Natural Variation of Canine Parvovirus

Abstract. *Canine parvovirus was first recognized during 1978. Analysis of isolates collected since its emergence revealed that viruses circulating after 1980 were antigenically different from earlier isolates. Monoclonal antibodies clearly distinguished the two strains, some being specific for either the old or the new viruses. Restriction enzyme analysis of viral DNA's showed that the post-1980 viruses were similar to earlier isolates, but some restriction site differences were present in the new strain. These results suggest that the canine parvoviruses infecting dogs in the seven areas of the United States that were sampled derive from a variant virus that replaced the original strain during 1980.*

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Knowledge of the evolution of viruses is important for understanding the ways in which they adapt to their hosts. We report studies of the variation of a newly recognized parvovirus during the first 7 years after its emergence in dogs. Canine parvovirus (CPV) was recognized in 1978 as the cause of two previously unrecognized disease syndromes of dogs, myocarditis and enteritis (*1, 2*). Canine sera collected in several countries before 1978 showed no evidence of infection by the virus before late 1976 or 1977 (*3*). Since 1978 the virus has been widely disseminated and is now found throughout the world.

The emergence and panzootic spread of CPV suggest that it arose from a single source and that it has been infecting dogs for a short time. Canine parvovirus is genetically and antigenically similar to the long recognized feline panleukopenia virus (FPV) and to similar viruses of mink or other animals (*4, 5*) and is believed to be a host range variant of one of those viruses. We examined CPV isolates obtained since 1978 and determined the extent to which the virus had evolved antigenically and genetically during that time.

Two CPV isolates obtained from various locations in the United States between 1978 and 1984 were examined in detail. These isolates were selected to represent as wide a geographic distribution as possible, although no attempt was made to sample the whole of the United States for each year. Viruses that had been passaged the fewest times in tissue culture were examined preferentially to minimize the effects of variation due to passaging in vitro. All of the isolates were cultured from original clinical specimens submitted either to diagnostic laboratories (*6*) or to our laboratories, and all were passaged fewer than nine times in cell culture before being analyzed. Some isolates or the disease outbreaks they were associated with have been described (*5, 7*).

Viruses were initially examined for antigenic type by testing with previously described monoclonal antibodies (mAb's) produced against CPV-a or FPV-c (*5*) (Fig. 1A). Before 1981 most isolates were of one antigenic type. None of the viruses studied that were isolated after 1980 reacted with a group of five mAb's—four directed at sites common to both CPV and FPV, and one recognizing a CPV-specific epitope. CPV-39, a recent isolate, also did not

CPV Isolates from years between 1978 and 1984

mAb	1978		1979		1980		1981		1982		1983		1984		FPV-c
	b	17	d	18	21	22	43	44	25	26	14	31	15	39	
A)															
7	512	256	512	1024	512	256	512	128	128	128	128	128	128	128	<2
14	128	64	32	64	64	64	256	128	128	256	128	256	128	256	<2
25	64	64	32	64	64	64	4	4	2	4	2	2	4	<2	2
C	128	64	64	64	128	128	<2	<2	<2	<2	<2	<2	<2	<2	64
D	256	256	256	256	256	256	2	4	4	4	2	2	2	2	256
E	256	256	256	256	128	256	8	8	8	8	8	4	8	4	128
J	128	64	128	32	128	128	<2	<2	<2	<2	<2	<2	<2	<2	64
6	64	64	128	128	128	256	256	128	128	256	64	128	256	64	64
12	64	128	64	64	64	64	64	32	64	64	32	64	64	64	64
A	32	64	64	64	128	256	256	128	128	256	256	256	256	<2	256
I	32	64	32	32	32	32	64	32	64	64	64	64	64	<2	64
1	1024	512	1024	1024	512	512	512	256	256	256	512	256	256	512	256
8	128	256	256	256	256	256	256	256	128	256	256	256	256	256	256
16	128	256	256	256	256	256	128	64	64	128	64	64	64	64	128
15	64	256	128	256	256	256	256	128	128	256	32	32	128	32	64
F	2048	1024	1024	2048	1024	2048	1024	512	1024	512	512	512	512	1024	1024
B)															
1D1	<2	<2	<2	<2	<2	<2	2048	1024	1024	1024	512	512	512	512	4
7D6	4	2	2	2	<2	<2	1024	2048	1024	1024	1024	2048	2048	1024	4
7E2	<2	<2	<2	<2	<2	<2	128	128	128	64	128	128	128	64	2
2A9	64	64	32	64	64	128	64	64	64	128	32	32	32	32	2
2E2	16	32	16	16	16	16	32	32	32	32	16	16	32	32	16
2E12	128	256	64	128	128	256	64	128	256	128	64	64	64	128	256
3G6	64	64	32	64	64	64	64	64	32	32	64	64	32	128	32
4A12	128	256	128	256	128	256	128	128	128	256	64	64	128	128	256
4E9	64	128	32	128	64	128	64	128	128	128	64	32	128	256	64

