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NOTES

Comparison of Canine Parvovirus with Mink Enteritis Virus by Restriction Site Mapping

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The genomes of canine parvovirus and mink enteritis virus were compared by restriction enzyme analysis of their replicative-form DNAs. Of 79 mapped sites, 68, or 86%, were found to be common for both types of DNA, indicating that canine parvovirus and mink enteritis virus are closely related viruses. Whether they evolved from a common precursor or whether canine parvovirus is derived from mink enteritis virus, however, cannot be deduced from our present data.

In 1978, outbreaks of an apparently new contagious enteric disease in dogs were observed almost simultaneously throughout the world (1, 2, 4, 10, 11, 22). The clinical and pathological picture of the disease closely resembled panleukopenia in cats and enteritis in mink. In agreement with this observation, the causative agent was found to be a parvovirus (referred to as canine parvovirus [CPV]) which antigenically proved to be closely related to feline panleukopenia virus (FPV) (5, 10, 13).

The origin of CPV is a matter of speculation. Dog sera collected before 1978 and examined by hemagglutination inhibition and serum neutralization tests proved to be free of antibodies to the virus (5, 10). Therefore, the disease was not endemic in dogs before 1978. It has been hypothesized that wild-type FPV was adapted to canine cells either deliberately during the production of attenuated live FPV vaccine or accidentally by contamination of canine cell cultures in laboratories (10, 13). After mutating to dog virulence, the virus then could have been spread worldwide with any vaccine produced in canine cells. As an alternative, Moraillon et al. (17) recently suggested that CPV developed from mink enteritis virus (MEV). MEV is assumed to be closely related, or even identical, to FPV and frequently is referred to as a strain of the latter virus (3, 8, 9).

Whether CPV can be distinguished from MEV and FPV by serological means is still in dispute. Therefore, we decided to compare the genomes of CPV and MEV by restriction enzyme analysis. These enzymes recognize specific oligonucleotide sequences and cut the double-stranded DNA at their sites. The resulting DNA fragments can be separated by gel electrophoresis. A restriction enzyme map which gives information on nucleotide sequences and genome organization can thus be constructed. Closely related viruses have similar maps, whereas those of distant viruses have no common patterns.

The MEV strain used has been described previously (9). CPV strain Ka/BE was isolated from the feces of an infected dog in Switzerland (Siegl et al., unpublished data). Both viruses were propagated in a permanent line of feline kidney cells (NLFK) (9). Double-stranded, replicative-form (RF) viral DNAs were isolated from cell cultures at about 48 h after infection, by which time viral replication had led to a cytopathogenic effect. RF DNAs were extracted by a modified Hirt procedure (7) as described elsewhere (15). RF DNAs were purified further either by sedimentation in a neutral 5 to 20% sucrose gradient or by selection for a spontaneously reannealing hairpin structure, followed by gel purification as previously described (15). Mature virions were isolated by banding in a CsCl step gradient. Single-stranded viral DNAs were extracted from the particles as described by Koczot et al. (12).

The molecular sizes of single-stranded DNAs extracted from virus particles were estimated by gel electrophoresis under denaturing conditions (16), using DNAs of known sizes as markers. Denaturing conditions were needed because the parvoviral DNA normally has double-stranded hairpin structures at both ends. For both CPV and MEV, a size of 4,900 ± 100 bases was determined (data not shown). To determine the sizes

