Pathogenesis of SIV Encephalitis

Selection and Replication of Neurovirulent SIV

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To investigate the viral and host factors that contribute to neurological disease, nine macaques were intravenously co-inoculated with SIV/DeltaB670, a primary isolate of SIV consisting of at least 21 different genotypes, and SIV/17E-Fr, a neurovirulent recombinant clone. CD4+ cell counts and antigenemia were measured throughout infection. The SIV env V1 region was amplified from brain and peripheral blood mononuclear cell DNA to compare the genotypes present in brain and blood. Seven of the 9 macaques (78%) developed typical SIV-associated neurological lesions classified as severe (4 macaques), moderate (2 macaques), or mild (1 macaque) with a mean time to euthanasia of 7 months. Macaques with severe neurological lesions progressed more rapidly, with a mean time to euthanasia of 3.6 months. SIV/17E-Fr was detected in brain homogenates from all four macaques with severe encephalitis, and in three of the four, SIV/17E-Fr was the only genotype identified in the central nervous system. Macaques with less severe or no neurological lesions usually had one of various genotypes of SIV/DeltaB670 in brain. A variety of genotypes of SIV/DeltaB670 and SIV/17E-Fr were detected in peripheral blood mononuclear cells throughout infection. Macaques with severe neurological lesions had the most precipitous declines in CD4+ cell counts, the highest levels of antigenemia, and the greatest expression of viral RNA and protein in the central nervous system. Macaca nemestrina were more likely to develop severe neurological lesions than M. mulatta or M. fascicularis (P = 0.048). This study demonstrated that neurovirulent strains within the virus swarm can selectively enter and become established in the central nervous system and that the neurological lesions that develop are correlated with the development of host immunosuppression. The species differences in severity of neurological lesions seen in this study suggest that host factors are also important in determining the outcome of lentiviral infection. (Am J Pathol 1997, 151:793–803)

Although a large proportion of HIV-infected individuals develop AIDS dementia,1 the viral and host factors that lead to the development of CNS disease are not well understood. Productive replication of HIV in the brain occurs mainly in cells of macrophage lineage, suggesting that the ability of the virus to replicate in monocytes/macrophages is a prerequisite for neurovirulence.2–4 Recent studies suggest that regardless of the route of transmission (mucosal, intravenous drug use, transfusion of blood products), the transmitted viruses are genotypically homogeneous5–7 and phenotypically macrophage-tropic in contrast to the lymphocyte-tropic viruses that predominate later in infection.5–11 However, infection with a macrophage-tropic strain separately is not sufficient for the development of neurological disease because many HIV-infected individuals remain free of neurological disease.

Simian immunodeficiency virus (SIV) infection of macaques provides an excellent model to examine the pathogenesis of HIV CNS disease.12,13 The pathological changes in SIV-infected macaques with encephalitis are similar to those of humans with AIDS encephalitis.10,14,15 In addition, macaques can be inoculated with well-characterized viruses or molecular clones of SIV to identify specific viral sequences that are responsible for organ-specific manifestations of disease. Further, infected animals can be euthanized at different stages of infection enabling tracking of the virus throughout the body and

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The purpose of this study was to compare viral genotypes present in neural and non-neural tissues of infected macaques to ascertain whether certain strains of the virus swarm preferentially enter the CNS (neuroinvasiveness), replicate there (neurotropism), and cause neurological lesions (neurovireulence). Nine macaques were co-inoculated with a virus mixture consisting of a biological isolate, SIV/DeltaB670, and a cloned recombinant virus, SIV/17E-Fr.\textsuperscript{16–18} The immune status of the macaques was monitored throughout infection by measuring CD4\(^+\) cells in peripheral blood. Antigen levels in plasma were determined by antigen capture enzyme-linked immunosorbent assay using antibody to detect viral p27. DNA was isolated from perfused brain and peripheral blood mononuclear cells (PBMC) of infected animals, and the SIV env V1 region was amplified and sequenced to identify the viral genotypes present. Viral genotypes present in the brain and PBMC were correlated with severity of neurological lesions and immune status of the macaques.

