Cytopathologic and Neurochemical Correlates of Progression to Motor/Cognitive Impairment in SIV-Infected Rhesus Monkeys

DIANNE M. RAUSCH, PH.D., MELVYN P. HEYES, PH.D., ELISABETH A. MURRAY, PH.D., JUDIT LENDOVAY, M.D., LEROY R. SHARER, M.D., JERROLD M. WARD, D.V.M., PH.D., SABINE REHM, DR. MED. VET, DONATUS NOHR, PH.D., EBERHARD WEIHE, PH.D., AND LEE E. EIDEN, PH.D.

Abstract. Neurochemical, pathologic, virologic, and histochemical correlates of simian immunodeficiency virus (SIV)-associated central nervous system (CNS) dysfunction were assessed serially or at necropsy in rhesus monkeys that exhibited motor and cognitive deficits after SIV infection. Some infected monkeys presented with signs of acquired immunodeficiency disease (AIDS) at the time of sacrifice. Seven of eight animals exhibited motor skill impairment which was associated with elevated quinolinic acid in cerebrospinal fluid (CSF). Examination of the brains revealed diffuse increases in glial fibrillary acidic protein immunoreactivity in cerebral cortex in all animals, regardless of evidence of immunodeficiency disease. Reactive astrogliosis preceded or was coincident with the onset of neuropsychological impairments. Virus rescue from CSF of six of eight infected animals showed that one of three animals with AIDS and none of three animals without AIDS at necropsy had virus rescue-positive CSF. Multinucleated giant cells were seen in the brain of only one animal with end-stage AIDS and high systemic virus burden at death. Neither systemic nor CNS virus burden was associated with the onset of CNS dysfunction. SIV-associated motor/cognitive impairment is associated with subtle, widespread changes in CNS cytology and neurochemistry, rather than with large increases in brain virus burden or widespread virus-associated brain lesions.

Key Words: AIDS; Astrogliosis; Motor/cognitive dysfunction; Neuropathology; Quinolinic acid; SIV.

INTRODUCTION

Central nervous system (CNS) dysfunction occurs during lentivirus-induced immunodeficiency disease in humans and nonhuman primates (1–4). How these motor and cognitive impairments are linked to the array of neuropathological changes occurring in the brain and the immune system during the course of immunodeficiency disease is not yet known. The sequence in which these changes occur, however, can be determined by examination of the rhesus macaque, experimentally infected with simian immunodeficiency virus (SIV) at various stages of CNS and immune disease, and related to the severity and temporal course of observed motor and cognitive deficits. Sequencing the immunological, virological, and neuropathological changes occurring in SIV disease relative to neurocognitive impairment may provide insight into their potential causal relationship to CNS dysfunction. Whether the seemingly irreversible pathologic alterations in the brain, including neuronal loss (5–7) and white matter pallor (8), seen at autopsy in end-stage acquired immunodeficiency syndrome (AIDS) are causal or consequential to CNS dysfunction, and whether reversible cellular responses or alterations in brain neurochemistry occur prior to the onset of motor and cognitive impairments are key questions for understanding AIDS dementia complex and other manifestations of human immunodeficiency virus (HIV) infection of the CNS in humans.

Diffuse poliodystrophy (9–12) and elevation of cerebrospinal fluid (CSF) quinolinic acid (QUIN) (13, 14) are two such potentially reversible alterations that have been observed in humans and rhesus macaques infected with the primate immunodeficiency viruses HIV and SIV which could precede the onset of motor and/or cognitive impairment. In the present study, plasma and CSF levels of QUIN, brain histopathology, and intrathecal and systemic virus burden were assessed in animals with previously documented evidence of motor and cognitive impairment following inoculation with SIV (1) and sacrificed at various stages of SIV disease. It was anticipated that longitudinal analysis would provide information about the relative contributions of candidate mechanisms for CNS insult in primate immunodeficiency virus infection leading to motor and cognitive impairment.