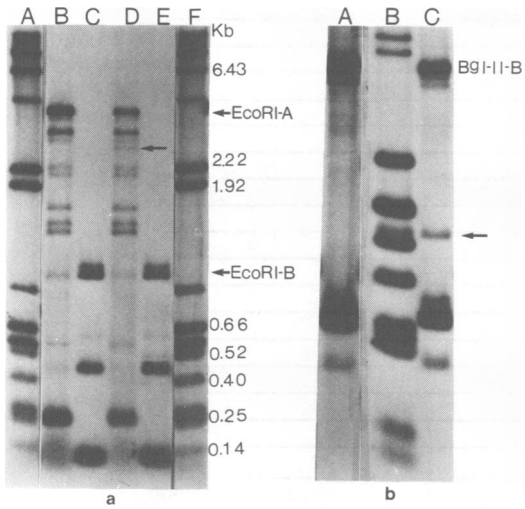

FIG. 1. *Restriction enzyme site mapping by partial digestion of 5'-end-labeled RF DNAs from MEV and CPV. (a) MEV and CPV RF DNAs were digested with EcoRI (20 MU) and then labeled at the 5' ends with ^{32}P. Two fragments were generated (EcoRI fragments A and B). The EcoRI B fragments (lanes C and E) are doublets due to a mixture of linear and hairpin structures of their 3' termini (23). Full digestion with EcoRII (20.4 MU) or TacI (19 MU) removed the 5'-end label from either EcoRI fragment A or B, respectively. These fragments were then subjected to partial digestion with MboI and electrophoresed for 4.5 h at 100 V in a 2% agarose gel at pH 7.8. (A and F) Bacteriophage lambda HindIII and Pbr322 AluI size markers (18, 20). (B) EcoRI fragments from CPV fully digested with TacI and then partially digested with MboI. (C) EcoRI fragments from CPV fully digested with EcoRII and then partially digested with MboI. (D) EcoRI fragments from MEV fully digested with TacI and then partially digested with MboI. (E) EcoRI fragments from MEV fully digested with EcoRII and then partially digested with MboI. Partial digestion of the EcoRI B fragments (lanes C and E) with MboI produced the same bands for both viruses. However, when the EcoRI A fragments were partially digested with MboI, a band present in MEV (lane D, arrow) was absent in CPV (lane B). This restriction site mapped 2,700 bases to the right of the EcoRI site, or at 76 MU. (b) MEV and CPV RF DNAs were digested with BglII and then labeled at the 5' ends with ^{32}P. Full digestion with PstI (59 MU) removed the 5' label of the BglII A fragments (0 to 66 MU) of MEV and CPV. The MEV and CPV BglII B fragments (66 to 100 MU) were partially digested with HphI and electrophoresed in a 2% agarose gel (pH 7.8) at 100 V for 4.5 h. (A) CPV BglII fragment B partially digested with HphI. (B) Bacteriophage lambda HindIII and Pbr322 AluI size markers (18, 20). Their sizes (in kilobases [Kb]) in order from the top were: 2.22, 1.92, 0.910, 0.659/0.655, 0.540, 0.521, 0.403, 0.281, 0.257, 0.226, 0.136, and 0.100. (C) MEV GblII fragment B partially digested with HphI. A band of 540 bases was detected in MEV (arrow) but not in CPV. This extra site in MEV mapped 540 bases to the right of BglII fragment B, or at 76 MU. The CPV sample in (A) was purposely exposed longer to determine whether the 540-base-paired fragment in MEV could be detected.*

of double-stranded RF DNAs, they were electrophoresed in denaturing gels after digestion by either *Eco*RI or *Bgl*II restriction enzyme. The pattern was identical for both viruses (data not shown), and the sum of the fragment sizes confirmed the value given above for the denatured viral DNAs. We concluded that neither the virion nor the double-stranded RF DNAs of the two viruses differ in size.

To produce the restriction maps, we always analyzed RF DNAs of CPV and MEV in parallel. The method of Smith and Birnstiel (19) was used, which consists of radioactively labeling the DNA at one end and then submitting it to partial digestion by a restriction enzyme, followed by gel electrophoresis and autoradiography. A DNA band corresponding to each restriction site became apparent (Fig. 1). We have previously found that the RF DNA of the minute virus of mice, another parvovirus, could not be efficiently labeled at its 5' end with polynucleotide kinase and [γ-^{32}P]ATP by the method of Maxam and Gilbert (14, 15). The same is true for RF DNAs of CPV and MEV. RF DNAs were therefore cut with *Eco*RI (at 20.4 map units [MU]), yielding two fragments, the larger one containing the 5' end of the genome (see below). Both fragments were then labeled at the 5' ends generated by the *Eco*RI cut. Full digestion with *Tac*I then removed the label from the short fragment, leaving the end-labeled large *Eco*RI fragment intact. This was subjected to partial digestion with the different enzymes, and the restriction sites were determined. For the study of the small *Eco*RI fragment, the large fragment was removed by complete digestion with *Eco*RII. Mapping by partial digestion was then performed on the small fragment. The results are shown in Fig. 2. The same mapping procedure was repeated with fragments produced by *Bgl*II, which cuts at 65 MU. This allowed a better resolution in the right half of the map.

To determine which side of the map represents the 5' end, the single-stranded DNA extracted from virions of CPV and MEV was labeled at the 5' terminus with polynucleotide kinase (14). This DNA was then hybridized to separated strands of unlabeled RF DNA which was previously digested with *Eco*RI. Digestion with restriction enzyme *Bgl*II (65 MU) showed that this site is approximately 1,750 bases from the viral 5' end (data not shown).

