Latent equid herpesviruses 1 and 4: detection and distinction using the polymerase chain reaction and co-cultivation from lymphoid tissues

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The polymerase chain reaction (PCR) and co-cultivation were used to identify the lymphoreticular system as the site of latency of equid herpesvirus 1 (EHV-1). Primers for PCR were designed from aligned nucleotide sequences of the glycoprotein gB genes to amplify the same region of both the EHV-1 and EHV-4 genomes. Subsequent restriction digests using specific enzymes distinguished the amplified fragments of the EHV-1 genome from those of the EHV-4 genome.

Ten weeks following an experimental infection of five ponies with EHV-1, latent virus was detected by PCR and recovered by co-cultivation, predominantly from lymphoid tissues draining the respiratory tract. Significantly, latent EHV-1 also persisted in peripheral blood leukocytes (PBL). Latent EHV-4, presumably from a preceding natural infection, was also detected in some tissues, including PBL, from all animals. Of additional interest was the recovery of EHV-1 and -4 only in the presence of the ubiquitous EHV-2.

Introduction

Latency is an important component in the pathogenesis of the herpesviruses. It is established following an initial infection and consequently the host remains a source for future infections. The equid herpesviruses 1 (EHV-1, formerly EHV-1 subtype 1) and 4 (EHV-4, formerly EHV-1 subtype 2) are included in group A of Melnick's classification (1971). They are classed as alphaherpesviruses on the basis of their genome structure and biological behaviour (Studdert et al., 1981; Roizman, 1982; Honess, 1984).

Frequently, infection with either virus results in rhinopneumonitis, although dissemination of EHV-1 may result in paresis or abortion in susceptible mares. In addition, EHV-4 has been isolated from aborted foetal tissue (Shimuzu et al., 1959; Studdert, 1983; Allen & Bryans, 1986). During acute infection both viruses can be isolated from nasal swabs. EHV-1 causes viraemia and can be isolated from peripheral blood mononuclear cells (PBMCs) for up to 3 weeks post-infection (P.I.). Isolation of EHV-1 from predominantly T lymphocytes and monocytes during an acute infection led Scott et al. (1983) to propose that EHV-1 is T lymphotropic and possibly establishes latency in T lymphocytes.

Neurotropism and latency in neurons are characteristic of alphaherpesviruses (reviewed by Wildy et al., 1982; Hill, 1985), yet failure to recover virus from the cranial ganglia of experimentally infected horses is well documented (Burrows & Goodridge, 1984; Edington et al., 1984; Allen & Bryans, 1986). This apparently contradicted the neurological disorders associated with EHV-1 (Manninger, 1949; Saxegaard, 1966; Jackson et al., 1977), until it was recognized that the lesion in the central nervous system was a vasculitis resulting from virus replication in endothelial cells (Patel et al., 1982; Edington et al., 1986). Clear evidence of latent EHV-1 and EHV-4 was shown by using high levels of corticosteroids (Edington et al., 1985; Browning et al., 1988). Additionally, recovery of virus from explanted tissues has indicated that lymphoid tissues are the sites of latency (N. Edington, unpublished data), but this remains to be confirmed.

Considerable similarity and gross collinearity between the genomes of EHV-1 and -4 have been demonstrated (Cullinane et al., 1988). Accordingly, in situ hybridization is inappropriate for the study of EHV's, particularly for studying naturally infected animals. Southern hybridization (Southern, 1975) is an alternative method, distinguishing viruses by their restriction pattern. Applying this method, Efthathiou et al. (1986) successfully detected latent herpes simplex virus type 1 in human ganglia. However, it is insufficiently sensitive to detect latent EHV's (H. M. Welch, unpublished data).