MATERIALS AND METHODS

Subjects and Clinical Assessments

Fifteen male rhesus macaques were obtained from the Texas Primate Center at approximately 1 year of age (12.0 ± 0.8 months [mean ± SD]). The animals were screened and determined negative for SRV-1, SRV-2, SRV-5, SIV, and B-virus (Herpes virus simiae). Animals were trained on a battery of
tasks designed to assess cognitive and motor function, inoculated with the SIVsmi isolate SIVsmi/Deer, then evaluated for their performance on the same tasks for approximately 1 year after inoculation as described previously (1, 15). These included three tests for assessment of cognitive function administered on an automated apparatus: i) delayed matching-to-sample with unique stimuli on every trial, a test of visual recognition memory; ii) delayed matching-to-sample with two repeatedly used stimuli, a test of recency memory; and iii) visual discrimination learning and retention, a measure of stimulus-response association. Motor skill was assessed by determining the speed at which the animal successfully retrieved food 50% of the time from a rotating turntable. When the animals reached an established criterion, ten animals were inoculated with 10 rhesus infectious doses of SIVsmi/Deer, and five animals were sham inoculated. Weekly physical examinations of all 15 animals were performed under acepromazine/ketamine anesthesia and included palpation of all lymph nodes, spleen, liver, and abdomen, recording of temperature, heart rate and respiration, and examination of skin and mouth for abnormalities (rash, lesions, or discoloration). Blood samples were obtained for analysis of SIV p26 antigen levels using the Coulter Corp. SIV p26 ELISA assay; hematology and chemistry profiles; FACS analysis of lymphocyte subsets including CD4/CD8 ratios using a panel of monoclonal antibodies directed to specific epitopes on B cells and T cells; and virus isolation by co-cultivation of rhesus peripheral blood mononuclear cells (PBMC) with uninfected PHA-stimulated, polybrene-treated (2 μg/ml) human PBMC in RPMI/15% fetal calf serum/10% IL-2, with assay of culture supernatants for SIV p26 (ELISA, Coulter Corp.). Cerebrospinal fluid was obtained monthly by cisternal tap.

**Quinolinic Acid Levels**

Cerebrospinal fluid and plasma sampled monthly were assayed in blinded analysis for QUIN levels as previously described in detail (16). Cerebrospinal fluid or plasma (20 or 50 μl) and QUIN standards (3–150 pmol in deionized water) were diluted with deionized water containing 30 pmol of [3H]QUIN as internal standard. Samples were freeze-dried overnight and QUIN and [3H]QUIN esterified to their dihexadecylurosopropyl esters. After extraction into heptane, samples were analyzed using a Hewlett Packard magnetic sector mass spectrometer (model 5998) with QUIN- and [3H]QUIN-monitored molecular ions.

**Necropsy**

Clinical illness did not usually occur until 1–2 weeks before animals became moribund. Animals that became moribund during the study were euthanized. The remaining clinically healthy animals were sacrificed at 12 months post-inoculation. After an overdose of anesthesia with acepromazine maleate and ketamine hydrochloride, CSF was taken by cisternal tap, and approximately 60 ml of blood were removed. Both blood and CSF were used for virus rescue as described above. The animals were then perfused transcardially with 2 liters of phosphate buffered saline (PBS) containing heparin 2,500 u/liter, followed by 2 liters of 4% formaldehyde/PBS. A complete necropsy was performed, and tissues (peripheral nerve, lung, heart, esophagus, stomach, intestine, colon, kidney, liver, spleen, pancreas, skin, and lymph nodes, adrenal, thymus, tongue, tonsils, sex organs, and skeletal muscle) were stored in 10% formalin/PBS and examined by light microscopic techniques. Blocks of brain and spinal cord were removed and post-fixed in 4% formaldehyde/PBS at 4°C, then embedded in paraffin. Sections were stained with hematoxylin and eosin.

**Immunohistochemistry**

Glia fibrillary acidic protein (GFAP) immunohistochemistry was performed on deparaffinized sections of cerebrocortical tissue incubated overnight with a guinea pig polyclonal antibody (Progen, Heidelberg, FRG, 1:1,000) against GFAP at room temperature and for an additional 2 hours at 37°C. Sections were rinsed in 1% bovine serum albumin (BSA)/PBS for 30 minutes (min). Species-specific, biotinylated secondary antisera (Amersham, 1:100) were applied for 45 min at 37°C, and the sections were rinsed again for 30 min. A streptavidin-biotin horseradish peroxidase complex (Amersham, 1:200) was applied for 2 hours at 37°C. All antisera and the horseradish peroxidase complex were diluted with PBS/1% BSA. Immunoreactions were visualized with 3,3′-diaminobenzidine (DAB; Sigma; 1.25 mg/100 ml PBS, nickel-enhanced) as a bluish-black reaction product. The sections were not counterstained. Sections were analyzed and photographed on a Zeiss Axiophot photomicroscope. Specificity of immunoreactions was tested by omitting primary or secondary antisera and by substituting a non-species-specific secondary antisera which resulted in a loss of immunostaining. Simian immunodeficiency virus immunohistochemistry was performed on deparaffinized brain sections with an affinity-purified rabbit polyclonal antiserum to SIV as previously described (10).

