VIROLOGY 215, 186–189 (1996)
Article No. 0021

SHORT COMMUNICATION

Evolution of Canine Parvovirus Involved Loss and Gain of Feline Host Range

UWE TRUYEN,* JAMES F. EVERMANN,† ELKE VIELER,‡ and COLIN R. PARRISH§,[1]

*Institute for Medical Microbiology, Ludwig Maximillians University, Veterinaerstrasse 13, 80539 Munich, Germany; †Washington Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Washington State University, Pullman, Washington 99164; ‡Institute for Hygiene and Infectious Diseases of Animals, Justus Liebig University, Frankfurterstrasse 89-91, 35392 Giessen, Germany; and §James A. Baker Institute for Animal Health, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853

Received June 22, 1995; accepted November 7, 1995

Canine parvovirus (CPV) type-2 emerged as a new virus infecting dogs in 1978, and it was probably derived as a variant of feline panleukopenia virus or of a closely related virus infecting another carnivore. CPV type-2 was subsequently replaced in nature by antigenically variant viruses (CPV type-2a and CPV type-2b) which now coexist in dog populations worldwide. We show that CPV type-2 isolates did not replicate in cats, but that both CPV type-2a and CPV type-2b isolates replicated efficiently. About 10% of the viruses isolated from cats with natural parvovirus disease were antigenically indistinguishable from CPV type-2a or type-2b. The capsid protein gene sequence of a 1990 feline parvovirus isolate ("FPV-24") was essentially identical to the sequence of CPV type-2b viruses from dogs. The loss and reacquisition of the feline host range in CPV was most likely due in each case to small numbers of changes in a region of the virus capsid where three protein monomers interact.

The natural restriction and evolution of animal virus host ranges is a complex and poorly understood phenomenon. However, viruses must have often gained altered host ranges during the evolutionary events which gave rise to the current range of animal viruses. Most of those events happened in prehistoric times, although there are several recent examples of newly recognized viruses emerging in a host species, or of viruses apparently gaining new host ranges — including human immunodeficiency viruses, influenza viruses, hantaviruses, or morbilliviruses (*1–4*). The origins of those viruses and the evolutionary pathways leading to the virus strains now recognized are often only incompletely understood.

Parvoviruses infect many different animals, including humans (*5*). The 25-nm-diameter canine parvovirus (CPV) particle comprises a total of 60 copies of two overlapping proteins (VP1 and VP2), and it encapsidates a single-stranded DNA genome of about 5100 bases, encoding three or four viral proteins from two promoters (*5*). CPV was first recognized worldwide in 1978, although serological evidence suggests that a similar virus began infecting dogs in Europe during the mid-1970s (see *6* and *7* for review). Antigenic and genomic comparisons indicate that CPV is a host range variant of a closely related virus from another carnivore — cats, mink, raccoons, or foxes (*8, 9*). Two or three sequence differences between the capsid proteins of CPV and feline parvovirus (FPV) determine most CPV-specific properties, including canine host range (*9–11*).

The original strain of CPV [designated CPV type-2 (CPV-2) to distinguish it from an unrelated parvovirus, minute virus of canines] was replaced throughout the world between 1979 and 1985 by two different but closely related antigenic variants, designated CPV type-2a (CPV-2a) and CPV type-2b (CPV-2b) (*12–14*). Variable proportions of CPV-2a and CPV-2b are now prevalent in different countries, with >80% of isolates being CPV-2b and <20% CPV-2a in the United States, while 60% are CPV-2a and 40% CPV-2b in Germany (U. Truyen and C. R. Parrish, unpublished results). Our previous studies showed that a CPV-2 isolate replicated in cultured feline cells, but not in cats, and that the feline host range was determined by at least two separate regions of the genome within the capsid protein gene (*15, 16*). However, other studies have indicated that a 1982 canine isolate from Japan (most likely CPV type-2a) replicated in cats and also that viruses resembling CPV were isolated from cats with parvovirus disease (*17, 18*).

To determine the basis for the reported differences in feline host range, here we inoculated cats with either of two low-passage isolates of CPV-2 collected in 1978 (CPV-a) and 1979 (CPV-d) (*14*), with a nonhemagglutinating isolate of CPV type-2 [vBI307 (*19*)], or with isolates of CPV-

[1] To whom correspondence and reprint requests should be addressed. Fax: (607) 256-5608; E-mail: crp3@cornell.edu.

