Stability of the Pseudorabies Virus Genome After in vivo Serial Passage

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SUMMARY

Restriction endonuclease patterns of pseudorabies virus (PRV) DNA were examined after each of 11 serial passages of the virus through pigs. Minor variations in the electrophoretic mobility of certain restriction enzyme fragments were observed by the sixth passage. This variability was similar to some of the minor variability observed in field isolates. The variable fragments were mapped to three locations on the PRV genome: the junction between the short unique sequences and the repeat sequences, the terminus of the long unique region, and an internal area of the long unique region.

Restriction endonuclease analysis of viral DNA has been shown to be a useful epidemiological tool in the investigation of herpesvirus infections (Buchman et al., 1978; Maitland et al., 1982; Paul et al., 1982; Whitley et al., 1982; Allen et al., 1983b; Chaney et al., 1983). The restriction enzyme profiles of herpesviruses are stable through many in vitro passages in cells from the natural host of the virus (Buchman et al., 1979; Zweerink et al., 1981; Allen et al., 1983a). However, in vitro serial passage of equine herpesvirus type 1 (EHV-1) in non-equine cells or in vivo serial passage in hamsters resulted in alterations in the migration of certain restriction enzyme fragments (Allen et al., 1983a). Previous studies with a non-plaque-purified, genetically mixed population of pseudorabies virus (PRV) indicated that variations in the viral DNA cleavage patterns can be detected after serial passage in swine due to either selection of a minor variant in the population or alterations in the migration of certain restriction enzyme fragments during passage in vivo (Mengeling et al., 1983). In this report, we further examine the effects of in vivo serial passage of PRV in swine, its natural host.

Initially, two 3-month-old seronegative pigs were infected intranasally with \(1 \times 10^7\) p.f.u. of PRV strain IA 62/26. The virus had been plaque-purified twice and then passed twice in cell culture prior to its use. When the pigs exhibited maximum clinical signs (about 5 days post-inoculation), nasal swabs were taken from each pig and mixed in 4 ml Eagle's MEM containing 2% fetal calf serum. This inoculum was then used to infect intranasally two more 3-month-old pigs (1 ml/pig). The virus was passed serially in pigs a total of 11 times. After two and eight passages, insufficient virus was recovered to produce clinical disease in the next series of pigs. Therefore, the recovered virus was passed once in cell culture to increase the virus titre.

Virus isolated from each pig passage was passed twice in porcine MVPK-1 cells (Swaney, 1976) at an m.o.i. of approximately 0-01 p.f.u./cell. PRV DNA was isolated from cytoplasmic virions as previously described (Paul et al., 1982). Purified PRV DNA (about 1 \(\mu\)g) was digested with various restriction enzymes and fractionated by electrophoresis in 0-8% (KpnI and BamHI), 1% (SalI) or 1.5% (HinfI) agarose gels. Restriction enzymes were from Bethesda Research Laboratories and the conditions of digestion were those recommended by the manufacturer. Following electrophoresis, the gels were stained for 1 h with 1 \(\mu\)g/ml ethidium bromide and photographed under u.v. light.

