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Canine and Feline Host Ranges of Canine Parvovirus and Feline Panleukopenia Virus: Distinct Host Cell Tropisms of Each Virus In Vitro and In Vivo

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Canine parvovirus (CPV) emerged as an apparently new virus during the mid-1970s. The origin of CPV is unknown, but a variation from feline panleukopenia virus (FPV) or another closely related parvovirus is suspected. Here we examine the in vitro and in vivo canine and feline host ranges of CPV and FPV. Examination of three canine and six feline cell lines and mitogen-stimulated canine and feline peripheral blood lymphocytes revealed that CPV replicates in both canine and feline cells, whereas FPV replicates efficiently only in feline cells. The in vivo host ranges were unexpectedly complex and distinct from the in vitro host ranges. Inoculation of dogs with FPV revealed efficient replication in the thymus and, to some degree, in the bone marrow, as shown by virus isolation, viral DNA recovery, and Southern blotting and by strand-specific in situ hybridization. FPV replication could not be demonstrated in mesenteric lymph nodes or in the small intestine, which are important target tissues in CPV infection. Although CPV replicated well in all the feline cells tested in vitro, it did not replicate in any tissue of cats after intramuscular or intravenous inoculation. These results indicate that these viruses have complex and overlapping host ranges and that distinct tissue tropisms exist in the homologous and heterologous hosts.

Canine parvovirus (CPV) and feline panleukopenia virus (FPV) are naturally derived viruses which are classified according to the host from which they were isolated. CPV and FPV are considered to be host range variants among the feline parvoviruses in the genus *Parvovirus* (51).

CPV and FPV isolates differ in less than 2% of their genomic DNA sequences (27, 35, 44, 45), and they are very similar antigenetically. However, they can be distinguished by using specific monoclonal antibodies (MAbs) (38, 40, 53), and they do show differences in their pH dependence of hemagglutination. CPV hemagglutinates rhesus macaque or pig erythrocytes over a broad pH range, between pH 6.0 and pH 8.0, whereas FPV isolates hemagglutinate these erythrocytes only below about pH 6.8 (11, 31, 36).

A further striking difference is in host range, since FPV strains have been shown to replicate in feline cells and cats but not in cultured canine cells. In contrast, CPV isolates have been shown to replicate in canine and feline cells in culture as well as in dogs (3, 10, 36). As with other autonomously replicating parvoviruses, these viruses have a tropism for tissues containing dividing cells, and in older animals both viruses replicate in cells in the lymphoid tissues, as well as in the rapidly dividing cells of the epithelium of the small intestine. There may be differences in the cell tropism of the two viruses, because FPV causes a profound leukopenia in cats whereas CPV infection of dogs results in only a relative lymphopenia.

The in vivo host ranges of the viruses have not been well defined. CPV-like isolates have been isolated from domestic dogs, wolves, and coyotes, as well as from other members of the family Canidae (16, 25, 34). FPV and related virus isolates replicate well in cats (many members of the family Felidae), as well as in common raccoons, mink, and also probably in foxes (2, 5, 34). However, little is known about the heterologous host ranges of the viruses. Previous studies suggest that FPV isolates probably replicate to some degree in dogs but that the virus is not shed in the feces (43). Little is known about CPV infection of cats, although one study has indicated that CPV replicated in cats in a pattern similar to FPV (17).

A detailed knowledge of the host ranges of these viruses is important for understanding the origin and evolution of CPV. CPV was first recognized around 1978, and the virus spread rapidly into most populations of domestic and wild canids (4, 20, 33, 41, 42, 48, 58, 59). Retrospective serological studies indicate that CPV first infected dogs in the mid-1970s (20, 37, 48) and subsequently spread widely among dogs during 1978 (for a review, see reference 34). The origin of CPV is still unknown, but it is possible that CPV is a variant of FPV or a related virus which mutated to gain the ability to efficiently replicate in and spread among dogs.

Here we examine the in vivo and in vitro canine and feline host ranges of CPV and FPV. Virus replication in animals was defined by virus recovery and by demonstration of viral DNA in tissues by Southern blot analysis or by strand-specific in situ hybridization. Virus replication in a number of canine and feline cultured cells was also examined by replicative-form (RF) DNA isolation and by virus titration of tissue culture supernatants. The data contribute to a further understanding of the relationships between the two viruses and of the changes which were necessary for the emergence of CPV as a new canine pathogen.

MATERIALS AND METHODS

Cell lines. The A72 canine fibroma-derived cell line (approximately passage 150) (6) and the NLFK feline cell line (derived from the Crandell feline kidney cell line [CRFK] [13]) were grown as previously described (39). The CRFK

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TABLE 1. PFU titers of supernatants of various permanent cell lines inoculated with FPV or CPV

Virus	Days p.i.	PFU titer[a] in:											
		Feline cells (MOI, 0.1 PFU/cell)						Canine cells (MOI, 0.1 PFU/cell)			Canine cells (MOI, 100 PFU/cell)		
		NLFK	CRFK	3201	Fc2Lu	AK-D	Fc3Tg	A72	Cf2Th	CT45-S	A72	Cf2Th	CT45-S
FPV	0[b]	1.0	<1	<1	<1	<1	<1	1.3	<1	<1	2	<1	<1
	3	6.0	5.0	4.3	4.0	5.8	2.6	2.3	<1	1.8	2	<1	<1
CPV	0	1.0	<1	<1	<1	<1	<1	<1	<1	<1	2.3	2.3	<1
	3	5.6	5.9	4.0	3.9	4.6	2.8	5.3	4.3	1.6	6	4	2

[a] Determined by the immune-staining plaque assay in NLFK cells. Expressed as $\log_{10}$ of PFU/0.3 ml of supernatant.
[b] Day 0 indicates 1 h postinoculation (p.i.).

cells (CCL 94), feline tongue cells Fc3Tg (CCL 176), feline lung cells Fc2Lu (CCL 217), and feline lung cells AK-D (CCL 150) were obtained from the American Type Culture Collection. The canine thymus epithelium cell line Cf2Th, the feline lymphoma cell line 3201 (46), and the canine T-cell line CT 45-S (21) were kindly provided by, respectively, F. W. Quimby, R. J. Avery, and M. J. G. Appel, New York State College of Veterinary Medicine.