The polymerase chain reaction (PCR; Saiki et al., 1985; Mullis & Faloona, 1987) has the advantages of sensitivity and accuracy. It will detect a single copy of target DNA in 10 μg of DNA (equivalent to approxima-
H. M. Welch and others

(a) Location of the EHV-1 and EHV-4 gB genes [from Whalley et al. (1989) and Riggio et al. (1989) respectively]. Relative positions of gB and some restriction sites (B, BamHI; E, EcoRI) are shown. (b) Regions of aligned gB genes. Positions of sense (→) and antisense (←) primers are shown, as are the regions amplified. Restriction enzymes used to distinguish EHV-1 from EHV-4 were D, DdeI; G, BglII; C, ClaI; E, BstEII; H, HhaI.

Fig. 1. Collinear genomes. (a) Location of the EHV-1 and EHV-4 gB genes [from Whalley et al. (1989) and Riggio et al. (1989) respectively]. Relative positions of gB and some restriction sites (B, BamHI; E, EcoRI) are shown. (b) Regions of aligned gB genes. Positions of sense (→) and antisense (←) primers are shown, as are the regions amplified. Restriction enzymes used to distinguish EHV-1 from EHV-4 were D, DdeI; G, BglII; C, ClaI; E, BstEII; H, HhaI.

tely 1.5 x 10^6 diploid cells; Saiki et al., 1988) and furthermore, the products can be subjected to analytical manipulations (reviewed by White et al., 1989). Primers capable of amplifying the same regions from both EHV-1 and -4 (see Fig. 1) were designed by aligning the nucleotide sequences of the glycoprotein gB genes of EHV-1 and -4 (Whalley et al., 1989; Riggio et al., 1989). By designing primers from highly conserved regions, the risk of sequence variation among natural populations and variation resulting from laboratory adaptation was reduced. Although 84% of the bases were identical, sufficient base mismatches were within the recognition sequences of restriction enzymes to allow restriction fragment length polymorphisms (RFLPs) specific for each virus (see Fig. 2) to be identified. In addition, should amplification of spurious sequences occur, particularly of those from the ubiquitous EHV-2 (Kemeny & Pearson, 1970), they would be readily identified.

In this paper we report the successful application of PCR, RFLP analysis and co-cultivation to the study of experimentally infected equidae.

Methods

Experimental animals
(i) Infection. Five Welsh mountain ponies from a closed herd, at 6 months of age, were infected intranasally using a swab and nebulizer as described previously (Edington et al., 1986). The inoculum contained 10^6 TCID₅₀/ml of the 11th passage (in equine cells) of the Ab-4 (p2) isolate of EHV-1 (Patel & Edington, 1983). Virus recovery from nasal swabs and PBMCs was analysed daily as described previously (Patel et al., 1982). Serum samples were taken weekly for 4 weeks and subsequently at monthly intervals, and used to isolate complement-fixing (CF) antibody (Thomson et al., 1976).

Ten weeks after the primary infection, all five ponies were killed with intravenous barbiturate, exsanguinated, and samples of PBMCs, retropharyngeal (RPLN), submandibular (SMLN), bronchial (BLN), tonsillar (TON) and popliteal (PLN) lymph nodes, samples of thymus (THY), spleen (SPL) and lung, cells from pulmonary lavage (AL) and nasal mucosa (NM), and ganglia of the Vth cranial nerve (SLG) were taken. These were examined for virus by conventional isolation methods, and by co-cultivation and explant as detailed below.

(ii) Explant and co-cultivation. These were essentially as described by Stevens & Cook (1971). Dissected 1 cm² fragments of SLG, NM and lung were adhered directly onto 25 cm² flasks and immediately overlaid with equine embryonic kidney cells (EEK) or rabbit kidney (RK13) cells which served as detectors of reactivated virus. Duplicate preparations were made of ground tissue. Teased suspensions of lymph nodes were seeded at 6 x 10^6 cells onto confluent monolayers of 25 cm² flasks of EEK and RK13 cells. Duplicate PBMC preparations were made after disruption by freezing and thawing.