0042-6822/96 $12.00

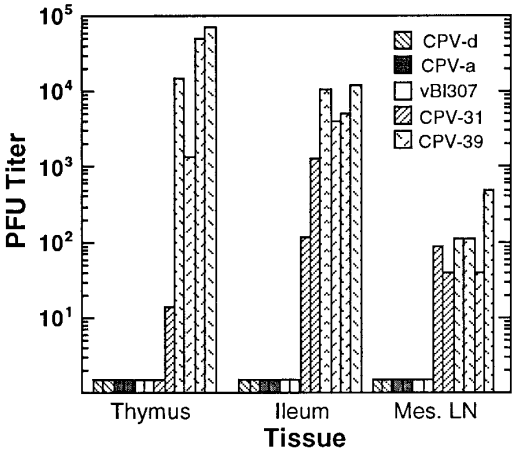

FIG. 1. Virus titers in tissues of cats inoculated with CPV type-2 (CPV-a, CPV-d), CPV type-2a (CPV-31), and CPV type-2b (CPV-39) isolates and vBI307, a nonhemagglutinating mutant of CPV type-2. Viruses have been previously described and characterized (*14, 19*). Two cats were inoculated with each virus except CPV-39, with which four cats were inoculated. Between 0.5 and 1 $\times$ 10^6 PFU of each virus was inoculated by a combination of intranasal and intramuscular routes, and tissues were collected 4.5 days later. Plaque forming unit titers per 0.03 g of each tissue of each cat are shown. No PFU of CPV-a, CPV-d, or vBI307 were detected in any tissue tested. No clinical illness was detected during this short period of infection, and infections were performed in compliance with all applicable animal care and use regulations.

2a (CPV-15) or CPV-2b (CPV-39) which were both collected in 1984 (*14*). As shown in Fig. 1, none of the CPV-2 isolates replicated in any of the cats, but the CPV-2a and CPV-2b isolates both replicated to high titers in lymphoid and intestinal tissues that are targets of virus replication. CPV-2a or CPV-2b virus antigens were also detected by immunoperoxidase staining in sections of the thymic and ileal tissues of cats inoculated with those viruses, but not in tissues of CPV-2-inoculated cats (results not shown). FPV titers in cats under these conditions are similar to those seen for CPV-39 replication (*15, 16*).

Parvoviruses collected from cats in various regions of the United States or Germany were antigenically typed using a panel of monoclonal antibodies which distinguish between FPV, CPV-2, CPV-2a, or CPV-2b strains (*12–14*). Two of 20 United States isolates and 1 of 36 German viruses had the antigenic type of CPV-2b, while 2 German viruses were the CPV-2a antigenic type. All other feline isolates were antigenically indistinguishable from FPV.

The VP1/VP2 protein gene of a feline isolate from a cat in Idaho (designated "FPV-24") that antigenically typed as CPV-2b was cloned and sequenced. That gene differed in a single silent nucleotide from the sequence of a CPV-2b isolate collected in Georgia in 1990 [CPV-133 (*14*)], but it was quite distinct from the sequence of FPV (Fig. 2A).