Measurement of CD4\(^+\) Cell Counts and Antigenemia

Peripheral blood was taken from each macaque weekly for the first month after inoculation and approximately monthly thereafter. Complete blood counts with differentials were performed and the absolute lymphocyte number determined. Absolute CD4\(^+\) cell counts were determined by multiplying the percentage of CD4\(^+\) cells by the absolute lymphocyte count. Mononuclear cells were separated on Ficoll-Hypaque discontinuous gradients and labeled with fluorochrome-conjugated monoclonal antibodies (Becton Dickinson, Bedford, MA) to identify CD4\(^+\) lymphocytes as previously described.\textsuperscript{22} Plasma p27 antigen was measured approximately monthly using an enzyme-linked immunosorbent assay kit (Coulter).

Polymerase Chain Reaction

Polymerase chain reaction (PCR) was performed on DNA isolated from PBMC and brain homogenates as previously described.\textsuperscript{16} PBMC were isolated from heparinized blood on Ficoll-Hypaque density gradients and cells were lysed in DNA extraction buffer. Brain homogenates from basal ganglia were prepared by mincing with scalpels in DNA extraction buffer. DNA from PBMC and brain was purified from lysates by solvent extraction and spooling on glass rods. One µg of DNA was amplified by nested PCR as previously described.\textsuperscript{16} The primers used for the first and second round amplification reactions amplified the V1 hypervariable region of gp120 from both SIV/DeltaB670 and SIV/17E-Fr. The first-round primers were (nucleotides 6709–6728) 5'-AGGAATGCCGACAT-TCCCCCT-3' and (nucleotides 7406–7385) 5'-TCCAT-CATCCTTGTCATGAAG-3'. The second-round primers were (nucleotides 6845–6868) 5'-CAAGTCACAGAAGG CAAATA-3' and (nucleotides 7327–7305) 5'-TAAG-CAAAGCATAACCTGGCGGT-3'.

Cloning and Sequencing

PCR amplification and direct sequencing of the V1 region was used to compare virus strains in PBMC and brain. PCR products from two to three independent amplifications with 1 µg of undiluted DNA (from PBMC or brain homogenates) were pooled and the PCR products were directly sequenced with the fmol DNA Cycle Sequencing System (Promega, Madison, WI). In various macaques with neurological lesions ranging from severe to none (N861, M692, M687, M698, and N865), the pooled PCR products were cloned into a TA cloning vector (Invitrogen, San Diego, CA). After transformation, colonies containing inserts of the appropriate size were selected and plasmid DNA was purified by alkaline lysis. Ten clones were sequenced by dideoxy sequencing with Sequenase (United States Biochemical, Arlington Heights, IL). In pre-
vious experiments, the rate of nucleotide misincorporation by Taq polymerase was determined to be similar to that reported by other laboratories.23 The predominant viral variant(s) in DNA were confirmed by serial dilution of the DNA before amplification by PCR. The PCR product obtained from the highest dilution of the DNA before amplification was directly sequenced by using 3 \mu l of the PCR product and the fmol DNA Cycle Sequencing System.

Viral envelope sequences were analyzed with a Sun sparkstation and the IG suite of programs (Intelligenetics, Mountain View, CA). Preliminary sequence alignments were performed with the IG program GENALIGN and the DOTS alignment program provided by the laboratory of J. Mullins (J. Shpaer and J. Mullins, unpublished data).

**In Situ Hybridization**

Streck-fixed (Streck Laboratory, Omaha, NE), paraffin-embedded tissue sections were deparaffinized in a graded alcohol series, and pretreated with 0.2 N HCl and 25 \mu g/ml proteinase K (Boehringer Mannheim, Indianapolis, IN). The tissues were then acetylated, dehydrated through a graded alcohol series and air-dried as previously described.20,24 Hybridization was performed using a 2.4-kb PCR product amplified from the 3' end of the \textit{env} (bases 6607-9000) of SIVmac239. The primers used to amplify this segment were 5'-AAGCTTGGATCCCTC-CAACGAGGCTTCTCAT-3' and 5'-TGAGATCCGAA-GAGAACCTGGCCTATACC-3'. The DNA was radiolabeled with \textsuperscript{35}SdCTP (Amersham, Arlington Heights, IL) by random priming using an oligolabeling kit (Pharmacia, Piscataway, NJ). Specific activities of the radiolabeled DNA probes were approximately $1 \times 10^6$ dpm/\mu g.

