Asymptomatic Infection of the Central Nervous System by the Macaque Immunosuppressive Type D Retrovirus, SRV-1

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SUMMARY

The aetiological agent of spontaneously occurring simian acquired immune deficiency syndrome (SAIDS) in rhesus monkeys (Macaca mulatta) at the California Primate Research Center is a type D retrovirus designated SAIDS retrovirus serotype 1 (SRV-1). SRV-1 DNA and RNA have previously been detected in the brains of rhesus monkeys with SAIDS in the absence of viral antigen or neuropathological lesions. In this study we further define the relationship between SRV-1 and the central nervous system (CNS) in rhesus monkeys by examining the CNS for infectious SRV-1, viral antigen and anti-SRV-1 antibodies. In addition, cerebrospinal fluid (CSF) was assayed for alterations in IgG and albumin levels, IgG/albumin ratios and cell count in comparison to uninfected control animals. No differences in CSF parameters were detected between infected and uninfected animals except for the presence of infectious SRV-1 which was isolated from the CSF from 13 out of 19 (68%) viraemic rhesus monkeys. The probable source of this virus was the choroid plexus, where approximately 1 in 1000 surface epithelial cells were found to contain viral antigen by immunohistochemistry. Antibodies against SRV-1 were not detected in the CSF even when present in the serum. Neither infectious virus nor viral antigen were found in the brain parenchyma of any animal examined. Thus infection of the CNS by SRV-1 appears to be subclinical without an intrathecal immune response. This may be related to the apparent restriction of productive infection in the CNS to cells of the choroid plexus.

INTRODUCTION

Central nervous system (CNS) infection by retroviruses has become a topic of great concern due to the recognition of severe neurological sequelae from infection with the human immunodeficiency virus (HIV) (Carne et al., 1985; Ho et al., 1985; Levy et al., 1985; Petito et al., 1985; Price et al., 1988; Resnick et al., 1985; Shaw et al., 1985; Vazeux et al., 1988). Encephalopathy and dementia occur frequently in association with HIV infection of the brain (Ho et al., 1987; Price et al., 1988) in children and in adults but many questions remain unanswered about the pathogenesis of this CNS infection in humans. Relevant animal models would help in understanding the pathogenesis of retrovirus CNS involvement. Lentivirus diseases of sheep and goats (Narayan & Cork, 1985) and a spongiform polioencephalomyelopathy in wild mice (Gardner, 1985) have set the precedents in nature for non-oncogenic retrovirus infections of the CNS. However, in none of these animal models is virus-induced immunosuppression a concomitant feature as seen in the human acquired immune deficiency syndrome (AIDS).

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Simian acquired immune deficiency syndrome (SAIDS) caused by type D retroviruses is a frequent, often fatal disease of macaque monkeys maintained at several National Institutes of Health regional primate centres (Daniel et al., 1984; Gardner & Marx, 1985; Henrickson et al., 1983; Marx et al., 1984, 1985; Stromberg et al., 1984). The clinical and pathological features resemble AIDS in humans (MacKenzie et al., 1986; Meyer et al., 1985; Osborn et al., 1984) although the causative retroviruses are not closely related (Bryant et al., 1985). At the California Primate Research Center (CPRC) the aetiological agent of SAIDS in rhesus monkeys (Macaca mulatta) is a type D retrovirus designated SAIDS retrovirus serotype 1 (SRV-1). This aetiology has been proven by the fulfilment of Koch’s postulates, including the induction of disease with molecularly cloned SRV-1 (Heidecker et al., 1987) and protection against SRV-1 challenge with a formalin-inactivated SRV-1 vaccine (Marx et al., 1986). Lentiviruses implicated in the immune suppression of macaques such as the simian immunodeficiency virus (SIV, formerly simian T-lymphotropic retrovirus III) (Daniel et al., 1985; Letvin et al., 1985; Murphey-Corb et al., 1986) have been excluded as aetiological agents of current SAIDS cases in rhesus macaques at the CPRC (Lerche et al., 1986, 1987; Maui et al., 1986). Neurological signs and lesions have not been associated with SAIDS caused by SIV (Ringler et al., 1988; Sharer et al., 1988). The reason for the lack of CNS sequelae in SRV-1-induced SAIDS is unknown. Previously, SRV-1 nucleic acid was demonstrated in the brains from four out of six rhesus monkeys infected with SRV-1 in the absence of detectable viral antigens or viral particles (Lackner et al., 1988). This finding suggested that the virus is latent in the CNS. In the present study we extend these observations by examining the CNS for infectious SRV-1, viral antigen and anti-SRV-1 antibody in a larger group of rhesus monkeys. We also looked for changes in the composition of the cerebrospinal fluid (CSF). Our findings indicate that SRV-1 establishes an asymptomatic infection in the CNS with latent infection of the brain parenchyma and productive infection of the choroid plexus in the absence of a detectable intrathecal immune response.