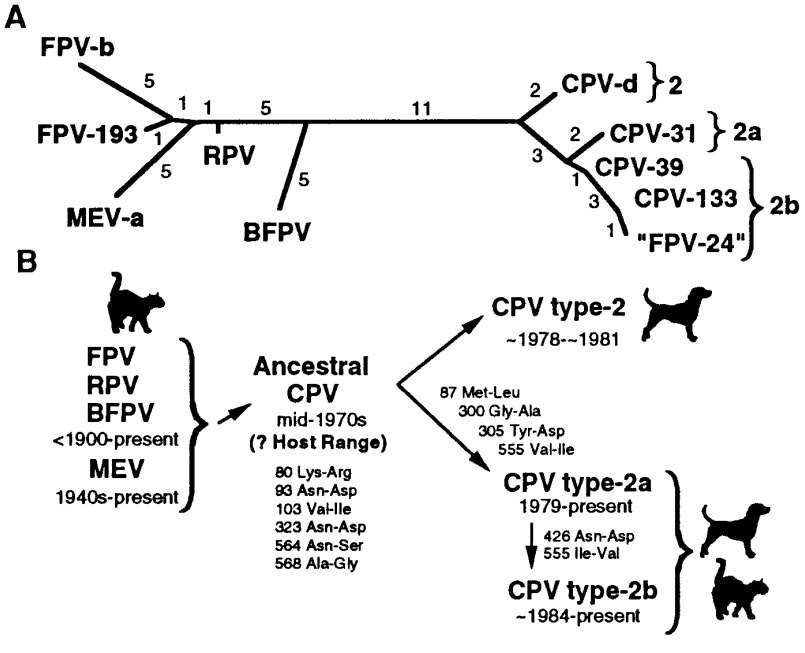

FIG. 2. (A) One of two most parsimonious phylogenies of the complete VP1/VP2 gene nucleotide sequences of viruses from cats [FPV-d, 1969 (U.S.A.), FPV-193, 1970 (Australia), "FPV-24", 1990 (U.S.A.)], mink [MEV-a, 1975 (U.S.A.)], raccoon [RPV 1980 (U.S.A.)], fox [BFPV, 1983 (Finland)], and dogs [CPV-d, 1979; CPV-31, 1984; CPV-39; CPV-133, 1990 (all U.S.A.)]. Numbers of nucleotide differences on each branch are given. Sequences have been reported (*8, 9, 14, 23*), except for "FPV-24" determined here (GenBank Accession No. U22896). The relationships between the sequences were determined by the Branch and Bound algorithm of the program PAUP 3.1.1 (*24*). (B) The apparent evolutionary processes which occurred. Viruses from cats, raccoons, foxes, and mink form a single group from which a CPV ancestor emerged by the 1970s. CPV-2 spread around the world during 1978, then was replaced by CPV-2a between 1979 and 1981. CPV-2b derived from CPV-2a or a closely related virus and spread globally in the mid-1980s. Amino acids in the VP2 protein which differ consistently between virus types are indicated below each branch.

