Persistent Epstein–Barr virus infection in the common marmoset (Callithrix jacchus)

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Epstein–Barr virus (EBV) infection of the common marmoset causes long-term infection, with production of antibodies to virus-induced antigens, without clinical illness. Attempts to show the presence of EBV DNA in saliva of infected animals by PCR were initially unsuccessful, although slot-blot hybridization analysis demonstrated that viral DNA was present. Further investigations showed that most samples of pilocarpine-induced saliva, and 33% of the samples of whole mouth fluids (WMF) tested, were inhibitory to PCR. Similar results were found using human WMF. A method of assessing samples of marmoset WMF for the presence of EBV, by PCR using an EBV BamHI W probe, and removing inhibition with Chelex 100, is described. A total of 202 samples from 21 EBV infected, and seven non-infected animals was tested. Five seropositive animals shed virus on every occasion, and 15 intermittently. Two marmosets, infected as neonates, showed progressively increasing humoral responses to viral antigens, and shed virus on every occasion tested over 3 years. When mated with uninfected animals, the latter seroconverted 4 and 6 weeks later, respectively, and later shed virus into their WMF. The naturally infected animals were paired with naive marmosets, and were able to pass on infection. These results establish that long-term, permissive EBV infection occurs in the common marmoset, and demonstrate again the similarities in the response to EBV between marmoset and man.

Introduction

Epstein–Barr virus (EBV), the causative agent of infectious mononucleosis (IM), is also associated with Burkitt's lymphoma (de Thé et al., 1978), nasopharyngeal carcinoma (zur Hausen et al., 1970; Ho, 1972), lymphoproliferative disease, oral hairy leukoplasia in transplant recipients and AIDS patients (Hanto et al., 1981; Thomas & Crawford, 1989; Greenspan et al., 1985, 1989) and Hodgkin's disease (Herbst et al., 1990), and is increasingly being identified in other malignancies (Imai et al., 1994; Labrecque et al., 1995).

Work on the development of a vaccine which could modify the effects of infection has continued since its initial proposal (Epstein, 1976). The efficacy of potential vaccines has been widely tested in the cotton-top tamarin, Saguinus oedipus. Preparations of EBV membrane glycoproteins gp340 and 220, together with various adjuvants, have proved successful in preventing strain B95-8-induced disease in this species (Epstein et al., 1985; Morgan et al., 1989; Finerty et al., 1992).

Infection of the common marmoset with the M81 strain of EBV, produced by a common marmoset lymphoblastoid line (Desgranges et al., 1976; de Thé et al., 1970), is followed by long-term production of antibody to viral antigens, without clinical disease (de Thé et al., 1980; Wedderburn et al., 1984). Similar results using the B95-8 strain have been reported, although larger doses are required for seroconversion (Emini et al., 1986; Felton et al., 1984). The fact that EBV infection was followed by responses commonly seen in man (Wedderburn et al., 1984) suggested that this marmoset would be a good model for testing EBV vaccines. Permissive infection was suggested by the seroconversion of virus capsid antigens (VCA)-negative, housed with seropositive, animals (see below). However, early attempts to demonstrate the presence of the EBV genome in tissues and saliva, by PCR, failed to give reproducible results (Arrand, 1992).

We now report that EBV DNA can be reliably demonstrated in samples of mouth fluids and tissues,
using slot-blot and PCR analysis. A method of overcoming inhibition of PCR, using Chelex 100, was adapted from Walsh et al. (1991). The sequential monitoring of natural infection of naive animals, housed with infected cage mates is described.

Methods

Animals: Callithrix jacchus. The common marmosets used in this study were part of a closed colony to which there have been no additions since 1970.

Epstein-Barr virus. EBV was obtained from the M81 cell line (Desgranges et al., 1976). Supernatants from 10–11 day cultures were concentrated by centrifugation (Johnston et al., 1990). Animals were infected with EBV either by the intramuscular (i.m.) and intraperitoneal (i.p.) routes, or ‘orally’, by injections into the palatine tonsils, the back of the tongue, and sublingually, under ketamine anaesthesia (10 mg/kg i.m.; Vetalar, Park Davis).

