Canine Parvovirus: Relationship to Wild-type and Vaccine Strains of Feline Panleukopenia Virus and Mink Enteritis Virus

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SUMMARY

Canine parvovirus (CPV), feline panleukopenia virus (FPLV) and mink enteritis virus (MEV) were compared serologically, by determination of their host range in cell cultures, as well as by restriction enzyme analysis. Maps of the virus genomes were established using seven different restriction enzymes cutting at a total of 56 sites. MEV and FPLV gave maps which were identical except for one restriction site. The map of CPV is closely related to those of FPLV/MEV since their DNAs share about 80% of the restriction sites tested. However, CPV is clearly distinct from FPLV/MEV since either eight (German isolate) or nine (Belgian, Swiss and American isolates) restriction sites are different. The DNAs of six vaccine strains of FPLV and MEV were also analysed. They gave maps which closely resembled those of the respective wild-type strains. CPV and FPLV/MEV also differed with respect to antigenicity, as well as to host range in cell cultures.

INTRODUCTION

Canine parvovirus (CPV) causes an acute, sometimes fatal, enteritis in dogs of all ages and myocarditis in puppies (for review, see Carmichael & Binn, 1981). Antigenically the virus is closely related to feline parvovirus (FPV) (Johnson & Spradbrow, 1979; Carmichael et al., 1980; Lenghaus & Studdert, 1980) of which two variant viruses, feline panleukopenia virus (FPLV) and mink enteritis virus (MEV), are known (Siegl, 1976). The first outbreaks of CPV disease were observed in 1978 when epidemics occurred almost simultaneously in all five continents (Kelly, 1978; Appel et al., 1979; Burtonboy et al., 1979; Van Rensburg et al., 1979; Azetaka et al., 1981). In dog sera collected before 1976 no antibodies against CPV have been found (Carmichael & Binn, 1981). However, the origin of CPV, as well as the mode by which it was spread so efficiently, are still matters for speculation.

It has been suggested that either a wild-type or live attenuated vaccine strain of FPLV or MEV may have mutated to dog virulence and then was spread in a biological product designed for veterinary use (Johnson & Spradbrow, 1979; Lenghaus & Studdert, 1980). To investigate this possibility, we compared CPV strains isolated in various geographical regions with each other, and with wild-type and live attenuated vaccine strains of FPLV and MEV. These studies were based on recent experiments (McMaster et al., 1981b) indicating that MEV and CPV can be readily distinguished by restriction enzyme analysis of their double-stranded replicative form (RF) DNA. The antigenic characteristics of CPV, FPLV and MEV were compared and the ability of the various virus strains to replicate in cell cultures of feline or canine origin was determined.
METHODS

Cell cultures. A permanent line of feline kidney cells (NLFK) (Johnson et al., 1974) was used throughout this study for virus production, for preparation of RF DNA, as well as for the plaque assay. Medium and growth conditions were as described by Siegl & Kronauer (1980). For the investigation of the host range in cell cultures two additional stable lines derived from canine kidney cells were used, namely the cell line A-72 recently established by Binn et al. (1980) and the Madin–Darby canine kidney (MDCK) cell line (ATCC).

CPV strains. CPV strains derived from faeces of infected dogs in four countries, i.e. Switzerland, Belgium, Germany and U.S.A. were included in this study. The Swiss strain (Ka/BE, 1979) and the German strain (Qu/FA, 1980) were isolated in our laboratory. The Belgian strain (Bu/BR, 1978) was a gift from G. Burtonboy, Université catholique de Louvain, Brussels, Belgium. The American strain (Ca/IT, 1978) was kindly provided by L. E. Carmichael, Cornell University, Ithaca, N.Y., U.S.A.

FPL V strain. The FPLV used in this study was a plaque-purified isolate of the 6th in vitro passage of the leopard virus strain isolated by Johnson (1964).

MEV strains. Three MEV strains were analysed, namely the 6th in vitro passage of the MEV strain described by Johnson et al. (1974) and two additional strains, V1 and V22, which were kindly provided by A. Moraillon, Ecole nationale vétérinaire d’Alfort, Maisons-Alfort Cedex, France. The V1 strain was isolated from a naturally infected mink, whereas the strain designated V22 was isolated from a dog which had been experimentally infected by feeding an intestine homogenate from a naturally diseased mink (Moraillon et al., 1980).

Live attenuated vaccine strains. Five commercially available live attenuated vaccine strains originating from FPLV, namely Féliniffa from Iffa-Mérieux (strain A), Felivax L from Norden (strain B), Panivax from Friesoythe/Wellcome (strain C), Dohyvac P from Philips Roxane (strain E) and Panocel from Dellen (strain F), besides one vaccine strain derived from MEV, namely Convac FD from Connaught (strain D), were used.

