Pathogenesis of Simian Immunodeficiency Virus Encephalitis: Viral Determinants of Neurovirulence

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To examine the relationship between macrophage tropism and neurovirulence, macaques were inoculated with two recombinant hybrid viruses derived from the parent viruses SIVmac239, a lymphocyte-tropic, non-neurovirulent clone, and SIV/17E-Br, a macrophage-tropic, neurovirulent virus strain. The first recombinant, SIV/17E-Cl, contained the portion of the *env* gene that encodes the surface glycoprotein and a short segment of the transmembrane glycoprotein of SIV/17E-Br in the backbone of SIVmac239. Unlike SIVmac239, SIV/17E-Cl replicated productively in macrophages, demonstrating that sequences in the surface portion of *env* determine macrophage tropism. None of five macaques inoculated with SIV/17E-Cl developed simian immunodeficiency virus (SIV) encephalitis. The second recombinant, SIV/17E-Fr, which contained the entire *env* and *nef* genes and the 3' long terminal repeat of SIV/17E-Br in the SIVmac239 backbone, was also macrophage tropic. Six of nine macaques inoculated with SIV/17E-Fr developed SIV encephalitis ranging from mild to moderate in severity, indicating a significant ($P = 0.031$) difference in the neurovirulence of the two recombinants. In both groups of macaques, CD4⁺ cell counts declined gradually during infection and there was no significant difference in the rate of the decline between the two groups of macaques. This study demonstrated that macrophage tropism alone is not sufficient for the development of neurological disease. In addition, it showed that while sequences in the surface portion of the envelope gene determine macrophage tropism, additional sequences derived from the transmembrane portion of envelope and/or *nef* confer neurovirulence.

Although a large percentage of people infected with the human immunodeficiency virus (HIV) develop neurological disease (13, 18), our understanding of the neuropathogenesis of HIV is incomplete. HIV exists in the host as a quasispecies, and strains within a single host may vary in cell tropism (6, 9). Lymphocyte-tropic strains replicate in both primary lymphocytes and lymphocytic cell lines, whereas macrophage-tropic strains replicate in both primary lymphocytes and cells of the macrophage lineage. These differences in tropism are thought to be in part responsible for the different presentations of disease. The consistent finding of viral gene products in cells of the macrophage lineage in the central nervous system (CNS) of HIV-1-infected individuals suggests that macrophage tropism plays an important role in the development of neurological disease (2, 9, 23).

Macaques inoculated with simian immunodeficiency virus (SIV) develop clinical neurological deficits and CNS lesions similar to those in patients infected with HIV-1 (16, 20). Because macaques can be inoculated with molecularly cloned, biologically characterized viruses and then examined at serial stages of disease progression, the SIV-macaque model of lentivirus encephalitis is valuable for pathogenesis studies. In a previous study, the lymphocyte-tropic, nonneurovirulent clone SIVmac239 was passaged in vivo in rhesus macaques. After three passages, the virus had shifted tropism and become macrophage tropic, but it remained nonneurovirulent. Fourth-

passage virus was, however, both macrophage tropic and neurovirulent; inoculated macaques developed typical SIV encephalitis (22). This suggested that macrophage tropism was necessary but not sufficient for neurovirulence.

To examine the relationship between macrophage tropism and neurovirulence, macaques were inoculated with two recombinant viruses derived from the parent viruses, SIVmac239, the lymphocyte-tropic nonneurovirulent clone, and SIV/17E-Br, a virus strain isolated from the brain of a macaque with neurological lesions after four in vivo passages of SIVmac239 (22). The first recombinant, SIV/17E-Cl, contained the portion of the *env* gene that encodes the surface glycoprotein and a short segment of the transmembrane glycoprotein of SIV/17E-Br in the backbone of SIVmac239. Unlike the parent clone, SIVmac239, SIV/17E-Cl replicated productively in macrophages, demonstrating that sequences in the surface portion of *env* determine macrophage tropism (1). The second recombinant, SIV/17E-Fr, contained the entire *env* and *nef* genes and the 3' long terminal repeat (LTR) of SIV/17E-Br in the SIVmac239 backbone (5). Like SIV/17E-Cl, this recombinant was macrophage tropic. Macaques were inoculated with these two macrophage-tropic recombinant clones and later examined for neurological lesions and virus gene expression in the CNS. SIV/17E-Fr caused neurological disease in six of nine inoculated macaques, whereas SIV/17E-Cl did not cause neurological disease in any of five inoculated macaques. This study demonstrated that macrophage tropism alone is not sufficient for the development of neurological disease. In addition, it showed that while sequences in the surface portion of the envelope gene determine macrophage tropism, additional se-