Blood and saliva samples. Blood for haematological investigations was taken under ketamine anaesthesia. Saliva was obtained following pilocarpine stimulation [1 mg/kg pilocarpine nitrate (Sigma) in saline, intradermally (i.d.)], under anaesthesia with alfadolone alfaxalone (1–1.5 mg/kg, Saffan Glaxovet). ‘Whole mouth fluid’ (WMF) was obtained by inserting 0.3 ml of distilled water (dH2O) into the mouth, under ketamine anaesthesia, and removing the fluids. WMF was collected from human subjects by requesting individuals to mix 5 ml dH2O with their mouth fluids.

EBV serology. Antibodies to virus capsid antigens (VCA) were estimated by direct immunofluorescence with P3HR1 cells (Henle & Henle, 1966). Antibodies to early antigens (EA, D + R) were estimated on Raji cells induced by TPA and BUDR by a modification of the methods of Henle et al. (1970, 1971).

BamHI W PCR. Primers used in PCR, LLW1 and LLW2 (Labrecque et al., 1995), were localized between bp 505 and 740 (Baer et al., 1995), were localized between bp 505 and 740 (Baer et al., 1995), were localized between bp 505 and 740 (Baer et al., 1995), and were localized between bp 505 and 740 (Baer et al., 1995) in the BamHI W EBV fragment. LLW1, 5′ CCGTATGTAAGCT-TGCGCTGGAG 3′, and LLW2, 5′ GCTCTAGATCGCTGCCCTTCTTG 3′ yielded a product of 236 bp.

Isolation of total chromosomal DNA. Following post-mortem examination, tissue samples were collected, and small pieces degraded in lysis buffer [10 mM-Tris·HC1 (pH 8.0), 1 mM-EDTA (pH 8.0), 5 mM-NaCl, 0.5% (w/v) SDS and 100 µg/ml Proteinase K] overnight at 37 °C. Saliva was treated in 2 x lysis buffer for 3–4 h at 37 °C. The mixture was extracted twice with phenol-chloroform and precipitated. The DNA was pelleted and resuspended in TE. Nucleic acid concentration was measured on a spectrophotometer at 260 nm.

Transfer of DNA to membranes (slot-blotting). Typically, 2 µg of DNA extracted from a tissue sample, or half the DNA from a saliva sample, was placed in 150 µl 5 x SSC and 50 µl 1 M-NaOH, and incubated for 5 min at 80 °C; 200 µl 1 M-Tris·HCl (pH 4.2) was added and the solution placed into the well of a slot-blot apparatus, containing a nylon membrane (Biodyne B, Pall) soaked in 5 x SSC, under suction. The membrane was then baked at 80 °C for 2 h.

PCR protocol. PCR was performed with 2.5 units Taq polymerase (Perkin-Elmer Cetus) in a 50 µl reaction containing 20 mM-(NH4)2SO4, 75 mM-Tris·HCl (pH 9.0), 1.5 mM-MgCl2, 0.01% (w/v) Tween 20, 100 µM of each primer and 25 mM-dNTPs. Following the addition of denatured sample DNA, the mixture was covered with two drops of sterile light mineral oil, and amplification carried out using a denaturation step of 95 °C for 1 min, and a combined annealing and extension step of 63 °C for 3 min; 35 such steps needed to be performed to detect the DNA from 10 M81 cells consistently. A final step at 63 °C for 5 min finished the program. The products were separated by electrophoresis on an agarose gel and transferred by Southern blotting to a nylon membrane (Biodyne B, Pall) after NaOH-denaturation; the membrane was then baked at 80 °C for 1 h.