**RESULTS**

**Clinical Findings**

Eight animals were productively infected with SIV (determined by virus rescue from rhesus PBMC). Generally, all the animals remained in good health, with food consumption, weight gain, and body temperatures within the range of the five controls (data not shown). Three of the animals had occasional episodes of fever, and two of the animals showed brief (1–2 day) periods of loss of appetite. Table 1 summarizes the clinical observations for each individual infected animal. All of the infected animals developed either intermittent or chronic lymphadenopathy. Four animals developed AIDS during the study, characterized by weight loss or opportunistic infection, and were euthanized. In each case, progression from the onset of clinical symptoms of disease to a moribund state was rapid, and the animal was euthanized within 1–2 weeks. Since the purpose of the study was to correlate CNS disease stage with stage of immune disease and neurochemical parameters, the criteria for euthanasia was set to minimize agonal changes in the brain. An animal was considered moribund if the fluid and/or food intake decreased sufficiently such that parenteral infusion of fluids was required or if opportunistic infections, including diarrhea, became clinically identifiable and failed to respond to antibiotic therapy. The remaining four infected...
animals, although clinically healthy, were sacrificed approximately 12 months after inoculation. The 1 year time point is the predicted half-life of survival for juvenile macaques infected with the B670-Delta isolate of SIV (17). Four uninfected animals were also sacrificed at the end of 1 year for controls for brain pathology.

As a measurement of systemic viral load, viral core protein was measured in weekly serum samples. All of the infected animals showed a detectable elevation of serum p26 antigen levels, of variable magnitude, within 1 month of inoculation (Fig. 1). Two animals (M014 and M023) showed persistent elevated serum antigen, and both of these animals developed AIDS during the course of the study. Four animals showed detectable serum p26 only once within 1-2 months of inoculation, then remained negative throughout the remainder of the study, and two of those (M007 and M025) also developed AIDS. The remaining two animals (M011 and M031) had an initial increase in serum p26 after inoculation, then remained negative until just prior to the termination of the study, at which time the serum antigen again became elevated. Both of these animals remained healthy during the course of the study. Two inoculated animals (M010 and M019), like the uninfected controls, were never virus rescue- or antigen-positive and were considered to be uninfected controls for the purpose of neuropathological comparisons.

CD4 and CD8 lymphocyte counts were measured monthly to obtain CD4/CD8 ratios for peripheral blood. Six of the eight infected animals showed a persistent decrease in CD4/CD8 ratio during the course of the experiment (Fig. 1; Table 1). Of these six, four animals became moribund during the study and were euthanized, and two animals remained clinically asymptomatic throughout the study. Each of these six animals had evidence of opportunistic infections upon postmortem examination, reflecting impaired immune function. By contrast, all of the control, uninfected animals maintained a CD4/CD8 ratio above 1:2 throughout the course of the study (data not shown).

Although four animals remained clinically healthy until the end of the study, pathological examination at necropsy revealed lesions characteristic of SIV (17, 18) in all of the infected animals, including primary SIV viral lesions (lung and intestines), and secondary infections (adenovirus, pneumocystis [PCP], and cytomegalovirus [CMV]) (Table 1). Diffuse lymphoid hyperplasia in various nonlymphoid tissues was evident in seven out of eight of the animals, and lymphoid follicular hyperplasia was found in four of the infected monkeys. Three of the monkeys developed lymphoma which did not involve the brain. All but two of the animals (M030 and M031) developed pneumonia of some type (CMV, SIV, bronchopneumonia, and PCP). Virus rescue from CSF taken at necropsy from six of the eight infected animals (excluding M014 and M023) was positive only from animal M025.