When PRV was serially passed in vivo through its natural porcine host, minor variations in the migration of specific DNA fragments were rapidly detected. Variability could be detected with all four restriction enzymes by the sixth virus passage (Fig. 1, arrows indicate variable fragments). The differences in migration ranged in size from 100 to 400 base pairs. Two of the more obvious changes were detected with HinfI (Fig. 1d) and SalI (Fig. 1c, second arrow).
Fig. 1. Restriction endonuclease analysis of PRV following serial passage through swine. PRV (IA 62/26) DNA (about 1 μg/lane) was analysed after passage of virus through pigs with the restriction enzymes \textit{KpnI} (a), \textit{BamHl} (b), \textit{SalI} (c) and \textit{Hinfl} (d). The stock virus (lane 1) and passages 1 (lane 2), 2 (lane 3), 3 (lane 4), 4 (lane 5), 5 (lane 6), 6 (lane 7), 7 (lane 8), 8 (lane 9), 10 (lane 10) and 11 (lane 11) are shown. Virus from passage 9 was unavailable for analysis. Arrows indicate restriction endonuclease fragments that varied in electrophoretic mobility. where restriction enzyme fragments lost a sufficient amount of DNA to co-migrate with the DNA band directly below it. The co-migration of the restriction enzyme fragments was determined by densitometric scanning which detected an increase in the molarity of the lower band, and by Southern blot hybridization (M. W. Wathen & E. C. Pirtle, unpublished data). This variation in mobility was probably due to the addition or deletion of short DNA sequences as has been observed for other herpesviruses (Hayward et al., 1975; Allen et al., 1983a; Straus et al., 1983). Alterations in the cleavage patterns due to loss or gain of restriction enzyme sites were
not detected after in vivo serial passage of the virus using the four restriction enzymes indicated above.

*BamHI* and *KpnI* restriction enzyme maps for the Shope strain of PRV have been published (Feldman *et al.*, 1979), and a map for *SalI* is known (T. Ben-Porat, personal communication). The strain IA 62/26 used in this study has the same restriction enzyme cleavage patterns and therefore the same restriction enzyme cleavage maps as the Shope strain (M. W. Wathen & E. C. Pirtle, unpublished data). The variable fragments in the *BamHI* and *KpnI* digests and the larger variable fragment in the *SalI* digest all have in common the junctions between the repeat sequences and the short unique sequences (Fig. 2). The smaller variable fragment in the *SalI* digest lies at the terminus of the long unique region of the PRV genome (Fig. 2). Although a *HinfI* restriction enzyme map is not available for PRV, the variable fragment in the *HinfI* digest of Fig. 1 has been mapped between 0.19 and 0.23 map units by hybridization to *KpnI*, *BamHI* and *SalI* digests of PRV (M. W. Wathen & E. C. Pirtle, unpublished data). Therefore, we were able to detect three regions of variability in the cleavage patterns of PRV DNA after serial passage in pigs.

Heterogeneity similar to that detected in this study has been observed in the repeat sequences of herpes simplex virus (HSV) after plaque purification and passage in vitro (Davison & Wilkie, 1981). This heterogeneity in HSV is probably due to continuous amplification or reduction of certain sequences in the repeats during viral DNA replication. Heterogeneity was not detected in a similar study when 10 PRV plaque-purified isolates were each plaque-purified a further 10 times and analysed after growth in cell culture (Mengeling *et al.*, 1983). However, such a phenomenon could account for the inconsistency in the size of the variable fragments from the PRV repeat region [Fig. 1a, b, c (upper arrow)]. These fragments appeared to be capable of varying in size from one pig passage to the next. It should be noted that the variable fragments from the terminus and the middle of the long unique region did not exhibit this inconsistency in size, indicating that the variability in these regions may be due to a different mechanism from the variability in the repeat sequences.

The map locations of the three variable regions were strikingly similar to the regions of the EHV-1 genome which vary during serial passage in hamsters (Allen *et al.*, 1983a). It is of interest to note that detectable differences in the EHV-1 DNA cleavage patterns required about six times more passages in hamsters than PRV required in pigs. Perhaps this is due to an enhanced capability of the virus to generate variability in its natural host.

The variability in the DNA restriction enzyme patterns detected during serial passage in pigs was similar to some of the minor differences detected in various field isolates. Although some field isolates have significantly different restriction enzyme cleavage patterns, many isolates only vary by minor differences in the migration of a few DNA fragments (Pirtle *et al.*, 1984). Since this variability can arise rapidly during passage in pigs, minor differences in the migration of DNA fragments such as those observed in this study should not be used as a criterion for differentiating PRV isolates.
Short communication

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REFERENCES


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