Detection of inhibition of PCR in WMF samples. WMF (25 µl) was diluted with 75 µl dH2O, boiled for at least 7 min and chilled on ice. Aliquots (20 µl) were added to three PCR reaction mixes. Two mixes were ‘spiked’ with DNA extracted from 10° and 10 M81 cells, respectively. The final mix was not spiked. If inhibition of PCR was detected the WMF sample was treated with Chelex 100.

Chelex 100 (Bio-Rad) extraction of WMF. WMF (25 µl) was diluted with 75 µl sterile dH2O. Depending on the degree of inhibition detected, either 25 µl 5% (w/v) Chelex 100 in dH2O (final concn 1%, w/v), or for strongly inhibitory samples 25–200 µl 25% (w/v) Chelex 100 (final concn 5–17%, w/v) was added to the sample. The WMF–Chelex 100 mixture was incubated at 56 °C for 30 min, vortexed for 10 s, boiled for 5 min, then spun down in a microfuge for 4 min at room temperature, and the supernatant aspirated to recover DNA for PCR.

Hybridization of membranes with a radiolabelled DNA probe. Hybridization of membranes was carried out with [α-32P]dCTP-labeled EBV DNA probes made from specific BamHI fragments of the EBV genome in Bluestrip vectors (Stratagene). DNA (25 ng) was labeled using the Random Primers DNA Labelling System (Gibco BRL) following the manufacturer’s instructions: 50 µCi [α-32P]dCTP (2–3000 Ci/mmol; Amersham) was routinely used for each probe. Hybridization was performed at 68 °C overnight in hybridization buffer consisting of 5 x Denhardt’s solution, 6 x SSC, 0.1% (w/v) SDS, 1.5 mM-sodium pyrophosphate, 25 mM-NaH2PO4, and 100 µg/ml of sonicated calf thymus DNA. Three 1 h washes with 0.3 x SSC, 0.1% SDS, at 68 °C were performed, followed by two 30 min washes using 0.1 x SSC, 0.1% SDS, before the membrane was left in contact with X-ray film (Fuji Japan) in an autoradiograph cassette for 1 h to 1 week at −70 °C, before developing.

Results

Detection of EBV DNA in tissues and saliva samples from marmosets

Marmoset 269.5 was orally infected with EBV (see Methods), at 1, 9 and 21 weeks. At 20 months the serum IgG VCA titre was 80, and the EA titre was negative. DNA was extracted from a pilocarpine-induced saliva sample, and from various tissues; 2 µg was slot-blotted onto a nylon membrane, and hybridized with an EBV BamHI W specific, radiolabelled DNA probe. EBV DNA was detected in saliva, nasopharynx, tongue, liver and mesenteric lymph node, but not in DNA from kidney, submandibular gland or small intestine (Fig. 1a). Hybridization of the blot with an EBV BamHI W specific, radiolabelled DNA probe, from a recombinant using Raji cell DNA, produced similar results, although at lower intensity, probably because the genome contains
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Fig. 1. Slot-blot of DNA (2 μg) extracted from tissues from marmoset 2693, hybridized with a BamHI W specific (a) and a BamHI Ib specific (b) radiolabelled DNA probe. 1, parotid; 2, saliva; 3, mesenteric lymph node; 4, kidney; 5, nasopharynx; 6, liver; 7, submandibular gland; 8, small intestine; 9, tongue; 10, Namalwa (2 μg), positive control (1–3 copies EBV per cell); 11, Ramos (2 μg), negative control; 12, blank.

BamHI W repeats, while BamHI Ib is present as a single copy (Fig. 1b). This indicated that the M81 strain of EBV did not contain the deletion found in B95-8. This form of analysis was repeated using tissues from other infected marmosets. EBV DNA was detected most strongly in tissue from nasopharynx, parotid, tongue, mesenteric lymph node, and in saliva.