Rate or extent of decline in the CD4/CD8 ratio did not appear to be related to extent of cognitive or motor impairment (as measured by number of different tasks im-
TABLE 2
Neuropathology of SIV-infected Animals Identified at Necropsy

<table>
<thead>
<tr>
<th>Animal number</th>
<th>M007</th>
<th>M008</th>
<th>M011</th>
<th>M014</th>
<th>M023</th>
<th>M025</th>
<th>M030</th>
<th>M031</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuropathology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perivascular inflammation (parenchymal)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Macrophage infiltrates (parenchymal)</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lymphocytic meningitis</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Choroid plexitis</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Multinucleated giant cells</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SIV immunoreactivity (meningeal)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SIV immunoreactivity (parenchymal)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

paired), but did appear to presage the development of opportunistic infections. Animal M025 showed impairment in four measures of cognitive function (Fig. 2), and a CD4/CD8 ratio that showed a steady decline after inoculation to a value of 0.38 at the time of euthanization due to pneumonia. In contrast, animal M014, with impairment in only one task, motor skill (Fig. 2), showed a precipitous drop in CD4/CD8 ratio immediately after inoculation and a persistent decline thereafter, until death from wasting, with evidence of CMV, PCP, and adenovirus infection identified at necropsy. Similarly, the extent or duration of elevated serum p26 did not relate to the severity of CNS impairments. Animals M011, M023, and M025 had the most extensive cognitive and motor impairments, but only M023 had persistently high serum antigen levels.

Quinolinic Acid Levels

All infected animals showed a variable but significant increase in CSF QUIN following infection compared to uninfected control animals (Fig. 2). The time of the highest measured value of CSF QUIN levels significantly correlated with onset of motor skill impairment (p<0.05, r = 0.777, n = 7, Spearman Correlation, a measure of correlation computed on the ranks and average ranks [19]) in individual animals. Elevation in CSF QUIN did not accompany the onset of cognitive impairment in any of the three animals (M011, M023, M025) so impaired (Fig. 2). All of the animals also showed variable but significant elevations in plasma QUIN compared to corresponding values of the control animals. The increase in plasma QUIN generally paralleled the increase in CSF QUIN throughout the period after inoculation.

Fig. 1. Serum antigenemia levels (filled circles) and CD4/CD8 ratios (open circles) in rhesus monkeys just prior to and for approximately 12 months following inoculation. Blocks refer to testing schedule, one block represents 4 weeks of testing, with 1 week rest every two blocks. Serum antigenemia was measured weekly and the highest level for each block is indicated on the graph. The first data point of CD4/CD8 ratio indicates the value measured immediately prior to inoculation; pre-inoculation range, consisting of 2–4 measurements, is indicated by vertical line over symbol. Asterisks indicate animals that developed AIDS, became moribund, and were euthanized prior to or near the termination of the study. Note different scales indicated on ordinate of both p26 antigenemia, levels and CD4/CD8 ratios for individual animals.
TABLE 3
GFAP Immunoreactivity in Cerebral Cortex of Infected Animals

<table>
<thead>
<tr>
<th>Animal</th>
<th>Cortical area</th>
<th>Lesion</th>
<th>GFAP immunoreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>M007</td>
<td>frontal cortex</td>
<td>none</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>superior parietal cortex</td>
<td>none</td>
<td>+</td>
</tr>
<tr>
<td>M008</td>
<td>frontal cortex, corpus callosum</td>
<td>none</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>foci of leptomeningitis</td>
<td>+</td>
</tr>
<tr>
<td>M011</td>
<td>frontal cortex, corpus callosum</td>
<td>leptomeningitis</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>frontal cortex, corpus callosum</td>
<td>small perivascular infiltrate in white matter</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>macrophages in leptomeninges</td>
<td></td>
</tr>
<tr>
<td>M014</td>
<td>occipital cortex, posterior (including calcarine region)</td>
<td>none</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>frontal cortex</td>
<td>none</td>
<td>+</td>
</tr>
<tr>
<td>M023</td>
<td>frontal cortex</td>
<td>white matter pallor, extensive</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>frontal cortex</td>
<td>white matter pallor, focal hemorrhage</td>
<td>+ +</td>
</tr>
<tr>
<td>M025</td>
<td>frontal cortex, corpus callosum</td>
<td>perivascular lymphocytes in leptomeninges</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>frontal cortex</td>
<td>collection of pleomorphic lymphoid cells</td>
<td>+</td>
</tr>
<tr>
<td>M030</td>
<td>frontal cortex</td>
<td>scattered infiltrates of lymphocytes and macrophages in leptomeninges</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>frontal cortex</td>
<td>lymphocytic infiltration into leptomeninges</td>
<td>+</td>
</tr>
<tr>
<td>M031</td>
<td>frontal cortex, corpus callosum</td>
<td>none</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>frontal cortex</td>
<td>inflammatory cells in leptomeninges</td>
<td>+ +</td>
</tr>
</tbody>
</table>