METHODS

**Animals and specimens.** Thirty-six rhesus monkeys in four groups were examined. The groups were (1) 15 SRV-1 viraemic animals with clinical signs of SAIDS (Gardner & Marx, 1985; Henrickson et al., 1984), (2) four SRV-1 viraemic, clinically normal animals (healthy carriers) (Lerche et al., 1986), (3) five clinically normal, SRV-1 antibody-positive, nonviraemic animals and (4) 12 control animals from the SAIDS-free colony at the CPRC that were seronegative for SRV-1 antibody and virus. Of the monkeys in group 1, five had been inoculated with plasma from SRV-1-infected monkeys, eight with tissue culture-grown SRV-1 and two with molecularly cloned SRV-1. Four of these rhesus monkeys had been viraemic for at least 42 months. All animals in group 2 (healthy carriers) were spontaneously occurring cases from an outdoor field cage with unknown dates of initial infection. All of these animals had been viraemic for at least 24 months since their first positive viral culture. Of the monkeys in group 3, two had been inoculated with viraemic plasma, two with tissue culture-grown virus and one with molecularly cloned SRV-1.

Serum, peripheral blood mononuclear cells (PBMC), CSF and saliva were collected while the animals were immobilized with ketamine hydrochloride (10 mg/kg intramuscularly). Saliva, CSF and PBMC were immediately cultured for SRV-1, and CSF was analysed for cells using a haemocytometer. For four animals cytospin preparations of 100 μl of CSF were also examined. Paired samples of serum and CSF were stored at −70 °C for future analysis. The choroid plexus, cerebrum (parietal cortex), cerebellum, spleen and axillary lymph node were collected at necropsy from nine viraemic animals and four control animals. Tissues were immersed in optimal cutting temperature compound (Miles Laboratories), snap-frozen in liquid nitrogen-cooled Freon and stored at −70 °C. Tissue sections were cut at 6 μm and examined by immunohistochemistry for SRV-1. In addition portions of cerebrum, cerebellum, spleen and skeletal muscle obtained at necropsy from three of these animals were cultured for SRV-1. A complete set of tissues was also collected from these animals for routine histological examination.

**Virus isolation.** Infectious SRV-1 was isolated from rhesus monkeys by cocultivation of Raji cells with PBMC (Daniel et al., 1984; Marx et al., 1985) and by inoculation of Raji cell cultures with CSF or saliva. All cultures were subcultured and examined for the presence of characteristic syncytia (Marx et al., 1985). The presence of SRV-1 was confirmed by indirect immunofluorescence using an anti-SRV-1 gp20 monoclonal antibody (MAb) (Kwang et al., 1987).
Saliva samples were obtained by saturating sterile cotton swabs with saliva from all areas of the mouth. These swabs were placed in sterile tubes containing 1 ml transport medium composed of RPMI 1640 (Gibco). Medium and saliva were expressed from the swab by rolling it along the inside of the tube. Each saliva sample was filtered through a sterile 0.45 µm filter (Gelman). Doubling dilutions of 50 µl of the filtrate in RPMI 1640 medium with 10% foetal calf serum were then inoculated onto 10^6 Raji cells.