Fig. 1. Hemagglutination inhibition titers of monoclonal antibodies reacted with an FPV isolate and with two CPV isolates collected during each year between 1978 and 1984. Four hemagglutinating units of each isolate were reacted in the hemagglutination inhibition test with a panel of mAb's produced against (A) CPV-a (indicated by numbers), FPV-c (indicated by letters) or (B) CPV-39. Titers are the reciprocal of the last antibody dilution inhibiting viral hemagglutination. The shading indicates reactions characteristic of the new (A) or the old (B) viruses. Monoclonal antibodies directed against CPV-a or FPV-c have been described (*5*). Those to CPV-39 were produced as described (*5, 15*), after BALB/c mice were immunized with purified virus. Spleen cells were fused with the Sp2/0-Ag 14 myeloma cell line. Hybridomas producing antiviral mAb's were detected with an ELISA and cloned by culture at limiting dilution. MAb's were prepared as tissue culture supernatants or as ascitic fluids in mice. Canine parvovirus strains, CPV-a, CPV-b, CPV-d, and feline parvovirus FPV-c have been described (*5*). Isolates were from Washington [CPV-17 (78-16); CPV-18 (79-600); CPV-21 (80-22); CPV-22 (80-139); CPV-25 (82-1315); and CPV-26 (82-2424)], New York [CPV-d and CPV-14], Illinois [CPV-b and CPV-15 (8407040)], New Jersey [CPV-44 (85702)], Pennsylvania [CPV-43 (79668-8)], Virginia [CPV-31 (179088)], and Texas [CPV-39 (C84176071, No. 2)]. Numbers in parentheses are the original accession numbers of isolates that have not been previously described.

react with two other mAb's that reacted with CPV and FPV (Fig. 1).

CPV-39 was purified and used to produce additional mAb's, nine of which were used to examine the panel of viruses (Fig. 1B). Three of these mAb's reacted with post-1980 CPV isolates, but not with earlier CPV isolates or FPV. Other mAb's reacted with isolates from all years. One mAb (4D7) bound only to CPV-39. This mAb reacted poorly in the hemagglutination inhibition test and was characterized with an enzyme-linked immunosorbent assay (ELISA).

Because the antigenic analysis revealed that CPV had changed since its first appearance in dogs and that around 1980 a new antigenic type of virus emerged, the extent of genomic variation in the virus was determined by restriction enzyme analysis of viral replicative form DNA (RF DNA). DNA's of viruses from 1979 (CPV-d) and 1984 (CPV-39) were digested with restriction enzymes and analyzed by agarose gel electrophoresis (Fig. 2). Differences were observed after digestion with Hph I, and possibly with Alu I.

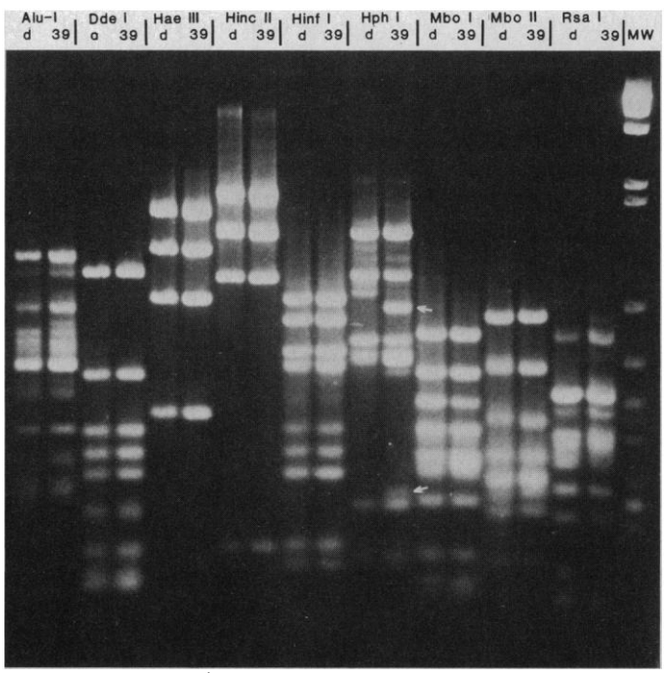
Analysis of the DNA of two isolates from each year with Hph I revealed that the change in genome type correlated with the change in antigenic type of the virus (Fig. 3). The significance of the difference between the Hph I digests of CPV-31 and the other post-1980 isolates is not known, but this is not associated with a difference in the antigenic type of that isolate.