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TABLE 1. Inoculum doses of virus received by macaques

Macaque	Species	Dose of SIV/17E-Fr (TCID ₅₀) ^a	Dose of SIV/17E-Cl (TCID ₅₀)
H178	<i>M. mulatta</i>	1.0×10^1	0
L859	<i>M. mulatta</i>	1.0×10^{-4}	0
L839	<i>M. mulatta</i>	1.0×10^{-2}	0
M069	<i>M. mulatta</i>	1.0×10^{-3}	0
G060	<i>M. mulatta</i>	1.0×10^1	0
M819	<i>M. nemestrina</i>	1.0×10^1	0
N907	<i>M. nemestrina</i>	1.0×10^{-5}	0
N868	<i>M. mulatta</i>	1.0×10^1	0
L745	<i>M. fascicularis</i>	3.3×10^1	0
L471	<i>M. mulatta</i>	0	1.0×10^1
M693	<i>M. nemestrina</i>	0	2.0×10^{-1}
M862	<i>M. fascicularis</i>	0	5.0×10^2
M684	<i>M. nemestrina</i>	0	1.0×10^3
M458	<i>M. fascicularis</i>	0	1.0×10^3

^a TCID₅₀, 50% tissue culture infective dose.

quences derived from the transmembrane portion of envelope and/or *nef* confer neurovirulence.

MATERIALS AND METHODS

Viruses. The macrophage-tropic strain SIV/17E-Br was obtained by four serial intracerebral passages of SIVmac239 in rhesus macaques (22). The recombinant molecular clone SIV/17E-Cl was constructed by inserting the surface glycoprotein and a small portion of the transmembrane protein of SIV/17E-Br (bp 6351 to 8742) into the backbone of SIVmac239 (1). The recombinant molecular clone SIV/17E-Fr was constructed by inserting the entire envelope gene, the *nef* gene, and the 3' LTR (bp 6351 to flanking sequences at the terminus of the 3' LTR) of SIV/17E-Br into the backbone of SIVmac239 (5). Since the sequence changes present in the LTR of SIV/17E-Br overlapped with *nef*, SIV/17E-Cl and SIV/17E-Fr were congenic except for the transmembrane portion of *env* and the *nef* gene. Recombinants were determined to be macrophage tropic by being cultured in primary rhesus blood-derived macrophages (5). Stocks of SIV/17E-Cl and SIV/17E-Fr were obtained by transfection into CEMx174 and amplification in rhesus peripheral blood mononuclear cells. Viral titers in supernatants were assayed by adding serial log dilutions of cell-free supernatant to CEMx174 indicator cells and looking for syncytia.

PCR. Frozen brain tissue from basal ganglia and thalamus was thawed, minced with scalpel blades, added to STE solution (0.1 M NaCl, 0.05 M Tris [pH 8.0], 1% sodium dodecyl sulfate, 1 mM EDTA [pH 8.0]), and then treated with proteinase K (to a final concentration of 250 µg/ml) overnight at 55°C. DNA was then extracted with phenol-chloroform and ethanol precipitated. RNase was added to a final concentration of 50 µg/ml, and the mixture was incubated for 2 h at 37°C. The DNA was then reextracted and quantitated. Hot-start PCR was performed with 1 µg of DNA and 1 µl of *Taq* polymerase. Following an initial cycle of 99°C for 15 min, 65°C for 1 min, and 72°C for 1 min, *Taq* polymerase was added to the reaction mixture and 49 cycles were performed at 95°C for 40 s, 65°C for 30 s, and 72°C for 30 s. These samples were amplified in two independent reactions with different primer sets. Primers (5'-AAGCTTGGATCCCTCCAACGAGCGTCC TTCAT-3' and 5'-CACTTAAAGCAAGATCGCGATAAGCA-3') complementary to bases 6343 to 6375 and 6652 to 6626 were used to amplify a 309-bp segment from the 3' end of the *env* gene. Primers (5'-AAGCTTCTCGAGGA CATAGAATCTTAGACATAT-3' and 5'-GGAAAGTCCCTGCTGTTTCAG CG-3') complementary to bases 28 to 48 and 401 to 424 were used to amplify a 396-bp product from the U3 region of the LTR.