Radiolabeled DNA (0.2 \mu g/ml) was denatured by heating and placed over the pretreated tissues. After hybridization (16 hours at 25°C; 50% formamide), the slides were washed, dehydrated, dipped in NTB3 autoradiographic emulsion (Eastman Kodak, Rochester, NY), air-dried, and developed after 3 to 10 days of exposure in the dark. Slides were examined by light microscopy; the presence of viral RNA was indicated by silver grains over cells. Uninfected and SIV-infected CEMx174 cells and macaque tissues, and a nonspecific radiolabeled probe (usually Borna disease virus cDNA) were used as controls.

**Immunohistochemistry**

A monoclonal antibody to the transmembrane portion of SIVmac239 envelope that cross-reacts with SIV/17E-Fr and SIV/DeltaB670 (kindly provided by Dr. K. Kent) was used in immunohistochemical reactions using the ABC method of signal amplification as previously described.21 Briefly, Streck-fixed, paraffin-embedded tissue sections were deparaffinized and rehydrated, and blocking serum was applied for 30 minutes. Primary antibody was applied to the tissues for 2 hours at room temperature, the tissues were washed in phosphate-buffered saline, and secondary biotinylated antispecies antibody was applied for 1 hour. The tissues were washed again, endogenous peroxide was quenched in absolute methanol with 0.02% H\textsubscript{2}O\textsubscript{2} for 30 minutes, and ABC was added for another 30 minutes. The sections were then washed and diaminobenzidine tetrahydrochloride in citrate buffer containing 0.01% H\textsubscript{2}O\textsubscript{2} was applied to the sections for 10 minutes. The sections were washed, counterstained with hematoxylin, dehydrated, mounted, and examined. If sections were to be double-labeled by \textit{in situ} hybridization, the tissues were dehydrated but not counterstained and \textit{in situ} hybridization was performed within 1 week.

**Lectin Histochemistry**

Biotinylated \textit{Ricinus communis} agglutinin-I lectin (RCA-I, Sigma, St. Louis, MO) was used to detect cells of macrophage lineage in Streck-fixed, paraffin-embedded sections as described.25 The tissue sections were pretreated with 0.025% trypsin (Sigma), then overlaid with RCA-I diluted 1:1000 in Tris-HCl (0.50 mmol/L, pH 7.6), incubated at room temperature for 1 hour, then washed in phosphate-buffered saline, and avidin-biotin complex applied for 30 minutes. The sections were washed again, diaminobenzidine tetrahydrochloride was applied for 10 minutes, and the sections were washed, dehydrated, mounted, and examined.

**Statistical Analysis**

To describe the trajectories of CD4$^+$ cell counts and levels of antigenemia, we modeled the outcome as a quadratic function of time in months from the date of infection. To calculate the three parameters describing the best quadratic curve for each animal, we used standard regression methods and quantified the adequacy of the fitted curve by the $R^2$ (ie, the percentage of variance of the data explained by the quadratic model).26 The individual curves using the fitted models were depicted grouping the animals by severity of lesions. To determine the correlation between the CD4$^+$ cell counts per mm$^3$ and the level of antigenemia in pg/ml, we calculated the Pearson's coefficient for each of the four severity levels and we tested the statistical significance by the normal approximation using the Fisher-Z transformation.26 To assess whether animals of different species had significantly different levels of severity of neurological lesions, we compared the frequency of severe or moderate versus mild or no lesions in different species using exact methods for $2 \times 2$ tables.27

**Results**

**Development of Neurological Lesions**

The macaques were euthanized when they showed signs of AIDS or neurological disease as judged by observation of unsedated macaques and clinical examination under ketamine anesthesia. Two of the macaques (N861 and M687) developed clinical signs of neurological disease
Table 1. CNS Lesions in Macaques Inoculated with SIV/DeltaB670 and SIV/17E-Fr