Preparation of virus. Virus particles were isolated from infected monolayers of NLFK cells as soon as a cytopathogenic effect was visible by extraction with an alkaline glycine buffer (Hallauer & Kronauer, 1965).

Preparation of virus RF DNA. RF DNA was extracted from infected NLFK cell cultures and purified by selection for spontaneous reannealing hairpin structures as described previously (McMaster et al., 1981 a).

Restriction enzyme analysis. To obtain the restriction enzyme patterns, purified virus RF DNA was completely digested by each of the following enzymes: AluI, HaeIII, HinII, HinfI, HphI, MboI and MbolI. These seven restriction endonucleases had previously given different restriction patterns for the CPV and MEV DNAs (McMaster et al., 1981 b). The restricted DNA samples were analysed by electrophoresis either on 2% or 2.5% agarose gels at pH 7.8 (40 mm-tris, 4 mm-sodium acetate, 2 mm-EDTA) or at pH 8.3 (100 mm-tris-borate). After ethidium bromide staining, the gels were photographed on a u.v. transilluminator. Restriction enzyme mapping by partial digestion of the 5'-end-labelled RF DNA was as described by Smith & Birnstiel (1976). The RF DNAs were purified from low-gelling agarose as described previously (McMaster et al., 1981 b).

Serological methods. Haemagglutination-inhibition (HI) tests were as described by Hallauer et al. (1971). The plaque reduction test was carried out according to Siegl & Kronauer (1980).

Sera. Canine antiserum against MEV was a generous gift of L. E. Carmichael, whereas canine antisera against FPLV and CPV were kindly supplied by G. Chappuis, Iffa-Mérieux, Lyon, France. Feline sera against MEV and FPLV were as described elsewhere (Siegl & Kronauer, 1980). The preparation of rabbit sera against parvoviruses was reported by Hallauer et al. (1971).
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**Host cell range.** Cells were infected at the time of seeding. Cultures were then incubated at 37 °C for 5 to 7 days, when both the medium and the alkaline extract of the cell sheets were tested for the presence of haemagglutinating virus. Negative cultures were trypsinized and subcultured one to three times.

**RESULTS**

**Restriction enzyme analysis**

Recently, we published a restriction enzyme map of the double-stranded RF DNA of a strain of MEV (Fig. 1 a) (McMaster et al., 1981 b). Now we have established maps for a series of CPV, FPLV and MEV isolates by comparing the electrophoretic pattern of their RF DNA to that of MEV. Complete digestion was performed with each of the following enzymes: AluI, HaeIII, HincII, HinfI, HphI, MboI and MboII. Fig. 1 (b) shows the results obtained. Each horizontal line represents the DNA of a parvovirus isolate. The only restriction enzyme sites indicated are those which are not present on the MEV map. Two separate isolates V1 and V22 cannot be distinguished from one another, while they differ from the previously analysed MEV by having an additional HinfI site at 37 map units and lacking a HaeIII site at 98 map units. The FPLV strain studied also lacks this HaeIII site, but otherwise is like MEV.

As previously found (McMaster et al., 1981 b), a Swiss isolate of CPV differs from MEV in 11 out of 79 restriction sites analysed. Fig. 1 (b) shows the comparative maps of three additional isolates of CPV. The Swiss, Belgian and American isolates have identical maps. The isolate from Germany has an additional MboI cleavage site at 76 map units, which is also present in the FPLV and MEV isolates studied. The existence of this site was first suggested by the absence of the third band from the top of the gel after complete MboI digestion (Fig. 2 a, lanes 1, 2, 5, 7 and 8). The site was subsequently mapped by the method of Smith & Birnstiel (1976) (see Fig. 2b). In Fig. 1 (a) different AluI sites are given for CPV and MEV in the region of 52 map units. This difference is not shown in Fig. 1 (b) because the technique used in the present work did not detect very small DNA fragments.

Six attenuated vaccine strains of FPLV or MEV origin were also analysed. Three of them (A, C and D, see Fig. 1b) gave maps like MEV except for size differences of DNA fragments near the 5'-end (see below). Strain F has two additional HinfI sites at 37 and 87 map units respectively and a third HinfI site is found in strain E at 49 map units. These three HinfI sites are present in all CPV isolates; therefore, CPV DNA after HinfI digestion gives the same gel pattern as vaccine strain E (see Fig. 3, lanes 2 and 7). Between map positions 0 and 70 vaccine strain B cannot be distinguished from FPLV/MEV, while the rest of the map is very different, suggesting that the DNAs in this region have little or no homology.