Although we have not mapped all Hph I sites of the two types of CPV, preliminary DNA sequence analysis of old and new CPV isolates has revealed that differences in two Hph I sites within the gene for the capsid protein are associated with differences in the predicted amino acid sequences. A site at 73 map units in the genome of the old virus is not present in the new virus, and a site at 77 map units in the new CPV isolates is not present in the old isolates (*8*). The relation between sequence differences in the capsid proteins of the viruses and the antigenic types of the viruses has not been defined.

In a total of 49 CPV isolates that we have examined (21 obtained between 1978 and 1980 and 28 obtained between 1981 and 1985), we have observed only two exceptions to the patterns presented here (*8*). One 1979 isolate (CPV-e) (*5*) that showed some similarities to the post-1980 isolates had been passaged in dogs after 1980 and may have been contaminated with the newer virus. A 1985 isolate (CPV-48) with the original antigenic type was obtained from a dog breeding facility that has been maintained as a closed colony since 1979 (*8*). That colony, which has been studied earlier (*9*), appears to have been continually infected with the original strain of virus.

Several explanations can be proposed for the change of the virus. First, antigenic drift may have occurred in response to immune pressure. The dog population of the United States, which was immunologically naive to CPV in 1977, was largely immune by 1980, and an altered antigenic type of virus may have had a selective advantage. There is, however, still cross-reactivity between the old and new strains of virus. Furthermore, many CPV vaccines in current use are derived from old strains and still appear to be efficacious. In our earlier studies of mink enteritis virus isolates, which differed antigenically to about the same degree as the CPV isolates described here, there was efficient cross-protection between the various antigenic types (*10*). For these reasons it seems unlikely that the change was favored solely by immune selection.

A second explanation could be that the new strain emerged in a vaccine and was



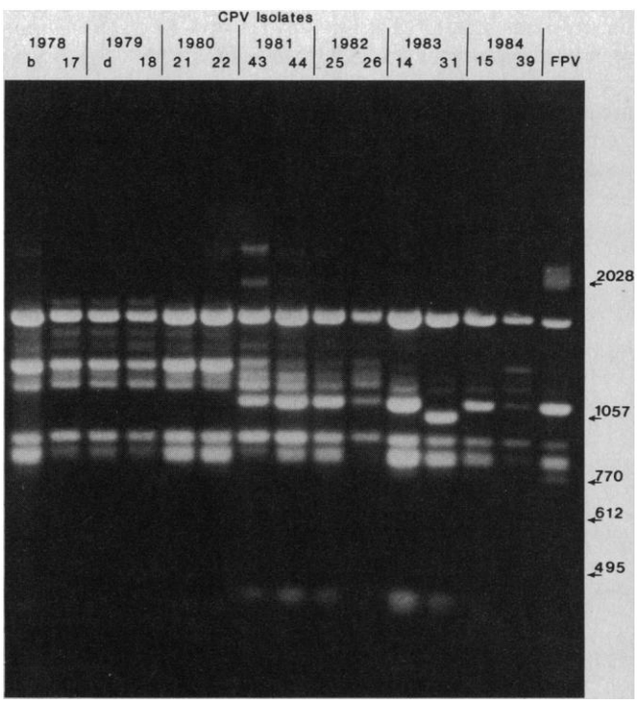


Fig. 2 (left). Restriction enzyme analysis of the DNA of CPV-d (isolated in 1979) and CPV-39 (isolated in 1984). RF DNA was prepared by a modification of the Hirt procedure and purified by electrophoresis in, and recovery from, low-melting-temperature agarose gels (*4, 9*). After the purified DNA was digested with various restriction enzymes, fragments were resolved by electrophoresis in a 2 percent agarose gel with tris-acetate buffer (0.02*M*, *p*H 7.6) and 0.001*M* EDTA, then stained with ethidium bromide (0.5 μg/ml) and photographed in ultraviolet light. The arrows indicate restriction fragments that differ in the two viruses. Molecular size markers were obtained from a Hind III digest of phage λ DNA and a Hinc II digest of φX174 RF DNA. Fig. 3 (right). Restriction enzyme digests of the DNA of two isolates of CPV for each year from 1978 to 1984 and a single isolate of FPV. Isolates have been described [(*5*) or legend to Fig. 1]. Purified viral RF DNA's were digested with the restriction enzyme Hph I (see Fig. 2), subjected to electrophoresis in 2 percent agarose gels in tris-acetate buffer (0.02*M*, *p*H 7.6), 0.001*M* EDTA, stained with ethidium bromide, and photographed in ultraviolet light. Sizes are in base pairs.

spread by this means throughout the country. However, modified-live vaccine viruses that we have tested had the original antigenic type (*11*), and the isolation of the new antigenic type of CPV from dogs in Europe (*12*) suggests that more than a simple vaccine spread was involved.