<table>
<thead>
<tr>
<th>ID</th>
<th>Species</th>
<th>Days post-inoculation</th>
<th>SIV-related CNS lesions</th>
<th>Severity of CNS pathology</th>
<th>Viral variant(s) in CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>N861 M. fascicularis</td>
<td>50</td>
<td>Mononuclear perivascular cuffs with syncytia, meningitis with syncytia, myelitis</td>
<td>Severe</td>
<td>SIV/17E-Fr</td>
<td></td>
</tr>
<tr>
<td>M699 M. nemestrina</td>
<td>205</td>
<td>Mononuclear perivascular cuffs with syncytia, vasculitis, meningitis with syncytia,</td>
<td>Severe</td>
<td>SIV/17E-Fr</td>
<td></td>
</tr>
<tr>
<td>M692 M. nemestrina</td>
<td>126</td>
<td>Mononuclear perivascular cuffs, mineralization of large vessels in the basal ganglia, meningitis with syncytia,</td>
<td>Severe</td>
<td>SIV/17E-Fr</td>
<td></td>
</tr>
<tr>
<td>M687 M. nemestrina</td>
<td>53</td>
<td>Mononuclear perivascular cuffs with syncytia, multifocal glial nodules, myelitis</td>
<td>Severe</td>
<td>SIV/DeltaB670 mixed clones</td>
<td></td>
</tr>
<tr>
<td>M690 M. nemestrina</td>
<td>239</td>
<td>Mononuclear perivascular cuffs, multifocal glial nodules, meningitis with syncytia, myelitis, optic neuritis, iriditis</td>
<td>Moderate</td>
<td>SIV/DeltaB670 mixed clones</td>
<td></td>
</tr>
<tr>
<td>M698 M. nemestrina</td>
<td>454</td>
<td>Mononuclear perivascular cuffs, multifocal glial nodules, meningitis with syncytia, myelitis, ganglioneuritis</td>
<td>Moderate</td>
<td>SIV/DeltaB670-CI 12</td>
<td></td>
</tr>
<tr>
<td>N863 M. fascicularis</td>
<td>350</td>
<td>Meningitis with syncytia, occasional perivascular cuffs</td>
<td>Mild</td>
<td>SIV/DeltaB670-CI 6</td>
<td></td>
</tr>
<tr>
<td>N865 M. fascicularis</td>
<td>229</td>
<td>None</td>
<td>None</td>
<td>SIV/DeltaB670-CI 3</td>
<td></td>
</tr>
<tr>
<td>N683 M. mulatta</td>
<td>249</td>
<td>None (progressive multifocal leukoencephalopathy)</td>
<td>None</td>
<td>SIV/DeltaB670-CI 2</td>
<td></td>
</tr>
</tbody>
</table>

including depression, ataxia, and blindness. At necropsy, neurological lesions were present in seven of the nine co-inoculated animals. The mean time from inoculation to euthanasia for all animals with neurological lesions was seven months (Table 1). The four animals with the most severe neurological lesions had the shortest mean time to euthanasia (3.6 months). The three animals with mild or moderate neurological lesions had a slower progression of infection, with a mean time to euthanasia of 11.5 months. The two animals without neurological lesions were euthanized with AIDS at months 7.6 and 8.3 post-inoculation.

Lesions in the CNS consisted of perivascular cuffs of mononuclear cells and occasional multinucleated giant cells, multifocal glial nodules, isolated multinucleated giant cells scattered throughout the brain parenchyma, mineralization of large vessels in the basal ganglia, mononuclear and multinucleated cell infiltrates in the meninges, multifocal myelitis, and ganglioneuritis of the cranial and cervical dorsal root ganglia (Table 1, Figure 1). The lesions were seen in the thalamus, basal ganglia, cerebrum, midbrain, cerebellar white matter, brain stem, and spinal cord, but were most severe in basal ganglia and thalamus. Lesions were classified as mild, moderate, or severe using the following semiquantitative system. Animals with more than 30 perivascular macrophage-rich cuffs in tissue sections through the basal ganglia and thalamus and with involvement of the frontal and temporal cortex, midbrain, and brainstem were listed as having severe encephalitis (4 macaques). Animals with 10 to 30 perivascular cuffs in sections through the basal ganglia and thalamus and with involvement of the frontal and temporal cortex were listed as moderate (2 macaques), and those with less than 10 perivascular cuffs in sections through the basal ganglia and thalamus and involvement limited to these areas were described as mild (1 macaque).

Non-neural lesions consisted mainly of marked thymic atrophy and lymphoid depletion of spleen and lymph nodes (Figure 2). The lymph nodes of all nine macaques had moderate to severe lymphocyte depletion, both of follicles and paracortex, and most had multinucleated giant cells in the medulla. Secondary opportunistic infection was noted only in macaque N683, which had classical histological lesions of progressive multifocal leukoencephalopathy. Sections of brain tissue from this animal were positive for SV40 (a papovavirus related to JC virus, the cause of progressive multifocal leukoencephalopathy) large T antigen by immunohistochemical staining (data not shown).