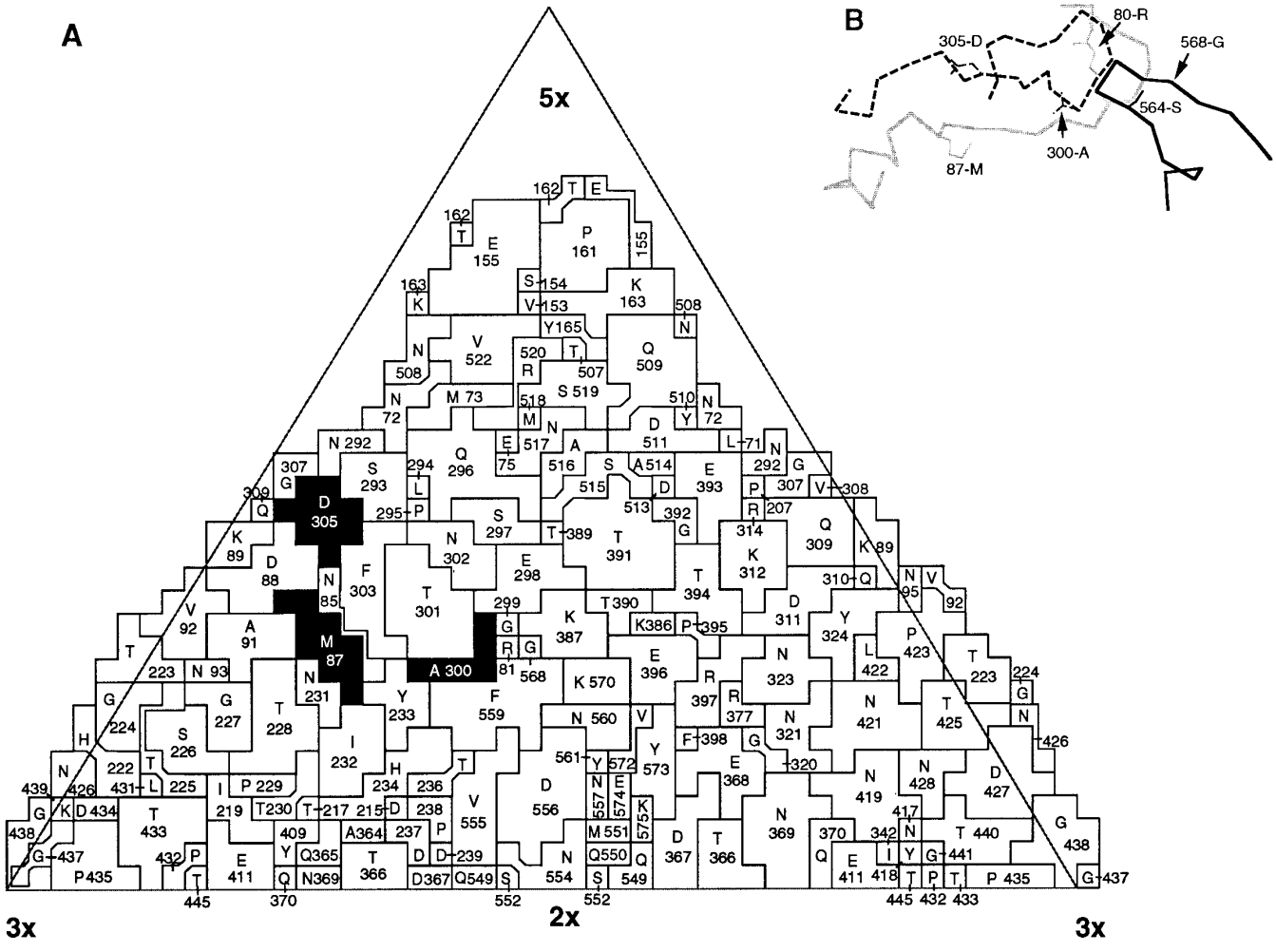

FIG. 3. Structural relationships between sequences which have been shown to influence feline host range of CPV and FPV (VP2 residues 80, 564, and 568) (*16*), and those which differ between CPV-2 and CPV-2a isolates in the shoulder region of the threefold spike (residues 87, 300, 305) (*14*). (A) A roadmap showing the surface of one asymmetric unit of the virus capsid, as determined by the method of Rossmann and Palmenberg (*25*). The numbers indicate the residue in VP2, and those which differ between CPV-2 and CPV-2a that are exposed on the capsid surface on the shoulder of the threefold spike are shaded. (B) A model showing the protein interactions and the locations of residues that differ between CPV and FPV or CPV-2a in the shoulder of the threefold spike. The alpha-carbon tracings of the three adjoining VP2 polypeptide chains are shown as solid, shaded, and dashed lines, and the positions of the side chains of residues 80, 87, 300, 305, 564, and 568 of CPV which are located in that region of the structure are indicated.

The mechanism restricting replication of CPV-2 in cats is unknown. CPV-2 differs from FPV in hemagglutination of feline erythrocytes, as it hemagglutinates those cells at 4°, although not at 37° (*16*). This raised the possibility that CPV-2 might have been binding to some sialic acid on feline tissues or erythrocytes. Erythrocyte binding does not affect virus replication in tissue culture (*16*), and here we show that the HA binding also did not determine *in vivo* feline replication, as the non-HA mutant vBI307 did not replicate in cats (Fig. 1). Mutant vBI307 differs from CPV-d in only a single Arg-Lys change of residue 377 in the VP2 protein (*19*).

These results indicate that the evolution of CPV was a multistep process, as diagrammed in Fig. 2B. A single CPV-like ancestor arose from a group of viruses infecting one or more of several carnivores, and it circulated in dogs in Europe during the mid-1970s (reviewed in *6*). That ancestor of CPV gave rise to CPV-2 which spread worldwide during 1978, but which had apparently lost the *in vivo* feline host range. CPV-2a also arose during the late 1970s from the CPV common ancestor, and it had regained the feline host range. However, CPV-2a and CPV-2b did not regain the FPV-specific sequences associated with the *in vivo* feline host range of FPV—including VP2 residues 80, 564, and 568 (*14, 16*). It is therefore likely that the changes in CPV-2a and CPV-2b

which allow replication in cats were one or more of the differences of VP2 residues 87, 300, and 305, in the same region of the CPV capsid structure as the differences which determine the FPV–CPV-2 difference in feline host range (*21, 22*) (Fig. 3).

Both of those sets of sequence differences alter an important antigenic epitope on the capsid that can be detected with strain-specific monoclonal antibodies (*12, 14, 16*), and antigenic selection may therefore have been involved in the emergence and replacement of the variant CPV types.

The isolation of CPV-2a and CPV-2b viruses from feline samples indicates that both can cause clinical disease in cats as well as dogs, and suggests the interesting possibility that the newer types of CPV may further adapt to cats and perhaps eventually replace FPV in nature.