Cells were grown in RPMI 1640 medium with 20% fetal bovine serum (CT 45-S), Dulbecco's minimal essential medium with 10% fetal bovine serum (AK-D, Fc2Lu, and Cf2Th), or a 50% mixture of McCoy's 5A and Leibowitz L15 media with 5% fetal bovine serum (3201, NLFK, CRFK, A72, and Fc3Tg).

Lymphocyte culture. Canine and feline peripheral blood lymphocytes (PBLs) were isolated from heparinized blood samples of parvovirus-seronegative animals by Ficoll-Paque density centrifugation. Freshly separated PBLs at a density of 2×10^6/ml were incubated with 5 μg of concanavalin A (ConA) per ml for 48 h and then cultured in the same medium with 100 U of human recombinant interleukin-2 (rIL-2; Boehringer-Mannheim) per ml without ConA.

[^{3}H]thymidine incorporation test. PBLs (1×10^5 cells) were incubated for 16 h with 0.5 μCi of [^{3}H]thymidine. After the cells were harvested with a Skatron cell harvester, the filter-bound radioactivity was counted.

Viruses. Viruses derived from the molecular clones of the CPV strain CPV-d (CPV type 2 antigenic type) and the FPV strain FPV-b (35, 40, 49) were used throughout the experiments. The uncloned FPV-b was also used in some studies.

Infection of cells. Cells of the various cell lines were seeded at a density of 2×10^4/cm^2 and then, after 20 h of incubation, inoculated with virus at a multiplicity of infection (MOI) of 0.1 PFU per cell. In some studies the canine cell lines were also inoculated with CPV or FPV at a MOI of 100 PFU per cell. The virus was allowed to adsorb to the cells for 1 h at 37°C, and the cells were washed once with Dulbecco's minimal essential medium before and after the virus adsorption. Culture supernatants collected 1 h and 3 days after inoculation were tested for virus by plaque titration in NLFK cells.

PBLs cultured at a cell density of 2×10^6/ml were inoculated with CPV or FPV at MOIs between 10^{-1} and 10^{-5} PFU per cell after 48 h of ConA stimulation. The cells were then cultured for a further 3 days in rIL-2-supplemented RPMI 1640 medium with 50 μM 2-mercaptoethanol and 20% fetal bovine serum without ConA. Unstimulated cells were treated the same way except the ConA was omitted.

Virus titer determination. Virus titers were determined by using an immune-staining plaque assay in NLFK cells (23, 39). Virus antigen was also assayed in the hemagglutination test by using rhesus macaque erythrocytes in barbital acetate buffer (pH 6.2) as described previously (39, 40).

Hemagglutination inhibition assay and virus antigenic typ-

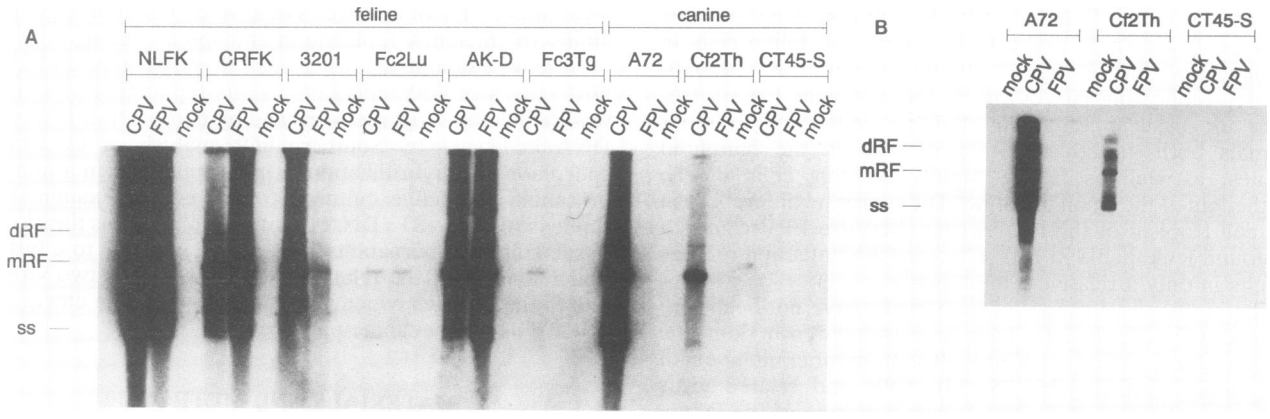

FIG. 1. Southern blot analysis of low-molecular-weight DNA recovered from the various cell lines 3 days after inoculation. Samples representing the DNA from approximately 2×10^4 cells were electrophoresed in a 1% agarose gel. The autoradiograph is exposed to make the smaller amounts of RF DNA in certain cell lines visible. (A) Feline and canine cells inoculated with CPV or FPV at a MOI of 0.1 PFU per cell. (B) Canine cell lines inoculated with CPV or FPV at a MOI of 100 PFU per cell. Abbreviations: dRF, dimer RF DNA; mRF, monomer RF DNA; ss, single-stranded virion DNA.