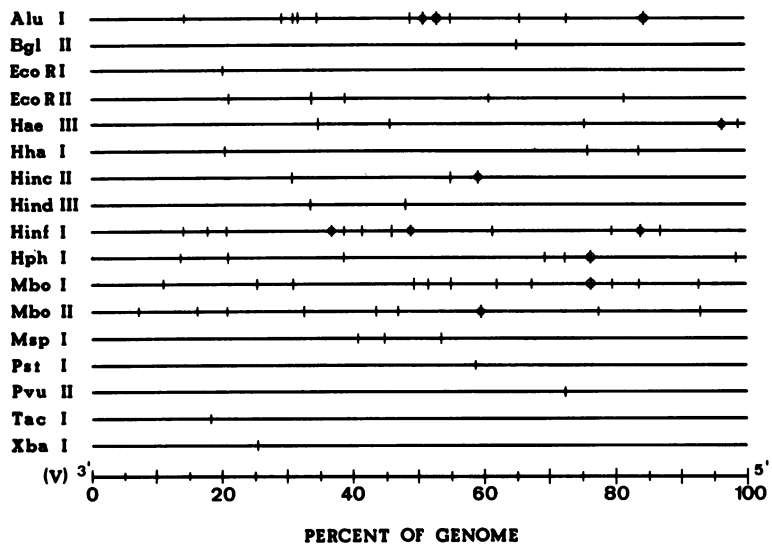

FIG. 2. *Comparative restriction maps of MEV and CPV. The maps represent the single-stranded virion DNA (hairpin structure denatured). Restriction sites were derived from the digestion of double-stranded RF DNAs. The scale is given in MU (percentage of the genome), starting at the 3' end (as conventional for parvoviruses). Symbols: |, sites present in both MEV and CPV; ○, sites present only in MEV; ●, sites present only in CPV. Restriction enzymes which did not digest either DNA were: AvaI, BglI, KpnI, PvuI, SacI, SalI, SmaI, and XhoI.*

Figure 1 shows examples for the mapping of the restriction sites for both viruses by partial digestion of end-labeled fragments. In Fig. 1a, the large *Eco*RI fragments were partially digested with *Mbo*I. A fragment of 2,700 base pairs present in MEV (lane D) was absent in CPV (lane B). The corresponding restriction site in CPV therefore mapped 2,700 bases to the right of the *Eco*RI site (76 MU). Another example is shown in Fig. 1b, where the smaller *Bgl*II fragments were partially digested with *Hph*I. A cleavage site mapping 540 bases to the right of the *Bgl*II site (76 MU) was detected in MEV but not in CPV (lanes A and C).

Complete digestion of RF DNAs by the different enzymes was also done, and the electrophoresed fragments were detected either by staining with ethidium bromide or by autoradiography (after 5' labeling of the fragments subsequent to complete digestion). The results obtained were in agreement with those shown in Fig. 2.

Of 25 enzymes used, 17 cleaved RF DNAs of both viruses at least once, and 79 restriction sites were mapped. These sites represent approximately 8% of the total nucleotide sequence. A total of 68 sites were common on both types of DNA, whereas 11 sites (14%) were present on only one type.

To illustrate the significance of these findings, we refer to our recent experiments with the minute virus of mice and the immunosuppressive virus (15). In this case, the two viruses which replicate in different cell types of the same animal were distinguished by 22 (20%) of 109 restriction enzyme sites. In addition, the immunosuppressive virus had a deletion of 60 bases. On the other hand, no common patterns appeared when the restriction map of the minute virus of mice was compared with those of CPV and MEV. Therefore, we concluded that CPV and MEV are closely related. Whether they evolved from a common precursor or whether CPV is derived from MEV by few genetic changes, however, cannot be deduced at present.

Data reported for other autonomous parvoviruses (6, 21) also suggest that most of the 11 restriction sites which are different for CPV and MEV are in regions coding for viral capsid proteins. Therefore, they may be responsible for the slight serological differences and for the difference in host cell range observed for the two viruses (5, 10, 13).

Our experiments provide a basis for comparison of isolates of CPV from different geographical regions with both wild-type and vaccine strains of FPV and MEV. This approach might yield insight into the relation of these viruses among themselves and could help to trace the origin of CPV. Such studies are in progress in our laboratories.

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