This group of simple single-stranded DNA viruses shows high conservation of DNA sequences, with less than 1.3% sequence variation between diverse isolates collected 30 years apart (8). However, the viruses have undergone a complex series of host range changes and adaptations which caused them to gain the canine and lose the feline host range and to subsequently regain the feline host range. Those changes were accompanied by a series of antigenic changes and accompanying replacements of virus types around the globe.

ACKNOWLEDGMENTS

We thank W. S. Weichert, G. Platzer, and A. J. McKiernan for expert technical support. Supported by NIH Grant AI 28585 and by Grant Tr282/2-1 from the Deutsche Forschungsgemeinschaft.

REFERENCES

1. Myers, G., MacInnes, K., and Korber, B., *AIDS Res. Hum. Retroviruses* 8, 373–386 (1992).
2. Barrett, T., Visser, I. K. G., Mamaev, L., Goatley, L., van Bressem, M.-F., and Osterhaus, A. D. M. E., *Virology* 193, 1010–1012 (1993).
3. Webster, R. G., Bean, W. J., and Gorman, O. T., *Microbiol. Rev.* 56, 638–179 (1992).
4. Morse, S. S., *Ann. N. Y. Acad. Sci.* 740, 199–207 (1994).
5. Cotmore, S. F., and Tattersall, P., *Adv. Virus Res.* 33, 91–17 (1987).
6. Parrish, C. R., *Adv. Virus Res.* 38, 403–450 (1990).
7. Parrish, C. R., *Semin. Virol.* 5, 121–132 (1994).
8. Truyen, U., Gruenberg, A., Chang, S.-F., Veijalainen, P., Obermaier, B., and Parrish, C. R., *J. Virol.* 69, 4702–4710 (1995).
9. Parrish, C. R., Aquadro, C. F., and Carmichael, L. E., *Virology* 166, 293–307 (1988).
10. Chang, S.-F., Sgro, J.-Y., and Parrish, C. R., *J. Virol.* 66, 6858–6867 (1992).
11. Horiuchi, M., Goto, H., Ishiguro, N., and Shinagawa, M., *J. Gen. Virol.* 75, 1319–1328 (1994).
12. Parrish, C. R., O'Connell, P. H., Evermann, J. F., and Carmichael, L. E., *Science* 230, 1046–1048 (1985).
13. Parrish, C. R., Have, P., Foreyt, W. J., Evermann, J. F., Senda, M., and Carmichael, L. E., *J. Gen. Virol.* 69, 1111–1116 (1988).
14. Parrish, C. R., Aquadro, C. F., Strassheim, M. L., Evermann, J. F., Sgro, J.-Y., and Mohammed, H. O., *J. Virol.* 65, 6544–6552 (1991).
15. Truyen, U., and Parrish, C. R., *J. Virol.* 66, 5399–5408 (1992).
16. Truyen, U., Agbandje, M., and Parrish, C. R., *Virology* 200, 495–503 (1994).
17. Goto, H., Uchida, E., Ichijo, S., Shimizu, K., Morohoshi, Y., and Nakano, K., *Jpn. J. Vet. Sci.* 46, 726–731 (1984).
18. Mochizuki, M. R., Harasawa, R., and Nakatani, H., *Vet. Microbiol.* 38, 1–10 (1993).
19. Barbis, D. P., Chang, S.-F., and Parrish, C. R., *Virology* 191, 301–308 (1992).
20. Strassheim, M. L., Gruenberg, A., Veijalainen, P., Sgro, J.-Y., and Parrish, C. R., *Virology* 198, 175–184 (1994).
21. Agbandje, M., McKenna, R., Rossmann, M. G., Strassheim, M. L., and Parrish, C. R., *Proteins* 16, 155–171 (1993).
22. Tsao, J., Chapman, M. S., Agbandje, M., Keller, W., Smith, K., Wu, H., Luo, M., Smith, T. J., Rossmann, M. G., Compans, R. W., and Parrish, C. R., *Science* 251, 1456–1464 (1991).
23. Martyn, J. C., Davidson, B. E., and Studdert, M. J., *J. Gen. Virol.* 71, 2747–2753 (1990).
24. Swofford, D. L., PAUP — Phylogenetic Analysis with Parsimony — Program Manual. Center for Biodiversity, Illinois Natural History Survey (1993).
25. Rossmann, M. G., and Palmenberg, A. C., *Virology* 164, 373–382 (1988).