Correlation Between Levels of Virus Gene Expression in the CNS and Severity of CNS Lesions

Abundant virus RNA and protein expression was demonstrated in brain tissue of macaques with SIV encephalitis by immunohistochemical staining using an antibody to SIV envelope gp41 and in situ hybridization (Figure 3). Macaques with the most severe lesions had the highest levels of viral protein and RNA expression, particularly in perivascular cuffs, gliotic nodules, and meninges, and the highest levels of gene expression were seen in areas with the greatest inflammation: the basal ganglia, thalamus, frontal and temporal cortex, rostral midbrain, and meninges. The majority of cells expressing viral protein had morphology consistent with macrophages or multinucleated giant cells.

Double-labeling using the lectin RCA-I to label brain macrophages was followed by in situ hybridization to identify macrophages containing viral RNA. Many large, activated macrophages containing viral RNA were identified in and around perivascular cuffs and in areas of focal gliosis (Figure 4).

Abundant viral RNA and antigen were also detected in a variety of non-neural tissues. Large numbers of macrophages and giant cells containing abundant viral RNA were identified in the parenchyma of the spleen and in the medullas of the majority of lymph nodes (Figure 5). Bone marrow also contained large numbers of cells with viral antigen and RNA.
Immune Status and Severity of CNS Lesions

The immune status of the macaques was monitored throughout infection by FACS analysis to determine the number of CD4+ cells in PBMC. In most animals the CD4+ T-cell counts fell precipitously during the first 2 weeks after inoculation and then recovered. CD4+ cell counts then either remained stable or declined more slowly. The CD4 counts after this initial drop were modeled as a function of time using a quadratic equation with $R^2$ values ranging from 20.4 to 88.7%, with a single outlier (macaque M687) of 2.5% (Table 2). The low $R^2$ value for M687 may be because this animal had an early rapid decline in CD4+ cell count that never recovered. This
macaque's infection progressed rapidly. When the graphs of the fitted curves were grouped according to severity of neurological lesions (Figure 6), the results showed that the macaques with the most severe neurological lesions had the most rapid decline in CD4 counts or very low levels overall suggesting a relationship between the development of immunosuppression and the severity of neurological lesions.

Viral Load in Peripheral Blood and Severity of CNS Lesions

Plasma viral gag antigen (p27) was measured to evaluate viral load in the peripheral blood. Data for p27 was modeled as for CD4⁺ cell counts (i.e., as a quadratic function of time) (Figure 7). R² values for goodness of fit ranged from 19.9 to 87.7%, with the same outlier (macaque M687, 3.8%). These results showed that the macaques with the most severe neurological lesions had the greatest increase in plasma antigenemia, suggesting a relationship between viral load in the peripheral blood and the severity of neurological lesions. There was a strong inverse correlation between plasma antigenemia and CD4 counts in all macaques (Table 3) with a P value of 0.070.

Selection of Viral Genotypes by the CNS

All animals were perfused before euthanasia to remove infected PBMC from the brain vasculature, thus allowing an accurate comparison of viral genotypes in brain tissue and peripheral blood mononuclear cells. Viral genotypes in the CNS were identified by sequence analysis of the V1 region of the SIV env gene after direct PCR amplification of DNA and pooling of a minimum of 2 or 3 independent amplification reactions. Sequence analysis of the V1 region was used because the V1 region is distinctive for SIV/17E-Fr and strains of SIV/DeltaB670.²³,²⁸ and thus can be used to identify the genotypes in a given tissue. Severity of neurological lesions was strongly associated with the presence of SIV/17E-Fr in brain homogenates (Table 1). In all four animals with severe neurological lesions (N861, N699, M692, and M687), SIV/17E-Fr was detected in brain homogenates, and in three of these animals SIV/17E-Fr was the only genotype detected. SIV/17E-Fr was not detected in brain homogenates from macaques with moderate, mild, or no neurological lesions. These data suggest that under these conditions of dual infection specific neurovirulent strains can selectively enter the CNS and once there will replicate and cause neurological lesions.