A third possibility is that the new virus is better adapted for replication in dogs, or that it spreads more efficiently. The surface antigenic structures of the viruses appear to be important in determining the host ranges of CPV or FPV in cats and dogs (*8*). It is possible that the original CPV was the first virus infecting dogs, and the virus has since evolved to better fit its new host. The loss of a CPV-FPV type-common epitope from the early isolates and the acquisition of a new CPV type-specific epitope by the new strain suggests that CPV may be evolving away from FPV to a form better adapted to dogs.

Small antigenic changes detectable by mAb's are common among viruses (*13*). However, the emergence of a new antigenic type only 2 or 3 years after the first recognition of CPV and the apparent replacement of the original virus by the variant are unusual. Further studies of this phenomenon will allow exploration of the factors important in the evolution of parvoviruses. As a practical matter these results suggest that it is unwise to regard parvoviruses as genetically invariant. For example, field CPV strains are now antigenically different from most vaccine strains in current use, and diagnostic tests that depend on mAb reactivities (*14*) could be invalidated by small antigenic changes in the virus.

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Detection of Human Cytomegalovirus in Peripheral Blood Lymphocytes in a Natural Infection

Abstract. *In situ hybridization was used to detect human cytomegalovirus (HCMV) in the peripheral blood mononuclear cells of some naturally infected (seropositive) individuals. A subpopulation of cells hybridized specifically to a portion of the HCMV genome that is heavily transcribed during the immediate-early period of infection. The hybridization signal was markedly reduced by base hydrolysis and ribonuclease, and therefore the probe appears to be detecting viral RNA. A fluorescence-activated cell sorter was used to select lymphocytes bearing the OKT4 and OKT8 markers. Hybridization with the HCMV probe revealed a higher proportion of positive cells in the OKT4 than in the OKT8 subset. This observation specifically identifies lymphocytes as a cell population involved in natural HCMV infection and suggests that lymphocytes may be a reservoir for maintaining infection and may also serve as a vehicle for its spread by blood transfusion.*

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A characteristic of the members of the herpesvirus family is their ability to remain in host tissue for many years after the initial infection and then to be reactivated, occasionally causing disease. Herpes simplex 1 and 2 and varicella zoster virus are maintained in sensory ganglia (*1*), and Epstein-Barr virus is maintained in B lymphocytes (*2*).

Human cytomegalovirus (HCMV) also establishes a latent infection, frequently after an asymptomatic infection in a healthy individual. It can be reactivated, usually in association with immunosuppression and transplantation surgery, to cause interstitial pneumonia and systemic disease (*3–6*). The site of latency and the molecular mechanisms of the establishment and maintenance of HCMV have yet to be determined. There is evidence that lymphocytes may play a role in HCMV infection. Individuals with HCMV mononucleosis have atypical lymphocytes, and in vitro tests reveal depression of lymphocyte functions (*7–9*). Approximately 70 percent of the adult population have antibodies to HCMV, indicating previous exposure, and seronegative individuals have been infected via blood transfusions from healthy seropositive donors (*10*). Depletion of leukocytes from seropositive blood decreases the incidence of transfusion-mediated transfer of HCMV infection, and some evidence points to the involvement of polymorphonuclear leukocytes (*11*). However, although HCMV can be isolated from the buffy coat of acutely infected patients (*7, 9–11*), attempts to detect HCMV infection in peripheral blood mononuclear cells of asymptomatic individuals have seldom been successful (*12*).

We (*13*) and others (*14*) independently found that a small percentage (average, 2 to 3 percent; range, 1 to 15 percent) of lymphocytes could be infected in vitro, as judged by expression of immediate-early (IE) and early HCMV proteins detected by monoclonal and monospecific antibodies. Past attempts to define the infection of lymphocytes with HCMV have been hampered by several factors including (i) the use of laboratory strains of HCMV that appear to be less lymphoinfectious than recent HCMV clinical isolates (*13*), (ii) the cryptic nature of the infection (*15*), and (iii) the difficulty of detecting low levels of virus in a small percentage of cells.

Our strategy for detecting viral nucleic acid in lymphocytes was to select as probes cloned viral fragments from regions of the HCMV genome that might be heavily transcribed in these cells. The RNA transcripts produced during the IE stage of HCMV infection have been mapped, characterized, and cloned (*16*).