All sections from the frontal cortex were taken from dorsal medial pre-motor frontal cortex, including superior frontal gyrus and cortex dorsal to the cingulate. Sections for staining were adjacent to sections stained with hematoxylin and eosin and examined for lesions. Amount of staining was determined as (−) normal background, (+) weak reactivity, and (++) strong reactivity examined by two investigators in a double blind study. Two sections of cerebral cortex taken from each of four uninfected animals were examined as controls. All of these sections were negative for GFAP with the exception of one sham-inoculated animal (M024) that showed weak reactivity in one of the two sections.

reactive astroglialosis as evidenced by increased GFAP staining. Tissue blocks that included lesions and tissue blocks in which lesions were not detected were examined for GFAP immunoreactivity. Diffuse, variable astroglialosis was observed in layers 2–6 of cerebrocortical gray matter from all the SIV-infected monkeys, both with and without AIDS at the time of sacrifice but all with neuropsychological impairments (Table 3; Fig. 3, [20]). Reactive astrogliosis was observed in sections taken from tissue blocks containing SIV-induced lesions as well as from sections without apparent lesions. However, GFAP staining was greater in areas of gray matter with lesions suggesting that diffuse poliodystrophy is widespread and not confined to regions surrounding an inflammatory locus.

DISCUSSION

The SIV-infected rhesus macaque has been used for the correlative staging of motor/cognitive impairment, immune disease and CNS neuropathology accompanying SIV infection during the 1 year period of observation after inoculation. Three of four animals showed early and pro-

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**Fig. 2.** Elevation of QUIN in the CSF (closed circles) and plasma (open circles) of infected animals relative to control values. Bars indicate onset and duration of cognitive or motor deficits (1). Significant impairments were determined by the method of tolerance limits, a statistic that is based in part on the mean and SD of the scores of the control animals, which indicate a range of scores within which a specified percentage of a statistical population can be expected to fall. Impairments here indicate animals that are outside the 95% tolerance limit, which as calculated here are well outside two SD of the mean of the control scores (1). The CSF QUIN levels were determined (nM) and expressed as the increase over the 95% tolerance limit (indicated as 0 by dashed line) of the control animals for each block over the same period of time. The mean and SD of the controls for each block ranged from 15.7 ± 2 to 28.4 ± 14. Note different scales on ordinate for different animals. The plasma QUIN levels were determined (nM) and expressed as the increase over the 95% tolerance limit (indicated as 0 by dashed line) of the control animals for each block over the same period of time. The mean and SD of plasma QUIN of the control animals for each block ranged from 503.4 ± 70.7 to 687.2 ± 173 nM. The scale for plasma QUIN is proportional to the scale for CSF QUIN, so the relative increases of both measurements over control are equivalent. Measures of QUIN at or below the td are expressed as 0. MS, motor skill; DL, discrimination learning; DR, discrimination retention; Rec, recency memory; Recog, recognition memory.

found motor/cognitive impairment, but only one of four animals with late motor impairment progressed to AIDS. Diffuse gray matter astroglisis was present in all animals with motor and/or cognitive impairments regardless of the presence or absence of signs of AIDS, and thus appeared to be an early sign of CNS involvement. In general, CSF QUIN levels increased progressively throughout the disease course, with the occurrence of the highest measured value correlating significantly with onset of motor impairment. Multinucleated giant cell formation, described previously to occur late in SIV and HIV disease (21–24), was found in the single animal that died of complications of AIDS while moribund prior to euthanasia. This animal demonstrated white matter pallor and viral antigen in the CNS, with multinucleated giant cells in spinal cord white matter. Thus, both motor and cognitive impairments occurred without multinucleated giant cell formation in all animals except for this one, implying that multinucleated cells may be correlated with but not causal for CNS dysfunction.