CSF was collected from the cerebellomedullary cisterna by sterile technique. The CSF was cultured by inoculating 10^5 Raji cells with serial twofold dilutions of 50 µl of CSF in RPMI 1640 medium with 10% foetal calf serum. In three animals (20249, 21903, 21915) the second sample of CSF examined (Table 1) was centrifuged at 100 g for 10 min to remove debris and then incubated on ice with 10^5 Raji cells for 30 min. The cells were pelleted by centrifugation at 100 g for 10 min and cultured in a 1.5 ml well of a 24-well plate with RPMI 1640 medium plus 10% foetal calf serum.

Albumin and IgG levels in CSF and serum. CSF and serum IgG and albumin concentrations were determined by radial immunodiffusion using commercially prepared plates with anti-human antisera (Tago). Antibodies to human immunoglobulin and albumin cross-react with those of macaques (Maul et al., 1985; Stone, 1975). The concentrations of albumin and IgG in the serum and CSF were used to calculate the CSF IgG/albumin ratio and the albumin CSF/serum ratio to assess the permeability of the blood–brain barrier. Statistical significance of observed differences between groups was determined by analysis of variance (Daniel, 1983).

ELISA for the detection of SRV-1. Samples of serum (diluted 1:100) and CSF (undiluted) were tested for antibodies to SRV-1 by an ELISA using whole SRV-1 and peroxidase-conjugated anti-macaque immunoglobulin as previously described (Marx et al., 1985).

Immunohistochemistry. Viral antigen was detected with a MAb against the transmembrane envelope glycoprotein of SRV-1 (gp20) (Kwang et al., 1987) using an avidin–biotinylated horseradish peroxidase complex (ABC) technique (Hsu et al., 1983). Briefly, frozen sections were fixed in reagent grade acetone at room temperature for 5 min, washed in phosphate-buffered saline (PBS) for 15 min and sequentially incubated with 10% normal heat-inactivated horse serum for 30 min, 1 µg/ml of anti-SRV-1 gp20 MAb with 1% normal monkey serum for 1 h, 1% hydrogen peroxide in methanol for 20 min, a 1:100 dilution of an ABC (Vector Laboratories) for 30 min and 0.4 mg/ml 3-amino-9-ethylcarbazole containing 0.015% hydrogen peroxide in a 0.1 M-acetate buffer pH 5.2 for 15 min. All steps were done at room temperature unless otherwise specified. Between each step the slides were washed in three changes of PBS for a total of 15 min. Sections were counterstained with Mayer's haematoxylin and mounted in Aquamount (Lerner Laboratories).

Control procedures included the substitution of equivalent concentrations (1 µg/ml) of a heavy chain-matched MAb of irrelevant specificity and elimination of the primary antibody. Additional controls included infected and uninfected Raji cells and matched tissues from normal, uninfected rhesus monkeys. In order to assure reproducibility each tissue was tested three separate times.

RESULTS

Type D retrovirus isolation from CSF, PBMC and saliva

Virus isolation was performed on CSF, PBMC and saliva collected at the same time. CSF was cultured to determine whether the CNS was infected by SRV-1. Viral culture of PBMC confirmed viraemia and the number of infected cells was determined by endpoint dilution to compare viral load between animals. Saliva was cultured to determine whether salivary shedding of SRV-1 correlated with the presence of virus in PBMC or in CSF.