All preparations were kept for 10 days in RPMI 1640 medium supplemented with 10% foetal calf serum (FCS), after which they were trypsinized and passaged twice if no c.p.e. was observed. Cytospin preparations of cultures showing c.p.e. were made as described previously (Patel et al., 1982). EHV-1, -4 and -2 were distinguished from each other by indirect immunofluorescence (IIF) using rabbit polyclonal antisera for screening (Thomson et al., 1976), or by using specific monoclonal antibodies (MAbs) (Yeargen et al., 1985; Edington et al., 1987).

(iii) Virus isolation. This was performed on EEK or RK13 cell monolayers as described previously (Patel et al., 1982).
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(iv) Tissues for PCR. Triplicate samples were diced, snap-frozen in liquid nitrogen and stored at −70 °C until required. Samples (10 ml) of heparinized (10 units/ml) whole blood were immediately frozen until required.

Establishment and application of the PCR


(ii) Oligonucleotide primers. The sequences chosen were from aligned regions showing >85% similarity (see Fig. 2), the following guidelines. Optimal length 20 to 25 bases; amplified fragment, 200 to 800 bp; approximate GC content, 50%; non-complementary 3' ends; minimum degeneracy at the 3' ends. Each primer pair was assessed for optimum temperature for each primer pair (see Table 1), polymerization cycles of denaturation at 94 °C for 1 min, annealing for 2 min at the 5 °C for each primer pair. The optimal annealing temperature for each primer pair agreed with that predicted by Oligo (see Table 1). To reduce the risk of contamination, the following precautions were taken. Positive displacement pipettes were used for all manipulations. High M, (latent) DNA was isolated from acutely infected tissues; NM and lung acutely infected with the EHV-1 isolate Ab-4 (P2) were obtained as described by Edington et al., 1986. Similar tissues from a gnotobiotic foal acutely infected with the EHV-4 isolate MD (Pate et al., 1982), were obtained essentially as above. For use as a negative control, DNA was isolated from tissue culture monolayers of E cell lines by standard proteolytic digestion with the addition of a pre-wash with 0-9% saline. (v) Amplification. To facilitate handling prior to amplification, 10 μg of high M, DNA was digested with EcoRI, precipitated and taken up in H2O to a final concentration of 100 ng/ml. Amplification was performed using the Perkin-Elmer Cetus DNA Thermal Cycler Kit and AmpliTaq Polymerase. The optimal viral DNA concentration was determined to be 200 ng/ml and the Mg2+ ion concentration to be 1.5 mM for each primer pair. The optimal annealing temperature for each primer pair agreed with that predicted by Oligo (see Table 1).

(iii) Evaluation of primers and restriction sites. To assess the efficacy of the primers and conservation of restriction sites, a number of virus isolates were analysed. For EHV-1, the isolates were Ab-4 (P2), 9524, W15, A183 and RACH (Pate & Edington, 1983), and Lord P isolated from an aborted foetus and kindly provided by R. Burrows. EHV-2 isolates were 2252 and H45 (Pate et al., 1983), Ky-D (Doll & Wallace, 1954), and 198, 1511, 1999, 1363 and 1193, kindly provided by Dr J. Mumford (The Animal Health Trust, Newmarket, U.K.). An EHV-2 isolate (C9) was recovered from the RPLN of an abortoir horse. All isolates were grown in equine dermal (ED) (NBL-5; Flow Laboratories) or EK cells. Cells were maintained in MEM containing Earle’s salts, supplemented with 10% FCS, 2 mM-glutamine and non-essential amino acids in 80 cm2 Nunc flasks (Gibco/BRL) in the presence of 5% CO2 at 37 °C. (iv) Isolation of DNA. Viral DNA was extracted from infected cells showing >85% c.p.e. according to the method of Tym et al., 1984, with an additional pretreatment with DNase I (20 μg/ml) at 37 °C for 20 min.