Slot-blot analysis of sequential saliva samples

Saliva samples from infected and non-infected animals were similarly analysed. EBV DNA was detected in 12 of 39 samples (31%) from 14 infected marmosets; 38% of the samples from marmosets with IgG VCA titres ≥320, and 20% of those from animals with titres of 5–160 gave positive results. EBV DNA was never detected in every sample, taken at different intervals, from a single animal. In two of eight animals with higher VCA titres, and three of six with lower titres, EBV DNA was not detected in any sample. No sample from a non-infected animal was positive.

Buccal fluids contain an inhibitor of PCR

Slot-blot analysis is considerably less sensitive than PCR (Pedneault & Katz, 1993). However PCR, using BamHI W primers, on DNA from 12 samples of saliva positive on slot-blot analysis (see above), gave negative results. Efforts to remove impurities from extracted DNA, by more stringent washing, removal of traces of phenol etc., did not overcome this problem. Diluting the sample 1:4 in dH₂O, followed by boiling for 7 min was partially successful in removing inhibition.

When the products of PCR were separated by gel electrophoresis, the primer dimers observed with control M81 DNA were often absent in reactions with DNA extracted from oral samples. As active *Taq* polymerase is required for the formation of primer dimers, their absence suggested that the integrity of the enzyme was affected. This hypothesis was confirmed by ‘spiking’ DNA extracted from oral samples with DNA from M81 culture cells. In many cases, the latter control EBV DNA then became undetectable, indicating that an inhibitor was co-purifying with the salivary DNA.

Removal of inhibitors of PCR by the chelating resin Chelex 100

Samples were tested for inhibitors by ‘spiking’ with DNA extracted from a standard number of M81 cells. Samples were diluted and boiled, and aliquots of each ‘spiked’ with DNA from 10⁶ and 10 M81 cells (see Figs 2 and 3). If the aliquot ‘spiked’ with the DNA from 10 M81 cells produced a signal of approximately the same intensity as that seen with a similar amount of M81 DNA added to dH₂O, the sample was considered not to contain inhibitors. If the signal was reduced, the sample was treated with Chelex 100 by a modification of the procedure of Walsh et al. (1991), as described. Because WMF was easier to collect and less inhibitory than saliva (nearly all saliva samples, compared to approximately 30% of WMF samples, were found to contain inhibitors), subsequent experiments used this substrate.

In some cases, the signal produced from a WMF sample ‘spiked’ with DNA from 10⁶ M81 culture cells was also markedly reduced. These samples required treatment with 25–200 μl 25% (w/v) Chelex 100 to remove inhibition. Levels of inhibitors had to be determined before treatment, as use of excess Chelex 100 could itself be inhibitory. This method allowed a semi-quantitative estimate of the amount of EBV DNA present to be made. The intensity of the signal from the ‘unspiked’ aliquot of WMF was compared with that produced by DNA from 10 M81 cells. No signal indicated that the sample was negative. Signals less intense than, roughly equal to, or stronger than that found with DNA from 10 M81 cells in dH₂O, were given values of 1, 2 or 3 respectively.

Fig. 2 shows tests for inhibition on WMF (diluted and boiled as described) from marmosets 160_; 184_; 724_; and 742_; (see below). WMF from 160_; 184_; and 724_; contains inhibitors (lanes 6–8 and 9–11), whereas WMF from 724_; and 742_; does not. It was noted that although streaks of DNA were seen in lanes 6 and 7, subsequent Southern blotting and hybridization with a radiolabelled, BamHI W specific DNA probe did not reveal positive signals in either of these lanes.
Fig. 2. Electrophoresis of amplified EBV sequences (BamHI W specific primers). Lanes 1–3, serial dilution of DNA from M81 cells (10³, 10² and 10 cells); lane 4, DNA from 10³ Ramos cells; lane 5, dH₂O control; lanes 6–8, WMF from 160roids6 spiked with the DNA from 10³ M81 cells (lane 6), with DNA from 10 M81 cells (lane 7) and with no added M81 DNA (lane 8); lanes 9–11, as lanes 6–8 except using WMF from 184oids6; lanes 12–14, as lanes 6–8 except using WMF from 724oids6; lanes 15–17, as lanes 6–8 except using WMF from 742oids6.