TABLE 2. PFU titers of supernatants of ConA-stimulated canine or feline PBLs inoculated with FPV or CPV

MOI	Virus	PFU titer[a] in:	
		Canine PBLs	Feline PBLs
0.1	CPV	6.3	5.3
	FPV	1.8	6.3
0.01	CPV	5.6	5.0
	FPV	1.6	6.0
0.001	CPV	6.3	<1
	FPV	1.4	5.8

[a] Expressed as $\log_{10}$ PFU/0.3 ml. Titers of cell culture supernatants 3 days after inoculation as determined by the immune-staining plaque assay in NLFK cells.

ing. The inhibition assay was performed with heat-inactivated and erythrocyte-preincubated sera or with MAbs, using rhesus macaque erythrocytes and 8 hemagglutination units of virus (11). The MAbs used for antigenic typing of the viruses (MAb 7, MAb 14, MAb G, and MAb H) have been described previously (38).

Isolation and analysis of viral DNA. Genomic and low-molecular-weight DNA was isolated from infected-cell cultures or animal tissue samples by standard procedures (18, 28, 47). For total DNA preparation, high-molecular-weight DNA was digested with *Bam*HI prior to electrophoresis in 1% agarose gels in Tris-acetate-EDTA (TAE) buffer, with 1 μg of ethidium bromide per ml. Low-molecular-weight DNA was not digested but was electrophoresed under the same conditions. DNA was transferred to nylon membranes and then hybridized with a ^{32}P-labeled probe representing map units 59 to 98.5 (ca. 2,000 bases) of the CPV genome.

Animal infections and analysis. Twelve-week-old specific-pathogen-free beagles (J. A. Baker Institute) or 12-week-old specific-pathogen-free kittens (Harlan-Sprague-Dawley Inc., Indianapolis, Ind.) were inoculated intranasally, intramuscularly, or intravenously with 0.5×10^6 to 1.0×10^6 PFU of either FPV or CPV. Animals were bled, and their temperatures were monitored daily. They were killed between days 4 and 6 postinfection (see Table 3). Tissue samples were snap-frozen in liquid nitrogen or immediately fixed in ice-cold periodate-lysine-paraformaldehyde-glutaraldehyde (PLPG) solution (1). Aspirates of bone marrow cells collected from the femur were spun onto poly-L-lysine-coated microslides, fixed in ice-cold PLPG solution, and stored at −20°C. Frozen tissues were stored at −70°C, and PLPG-fixed tissues were paraffin embedded.

In situ hybridization. In situ hybridization of frozen tissue sections or PLPG-fixed cytospins was performed essentially as described elsewhere (1, 56) with strand-specific RNA probes. The probes were transcribed, by using either T7 or SP6 polymerase, from plasmid pBI264, which represents about 2,000 bases of the CPV genome (map units 59 to 98.5) in the vector pGEM3Z (Promega). The strand specificities of the probes were confirmed by Southern blotting with viral RF DNA and virion single-stranded DNA preparations of CPV (data not shown). Since the viral genome of FPV and CPV represents a single-stranded DNA of negative polarity, the minus-sense probe hybridizes with both mRNA and positive-sense DNA. Both are synthesized only in an infected cell, and a hybridization signal with the minus-sense probe therefore indicates DNA replication and/or transcription.

RESULTS

In vitro studies. (i) Virus replication in permanent cell lines. FPV and CPV replicated in all feline cell lines to similar PFU titers (Table 1). NLFK, CRFK, and AK-D, 3201, and Fe2Lu cells replicated FPV and CPV efficiently, as measured by virus titers in the supernatant (Table 1), and FPV and CPV