High M, DNA was extracted from frozen tissues by standard proteolytic digestion; approximately 80 mg of tissue was ground under liquid nitrogen using a pestle and mortar, suspended in STE (150 mM-NaCl, 10 mM-Tris–HCl pH 8, 10 mM-EDTA) in the presence of protease K (200 μg/ml) and 0.1% SDS, incubated overnight at 37 °C, extracted twice with phenol and once with chloroform, precipitated with ethanol and taken up in TE (1 mM-Tris–HCl pH 8, 0.1 mM-EDTA) to give 500 μg/ml DNA. DNA from frozen blood samples (referred to as PBL) was extracted similarly following thawing and pellet isolation using 0.5% NP40 (BDH) (M.B. Davis, personal communication).

In addition to that from latently infected tissues, DNA was extracted from acutely infected tissues; NM and lung acutely infected with the EHV-1 isolate Ab-4 (P2) were obtained as described by Edington et al., 1986. Similar tissues from a gnotobiotic foal acutely infected with the EHV-4 isolate MD (Pate et al., 1982), were obtained essentially as above. For use as a negative control, DNA was isolated from tissue culture monolayers of E cell lines by standard proteolytic digestion with the addition of a pre-wash with 0.9% saline.
Table 1. Primer details

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'-3')†</th>
<th>Length</th>
<th>GC (%)</th>
<th>Tr (°C)‡</th>
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</thead>
<tbody>
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<td>1L</td>
<td>GGA AAG GAT ACA GCC ATA CGT C</td>
<td>22-mer</td>
<td>50</td>
<td>51</td>
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<tr>
<td>2L</td>
<td>TC[CG]§ ACC GGT GAT ATT GTG TAC</td>
<td>21-mer</td>
<td>48</td>
<td>47</td>
</tr>
<tr>
<td>3L</td>
<td>C[CG]T CTC CGT TTT ACG GCC T</td>
<td>19-mer</td>
<td>58</td>
<td>45</td>
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<tr>
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<td>22-mer</td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td>2R</td>
<td>TAG TAA ATC TGT AGG ACC CGC G</td>
<td>22-mer</td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td>3R</td>
<td>GTA TAT CGA GTC TAT GGC TTC</td>
<td>21-mer</td>
<td>43</td>
<td>45</td>
</tr>
</tbody>
</table>

† Primer sequences were identical for EHV-1 and -4 except at those positions marked with an asterisk, for which the EHV-1 base is given.
‡ Tr, Optimal annealing temperature.
§ [CG], Mixed base site.

Restriction enzyme digestion and gel electrophoresis. Restriction enzymes were purchased from Anglian Biotech or Gibco/BRL and used according to their recommendations, with the addition of spermidine-HCl to 4 mM. Digests were performed on approximately 0.3 µg (one-twelfth) of the amplified product, electrophoresed, using a Pharmacia mini-gel apparatus, through 4% NuSieve GTG agarose gels (FMC BioProducts) in TBE buffer (90 mM-Tris pH 8, 90 mM-boric acid, 8 mM-EDTA, 0.5 µg/ml ethidium bromide) at 75 V for 1.5 to 2 h and visualized using a 254 nm u.v. transilluminator.

Membrane hybridization. Equivocal results were analysed further by Southern hybridization (Southern, 1975). Amplification products were digested with DdeI, transferred from 4% NuSieve-TAE (40 mM-Tris-HCl pH 8, 20 mM-sodium acetate, 1 mM-EDTA) gels onto GeneScreen Plus and hybridized according to the manufacturer’s recommendations (DuPont). An EHV-4 gB gene probe was labelled according to the method of Feinberg & Vogelstein (1983) and used to probe the filters under conditions of high stringency.

Results

In vivo experimental infection

During acute infection, virus was consistently recovered from nasal swabs between 1 and 4 days p.i., and sporadically up to 12 days p.i. Co-cultivation of PBMCs yielded virus from four ponies between 4 and 8 days p.i., with sporadic isolation at 10 and 12 days p.i. from two of them, but only on day 11 p.i. from the fifth pony. No virus was isolated from any sample in the third week after infection. CF antibody titres were all less than 1:20 on day 1; 8 days p.i. three of the five showed a fourfold increase in titre and all had a titre > 1:80 by 14 days p.i. and thereafter.