Fig. 3. Electrophoresis (a) and Southern blot (b) of amplified EBV sequences (BamHI W specific primers). Lanes 1–3, serial dilution of DNA from M81 cells (10³, 10² and 10 cells); lane 4, DNA from 10³ Ramos cells; lane 5, dH₂O control; lanes 6–8, WMF from 160oids6 (treated with Chelex 100) spiked with the DNA from 10³ M81 cells (lane 6), with DNA from 10 M81 cells (lane 7) and with no added M81 DNA (lane 8); lanes 9–11, as lanes 6–8 except using WMF from 184oids6 (treated with Chelex 100); lanes 12–14, as lanes 6–8 except using WMF from 724oids6 (untreated); lanes 15–17, as lanes 6–8 except using WMF from 742oids6 (untreated).

Table 1. PCR analysis of WMF samples collected at different time intervals from infected and non-infected common marmosets

<table>
<thead>
<tr>
<th>IgG VCA titre</th>
<th>No. of animals in group</th>
<th>No. of samples collected</th>
<th>No. of positive samples</th>
<th>No. of positive samples inhibitory before treatment</th>
<th>No. of negative samples inhibitory before treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>High (≥ 320)</td>
<td>14</td>
<td>105</td>
<td>91</td>
<td>36</td>
<td>4</td>
</tr>
<tr>
<td>Low-medium (&lt; 320)</td>
<td>7</td>
<td>34</td>
<td>19</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Negative (&lt; 5)</td>
<td>7</td>
<td>63</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
</tbody>
</table>

Samples from 160oids6 and 184oids6 were treated with 25 μl of 5 % (w/v) Chelex 100. The PCR products were separated, and visualized under UV light (see Fig. 3a). The signal detected from the samples 'spiked' with DNA from 10 M81 cells (lanes 7, 10, 13 and 16) is now equal to or greater than the signal detected from 10 M81 cells and dH₂O (lane 3). Therefore 160oids6, 184oids6, 724oids6 and 742oids6 were given WMF positivity values of 1, 3, -ve, and 1, respectively (lanes 8, 11, 14 and 17). The results obtained in this experiment were confirmed by transfer of the DNA to a membrane by Southern blotting, and hybridization of the membrane with a radiolabelled, BamHI W specific DNA probe (see Fig. 3b).

PCR analysis of WMF samples from EBV-infected marmosets

Using the techniques outlined above, 202 WMF samples from 21 EBV-infected, and seven non-infected marmosets were tested (see Table 1). EBV DNA was now detected in 79 % (110 out of 139) of the samples from infected marmosets, and in 87 % (91 out of 105) samples.
from animals with high VCA titres (≥320). Of the 14 animals in the latter group, every animal had EBV DNA detected in at least one sample taken at intervals during the experiment. Four had detectable levels of EBV DNA in every sample. EBV DNA was detected in 56% of WMF from marmosets with VCA titres of 5–160. One of seven animals in this group had EBV DNA in every sample, while one never produced a positive sample. No VCA negative animal produced a positive signal. Overall, 33% (67 out of 202) were found to contain PCR inhibitors. Failure to test for inhibitors would have led to 39% (43 out of 110) of the positive samples being considered negative, reducing the overall incidence of EBV DNA in WMF to 48% (67 out of 139).

These results indicate that a majority of infected animals shed virus intermittently into the oral cavity; in a minority every sample was positive. They also highlight the importance of testing samples for inhibition of PCR.

**PCR analysis of human WMF**

To determine whether the inhibition described was a property of marmoset fluids, eleven WMF samples were collected from eight human subjects (two separate samples were collected from three of the subjects). Samples from one seronegative and the remaining seven seropositive individuals were tested, as described for marmoset WMF. WMF from the seronegative individual tested negative for EBV DNA and was not inhibitory. Of the ten samples collected from seropositive individuals four were inhibitory; after Chelex 100 treatment seven scored positive and three negative for EBV DNA. A PCR test without prior testing and treatment of inhibition would therefore have recorded a lower percentage (40%) of positive samples. Analysis of the two samples collected from each of three seropositive individuals showed that both samples were positive in two cases, while only one of two samples was positive in the third case.