Virus was isolated from the CSF of 13 out of 19 (68%) rhesus monkeys that were viraemic (Table 1) including the three samples of CSF that were centrifuged prior to culture. In all cases the cell count in the non-centrifuged CSF was less than 1 cell/µl using a haemocytometer and no more than 10 cells/100 µl were seen in cytospin preparations. Of the 13 CSF samples from which SRV-1 was isolated, 11 were from group 1 (virus-positive with clinical signs of SAIDS) and two were from group 2 (virus-positive without clinical signs of SAIDS). Seven of the 11 animals in group 1 with positive CSF cultures were sampled three times over a period of up to 13 months.
Table 1. *SRV-1* isolation and antibody status in peripheral blood and cerebrospinal fluid

<table>
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<tr>
<th>Disease status</th>
<th>Animal identity no.</th>
<th>Time p.i. (months)</th>
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<th>SRV-1 antibody determined by ELISA</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PBMC†</td>
<td>CSF‡</td>
</tr>
<tr>
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<td>8016</td>
<td>3</td>
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</tr>
<tr>
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</tr>
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<td>20</td>
</tr>
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<td>1-25 × 10³</td>
<td>80</td>
<td>640</td>
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<td>40</td>
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<td>320</td>
<td>–</td>
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<td>48</td>
<td>3-2 × 10³</td>
<td></td>
<td>640</td>
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</tr>
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<td>1-25 × 10³</td>
<td>ND‡</td>
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</tr>
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<td>1-6 × 10³</td>
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<tr>
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<td>6-4 × 10³</td>
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<td>21954</td>
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<td>200</td>
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<tr>
<td>30</td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Virus-positive without clinical signs</td>
<td>17500</td>
<td>30</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>of SAIDS*</td>
<td>17569</td>
<td>2</td>
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<td>80</td>
</tr>
<tr>
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<td>2560</td>
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<td>+</td>
</tr>
<tr>
<td>Transiently viraemic without clinical</td>
<td>21315</td>
<td>33</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>signs of SAIDS</td>
<td>21913</td>
<td>24</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<tr>
<td></td>
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<td>–</td>
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<tr>
<td></td>
<td>20325</td>
<td>40</td>
<td>–</td>
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<tr>
<td></td>
<td>44</td>
<td>–</td>
<td>–</td>
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</tr>
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</table>

* All are spontaneous cases. Months p.i. is time from first positive viral culture.
† Number of *SRV-1* infected cells/10⁶ PBMC. Virus-negative (−) represents < 1 syncytium-inducing unit per 8-0 × 10⁴ PBMC.
‡ Number of syncytium-inducing units/ml of CSF or saliva.
§ Samples were centrifuged at 600 g for 10 min prior to culture. All are spontaneous cases.
|| Not determined.
Two of these seven animals were positive in the CSF all three times, four were positive twice and one was positive only once. This suggests that the presence of SRV-1 in the CSF may be persistent or intermittent. None of the five transiently viraemic animals in group 3 nor the 12 control animals had virus cultured from PBMC, CSF or saliva. No correlation was seen between months post-inoculation (p.i.) or titre of SRV-1 in the PBMC and the titre of SRV-1 in CSF or saliva. None of the animals in any group had any neurological signs or histological lesions in the CNS.

In group 1, animals with SRV-1 in the CSF also had SRV-1 in their saliva except for one animal (16416). One other animal (20132) was negative in the CSF but positive in saliva. Positive saliva cultures were obtained from 11 out of 15 (73%) animals in this group. Viral isolation from saliva in group 1 with the exceptions noted above correlated with isolation of SRV-1 from the CSF. There was no correlation between the presence of SRV-1 in the CSF and saliva in group 2. All four animals had SRV-1 cultured from saliva whereas only two out of four had SRV-1 cultured from the CSF.