A number of neurotoxic factors have been implicated in HIV-associated motor/cognitive complex. These include the HIV envelope glycoprotein, gp120 (25), which shows direct neuronal cell killing in culture, altered cytokine expression as a result of immune activation in the brains of infected individuals (26), and arachidonic acid metabolites plus altered cytokines produced by activated macrophage interaction with astrocytes in culture (27). Furthermore, unidentified substances secreted from HIV-infected macrophages have been shown to have toxic effects on neurons in vitro (28, 29).

One factor potentially involved in HIV-associated dementia is quinolinic acid. Quinolinic acid, an endogenous metabolite of L-tryptophan and an excitotoxic agonist of N-methyl-D-aspartate (NMDA) receptors (30, 31), causes neuronal cell death upon acute or chronic exposure both in vivo and in vitro (28, 32, 33). N-methyl-D-aspartate receptors are found in the basal ganglia, thalamus, hippocampus, and cerebral cortex, and mediate excitatory transmission in these regions. Disruption of NMDA receptors impairs such diverse behavioral functions as motor skill, learning, and memory, depending on the brain

Fig. 3. GFAP immunoreactivity in layers 1–6 of the cerebral-cortical gray matter of a) animal M010, an uninfected control; b) animal M011, showed early onset of motor and cognitive impairment but remained healthy until sacrificed at the end of the study; c) animal M014, showed early onset of motor skill impairment, developed AIDS and was euthanized 8 months post-inoculation; d) animal M023, an animal with early onset of both motor and cognitive impairments, also developed AIDS and was euthanized at 8 months post-inoculation. GFAP was stained with polyclonal anti-GFAP antibody raised in guinea pig, and the antibody visualized using biotinylated goat anti-guinea pig IgG (Amersham).
region affected (34). HIV-infected patients show early and sustained increases in CSF QUIN levels (35). Elevated QUIN in the CSF has been shown to correlate with the severity of dementia in late stage (WR 4–6) HIV-1-infected adults (14), in HIV-infected pediatric patients (36), and in SIV-infected macaques with overt neurologic defects (13). Mild psychomotor slowing has also been reported in a subpopulation of asymptomatic HIV-infected individuals (CD4 > 400 cells/mm³) who showed significantly slower simple and choice reaction times compared to healthy HIV-negative controls (37). However, the temporal relationship between CSF QUIN levels and the onset of motor deficits has not been examined. In this study, motor skill impairment was seen well before signs of disease, and in some cases even in the absence of retrograde pathologic evidence of incipient immune disease (Table 1; Fig. 2). The correlation between motor impairment and CSF QUIN levels in these subjects led us to undertake a rigorous attempt to correlate the actual onset of motor impairment with increased CSF QUIN in this study.

Serial sampling of the CSF throughout the period after inoculation allowed determination of QUIN levels during asymptomatic and symptomatic stages of disease, and at the onset of cognitive/motor impairments. Although CSF QUIN showed a significant increase in all infected animals compared to the uninjected controls, none of the CSF QUIN levels in these animals reached the magnitude (1,000–10,000 nM) seen in late stage AIDS patients (WR 4–6 with dementia, opportunistic infections, or aseptic meningitis) (14) or in overtly neurologically impaired macaques (13). This is likely due to the fact that in our study the animals with AIDS were euthanized when their health deteriorated and could not be maintained by routine veterinary care. However, one animal in this study, animal M023, did reach a state comparable to end-stage AIDS, and this animal expired spontaneously with a CSF QUIN level of 823 nM at the time of death. The CSF QUIN levels in the remaining animals more closely paralleled the elevation seen in the HIV-infected patients with impaired reaction times during early, asymptomatic stages where levels ranged up to 300 nM (37). This suggests that elevated CSF QUIN may be an early consequence of HIV and SIV infection leading to neurological impairments. Although the specific neuronal populations susceptible to the excitotoxic effects of QUIN have not been determined in the present study, data presented here are consistent with the premise that elevated QUIN affects neurons in the basal ganglia, reflected in motor dysfunction (37, 38) or discrimination learning/retention (39).