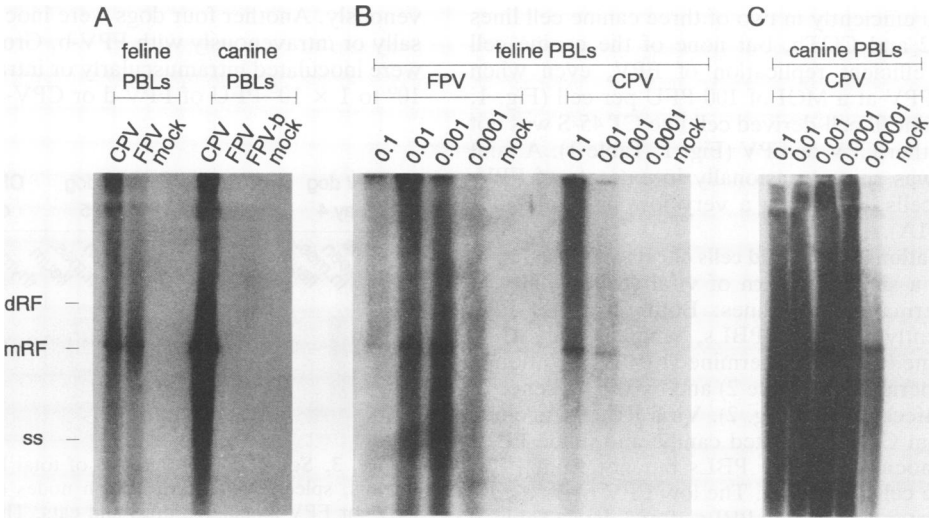

FIG. 2. Southern blot analysis of low-molecular-weight DNA of FPV- or CPV-inoculated ConA-stimulated canine or feline PBLs. Cells were inoculated with the MOIs (PFU per cell) indicated, and the DNA was harvested 3 days later. All DNA recovered from PBLs showed a certain degree of DNA degradation. Abbreviations: dRF, dimer RF DNA; mRF, monomer RF DNA; and ss, single-stranded virion DNA. (A) Feline and canine PBLs inoculated with CPV or FPV at a MOI of 0.1 PFU per cell. DNA from mock-inoculated control cultures and that from canine PBLs inoculated with the uncloned FPV-b isolate are also shown. (B) Titer determination of FPV and CPV in feline PBLs by inoculation with MOIs between 0.1 and 0.0001 PFU per cell or by mock inoculation. (C) Titration of CPV in canine PBLs by inoculation with MOIs between 0.1 and 0.00001 PFU per cell or by mock inoculation.

TABLE 3. Summary of the inoculations used in this study

Host and virus	No. of animals	Hours p.i.[a]	Inoculation route[b]	Seroconversion[c]	PFU titer (log_{10} PFU/0.3 ml) in [d,e]:			
					Ileum	Mesenteric lymph node	Spleen	Thymus
Dogs								
CPV	2	144	i.n.	<320	4.7	2.3	<1	2.0
				<320	4.9	2.6	<1	2.2
	1	96	i.v.	<10	6.0	6.3	4.0	5.9
	2	108	i.m.	ND[f]	<1, <1	1.5, 3.0	<1, <1	2.0, 5.0
	2	120	i.v.	2,560	4.7	1.0	<1	3.6
				5,120	5.0	3.3	<1	3.8
FPV	2	120	i.n.	40	<1	<1	<1	<1
				80	1.8	<1	2.0	6.0
	2	120	i.v.	160	<1	<1	1.4	5.9
				320	1.4	1.0	2.3	6.3
Cats								
CPV	2	96	i.v.	<10	<1	<1	<1	<1
				<10	<1	<1	<1	<1
	2	108	i.m.	<10	<1	<1	<1	<1
				<10	<1	<1	<1	<1
FPV	2	96	i.v.	40	5.5	2.8	3.3	7.0
				80	6.3	4.5	4.0	7.3
	2	108	i.m.	ND	4.7, 5.7	4.0, 4.9	2.6, 3.0	5.2, 5.4

[a] The animals were killed at the times indicated postinoculation (p.i.).
[b] Abbreviations: i.v., intravenous; i.m., intramuscular; i.n. intranasal.
[c] Virus-specific antibody titer as determined by the hemagglutination inhibition test. All animals were seronegative before inoculation.
[d] Titer determination of 10% tissue homogenates in the immune-staining plaque assay in NLFK cells.
[e] The PFU titer of the tissue from each animal is given separately.
[f] ND, not determined.

RF DNAs could be demonstrated in extracts of feline cells collected 3 days after inoculation (Fig. 1A). The amount of RF DNA in Fc2Lu cells was comparatively small (Fig. 1A), but this cell line clearly supported replication of both CPV and FPV.

CPV replicated efficiently in two of three canine cell lines investigated, A72 and Cf2Th, but none of the canine cell lines supported efficient replication of FPV, even when inoculated with FPV at a MOI of 100 PFU per cell (Fig. 1; Table 1). The canine T-cell-derived cell line CT 45-S was not permissive for either CPV or FPV (Fig. 1; Table 1). A faint RF DNA band was seen occasionally in extracts of FPV-inoculated A72 cells, indicating a very low level of DNA replication (Fig. 1A).

(ii) Virus replication in lymphoid cells of cats and dogs. PBL cultures showed a similar pattern of viral susceptibility to that seen for permanent cell lines. Both CPV and FPV replicated efficiently in feline PBLs, whereas only CPV replicated in canine PBLs, as determined by PFU titration of tissue culture supernatants (Table 2) and by the presence of RF DNA from infected cells (Fig. 2). Viral RF DNAs could be recovered from CPV-inoculated canine and feline PBLs and from FPV-inoculated feline PBLs but not from FPV-inoculated canine cells (Fig. 2A). The low FPV titers recovered from supernatants of canine PBLs after 3 days (Table 2) are most probably residual inoculum, since no RF DNA could be demonstrated in Hirt supernatants of those cells (Fig. 2A). Both inocula gave efficient infections of the homologous host cells even at very low MOIs, down to 10^{-4} PFU per cell (Fig. 2B and C). The [^{3}H]thymidine incorporation by the PBLs decreased during the course of infection, the degree of decrease being dependent on the inoculum titer (data not shown).

In vivo studies. A total of seven dogs were inoculated with 5×10^5 to 1×10^6 PFU of CPV-d as detailed in Table 3. Virus was inoculated intranasally, intramuscularly, or intravenously. Another four dogs were inoculated either intranasally or intravenously with FPV-b. Groups of two cats each were inoculated intramuscularly or intravenously with 0.5×10^6 to 1×10^6 PFU of FPV-d or CPV-d (Table 3).