**Long-term EBV infection in two marmosets**

Marmosets 160 and 184, infected at 4 weeks of age with M81 EBV (i.m./i.p. route) were seropositive for a period of 9 years. Marmoset 184 had received a second EBV challenge at 20 months, and both animals had been mated and held as part of an EBV positive group, remaining healthy throughout. It had been noted, prior to this experiment, that seronegative partners housed with these animals seroconverted after a few weeks. No seroconversion was observed in control, uninfected groups, held for long periods in the same room (e.g. Wedderburn et al., 1984). Following an early IgG VCA response, 160 and 184 produced IgG EA titres, and considerably later, low titres of antibodies in the IgA class (Fig. 4a, b). Over the period of WMF sampling (3
years) all samples tested positive for EBV DNA by PCR. Because of this high level of positivity these animals were used to demonstrate ‘natural’ EBV infection.

Transmission of EBV between paired common marmosets

In their ninth year, 160₃ and 184₃ were paired with naïve animals, 724₃ and 742₅. The course of infection of the latter is shown in Figs 5(a) and 6(a). VCA titres appeared 4 and 6 weeks after pairing, respectively, and later the animals became intermittently positive for EBV DNA in WMF; 11 weeks after the initial pairing, 724₃ and 742₅ were remated with 734₃ and 752₅ (Figs 5b and 6b). Again, the naïve animals seroconverted, although only after a longer period (16 and 9 weeks, respectively), and began to shed virus. In each case, EBV DNA positivity occurred after seroconversion, but in the case of 724₃, only after the animal had induced seropositivity in her new partner, 734₃ (see Fig. 5b). Titres of antibody to VCA were originally low in naturally infected animals, but rose over the succeeding year.

Discussion

Our early attempts to analyse marmoset buccal fluids by PCR gave inconsistent results, although the presence of EBV DNA in the samples, and in the tissues of long-term infected animals could be demonstrated by slot-blot analysis.

Other authors have reported problems with PCR of clinical specimens. A potent inhibitor of Taq polymerase has been noted to co-purify with genomic DNA from blood samples (de Franchis et al., 1988). Urine samples contain PCR inhibitors (Khan et al., 1991), as do semen (Crouse et al., 1993) and faeces (van Zwet et al., 1994). Ochert et al. (1994a, b), who found similar problems to those described here with human mouth fluids, also successfully removed inhibitors by treatment with Chelex 100, and hypothesized that the inhibitory factor present might be a polysaccharide. In this study, any WMF samples found to contain traces of blood tended to have particularly high levels of inhibition. The large excess of ions in saliva is likely to be inhibitory, and these would be removed by Chelex 100. Whatever the inhibitors, a modification of the method of Walsh et al. (1991), described, allowed for their removal, and for semi-quantitative estimates of viral DNA to be made.

Failure to include suitable controls for both false positives and negatives can result in seriously inaccurate results (e.g. Noordhoek et al., 1994), and such difficulties may be suspected when widely discrepant results are reported. For example, hepatitis C virus RNA has been found in the mouth fluids of infected patients in 0% (Fried et al., 1992), 20% (Puchammer-Stöckl et al., 1994), 48% (Liou et al., 1992) and 100% (Takamatsu et al., 1990) of samples tested. False positive results were not a serious problem in our study; suitable negative controls were always included, and the sensitivity of the PCR was reduced so that the technique only detected relatively large amounts of EBV DNA. As a result, no VCA negative animal produced a PCR positive saliva.