To compare the viral genotypes present in the brain with those in the periphery, PCR amplification was performed on DNA from paired samples of PBMC and brain homogenates from five randomly selected macaques: three of the four macaques with severe neurological lesions (N861, M692, and M687); one macaque with moderate neurological lesions (M698); and one macaque with no neurological lesions (N865). PBMC were obtained at various time points during infection and again at death. Most of the SIV/Delta B670 genotypes that were present in the inoculum virus mixture₁⁵ were detected in the PBMC at some
Table 2. Percent of Variance of CD4+ Cell Counts and p27 Levels Explained by Quadratic Models Fitted Separately for Each Animal (R²)

<table>
<thead>
<tr>
<th>Macaque</th>
<th>R² for CD4</th>
<th>R² for p27</th>
</tr>
</thead>
<tbody>
<tr>
<td>N681</td>
<td>88.7%</td>
<td>66.3%</td>
</tr>
<tr>
<td>M699</td>
<td>43.7%</td>
<td>52.0%</td>
</tr>
<tr>
<td>M692</td>
<td>20.4%</td>
<td>32.7%</td>
</tr>
<tr>
<td>M687</td>
<td>2.5%</td>
<td>3.8%</td>
</tr>
<tr>
<td>M690</td>
<td>42.1%</td>
<td>19.9%</td>
</tr>
<tr>
<td>M698</td>
<td>75.5%</td>
<td>35.2%</td>
</tr>
<tr>
<td>N863</td>
<td>80.9%</td>
<td>45.5%</td>
</tr>
<tr>
<td>N865</td>
<td>46.2%</td>
<td>87.7%</td>
</tr>
<tr>
<td>N868</td>
<td>73.7%</td>
<td>65.5%</td>
</tr>
</tbody>
</table>

Discussion

This study demonstrated that neurovirulent strains within the virus swarm selectively enter and become established in the CNS and that the neurological lesions that develop are correlated with the development of host immunosuppression. Our hypothesis is that throughout the course of infection SIV-infected activated macrophages and lymphocytes carry the virus into the CNS during routine trafficking (neuroinvasion). Viral strains are transmitted from trafficking cells to susceptible cells within the brain parenchyma (neuropathogenicity) and the virus begins to replicate. If the immune system is intact, as it is during the early stages of infection, viral replication in the CNS is quickly dampened by the immune response. However, once the host's immune system deteriorates virus replication in the CNS again ensues resulting in activation of cells and initiation of the inflammatory response with production of cytokines, influx of inflammatory cells, and alteration of the parenchymal microenvironment (neurovirulence).

This study confirmed selection of specific strains of virus at the level of the CNS. Although all 21 of the strains found in the virus inoculum were detected in PBMC at various times after inoculation, SIV/17E-Fr was the only genotype identified in the brains of all of the macaques with severe neurological lesions. Thus, SIV/17E-Fr contains the genetic determinants for neuroinvasion, neurotropism, and neurovirulence. SIV/17E-Fr is a dual-tropic molecular clone that contains the entire env, nef, and 3' long terminal repeat of a neurovirulent strain of virus, SIVmac239/17E-Br, derived by in vivo passage of SIVmac239.17,19 In vitro and in vivo studies of SIV/17E-Fr, its parental viruses, and other related recombinants have shown that the development of neurological lesions is dependent on the presence of an open nef gene and of a nucleotide change at base pair 8854 that results in a substitution of glycine for arginine at the N-terminal portion of the transmembrane glycoprotein.17,18

The mechanism for the selective replication of only certain viral genotypes in the CNS is not known. One
possibility is that virus infection may induce the expression of specific molecules on the surface of infected cells that allow them privileged access to the CNS. For example, HIV and SIV replication have been shown to activate lymphocytes and monocytes, a function that may depend in part on the Nef protein. This may result in the expression of cell adhesion molecules that selectively promote entry of infected cells to the CNS. Trafficking infected monocytes may then take up residence in the brain as perivascular macrophages and trafficking infected lymphocytes may transmit the virus to resident microglia.

That viral DNA was detected in the brain parenchyma of a macaque (N865) without neurological lesions indicates that a virus may be present in the CNS without inevitably resulting in encephalitis. This finding was unlikely to be due to a virus in contaminating peripheral blood leukocytes because the macaques were perfused with sterile saline before necropsy. It is possible that the source of the viral DNA might have been trafficking cells, although the expected paucity of these cells in nonepithelial brain makes it unlikely that they would have been sampled consistently for PCR. Another alternative is that DNA of non-neurovirulent strains was carried into the CNS in monocytes that then became perivascular macrophages. Unlike the macaques with neurological lesions, viral RNA was not detected in the CNS of the macaques without SIV-induced encephalitis. Together these data suggest that productive replication of specific strains of virus is a prerequisite for the development of neurological lesions.

