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Evolutionary Dynamics of Viral Attenuation

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The genetic trajectory leading to viral attenuation was studied in a canine parvovirus (CPV) strain grown on dog kidney cells for 115 transfers. Consensus sequences of viral populations at passages 0, 3, 30, 50, 80, and 115 were obtained from PCR products covering 86% of the genome; clones from each of the 80th and 115th passages were also sequenced, covering 69% of the genome. Sixteen changes were fixed in the 115th-passage virus sample. Levels of polymorphism were strikingly different over time, in part because of a plaque-cloning step at passage 112 that reduced variation: passage 80 had 19 variants common among the clones, but passage 115 had only a single common variant. Several mutations increased in the culture at the same time, with most reaching fixation only after the 80th passage. The pattern of evolution was consistent with recombination and not with separate selective sweeps of individual mutations. Thirteen of the changes observed were identical to or at the same positions as changes observed in other isolates of CPV or feline panleukopenia virus.

The most successful viral vaccines use live attenuated virus strains. For more than a century, attenuation has involved propagation of a virus under novel conditions so that it becomes less pathogenic to its original host as it evolves under the new conditions (9, 10). Despite the antiquity of this method and its remarkable success in generating many vaccines that are widely used in humans and other animals, little is known about the process by which the attenuating mutations arise and evolve. In addition, there are some drawbacks to this method: the outcome of an attempted attenuation is largely unpredictable, and a successfully attenuated virus may revert to virulence, depending on the nature of the attenuating mutation(s). Although newer approaches show promise, many contemporary vaccines are still being developed by these classic methods.

A better understanding of the classical attenuation process might thus suggest protocols to eliminate some disadvantages of these methods of attenuation. One of the obvious gaps in our understanding of attenuation is at the genetic level. Until recently, the molecular changes underlying the shift from virulence to attenuation were almost completely unknown for most attenuated vaccine viruses. Even now, the dynamics over an attenuation episode have yet to be described. To begin filling this gap, we examined the genetic evolution that occurred during the passaging of canine parvovirus (CPV) in tissue culture to give rise to a successfully attenuated vaccine that has been used effectively for 20 years.

CPV is a single-stranded DNA virus with a linear genome of ~5.15 kb. CPV appears to have derived from a single ancestral cat virus that emerged in dogs during the mid-1970s (24) and spread worldwide in 1978 (30). The initial 1978 strain of the virus (designated CPV type 2) was replaced during 1979 and 1980 by a genetically and antigenically distinct variant termed CPV type 2a (25). Those viruses have continued to evolve slowly in nature, but some sequence changes have swept through the global CPV population (30).

The source of CPV genomes for this study were the frozen or lyophilized archive stocks of the CPV type 2 strain (78-0916) that had been passaged in dog or cat cells in culture at 33 and 35°C, during which time the virus became attenuated (2). Passage 0 was the original canine feces from which the virus was isolated, while passages 1 through 115 had been grown in primary dog kidney cells. A second line of virus passages was started with dog cell passage 103 and was continued for another 9 passages in feline renal cell line CRFK (ATCC CCL-94). Virus titers were approximately 10^6/ml at the end of each passage, whereupon they were diluted 1:50 or 1:100 to start the next passage. The virus was passaged from an endpoint dilution culture at the 64th and 73rd passages and plaque cloned once in A72 canine cells at the 112th passage. The virus stocks were tested for virulence at passages 30, 50, 80, and 115 by inoculation of dogs. They proved to be significantly attenuated by the 80th passage and formed larger plaques in tissue culture by the 94th passage, as detailed previously (2, 3).

Consensus PCR sequences. In the first analysis, PCR products of archived viral suspensions were analyzed, thus representing a consensus of common changes in the viral population. Two overlapping PCR products were amplified with *Taq* DNA polymerase (between bases 480 and 2680 and between bases 2400 and 4889), spanning 86% of the 5,124-base genome. Those PCR products were completely sequenced for passages 1, 50, and 115 and for 103/9 virus (after 103 passages in dog cells and 9 passages in cat cells). Passages 3, 30, and 80 were sequenced only over the regions in which changes had been detected at passages 115 and 103/9. All sequencing reactions used ABI Big Dye chain-terminating reactions, with primers spaced every 300 to 400 bases. Reactions were read on an

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