For the vaccine strains, length differences were observed in the DNA fragments from the 5'-end (Fig. 3, arrow). The interpretation of the data is complicated by the fact that the 5'-end of the RF DNA of parvoviruses occurs in two forms, linear and hairpin (Ward & Dadachanji, 1978), the proportion of which can vary from one preparation to another. Thus, often two bands can be seen originating from the 5'-end (see Fig. 3, lanes 5 and 7). In lanes 6 and 8 the upper band is masked by another band. The differences in length in the fragments from the 5'-end were also observed after digestion with HaeIII, HincII or HphI (not shown). These could be explained by insertions of about 100 bases in strains A and C, and 50 bases for strains D and F, to the right of map position 87.

**Serological comparison**

The antigenic relationship between CPV, FPLV and MEV, as well as the two mink-related strains V1 and V22, was analysed by HI and plaque reduction tests. For this purpose
both dog sera collected in natural outbreaks of the disease and sera prepared in laboratory dogs against the viruses were used. The results, which are summarized in Table 1, showed that the antisera against MEV and FPLV did not distinguish between the five viruses in HI
Fig. 2. (a) Gel electrophoresis patterns of restricted DNA. RF DNAs of four CPV, three MEV and one FPLV isolate were digested to completion with MboI and then electrophoresed for 4 h at 120 V in a 2.5% agarose gel at pH 7.8. Lane 1, MEV; lane 2, FPLV; lane 3, CPV/Switzerland; lane 4, CPV/Belgium; lane 5, CPV/Germany; lane 6, CPV/U.S.A.; lane 7, MEV/V1; lane 8, MEV/V22. (b) Smith & Birnstiel (1976) mapping of virus RF DNAs. Gel-purified RF DNAs were digested to completion with BglII (single site at 66 map unit) and labelled at the 5'-ends with polynucleotide kinase after treating with bacterial alkaline phosphatase. The original virus 5'-ends are not labelled efficiently (McMaster et al., 1981b). The BglII-A 5'-end (0 to 66 map units) was shortened to 350 base pairs by digesting with PstI (59 map units). The BglII-B fragment (66 to 100 map units) was partially digested with MboI and analysed by agarose gel electrophoresis for 2 h at 50 V (tris–borate pH 8.3). The arrow indicates the band due to the additional MboI site by 530 base pairs or approx. 10 map units to the right of the BglII site (66 map units) at 76 map units (see text). Lane 1, SV40 HindIII DNA markers; lane 2, MEV; lane 3, FPLV; lane 4, CPV/Switzerland; lane 5, CPV/Germany; lane 6, CPV/Belgium; lane 7, CPV/U.S.A.; lane 8, MEV/V1; lane 9, MEV/V22; lane 10, SV40 HindIII. Numbers to the left of the gel represent base pairs.
Fig. 3. Gel electrophoresis patterns of restricted DNA. RF DNAs of six vaccine strains of FPLV/MEV as well as wild-type CPV and MEV were digested to completion with \textit{Hinf}I and then electrophoresed for 4 h at 120 V in a 2\% agarose gel at pH 7.8. Lane 1, MEV; lane 2, CPV/Switzerland; lanes 3 to 8, vaccine strains A to F respectively. The arrow indicates the fragment which varies in size (see text).

and plaque reduction tests. The anti-CPV serum, however, reacted with the homologous virus at a higher dilution than with the heterologous agents. The same was found when sera produced in cats were used (data not shown). This indicates that the higher affinity of homologous antibodies against CPV does not depend on the animal species of origin. HI tests were also performed with rabbit antisera against the following parvoviruses: minute virus of canines, porcine parvovirus, bovine parvovirus, rat virus, H-1 virus, RT virus, LuIII virus, TVX virus and minute virus of mice. All these sera failed to inhibit agglutination of pig erythrocytes by CPV.

\textit{Host cell range}

One feline (NLFK) and two canine kidney cell lines (A-72 and MDCK) were tested for their ability to support replication of the virus strains under investigation. It was shown that
Table 1. Serological comparison of CPV, FPLV, MEV, and strains V1 and V22 by haemagglutination inhibition and plaque neutralization

<table>
<thead>
<tr>
<th>Antiserum*</th>
<th>CPV</th>
<th>FPLV</th>
<th>MEV</th>
<th>V1</th>
<th>V22</th>
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<tbody>
<tr>
<td>CPV</td>
<td>2560†</td>
<td>1280</td>
<td>640</td>
<td>640</td>
<td>1280</td>
</tr>
<tr>
<td></td>
<td>&gt;40000‡</td>
<td>11000</td>
<td>5000</td>
<td>4000</td>
<td>10000</td>
</tr>
<tr>
<td>FPLV</td>
<td>640</td>
<td>640</td>
<td>640</td>
<td>160</td>
<td>640</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>2200</td>
<td>2000</td>
<td>1500</td>
<td>1800</td>
</tr>
<tr>
<td>MEV</td>
<td>160</td>
<td>320</td>
<td>160</td>
<td>160</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1500</td>
<td>1500</td>
<td>1200</td>
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</tbody>
</table>