In this study, there was a strong correlation between the severity of neurological lesions and the development of immunosuppression as determined by the decline in CD4+ cell counts and the concurrent rise in antigenemia. Macaques with severe neurological lesions also had the greatest viral gene expression in the CNS, possibly as a result of reduced immune surveillance in the brain. This association between immunosuppression and the development of neurological lesions is strengthened by another study in which SIV/17E-Fr separately was inoculated into macaques. These animals did not become immunosuppressed and developed only mild neurological lesions. An association between the development of clinical disease and the rapidity of decline of CD4+ T-cell counts in the peripheral blood is recognized in HIV infection. Recent studies also suggest that the rate of
neurological deterioration parallels the progression of immunosuppression.\textsuperscript{38-40}

Statistical analysis showed that the severity of neurological lesions in these co-inoculated animals correlated with the species of macaque. Pig-tailed macaques (\textit{M. nemestrina}) had a significantly higher prevalence of moderate or severe neurological lesions than rhesus (\textit{M. mulatta}) and cynomolgus (\textit{M. fascicularis}) macaques (\(P = 0.048\)). This suggests that host factors also contribute to development of neurological lesions. Development of AIDS and neurological disease has also been observed in pig-tailed macaques but not rhesus macaques after inoculation of a strain of SIVagm that was nonpathogenic in African green monkeys.\textsuperscript{41} Increased susceptibility of some species may be attributable to higher levels of expression of a cell surface molecule such as a viral coreceptor that contributes to the entry of virus-infected cells into the CNS or transmission of the virus from cell to cell in the brain. Alternately, there may be species differences in the constitution of the immune system that make pig-tailed macaques more susceptible to the development of lentivirus-induced immunosuppression thus allowing greater virus replication in the CNS. An interplay between virus strain and host factors probably determines the outcome of SIV infection. In this study one cynomolgus macaque had severe CNS lesions and two pig-tailed macaques had viruses other than SIV/17E-Fr in the CNS demonstrating that neither virus strain nor host factors separately exclusively determine the severity of CNS disease.

There may be common factors that allow SIV to cross the blood-brain barrier and the maternal-fetal barrier. In this study, SIV/DeltaB670-C12 and SIV/17E-Fr were detected only in the brains of macaques with severe or moderate neurological lesions. In a related study, SIV/DeltaB670-C12 and SIV/17E-Fr were preferentially transmitted from female macaques to their offspring across

\begin{table}
\centering
\caption{Correlation between CD4\textsuperscript{+} Cell Counts and Antigenemia}
\begin{tabular}{lcc}
\hline
CNS disease severity & N\textsuperscript{*} & Correlation \\
\hline
Severe & 21 & -0.284 \\
Moderate & 18 & -0.399 \\
Mild & 2 & -1.000 \\
None & 10 & -0.235 \\
Overall & 51 & -0.256 (\(P = 0.070\)) \\
\hline
\end{tabular}
\begin{flushleft}
N\textsuperscript{*}, number of CD4-p27 data pairs for all animals of a given severity.
\end{flushleft}
\end{table}
the placental membranes.\textsuperscript{16} In fact, SIV/17E-Fr was selectively transmitted \textit{in utero} to the infants of two of the macaques in this study (N861 and M692). Both of these dams had severe neurological lesions and SIV/17E-Fr was the only genotype identified in their brains. Recent studies of immune responses of humans during pregnancy have demonstrated down-regulation of Th1- and augmentation of Th2-mediated immune responses during pregnancy, presumably as a means of preventing rejection of the allogeneic fetus.\textsuperscript{42-44} Such alterations in the host cell-mediated immune responses might be expected to result in reduced cytotoxic T-cell activity and hence an increase in virus replication and/or spread. However, because activated T cells are more susceptible to infection with HIV, reduced T-cell activation might also restrict virus spread during pregnancy. At this time the majority of studies suggest that pregnancy has no apparent effect on the progression of HIV infection in women.\textsuperscript{45} Pregnancy may have contributed to the immunosuppression seen in these macaques. However, because all macaques were inoculated at the same stage of pregnancy, its contribution to immunosuppression should have been approximately equivalent in all animals.

This study demonstrating the selective entry and replication of specific strains of SIV in the CNS and the correlation between neurological lesions and immunosuppression has provided important information about the role that viral factors play in the development of neurological disease particularly in viral entry and establishment in the CNS. The challenge now is to dissect the interactions between these viral genes and the host cells that result in the combination of activation, inflammation, and neurodegeneration that characterizes lentiviral neurological disease.

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\section*{References}

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