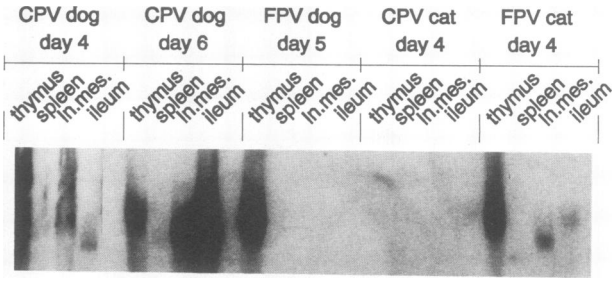

FIG. 3. Southern blot analysis of total DNA extracts from the thymus, spleen, mesenteric lymph nodes (ln.mes.), and ileum of CPV- or FPV-inoculated dogs and cats. The CPV-inoculated dogs were inoculated intravenously or intranasally and killed on day 4 or 6 postinoculation, respectively. The FPV-inoculated dog was inoculated intravenously and killed on day 5 postinoculation. Both cats for which tissue analyses are shown were inoculated intravenously and killed on day 4 postinoculation. The amounts of DNA loaded on each lane are equivalent to about 20 μg from the thymus, mesenteric lymph node, or ileum and about 50 μg from the spleen.

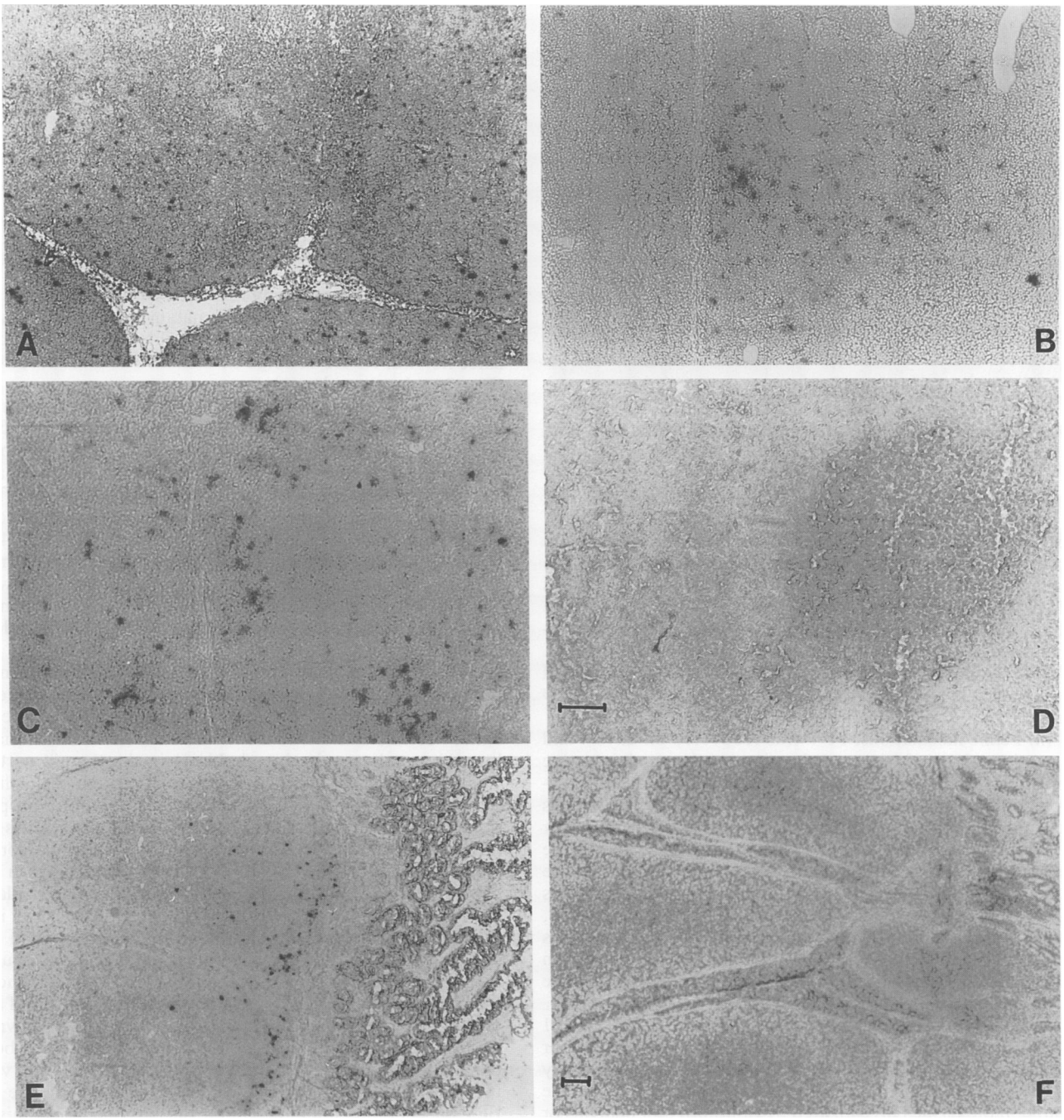

FIG. 4. In situ hybridization of thymus (A and B), mesenteric lymph nodes (C and D), and ileum (E and F) of CPV- (panels A, C, and E) or FPV (panels B, D, and F)-inoculated dogs. The animals shown were killed on day 4 (CPV) or day 5 (FPV) after inoculation. The minus-sense probe used hybridizes with viral RF DNA and mRNA, and positive signals therefore indicate active viral DNA replication and/or transcription, allowing the discrimination of virus-replicating cells. Panels containing the same tissue are at the same magnification. Bar, 100 μm.