**Type D retrovirus isolation from tissues**

Virus isolation was attempted on sections of brain from two animals in group 1 (20249, 21903), one animal in group 2 (19276) and two additional viraemic animals (21318, 21824) that died with SAIDS, to determine whether the brain parenchyma was productively infected with SRV-1. Cerebrum, cerebellum, spleen and skeletal muscle were collected at necropsy after the animals had been thoroughly exsanguinated. Spleen tissue served as a positive control and skeletal muscle as a negative control. Skeletal muscle has proven to be an excellent internal control in our experience, free of infectious virus or viral DNA even when adjacent tissues are positive for infectious virus and viral DNA (Bryant et al., 1986b). CSF, saliva and PBMC were also cultured. In all cases the only tissue positive for SRV-1 was the spleen. Terminal samples of PBMC and saliva were virus-positive in all five animals. The CSF was positive in 21903 and 19276 but negative in 20249. CSF samples from 21318 and 21824 were contaminated with blood and therefore were discarded. These two animals were excluded from the remainder of the study because of their contaminated CSF taps. Infectious virus could not be isolated from the brain parenchyma in viraemic animals even if the CSF was positive. Adjacent sections of cerebrum, cerebellum, spleen and axillary lymph node from 20249, 21903 and 19276 were also examined by immunohistochemistry as described below.

**Absence of antibodies to SRV-1 in the CSF**

Serum and CSF were examined by an ELISA to determine whether a humoral immune response in the blood correlated with a similar response in the CSF. Serum and CSF were collected at the same time as samples for viral cultures. None of the animals had detectable anti-SRV-1 antibodies in their CSF (Table 1), regardless of serum antibody status or the presence of virus in CSF. As measured by the ELISA, a humoral immune response directed against SRV-1 in the CSF does not occur.

**Albumin and IgG levels in CSF and serum**

Albumin and IgG concentrations and their ratios to each other in CSF and serum were determined to detect perturbations in the blood–brain barrier (Harbeck et al., 1979; Tourtellotte, 1971). The samples assayed were those tested for antibodies to SRV-1 by ELISA. The results, as determined by radial immunodiffusion, are presented in Table 2. No statistically significant differences were found between the four groups for absolute albumin and IgG levels in the CSF and serum, the CSF IgG/albumin ratio or the CSF/serum albumin ratio. These results suggest an absence of any perturbation in the blood–brain barrier.

**Immunohistochemistry for SRV-1**

Immunohistochemistry was done in an attempt to determine the source of the apparently cell-free virus in the CSF. Sections of choroid plexus, cerebrum, cerebellum, axillary lymph node and spleen were examined from nine viraemic animals and four uninfected control animals (Table
Table 2. Analysis of IgG and albumin levels and ratios in serum and CSF of macaques with and without SRV-1 infection*

<table>
<thead>
<tr>
<th>Disease status</th>
<th>CSF Serum</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>IgG (mg/dl)</td>
</tr>
<tr>
<td></td>
<td>(n = 12)</td>
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<tr>
<td>Control</td>
<td>1.56 ± 0.20</td>
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<tr>
<td>Virus-positive with clinical signs (n = 14)</td>
<td>1.48 ± 0.14</td>
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<td>Virus-positive without clinical signs (n = 3)</td>
<td>1.45 ± 0.06</td>
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<tr>
<td>Transiently viraemic without clinical signs</td>
<td>1.27 ± 0.15</td>
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* The results are expressed as the mean ± S.E.M. Observed differences were not statistically significant as determined by analysis of variance.

Table 3. Immunohistochemistry for SRV-1 transmembrane envelope glycoprotein (gp20)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Choroid plexus</th>
<th>Cerebrum</th>
<th>Cerebellum</th>
<th>Axillary lymph node</th>
<th>Spleen</th>
</tr>
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<td>Viraemic monkeys</td>
<td></td>
<td></td>
<td></td>
<td>ND†</td>
<td></td>
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<tr>
<td>8016*</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
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<td>16416</td>
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* All viraemic monkeys had SRV-1 cultured from PBMC and CSF except 8016 which was positive in the PBMC but negative in the CSF.
† ND, Not determined as tissue was not available for examination.
‡ All uninfected controls were negative for SRV-1 in PBMC and CSF.