PBMCs taken prior to infection (0 days p.i.) did not yield EHV-1 following a single passage of co-cultivation.

Establishment of the PCR

All possible combinations of primer pairs produced the expected fragments of identical size for each EHV-1 and EHV-4 isolate (see Fig. 3a and b). Restriction analysis of each fragment confirmed the predicted restriction sites in the genomes of all EHV-1 and EHV-4 isolates with the exception of a BanII site predicted for that of EHV-4 which was absent from all isolates (data not shown). This could have occurred because the isolate sequenced, 1942, was grown in non-equine cells (Cullinane & Davison, 1989) which could introduce errors. The restriction sites were highly conserved and therefore three enzymes were considered sufficient to distinguish EHV-1 from EHV-4 (see Fig. 1 and 3e). Single amplifications (using 1L and 1R) with modified parameters proved successful for some tissues (data not shown). However, the greatest precision and yield were achieved using two rounds of amplification with nested primer pairs, the most effective combination being 1L and 3R followed by 2L and 2R (see Fig. 4).

Spurious amplified fragments were occasionally seen with the primer pair 2L and 2R. They probably resulted from secondary amplification at the start of cycling when primers were in excess. They were more evident when the yield was low, but did not affect interpretation of the pattern of digested products. They were virus-specific; indeed, the presence of EHV-1 or -4 could often be predicted by the difference in mobility (see Fig. 4a, lanes 3 and 4).

All primer pairs were tested for spurious amplification of EHV-2 and equine genomic (ED cell) DNA sequences. No amplification was seen with any combination of primers.
Fig. 3. Amplified viral DNA. (a) EHV-1, (b) EHV-4. A 2% Nusieve gel showing amplification with different primer pairs: lane 1, 1L and 3R; lane 2, 1L and 2R; lane 3, 2L and 3R; lane 4, 3L and 3R; lane 5, 1L and 1R; lane 6, 2L and 2R; lane 7, 3L and 2R. (c) Restriction enzyme digests of EHV-1 (lanes 1, 3 and 5) and EHV-4 (lanes 2, 4 and 6) region VI, 2L and 2R with BstEII (lanes 1 and 2), Ddel (lanes 3 and 4) and HhaI (lanes 5 and 6). Lane L, 123 bp ladder (Gibco/BRL).

Fig. 4. Amplified tissue DNA. Initial amplification utilized 1L and 3R. Re-amplification using the primer pair 2L and 2R produced a fragment of 294 bp. (a) A 2% Nusieve gel showing a selection of positive tissues from pony 3. Lane 1, RPLN; 2, SMLN; 3, BLN; 4, TON; 5, PBL20; 6, PBL28; 7, PBL70; 8, THY; 9, AL; 10, Lung. (b) Selection of tissues from pony 3 showing latent EHV-1 (lanes 1, 4 and 7), EHV-1 and -4 (lanes 2, 5 and 8) and EHV-4 (lanes 3, 6 and 9) digested with BstII (lanes 1 to 3), Ddel (lanes 4 to 6) and HhaI (lanes 7 to 9). Lanes L, 123 bp ladder.