(i) Canine inoculations. All dogs inoculated with CPV acquired a systemic infection. Virus could be recovered from several regions of the small and large intestine and from a variety of tissues of the lymphatic system. The ileum and thymus consistently had the highest virus titers (Table 3). Viral DNA could be demonstrated by Southern blot analysis of total DNA recovered from these tissues (Fig. 3). Virus replication was also demonstrated in the thymus, mesenteric lymph node, and ileum by in situ hybridization with the minus-sense RNA probe (Fig. 4A, C, and E). At later times after inoculation, high virus titers could not be recovered from some CPV-inoculated dogs, probably as a consequence of the developing neutralizing-antibody response. However, DNA could be demonstrated in the tissues of those animals by Southern blot analysis (Fig. 3) and by in situ hybridization (data not shown), indicating an active infection.

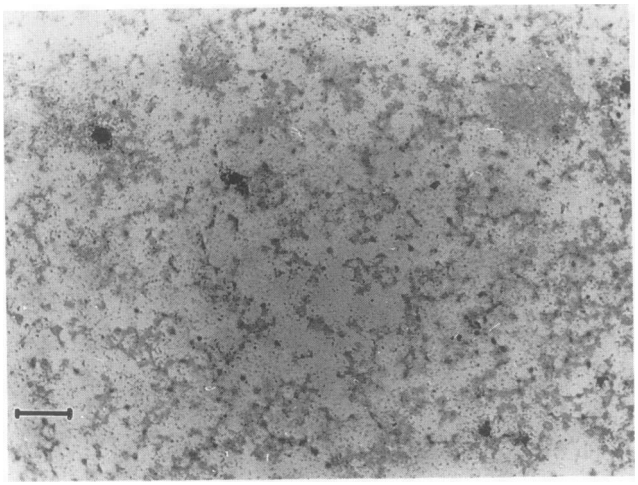

FIG. 5. In situ hybridization of bone marrow cells of a dog 4 days after inoculation with FPV. The minus-sense probe was used, allowing the detection of viral RF DNA or mRNA. Bar, 100 μm.

High titers of FPV could be recovered from the thymus of inoculated dogs, whereas much lower titers were recovered from the spleen (Table 3). The replication of FPV in the canine thymus was confirmed by both Southern blot analysis (Fig. 3) and in situ hybridization with the minus-sense probe (Fig. 4B). FPV-replicating cells could also be readily demonstrated in the bone marrow by in situ hybridization (Fig. 5). No virus or viral DNA could be detected in the small intestine or mesenteric lymph nodes (Table 3; Fig. 3 and 4D and F).

(ii) Feline inoculations. FPV was recovered on days 4 and 5 postinoculation from several regions of the small intestine and from lymphatic tissues of the cats after either intravenous or intramuscular inoculation. The identities of viruses reisolated from each animal were confirmed with CPV- or FPV-specific MAbs. The FPV replicated to high titers in the thymus and the small intestine, whereas lower titers were recovered from the spleen and mesenteric lymph nodes (Table 3). Viral DNA was recovered from the thymus, mesenteric lymph nodes, and ileum (Fig. 3), and cells containing FPV DNA or mRNA were also demonstrated in these organs by in situ hybridization with the minus-sense probe (Fig. 6B, D, and F).

Intramuscular or intravenous inoculation of 0.5×10^6 to 1×10^6 PFU of CPV-d did not result in infection of the four animals inoculated. No virus could be recovered from any tissue (Table 3), no viral DNA or virus-replicating cells were found in the tissues investigated by Southern blot analysis or by strand-specific in situ hybridization (Fig. 3 and 6A, C, and E), and no animal had seroconverted by day 4 after inoculation (Table 3).

DISCUSSION

CPV emerged suddenly in 1978 as a new pathogen of dogs. Current evidence suggests that CPV is a variant of FPV or some closely related virus which mutated to gain the canine host range. We have shown previously that only a small number of nucleotide differences between FPV and CPV determine the ability of the latter virus to replicate in canine cells in culture (35, 36). However, the relationship between the in vitro host ranges of the viruses and their abilities to replicate in various host animals is not well understood, and few studies have critically compared the in vitro and in vivo host ranges.

The present studies of the in vitro host ranges extend and confirm previous reports (3, 10, 36). We demonstrated that all feline cell lines tested replicated both FPV and CPV to comparable degrees, whereas two canine cell lines replicated CPV but not FPV. No FPV replication was detected in Cf2Th cells, and only a very low level of FPV DNA was detected occasionally in A72 cells by Southern blot analysis (Table 1; Fig. 1).

Lymphoid cells are a major target for CPV and FPV replication in dogs and cats (8, 9, 12, 14, 29, 30). CPV replicated efficiently in both feline and canine PBLs, whereas FPV replicated only in feline PBLs (Table 2; Fig. 2). The low virus titers in the supernatant of FPV-inoculated canine PBLs were probably derived from the inoculum, since no viral RF DNA could be demonstrated in those cells (Fig. 2A; data not shown).

In contrast to the in vitro results, the animal host ranges were unexpectedly complex. FPV, which did not replicate efficiently in any canine cell culture, replicated to very high titers in the thymus of intravenously (two of two) and intranasally (one of two) inoculated dogs (Table 3). The replication was confirmed both by Southern blotting of total DNA extracts (Fig. 3) and by in situ hybridization (Fig. 4B). Virus-replicating cells were also found in the bone marrow (Fig. 5) and occasionally in the spleen (results not shown). No FPV replication was detectable in the canine small intestine or mesenteric lymph nodes (Table 3; Fig. 3 and 4D and F).