Slot-blot analysis, using either BamHI W or BamHI Ib specific probes, revealed the presence of EBV DNA in several lymphoid tissues, and in the parotid, but not the submandibular, gland of infected marmosets. Niedobitek et al. (1994) have reported similar results for the cottontop tamarin. One unvaccinated animal, which was developing an EBV-induced lymphoma, had BamHI W transcripts in lymphoid tissues, detected by in situ hybridization. Three others, protected by a gp340 vaccine, did not. All four had small nuclear EBV-encoded RNAs (EBERs) in several lymphoid organs indicating that the vaccine, although protective, did not induce sterilizing immunity. The picture in the common marmoset resembles that in the unprotected tamarin, although the former remain healthy. EBERs can also be detected, and have been shown to be present in considerable numbers in the peripheral leukocytes of marmoset 160₃ (see above) (P.J. Farrell, personal communication).
The aim of this study was to develop a method adaptable to the very small samples available, by which EBV infection in vaccinated and non-vaccinated animals could be monitored sequentially over long periods. Collection of WMF, followed by PCR, is both minimally invasive, and more sensitive than other available methods. Previous attempts to transform cord blood lymphocytes with marmoset saliva were unsuccessful (Wedderburn et al., 1984); to obtain adequate sensitivity using this technique it is necessary to concentrate the virus in human mouth fluids by centrifugation (Yao et al., 1985). Attempts to demonstrate spontaneous transformation failed (unpublished results); only small amounts of blood were available, and since transformation depends on a two-step mechanism (Rickinson et al., 1984) and transformation of marmoset lymphocytes requires $10^3-10^4$ ID$_{50}$ of EBV (Desgranges et al., 1979, and personal communication), the virus released in cultures may be insufficient for secondary in vitro infection.

The finding that of 21 seropositive animals, five shed EBV DNA into WMF on every occasion, and 15 did so intermittently, resembles closely the results of Yao et al. (1985), who concentrated throat washings of healthy volunteers by centrifugation, before testing for EBV by transformation of cord blood lymphocytes. Of 24 subjects, six shed virus on every occasion, and 16 intermittently. These results are higher than are often reported, but our results with mouth washings from seropositive volunteers also gave a high level (70%) of positive samples.

The occurrence of natural EBV infection in the marmoset was confirmed using animals experimentally infected as neonates, which had produced EBV-positive WMF on every occasion tested over a period of 3 years. Serological evidence indicated that their virus load had progressively increased; VCA, followed by EA titres, rose, and finally IgA antibodies appeared. When these animals were mated with two naive animals, the latter seroconverted within 6 weeks; they later shed virus, although intermittently. In spite of the fact that neither antibody nor virus shedding rose so high as these indicators of infection in the original infected pair, the naturally infected animals were able to pass on infection, in one case (724G) before virus shedding could be detected. The reason for the latter observation may be that, since shedding was sporadic, its first appearance may have been missed, or possibly the method used was not sensitive enough to detect an infectious dose. Alternatively, saliva may not be the vehicle by which virus is passed. EBV has been found in the genital tract (Sixbey et al., 1986; Israe1 et al., 1991), breast milk (Junker et al., 1991) and urine (Landau et al., 1994) of human subjects.

The results presented here have allowed assessment of the efficacy of a gp340 vaccine in reducing viral shedding to be determined in marmosets experimentally infected with EBV (C. Cox and others, unpublished). They also open up the possibility of measuring the effect of vaccination on subsequent natural infection, although long-term observations would be needed because of variations in dose and the time of delivery of the virus. The data serve to emphasize the fact that the indicators of infection monitored in an experimentally EBV-infected animal may be modified by booster infections from its partner, and to strengthen the evidence that the common marmoset reacts to EBV infection in a way similar to that seen in man.

We thank J. L. Turk and A. Ochert for helpful advice and discussion, and L. Labrecque for supplying primer sequences. This work was supported by the Cancer Research Campaign of the United Kingdom and The Royal College of Surgeons of England.

References


(Received 12 October 1995; Accepted 29 January 1996)