3. Choroid plexus was chosen because it has been shown to be a target for systemic disease and a pathway to the brain (Levine, 1987). In addition, the choroid plexus has been shown to be a key site for replication of visna virus (Haase et al., 1977). Eight of the viraemic animals were from group 1 and one was from group 2. All of the viraemic animals had SRV-1 cultured from their CSF except 8016. Examination of axillary lymph node and spleen tissue provided internal positive controls. In these lymphoid tissues viral antigen was localized primarily to germinal centres (data not shown) consistent with previous findings (Lackner et al., 1988). Eight of the nine (88%) viraemic animals were positive for SRV-1 in the choroid plexus whereas no animal had detectable SRV-1 in the cerebrum or cerebellum. The incidence of SRV-1-positive cells in the choroid plexus was approximately 1 in 1000 (Fig. 1). No evidence of inflammation of the choroid plexus was found by routine histology. The cells which were positive for SRV-1 were morphologically identical to adjacent choroid plexus epithelial cells. The absence of detectable SRV-1 in the brain parenchyma correlates with the absence of detectable infectious virus in
Fig. 1. Localization by immunohistochemistry of SRV-1 in the choroid plexus of rhesus monkeys infected with SRV-1. Approximately 1 in 1000 choroid plexus epithelial cells (arrowhead) were found to be positive. Bar marker represents 50 μm.

SRV-1 in the central nervous system

SRV-1 infection of the CNS has been demonstrated in 13 out of 19 rhesus monkeys without histological or clinical evidence of neurological dysfunction. Infectious virus in the CNS was limited to the CSF and was not detected in the brain parenchyma by culture or immunohistochemistry, suggesting a restricted cellular distribution of SRV-1 in the CNS. The presence of SRV-1 in the CSF appears to be a common occurrence in viraemic rhesus monkeys. The duration of CSF viraemia could not be determined because the onset of CSF infection was unknown. However, SRV-1 was isolated from the CSF as early as 2 months p.i. and as late as 48 months p.i. in different animals. Two animals had three SRV-1-positive cultures from CSF, PBMC and saliva over a period of 13 months and four others were positive two out of three times over this same time period. This indicates that the virus may be persistent in the CSF of some animals and intermittently shed into the CSF of others.

Two related type D retroviruses, Mason–Pfizer monkey virus (MPMV), the prototype type D retrovirus (Chopra & Mason, 1970) and SAIDS-D/Washington (an isolate from the Washington Primate Research Center) (Stromberg et al., 1984), have previously been detected in the CNS (Fine et al., 1975; Tsai et al., 1987). Each of these type D retroviruses was isolated from the CSF of one animal. None of these animals were reported to have shown any neurological signs. Unlike in the present study MPMV was isolated from the brain parenchyma from eight out of nine animals and SAIDS-D/Washington was isolated from the brain of one animal. In neither report were negative tissue culture controls described to eliminate the possibility of contamination of cultured tissues by infected PBMC. Apart from possible contamination of tissues by infected PBMC or inclusion of choroid plexus in the homogenates, several other possibilities exist to explain the differences in the ability to isolate type D retroviruses from the brain. The most relevant include the use of different strains of type D retrovirus and the age group of animals infected. SRV-1, MPMV and SAIDS-D/Washington each belong to a different serogroup (Bryant et al., 1986a, b; Marx et al., 1985; P. Marx, unpublished results) and are
genetically distinct (Power et al., 1986; Sonigo et al., 1986; Thayer et al., 1987). SRV-1 and MPMV are closely related serotypes whereas SAIDS-D/Washington is a more distant relative (serotype 2) showing only about 60% similarity in predicted amino acid sequences of the external envelope protein gene. This difference in envelope genes may account for the unique ability of SAIDS-D/Washington and other type D retroviruses in serogroup 2 to induce retroperitoneal fibromatosis, an aggressive proliferation of fibrous tissue in the abdominal cavity (Giddens et al., 1985; Stromberg et al., 1984). Differences in the envelope could also account for the ability of this virus to infect the brain parenchyma. Genetic differences could also account for the ability to isolate MPMV from the brain, but in addition a different age group of animals was used. In the study of MPMV pathogenesis by Fine et al. (1975), newborn rhesus monkeys were used whereas juvenile rhesus monkeys were used in this study. The brain of newborn macaques may be more permissive to viral replication.