The histological pattern of FPV replication in the canine thymus, with positive cells mainly in the cortex, suggests some differentiation-dependent T-cell tropism of FPV replication. Stem cells derived from the bone marrow are initially located as subcapsular lymphoblasts in the thymic cortex. These $CD4^-$ $CD8^-$ cells divide rapidly; most of them differentiate into $CD4^+$ $CD8^+$ progeny cells, and a proportion finally mature into $CD4^+$ or $CD8^+$ lymphocytes. As the immature progenitor cells differentiate in the thymic cortex (26, 57), it is possible that one of those populations in the canine thymus ($CD4^-$ $CD8^-$ or $CD4^+$ $CD8^+$) is particularly permissive for FPV. The pathway of entry of the virus into the thymus is unknown. The blood-thymus barrier in the cortex region is relatively impermeable for high-molecular-mass molecules. Since FPV-replicating cells were found in the bone marrow (Fig. 5), and since both feline myeloid and erythroid progenitor and precursor cells have been shown to be susceptible to FPV infection in vitro (22), infected bone marrow-derived cells could be responsible for the viral invasion of the thymus. Experiments to further define the FPV-susceptible canine cells and the possible mechanisms of entry are in progress.

The molecularly cloned CPV-d strain replicated efficiently in dogs, with the typical tissue tropism described for CPV (24, 29, 30). The virus replicated in a variety of lymphoid tissues and in the small and large intestine, and an antibody response was observed as early as 4 days postinfection (Table 3). The virus PFU titers recovered from inoculated animals varied greatly depending on the degree of seroconversion, indicating that virus-neutralizing antibodies affected the recovery of infectious virus from ground tissues. The demonstration of RF DNA in these tissues (Fig. 3) and of positive signals by the in situ hybridization was a more reliable criterion for viral replication at later times after infection.

CPV inoculation of cats also revealed unexpected results.

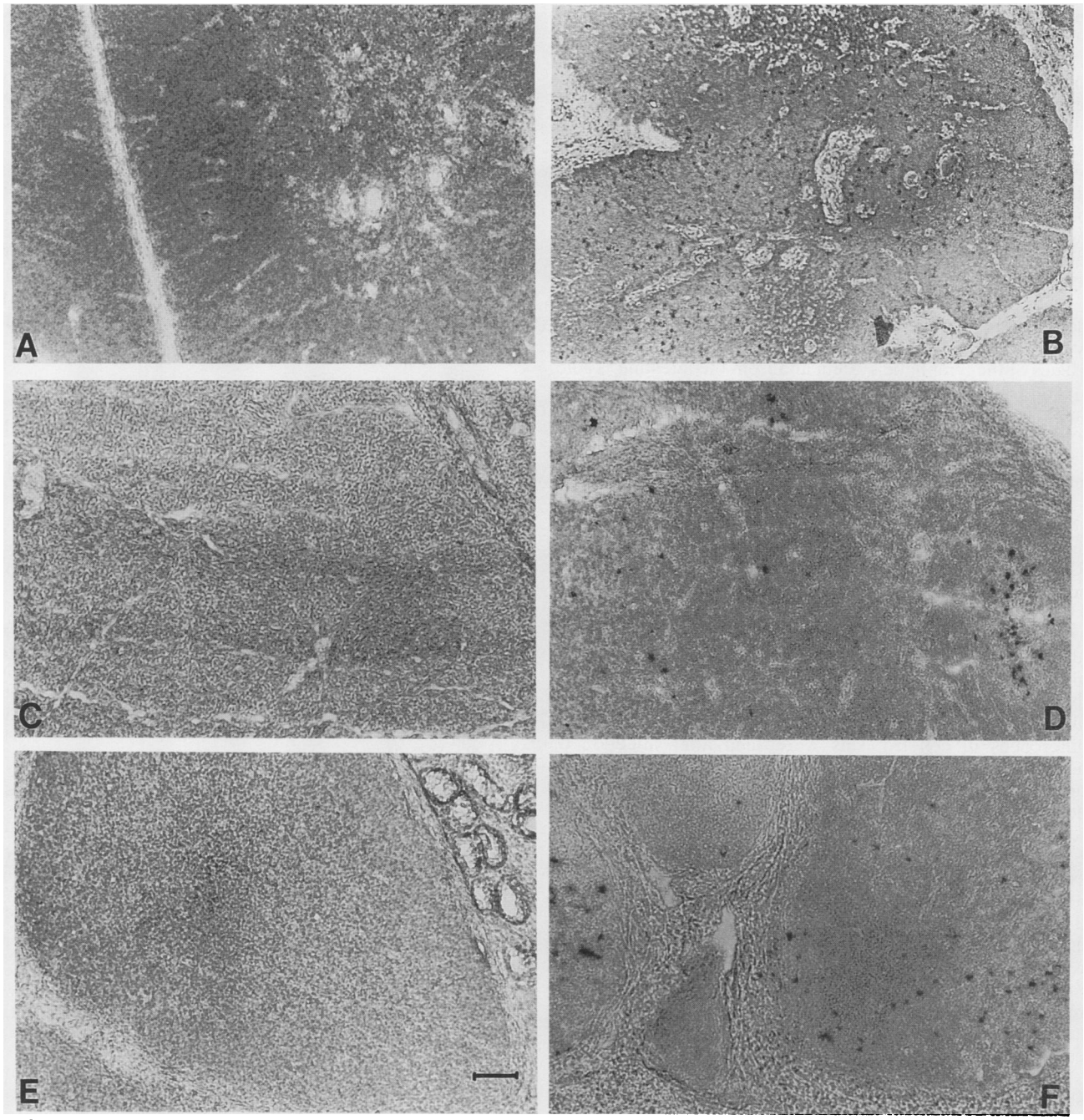

FIG. 6. In situ hybridization of thymus (A and B), mesenteric lymph nodes (C and D), and ileum (E and F) of CPV- (panels A, C, and E) or FPV (panels B, D, and F)-inoculated cats (intravenous inoculation). Animals were killed 4 days after inoculation. The minus-sense probe was used, allowing the detection of viral RF DNA and mRNA. Positive signals indicate active viral DNA replication and/or transcription, allowing the discrimination of virus-replicating cells. Panels containing the same tissue are at the same magnification. Bar, 100 μm.