Co-cultivation and IIF

Ten weeks after infection, EHV-1 was detected by IIF on co-cultivated explanted lymphoid tissues from four ponies and PBMCs of two ponies (Table 2). EHV-1 was not recovered from SLG, lung or NM, although cells
Table 2. Detection of latent virus by co-cultivation of explants 10 weeks following an experimental infection with EHV-1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Animal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5</td>
<td>EHV-1 EHV-2</td>
</tr>
<tr>
<td>RPLN</td>
<td>1.2 1.2 1.2* 1.2 2</td>
<td>4 5</td>
</tr>
<tr>
<td>SMLN</td>
<td>1.2 1.2 2 2 2 2</td>
<td>2 5</td>
</tr>
<tr>
<td>BLN</td>
<td>1.2 1.2* 1.2† 1.2 2</td>
<td>4 5</td>
</tr>
<tr>
<td>TON</td>
<td>2 2 1.2 1.2 2</td>
<td>2 5</td>
</tr>
<tr>
<td>PLN</td>
<td>2 2 2 2 2</td>
<td>2 5</td>
</tr>
<tr>
<td>PBMC70†</td>
<td>NT§ NT 1.2 1.2 2</td>
<td>2 3</td>
</tr>
<tr>
<td>SPL</td>
<td>1.2 2 1.2 1.2 2</td>
<td>3 5</td>
</tr>
<tr>
<td>THY</td>
<td>2 1.2 1.2 1.2 2</td>
<td>3 5</td>
</tr>
<tr>
<td>AL</td>
<td>2 1.2 2</td>
<td>1 3</td>
</tr>
<tr>
<td>Lung</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>NM</td>
<td>2 2</td>
<td>2 2</td>
</tr>
<tr>
<td>SLG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Presence of EHV-4 identified by PCR.
† Presence of EHV-4 confirmed using virus-specific MAbs.
‡ PBMC70 PBMCs 10 weeks after infection.
§ NT, Not tested.

Table 3. Detection of latent EHV-1 and -4 by PCR and RFLP analysis 10 weeks following an experimental infection with EHV-1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Animal</th>
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<tr>
<td></td>
<td>1 2 3 4 5</td>
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</tr>
<tr>
<td>RPLN</td>
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<tr>
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<tr>
<td>Lung</td>
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<td>2 1</td>
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<tr>
<td>NM</td>
<td>4 NT</td>
<td>4 1</td>
</tr>
<tr>
<td>SLG</td>
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</table>

* cc, Virus detected by co-cultivation but not PCR.
† PBL18, PBMCs pre-infection.
‡ PBL18, PBMCs 4 weeks p.i.
§ PBL70, PBMCs 10 weeks p.i.
|| NT, Not tested.

EHV-2 was recovered from all lymphoid tissues of all animals and also from three lung washings (AL), two NMs and one lung sample; it was not recovered from SLG, or ground tissues or frozen duplicates.

Amplification and RFLP analysis of tissue DNA

The PCR proved more sensitive than co-cultivation and all ponies were shown to be latently infected with both EHV-1 and EHV-4 (Table 3). Amplified fragments were seen in 45 samples and subsequent digestion showed that 40 were from EHV-1 and 10 were from EHV-4; both viruses were detected in five tissue samples, RPLN, BLN, TON, PBL and SPL, clearly demonstrating the efficacy of PCR and RFLP analysis (see Fig. 4). EHV-1 was detected in all tissues except NM; it was also detected in SLG of pony 4. Amplification of EHV-4 was more sporadic, but it was also present in the PBL (pre-infection) of pony 4.

Negative amplifications were repeated at least once. Latent virus was detected by co-cultivation, and not PCR, in five samples: two SMLN, two THY and one TON. Southern hybridization of these PCR products also failed to detect virus. Weak amplifications were confirmed using Southern hybridization.

The control for each group of amplifications remained negative throughout all reactions.

Discussion

Latency was established in five ponies following an experimental infection with EHV-1. Latent virus was recovered by co-cultivation and detected by PCR predominantly, but not exclusively, in lymphoid tissues draining the upper respiratory tract. This supported earlier observations (N. Edington, unpublished results), but was in contrast to the low recovery from and detection in tissues at the site of primary infection. Significantly, latent virus persisted in PBL, supporting the T lymphotropism proposal of Scott et al. (1983). The persistence of latent virus in leukocytes allows rapid dissemination through the vascular and lymphatic systems should reactivation of latent virus occur.

