Genomic Alterations Associated with Persistent Infections by Equine Infectious Anaemia Virus, a Retrovirus

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SUMMARY

The unique periodic nature of equine infectious anaemia (EIA) is believed to result from the ability of the infecting virus, EIAV, to undergo relatively rapid antigenic variations which circumvent host immune responses resulting in distinct virus populations in sequential clinical episodes in the persistently infected horse. This model was examined by oligonucleotide mapping comparisons of the RNA genomes of selected isolates of EIAV. Variations in oligonucleotide maps could be reproducibly demonstrated (i) after adaptation of the laboratory strain of EIAV to replication in a pony, (ii) after serial passage of virus between two ponies, and (iii) after a prolonged persistent infection in a single pony. In the latter case, the two isolates examined were recovered from different clinical episodes and were shown to be antigenic variants. In contrast, no variations in RNA structure could be detected in oligonucleotide maps of virus isolated after prolonged passage in tissue culture. Thus, these results support our concept that EIAV is a highly mutable virus, which may give rise to antigenic variants in the presence of immune pressures. The degree of variation observed between oligonucleotide maps is similar to that observed previously between variants of visna virus. These similarities between EIAV and visna virus suggest that genomic point mutations producing antigenic variants may be a more important mechanism of retrovirus persistence than was previously recognized.

Equine infectious anaemia virus (EIAV), a type C retrovirus, causes a persistent infection in horses. The chronic form of the disease caused by EIAV is typified by periodic clinical episodes and associated bursts of viraemia at irregular intervals following the initial infection (Kono, 1973; Crawford et al., 1978; Issel & Coggins, 1979). Horses infected with EIAV develop neutralizing antibody against the predominant virus while continuing to experience bursts of viraemia. Previous neutralization studies have indicated that the periodic and persistent nature of the disease may be due to the sequential production and release of novel antigenic strains of the virus capable of escaping host immunosurveillance systems (Kono, 1973; Kono et al., 1971, 1973). However, there have been no published studies confirming these neutralization studies or characterizing the nature of the viral variants. This lack of progress is due in part to the difficulty of propagating EIAV in tissue culture and in isolating virus from sequential febrile episodes in experimentally infected animals.

In recent years we have developed procedures for the propagation of EIAV in tissue culture in quantities sufficient for detailed biochemical analysis of the virion proteins (Parekh et al., 1980; Montelaro et al., 1982). In addition, an animal model system was developed in which numerous febrile episodes were routinely generated in Shetland ponies, and most importantly, virus isolates could be recovered from sequential febrile episodes and propagated in tissue culture (Orrego et al., 1982; Orrego, 1983). Neutralization data on certain isolates demonstrated the
serological uniqueness of virus isolates recovered from sequential febrile episodes (Orrego, 1983; Montelaro et al., 1984), thereby confirming the reports of Kono et al. (1971, 1973). Biochemical analyses indicated structural variations in the glycoproteins of the virus isolates examined, presumably correlating with the antigenic variations revealed in neutralization studies (Montelaro et al., 1984). In this report we have performed oligonucleotide mappings of the RNA genomes of selected virus isolates in order to determine whether antigenic variation can be detected in altered RNA sequences and whether the variation is due to point mutations or recombinational events.

Shetland ponies were infected with the equine cell-adapted Wyoming strain (Malmquist et al., 1973) of EIAV with subsequent recovery from these 'first passage' ponies of a host-adapted isolate designated PI-1 as outlined in Fig. 1. Strains P2-1 and P2-6 were isolated by endpoint titration from the first and sixth sequential febrile episodes from a 'second passage' pony (no. 82) inoculated with host-adapted EIAV (Orrego et al., 1982). Serum collected from pony 82 sixteen days after the first febrile episode displayed a log₁₀ neutralization index of 4 against P2-1, but failed to reduce the infectivity of P2-6 (Orrego, 1983; Montelaro et al., 1984). This difference in neutralization properties of P2-1 and P2-6 indicates a change in the antigenic properties of the virus over the time course of the infection, in agreement with the data of Kono (1973) and Kono et al. (1971, 1973). Virus isolates were propagated in foetal equine kidney cells, essentially as described previously (Montelaro et al., 1982).
The four virus isolates (cell-adapted, P1-1, P2-1 and P2-6) were compared biochemically by SDS–PAGE, immunoblot analysis, and oligonucleotide mapping. The purification of virus and viral proteins has been described in detail (Montelaro et al., 1982, 1983). SDS–PAGE analysis of viral proteins has also been described (Montelaro et al., 1984; Parekh et al., 1980). Immunoblot analysis was performed according to procedures described by Burnette (1981) using reference serum from a naturally infected horse and \(^{125}\)I-labelled Protein A. For the recovery of genomic RNA, virus was harvested at 4-h intervals and purified immediately by isopycnic centrifugation. The purified virus was then treated with proteinase K and SDS, and the RNA was recovered by phenol–chloroform extraction and ethanol precipitation. Genomic RNA was recovered by centrifugation on a 10 to 30% sucrose gradient (1-75 h at 4 °C in an SW50.1 rotor) followed by ethanol precipitation. Oligonucleotide mapping was performed essentially as described by Pedersen & Haseltine (1980a, b). Briefly, 400 to 500 ng of RNA was digested with RNase T1 and the resulting oligonucleotides were labelled at the 5' end with \(\gamma^{32}\)P]ATP and polynucleotide kinase. Two-dimensional gel electrophoresis was performed to generate oligonucleotide maps of each virus isolate.