In two separate experiments, intramuscular or intravenous inoculation of CPV into cats resulted in no detectable infection. No virus could be isolated from any tissue (Table 3), no RF DNA could be demonstrated by Southern blotting in DNA extracts (Fig. 3), and in situ hybridization was negative for all tissues (Fig. 6A, C, and E). The reason for this lack of replication is unknown. However, one possibility is that the virus is cleared or masked before it can adsorb to a susceptible target cell. For example, since CPV, but not FPV, hemagglutinates feline erythrocytes under physiological conditions (37°C and pH 7.4 [data not shown]), it is possible that binding by erythrocytes or some other ligand in the tissues removes CPV from the circulation after parenteral application. Further experiments are necessary to address this phenomenon.

Our findings on the feline replication of CPV differ from those of a study published by Goto et al. (17). They described a systemic infection of specific-pathogen-free cats

with CPV after subcutaneous inoculation with virus doses similar to those used here. Virus was recovered from several organs between days 3 and 5 postinoculation. In that study a CPV isolate from 1982 was used, most probably the antigenic type CPV-2a (37, 41, 42). Our study was done with a CPV-2 strain. Whether the different strains might have resulted in the different findings has not been determined. Also, owing to the high environmental resistance of parvoviruses, cross-contamination by a contaminated environment is a potential threat. In the present study we confirmed the virus types used for the inoculation and also those reisolated from inoculated animals, and we can exclude any contamination with FPV in our experiments.

Infections of cats with FPV-b derived from the infectious clone induced a systemic infection with a tissue tropism typical of FPV (8, 14). Virus was recovered from various tissues of the lymphatic system and from the intestine (Table 3), and DNA analysis revealed active virus replication in the ileum, mesenteric lymph node, thymus, bone marrow, and spleen (Fig. 3 and 6B, D, and F; data not shown). The cats developed virus-specific antibodies when tested 4 days after inoculation (Table 3).

The tissue tropism of CPV in dogs and FPV in cats was very similar to that seen in mink infected with the closely related mink enteritis virus (56). In that study, viral DNA or RNA was observed by in situ hybridization up to 8 days postinoculation, with the peak of DNA being detected at day 4.

These distinct tissue tropisms of virus replication in different hosts have not been clearly defined for other parvoviruses. However, mouse infection studies with biotypes of the minute virus of mice MVM(p) and MVM(i), which have distinct host ranges in vitro (15, 52, 54), showed that differences in cell tropisms were seen after infection of neonatal animals (7, 19). The importance of the nonstructural protein NS2 for efficient in vivo replication was also demonstrated (7), although it is not required for efficient replication in certain cell lines in vitro (32).

The results of our studies require a reassessment of the events that were necessary for the emergence of CPV as a new canine pathogen. Because FPV was able to replicate in the canine thymus and CPV did not replicate in cats, the emergence of CPV as an efficient canine pathogen may have required a change of tissue tropism but not of animal host range. The ancestor of CPV (probably FPV or a closely related virus) had to gain the ability to infect dogs efficiently and also the ability to replicate in cells of the small intestinal epithelium to allow efficient shedding and transmission.

Analysis of recombinants between FPV and CPV has shown that the canine in vivo and in vitro host range of CPV maps within the capsid protein gene, and the major determinant is located between 59 and 73 map units. There are four coding amino acid changes between FPV-b and CPV-d in that region of the VP1/VP2 gene (35, 36). Recent genetic studies have further defined the necessary changes to as few as three amino acids (VP2 residues 93, 103, and 323 [11a]). Some of these coding changes affect amino acids which are exposed on the surface of the viral capsid (55), and residue 93 also affects the CPV-specific epitope.

Hypotheses put forward to explain the emergence and selection of CPV from an FPV-like ancestor have suggested that it was derived from an FPV variant strain which contaminated canine vaccines (50). Our results do not exclude this hypothesis but suggest that such mechanisms were not necessarily required, because FPV can already replicate in some canine tissues even after oronasal infection. Only a small number of changes were apparently required to allow FPV to efficiently replicate in other canine tissues, including those of the small intestine (36).

CPV has changed rapidly since its emergence, giving rise to at least two distinct antigenic types (designated CPV type 2a and CPV type 2b), each of which largely replaced the previous antigenic type of virus (37, 41, 42). In the course of that evolution, the CPV DNA sequences continued to diverge from those of FPV (37). The host range of FPV defined here and the genetic and evolutionary studies of CPV and FPV support the possibility that CPV originated as a variant of an FPV-like virus and that the virus has subsequently evolved to become better adapted to dogs. In future studies we will seek to define the mechanisms which determine the altered tissue tropisms revealed here and the specific differences between the CPV variant types.

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