Plasma QUIN levels were also elevated to variable levels in all of the infected animals. Systemic levels of QUIN generally increase during systemic infection (40). The variability seen in these animals could be due to changes in the number or physiology of circulating infected monocytes and the resultant altered cytokine levels, it may correlate with altered numbers of CD4-positive cells, or it may be due to other physiological responses to infection as yet undetermined. Levels of CSF QUIN also increase with inflammation within the CNS, e.g. following poliovirus infection or ischemic brain injury (41, 42). In SIV-infected monkeys and HIV-infected humans, elevated plasma QUIN may contribute to the level of QUIN in the CNS following breakdown of the blood–brain barrier. Alternatively, since both SIV and HIV infection also result in inflammation within the CNS, it is also possible that CSF QUIN originates from macrophages/microglia in inflammatory loci within the brain.

All of the infected animals showed evidence of SIV infection in the brain, but the extent of the lesions in the brain was variable and not directly proportional to the extent of CNS dysfunction. This lack of quantitative correlation between viral neuropathology and neurologic signs has also been reported for the AIDS dementia complex in man (43, 44) and progressive encephalopathy in pediatric AIDS (45). Nevertheless, two of three of the animals with cognitive impairments and none of the animals without cognitive impairments exhibited signs, albeit not widespread, of SIV encephalitis. Thus, the cognitive impairments seen in SIV-infected monkeys may be caused by secondary effects of rare regions of viral encephalitis rather than direct viral-induced physical lesions, which become detectable only late in disease. This is evidenced by the widespread gliosis, as measured by increased staining for GFAP, observed in the cerebrocortical gray matter of all the infected animals. Regions of gliosis were not limited to areas of the brain immediately adjacent to SIV-infected cells or SIV-induced lesions, but extended well beyond the lesion sites, perhaps as a result of increased production of soluble mediators of glial activation. Increased GFAP immunoreactivity was not limited to animals with overt clinical signs of disease. All of the infected, CNS-impaired animals had a variable but increased intensity of GFAP staining in the cerebrocortical gray matter compared to uninjected animals and regardless of the stage of immune disease. Astrocytosis may thus represent an early sign of CNS involvement. We have since observed at least one infected animal that showed no CNS dysfunction and no apparent increase in GFAP immunoreactivity more than 1 year following productive infection with SIV (20). A quantitative analysis of changes in levels of GFAP expression using both immunohistochemistry and in situ hybridization is now under way.

Serial sacrifice studies have reported evidence of viral-induced inflammation in the brain following SIV infection of macaques, with perivascular infiltrates of macrophages as early as 4 weeks after inoculation (10, 46). Although it is more difficult to assess early viral effects in brains of HIV-infected humans, one report of neuropathological changes in HIV-infected, non-AIDS patients
following accidental death also revealed a high incidence of perivascular macrophages and microglial proliferation (22). Early infiltration of macrophages into the CNS may be a precipitating event in the onset of CNS impairment. Quinolinic acid is produced by macrophages (47), as are the cytokines IL-1 and TNF-α (48). Astrocytes have a low capacity to synthesize QUIN (49). Quinolinic acid has direct neurotoxic effects (28, 32, 33). IL-1 induces astrocytosis (50, 51), and activated astrocytes secrete both IL-1 and TNF-α (51, 53). Altered astrocyte function may indirectly affect neuronal function as a result of altered cytokine regulation (54, 55). Macrophage infiltration into the brain may contribute to both astroglialis and increased brain QUIN concentrations, and thereby be a major contributor to early CNS impairment.

The characteristics of neurological disease progression, frequency of CNS involvement, and neuropathology in SIV-infected monkeys parallel human neuro-AIDS in many respects (8). Neurochemical, virological, and cyto logical alterations in the lentivirus-infected host brain that actually precede or accompany the neurologic manifestations of HIV/SIV-associated motor/cognitive complex can therefore be assessed in SIV-infected macaques. The results presented here clearly demonstrate a sequence of CNS pathologies associated with magnitude of CNS impairment as well as stage of immune disease. Astrocystosis appears to be an early event which may be a response to inflammation and which may subsequently contribute to altered CNS function. We hypothesize that chronic elevation in QUIN levels in brain contributes to motor impairment and multiple factors, including but not restricted to QUIN elevation, contribute to cognitive dysfunction in SIV-infected macaques. Multinucleated giant cell formation can be postulated to occur late in end-stage AIDS and may correlate with, but not cause, motor/cognitive impairment. Elucidation of this sequence of CNS events should provide a basis for understanding in full the mechanisms mediating motor and cognitive deficits associated with lentivirus infection in primates.

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