Suitable assays performed prior to infection indicated that the animals appeared to be immunologically naive. Their response to antigen confirmed this (J. McCulloch, unpublished results), despite the presence of latent EHV-1 and -4. These results were not unexpected. The transient nature of the immune response to EHV-1 and -4 is well known (Allen & Bryans, 1986; J. McCulloch, S. Williamson & N. Edington, unpublished results). The detection by PCR of latent virus prior to infection was confirmed by the recovery of EHV-4 from the BLN and obtained by pulmonary lavage (AL) did yield virus from pony 2. Virus was not recovered from homogenized tissues or lymph node suspensions that had been subjected to freezing and thawing either. Although PCR confirmed the presence of EHV-1 in most tissues (see below and Table 3), it detected EHV-4 in four tissues: two BLNs, one RPLN and one TON. Explants from BLN and TON of pony 3 were re-passaged and tested for EHV-4 by IIF using an EHV-4-specific MAb; the presence of EHV-4 in both tissues was confirmed.

EHV-2 was recovered from all lymphoid tissues of all animals and also from three lung washings (AL), two NMs and one lung sample; it was not recovered from SLG, or ground tissues or frozen duplicates.
TON of pony 3. Although EHV-4 is a respiratory pathogen, the presence of latent virus in other tissues, including PBL, is not without precedence (Shimuzu et al., 1959; Allen & Bryans, 1986; J. Mumford, personal communication).

PCR and Southern hybridization failed to detect latent virus in five tissues, from which it was recovered by co-cultivation. This discrepancy is consistent with the low prevalence of virus and the fact that analyses were performed on duplicate samples. Southern hybridization of these products confirmed the stringency of the conditions employed. PCR also failed to amplify virus in PBL collected 28 days p.i. from ponies 4 and 5. The yield of DNA was low in these samples, such that \(<5 \times 10^5\) cells were analysed.

Recovery of EHV-2 by co-cultivation was widespread. Furthermore, growth always accompanied and preceded growth of EHV-1 and -4. A possible role for EHV-2 in trans-activation and reactivation is suggested, particularly because trans-activation of the immediate early gene promoter of EHV-1 by EHV-2 has been demonstrated (A. S. Purewal, A. V. Smallwood, A. Kausal, D. Adgeboyce & N. Edington, unpublished results). EHV-2 also was readily recovered from most tissues of pony 5 and whereas PCR detected latent EHV-1 and -4 in this animal, neither was recovered by co-cultivation. Host factors must also contribute to the state of latency and reactivation.

Latent virus in PBL of contaminating blood was possibly responsible for the PCR detecting latent EHV-1 in the SLG of pony 4. Alternatively, EHV-1 may have established a latent infection in SLG, but could not be reactivated. Both EHV-1 and -4 have been detected by PCR in a significant number of SLG taken from naturally infected animals (H. M. Welch, unpublished results), although they have never been recovered from this tissue; EHV-2 has been recovered only rarely (Edington et al., 1984).

As more data accumulate, the grouping of the family Herpesviridae into three classes on the basis of biological behaviour is becoming increasingly difficult. Although neurotropism is a characteristic of alpha-herpesviruses, it is not a constraint. Pseudorabies virus (suid herpesvirus) also establishes a latent infection in PBL (Wittmann et al., 1980; Rziha et al., 1986; Ohlinger et al., 1987) as well as trigeminal ganglia (Sabo & Rajcani, 1976; Beran et al., 1980; Ben-Porat et al., 1984). Conversely, Marek's disease virus is lymphotropic and therefore is considered by some authors to be a gammaherpesvirus; however, it has genomic homology to alphaherpesviruses, particularly varicella-zoster virus (Buckmaster et al., 1988).

We conclude that the techniques of PCR and co-cultivation together show that the alphaherpesviruses EHV-1 and EHV-4 become latent in the lymphoreticular system. This is consistent with aspects of their pathogenesis (Allen & Bryans, 1986). In situ hybridization should identify more precisely the particular cells involved and the specific expression of EHV-1 and EHV-4.

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References


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