Animals. Macaques were inoculated intravenously via the saphenous vein with either SIV/17E-Cl or SIV/17E-Fr at the doses listed in Table 1. The species inoculated included *Macaca mulatta* (rhesus macaques), *Macaca nemestrina* (pig-tailed macaques), and *Macaca fascicularis* (cynomolgus macaques), all of which are known to be susceptible to infection with SIV and to development of SIV-induced neurological lesions. All macaques seroconverted, and lysates of their peripheral blood leukocytes were positive for SIV by PCR amplification. The animals were observed daily for signs of clinical disease and sacrificed if they manifested any signs. Macaques that did not develop signs of clinical disease were sacrificed at 9 months postinoculation or later. Peripheral blood was taken from each macaque every week for the first month after inoculation and approximately monthly thereafter. Buffy coat cells were labeled with fluochrome-conjugated monoclonal antibodies against CD4 and CD8 (OKT4 [Coulter] and Leu2a [Becton-Dickinson]) to quantitate CD4⁺ and CD8⁺ lymphocytes as previously described (12). Antigen levels in plasma were determined during the

second week after inoculation by antigen capture enzyme-linked immunosorbent assay with antibody to detect viral p27 (Coulter).

Tissues. Prior to necropsy, animals were perfused with sterile 0.9% saline to remove SIV-infected peripheral blood leukocytes from the brain and other tissues. Transverse sections of brain (0.5 cm thick) were made through the frontal, temporal, parietal, and occipital cortex, the basal ganglia, the thalamus, the midbrain, the pons, the cerebellum, the medulla, and the cervical, thoracic, and lumbar spinal cord. Neural and nonneural tissues were fixed in tissue fixative (Streck Laboratory), embedded in paraffin, and sectioned for hematoxylin-and-eosin staining, in situ hybridization, and immunohistochemical and lectin histochemical staining. Other tissue samples were snap-frozen in liquid nitrogen and stored at -70°C for DNA isolation and subsequent PCR.

In situ hybridization. Streck-fixed, paraffin-embedded tissue sections were pretreated with 0.2 N HCl followed by 25 µg of proteinase K (Boehringer Mannheim) per ml, acetylated, dehydrated, and air dried as previously described (25). In situ hybridization was performed with a 2.4-kb PCR product amplified from the 3' end of the *env* gene of SIVmac239 (the primers used for amplification from bases 6607 to 9039 were 5'-AAGCTTGGATCCCTCCAACGAGCGCTC TTCAT and 5'-TGAGGATCCGAAGAGAACTGGCCTATACCC-3'). The DNA was labeled by random priming with ³⁵S-dCTP (Amersham Corp.). The specific activity of the radiolabeled probes was approximately 10⁹ dpm/µg. Radiolabeled DNA (0.2 µg/ml) was denatured, and hybridization was performed for 16 h at 25°C. The slides were washed, dipped in NTB3 autoradiographic emulsion (Eastman Kodak), air dried, and developed after 3 to 10 days of exposure. Uninfected and SIV-infected cell cultures and tissues and a nonspecific radiolabeled probe (Borna disease virus) were used as controls.

Immunohistochemistry and lectin histochemistry. The monoclonal antibody kk41 (a gift of K. Kent) was used to detect the SIV transmembrane protein, and the lectin *Ricinus communis* agglutinin-I (RCA-I; Vector) was used to identify macrophages. Immunohistochemistry was performed by the avidin-biotin complex technique with peroxidase as the substrate for the color reaction with 3,3'-diaminobenzidine tetrahydrochloride as described previously (11). Appropriate isotype controls were used. Lectin histochemistry was performed with biotinylated lectin on tissues predigested with trypsin (0.025% in phosphate-buffered saline) at room temperature for 5 min (22).

Data analysis. Fisher's exact test was used to investigate the relationship between the virus inoculum and the development of neurological disease. This test can be viewed as a test for homogeneity of proportions, to assess whether the proportion of animals that developed neurological disease is the same in each inoculation group. Due to the exact probability calculations used in this test, the results are appropriate regardless of the sample size. To visually explore the changes in CD4⁺ cell counts postinoculation, CD4⁺ cell counts from each individual macaque were plotted across time, resulting in 14 distinct scatter plots reflecting the pattern of CD4 change for each animal under observation. Least-squares methods were then used to estimate the straight line which best fit the data for each macaque, providing a linear summary of the CD4 trajectory for each animal. Estimates of the intercept (α) and the slope (β) describing the trajectory for each animal were then determined to establish the changes in CD4⁺ cell counts across time for each individual. Since our main interest was identifying animals that exhibited a significant postinoculation decline in CD4⁺ cell counts, significance levels for the estimated slopes were calculated based on a one-sided hypothesis test of no decline in CD4⁺ cell counts ($H_0: \beta = 0$) versus the alternative of decline in these measures ($H_a: \beta < 0$).