* Antisera produced in dogs.
† Dilution of serum giving 100% inhibition of 4 haemagglutinating units.
‡ Dilution of serum giving 50% inhibition of 50 p.f.u.

the feline cells are susceptible to infection with CPV, FPLV, MEV, V1, V22 as well as with the various strains of attenuated vaccine viruses tested. All CPV strains also replicated in A-72 and MDCK cells. Almost identical quantities of infectious virions (10^6 to 10^7 TCID<sub>50</sub>/ml) were recovered in harvests of all susceptible cell lines. On the other hand, neither wild-type nor attenuated viruses of FPLV/MEV could be grown in the canine cell lines even after repeated blind passages.

DISCUSSION

After the sudden appearance of MEV in Canada in 1947 (Schofield, 1949), the recent pandemic of canine parvovirus enteritis and myocarditis was the second serious episode in which a virus closely related to feline parvovirus (FPV) manifested itself as a pathogen in a previously unaffected species. In both instances it was important to identify the new variant virus and distinguish it from previously isolated strains of FPV.

It is not possible to discriminate serologically between FPLV and MEV (Table 1; for additional references, see Siegl, 1976). Analysis of the virus DNAs by restriction with seven endonucleases also revealed very similar maps which differ in a single restriction site. A useful criterion for distinguishing the two viruses, however, may be their haemagglutination behaviour (J.-D. Tratschin <i>et al.</i>, unpublished results; Johnson & Spradbrow, 1979; Moraillon <i>et al.</i>, 1980).

With respect to the identification of CPV, haemagglutination-inhibition, serum neutralization and agar gel precipitation (Table 1; Lenghaus & Studdert, 1980; Flower <i>et al.</i>, 1980) revealed some minor differences between CPV and FPLV/MEV. The haemagglutination behaviour of CPV closely resembles that of MEV. FPLV, MEV and CPV all grow in feline cell cultures; in canine cells, however, only CPV can be propagated. The genome of CPV can be unambiguously identified by its distinct restriction enzyme cleavage pattern which differs from that of FPLV/MEV in 11 out of 79 mapped restriction sites (McMaster <i>et al.</i>, 1981b). The characteristic restriction pattern is conserved in CPV isolates from distant geographical regions collected over a period of 2 years (Fig. 1b). The only exception was the one <i>MboI</i> restriction site in the genome of the German strain. These observations imply that all CPV strains stem from a common ancestor.

What is the origin of CPV? Moraillon <i>et al.</i> (1980) recorded enteritis in dogs after feeding the animals intestines of mink dead from natural infection with MEV. The authors postulated that canine disease could be caused either directly by MEV or after mutation of the latter
virus to dog virulence. According to our results, virus strain V22 isolated by Moraillon et al. (1980) from diseased dogs is indistinguishable from strain V1 of MEV recovered previously from affected mink in the same region of France. It lacks the characteristic restriction enzyme pattern of CPV and, unlike this virus, is also unable to replicate in permanent cultures of canine cells. We think that it should be verified whether V22 can proliferate and induce disease in dogs in vivo.

With regard to the possibility that CPV was derived from one of the many existing live modified FPLV or MEV vaccine viruses, our examination of only six strains necessarily allows no definite statement. However, we can conclude that canine parvovirus disease was not caused directly by one of the vaccine viruses under investigation. None of them was able to replicate in canine cell cultures and their restriction enzyme patterns resembled more closely those of the respective wild-type viruses than the one characteristic for CPV. On the other hand, comparison of the physical maps (Fig. 1b) indicates that in one case the process of attenuation apparently has led to the generation of restriction sites characteristic for CPV: vaccine strain E has all three Hinfl sites found in CPV. If this strain was a precursor to CPV, at least five additional specific mutations would be necessary to generate the canine virus.

There is sound reason to believe that the various wild-type viruses analysed in these studies underwent a great number of separate animal to animal passages before being isolated in cell cultures. The similar restriction enzyme data recorded for these isolates therefore indicate that the genomes of these viruses are stable under field conditions. Multiple genetic changes like those distinguishing CPV and FPLV/MEV may have developed under selective growth conditions, for example in the course of deliberate or accidental adaptation of FPV strains to replication in canine cells.

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Note added in proof: After submission of this paper, we have examined the genome of two CPV strains isolated in Sidney, Australia by Dr Margaret Sabine in 1978. The restriction site pattern of these viruses is indistinguishable from those isolated in the U.S.A., Belgium and Switzerland.

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