The isolation of SRV-1 from CSF cannot be due solely to the presence of leukocytes, since centrifugation of the CSF did not prevent virus isolation. In addition, less than 1 cell/μl of CSF was detected using a haemocytometer and no more than 10 cells/100 μl of CSF were detected in cytospin preparations. For the CSF to be virus-positive due to the cells alone, every cell would have to be infected. On the basis of the highest number of infected cells in the peripheral blood (1 in 40; Table 1) this is very unlikely.

The presence of SRV-1 in the CSF elicited no detectable antibodies, nor changes in albumin/globulin ratios. As shown by immunohistochemistry, the apparent source of the virus is relatively rare infected choroid plexus epithelial cells. Whether or not there is restriction of viral replication in other choroid plexus cells as there is for visna virus (Haase et al., 1977) awaits future studies involving combined in situ hybridization and immunohistochemistry. The lack of inflammation in the choroid plexus or any other part of the brain by routine histology, in conjunction with the morphological appearance of the labelled cells makes it very unlikely that these cells are other than choroid plexus epithelial cells. The presence of SRV-1 in such cells was not entirely unexpected since retroviral infection of the choroid plexus has been demonstrated in visna of sheep (Haase et al., 1977) and because it is a pathway into the brain (Levine, 1987). In addition, SRV-1 has a broad cellular tropism in vivo (Maul et al., 1988) and an affinity for secretory epithelium such as salivary gland acinar cells (Lackner et al., 1988; Lerche et al., 1986). Similar to the latter, choroid plexus epithelial cells are secretory cells with a high metabolic rate. We have also recently found evidence of viral infection in other secretory epithelial cells such as those of apocrine sweat glands of the skin, mammary glands and pancreatic acinar cells (A. Lackner, unpublished data).

The absence of neurological signs and lesions together with the lack of detectable antibodies or changes in CSF nucleic acid, viral antigens and viral particles were detected in lymph nodes and salivary glands of these same monkeys. This suggested that SRV-1 was latent in the brain parenchyma. Perhaps the quantity or quality of viral antigens in the brain parenchyma is such that an antiviral immune response is not initiated. The data from the current study in conjunction with our previous finding of viral latency in the brain parenchyma suggest that the pathogenesis of SRV-1 infection of the CNS may be as follows: infection with SRV-1 and viraemia, productive infection of choroid plexus epithelial cells, shedding of SRV-1 into the CSF by choroid plexus
epithelial cells without an immune response and latent infection of the brain parenchyma at a later date. Confirmation of this hypothesis will require in vivo time course studies of infection using immunohistochemistry and in situ hybridization to detect the virus at various levels of expression.

The pathogenesis of the neurological disease associated with HIV and the related lentivirus SIV in primates is largely unknown but is thought to be related to the combined effects of immunosuppression and monocyte/macrophage infection within the brain with local activation of these cells and the release of monokines (Price et al., 1988). However, SRV-1 is also immunosuppressive and infects macrophages (Legrand et al., 1985) but induces no detectable CNS abnormalities. Future studies will exploit the differences in neurovirulence between SRV-1 and SIV to understand better the pathogenesis of retrovirus-induced CNS disease. These differences may shed light on the neuropathogenesis of HIV. The presence of SRV-1 in the CSF also offers a good model for testing the efficacy of anti-retroviral drugs on virus in the CNS.

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REFERENCES


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