SDS–PAGE analysis of the proteins contained in the four virus isolates revealed virtually identical protein profiles for the respective virus samples; the relative amounts and apparent molecular weights of the major virion proteins (p26, pp15, p11 and p9) were identical (Fig. 2a). The viral glycoproteins can not be readily observed after SDS–PAGE of whole virus samples as they are minor components of the virus (Parekh et al., 1980; Montelaro et al., 1982). However, an immunoblot of the gel shown in Fig. 2(a) highlighted the two viral glycoproteins, gp90 and gp45, which are the primary immunogens during a persistent infection (Fig. 2b). The immunoblot revealed altered electrophoretic mobilities for the glycoproteins contained in different virus isolates. In contrast, the major internal protein (p26) from each isolate displayed a constant electrophoretic mobility. The observed shifts in glycoprotein mobilities of each of these virus isolates has remained constant after repeated passage of the virus in cell culture and apparently indicates the structural variation of EIAV glycoproteins during persistent infection in horses in the presence of immune responses.

Oligonucleotide mapping was performed to determine whether the apparent changes in glycoprotein structure could be detected at the level of the genome and to see whether these changes were the result of point mutations or recombinational events. The oligonucleotide maps shown in Fig. 3 demonstrate differences in the genomes of all virus isolates. Two or more maps were studied for each virus isolate, and the changes indicated reflect differences consistently observed in all maps. A number of changes have clearly occurred between each of the virus isolates. Additions and deletions between the laboratory strain virus and isolate P1-1 most likely represent changes that occurred during the adaptation from tissue culture to the host pony. Additions and deletions also occur between isolates P1-1, P2-1 and P2-6. Two additions and four deletions occur from P1-1 to P2-1. These changes are probably not ‘induced’ by immune pressures encountered in the infected pony, as isolate P2-1 was recovered during the first febrile episode in the animal, prior to the development of a significant immune response (Issel & Coggins, 1979; Orrego et al., 1982). Instead, these changes may indicate a highly mutable virus which undergoes mutation and selection when passaged laterally from host to host. P2-6, isolated during the sixth febrile episode, 180 days after the first febrile episode, has seven oligonucleotide changes when compared to P2-1. These changes have occurred in the long-term presence of neutralizing antibody against P2-1 and most likely represent viral variants selected specifically by their ability to escape neutralization. Although differences can clearly be seen in all maps, the overall similarities between the maps suggest that an accumulation of point mutations and not recombination with an endogenous virus are responsible for the generation of antigenic variants of EIAV. This appears to correlate with the reported absence of endogenous viruses related to EIAV in uninfected horses (Rice et al., 1978). Moreover, the characteristic oligonucleotide maps of each isolate of EIAV remained unaltered even after prolonged passage in tissue culture, suggesting that the alterations were stable under these growth conditions.

In summary, both neutralization data and biochemical analyses indicate that EIAV is a highly mutable virus. Mutation can be observed during re-adaptation to the host, during
Fig. 3. Oligonucleotide maps of EIAV 70S genomic RNA of cell-adapted Wyoming strain (a) and variants P1-1 (b), P2-1 (c) and P2-6 (d). Electrophoresis in the first dimension (8% polyacrylamide, pH 3.25) was from left to right and in the second dimension (22% polyacrylamide, pH 8.2) from bottom to top. Arrows (additions) and open circles (deletions) indicate differences in each pattern when compared to that isolate's predecessor. In this manner P1-1 is compared to the laboratory strain, P2-1 is compared to P1-1, and P2-6 is compared to P2-1.

horizontal passage between hosts, and during replication in a single host. The apparent stability of virus strains in tissue culture suggests that immune pressures in the host may be one selective force for the emergence of novel antigenic strains of EIAV. EIAV lends itself well to the study of antigenic variation, as the periodicity of the disease allows the recovery of the major antigenic species of virus present during recurrent febrile episodes. Studies similar to those presented here have been reported for another retrovirus, visna, which causes a progressive (rather than periodic) disease in sheep. In the case of visna, Scott et al. (1979) noted changes in the peptide maps of viral gp135 in antigenically distinct virus isolates, and in another study Clements et al. (1980) reported up to seven oligonucleotides with altered mobilities in RNA fingerprints between an infecting virus and serologically distinct isolates from a diseased animal. The degree of genomic mutation reported by Clements et al. (1980) for visna is similar to that reported here for EIAV isolates P2-1 and P2-6, between which seven changes were observed. Recently, there has been a recognition of the high mutation frequency associated with the replication of a variety of animal RNA viruses (Holland et al., 1982). The similarity between EIAV and visna with respect to variations in neutralization properties and the observed changes in viral glycoproteins and genomic RNA suggests that the development of novel antigenic strains in a diseased host may be a more common mechanism for the maintenance of persistent retrovirus infections than has been previously recognized.

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