specific neutralizing antibody by 3 days.

Animals that died 6 and 7 DPI succumbed before specific antibody response could manifest itself. The kitten that died 10 days after infection had a neutralizing antibody titer of 1:100 at 8 DPI. The cat in lateral recumbency at 14 DPI had circulating antibodies from 7 DPI, and the SN titer was 1:220 at 10 and at 14 DPI.

DISCUSSION

FPL caused subclinical infection in 19 out of 23 inoculated, susceptible newborn kittens. Bacteremia, secondary to viral infection, contributed significantly to the cause of death at least in three of four kittens. Two of the four animals died at 10 and 14 DPI, despite moderately high circulating panleukopenia antibody titer from the 8th DPI.

All infected kittens developed severe and prolonged leukopenia. This persisted until 10 to 12 DPI in most kittens. Inoculated offspring of FPL-immune queens did not develop leukopenia as a result of bleeding. The leukopenia observed in the susceptible infected kittens was the result of the virus acting on the lymphopoietic system.

Panleukopenia virus spread to all organs, regardless of the route of inoculation (intracerebral, intranasal, or oral). The respiratory and gastrointestinal tracts served as portals of entry for the virus. Virus was eliminated in feces and urine for at least 22 DPI and probably by droplets from the respiratory tract. Similar observations were made by the earlier investigators (5, 15) about the transmissible nature of the virus in postweaned cats.

Antibody production preceded recovery from leukopenia by 3 to 4 days in inoculated newborn kittens. The specific antibody titers that developed remained at a low level, approximately 2 logs, in seven of nine kittens that were kept longer than 10 days. The two other kittens developed about 3 logs of antibody titers.

By virtue of their housing in Horsfall isolation units, the kittens in this study were confined to a limited bacterial flora largely derived from the queen. The queens probably had antibodies in the colostrum against their normal flora which provided some protection to most of the FPL-infected kittens. In the absence of such protection, coupled with prolonged, severe leukopenia and any damage to the lining epithelium of the intestine, one would expect a much higher mortality rate. The FPL virus was shown to cause only a mild, nonfatal disease in 4.5- to 5.5-month-old gnotobiotic cats (13). The leukopenia was less severe and shorter in duration in the gnotobiotic cats in comparison to the infection in newborn kittens.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grants AI-265 and AI-06137, from the National Institute of Allergy and Infectious Diseases.

We acknowledge the technical assistance of E. Tompkins, C. Hoff, and J. Buck.

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