RESULTS

Pathological changes. Six of nine macaques inoculated with SIV/17E-Fr developed encephalitis, all within 280 days postinoculation (Table 2). The remaining three macaques examined at later times did not have any lesions in the brain parenchyma. Encephalitis was characterized by multifocal perivascular infiltrates of mononuclear cells and gliotic nodules predominantly in white matter (Table 3; Fig. 1). The lesions were most severe in the basal ganglia, thalamus, and cerebral cortex (frontal, parietal, and occipital lobes). Four of the six macaques had lesions extending into the spinal cord. The perivascular infiltrates were composed chiefly of activated macrophages, as demonstrated by lectin histochemical staining with the macrophage marker RCA-I (Fig. 2). All macaques inoculated with SIV/17E-Fr had meningitis, characterized by multifocal to diffuse infiltrates of predominantly macrophages. There was no correlation between the presence or severity of neurological lesions and the dose of virus with which the macaques were inoculated. The three macaques that did not have neurological lesions included one inoculated with the highest dose of virus and one inoculated with the lowest. All nine animals had his-

TABLE 2. Lesions and virus gene expression in macaques inoculated with SIV/17E-Fr and SIV/17E-CI

Macaque and SIV strain	Days postinfection	Meningitis	Encephalitis ^a	Myelitis	Spleen (ISH) ^b	Brain	
						ISH	PCR
SIV/17E-Fr							
H178	64	+	+	—	+	+	+
L859	117	+	++	+	+	+	+
L839	272	+	+	+	+	+	+
M069	272	+	+	—	+	+	+
G060	279	+	++	+	+	—	+
M819	280	+	++	+	+	+	+
N907	301	+	—	—	+	—	—
N868	359	+	—	—	+	—	+
L745	588	+	—	—	+	—	—
SIV/17E-CI							
L471	635	+	+ ^c	—	+	—	ND ^d
M693	651	—	—	—	+	—	+
M862	679	+	—	—	+	—	+
M684	813	+	—	—	+	—	—
M458	981	+	—	—	+	—	+

^a +, mild; ++, moderate.^b ISH, in situ hybridization.^c L471 had CNS lesions characteristic of cytomegalovirus infection but not of SIV encephalitis.^d ND, not determined.

tological lesions in lymph nodes including mild to moderate follicular hyalinosis and involution and lymphoid depletion of both follicles and paracortical zones. One animal inoculated with SIV/17E-Fr (N868) developed several opportunistic infections including cytomegalovirus pneumonia and *Mycobacterium avium-intracellulare* infection of the liver and small intestine; this animal also had multicentric lymphoma.

In contrast to the macaques inoculated with SIV/17E-Fr, none of the five macaques inoculated with SIV/17E-CI developed encephalitis, a number that was significantly lower than for the animals inoculated with SIV/17E-Fr ($P = 0.031$, Fisher's exact test), although all of these animals were infected for 635 days or longer (Table 2). With the exception of one macaque (L471) which developed cytomegalovirus encephalitis, neither perivascular inflammation nor focal gliosis was detected in the brain parenchyma of these animals despite extensive evaluation of CNS tissue sections from the cerebral cortex (frontal, temporal, parietal, and occipital lobes), basal ganglia, thalamus, brain stem, cerebellum, and cervical, thoracic, and lumbar spinal cord. Four of the five macaques did have mild, multifocal meningitis. All animals showed histological evidence of lymphoid depletion, most typically follicular involution and hyalinosis in cervical, mandibular, mesenteric, axillary, and inguinal lymph nodes. These lymphoid lesions were similar in severity and distribution to those observed in macaques inoculated with SIV/17E-Fr.

Virus gene expression and PCR. Viral RNA was detected by in situ hybridization in scattered cells in the CNS of five of the six SIV/17E-Fr-inoculated macaques with encephalitis (Fig. 3). Brain homogenates from the basal ganglia and thalamus from each of these animals were positive for viral DNA by PCR with primers that amplified sequences in the envelope gene and the LTR (Table 2). None of the three SIV/17E-Fr-inoculated macaques without neurological lesions was positive for viral RNA. One animal (N868) was, however, positive by PCR. The spleen and lymph nodes from all of the macaques inoculated with SIV/17E-Fr were strongly positive for SIV RNA by in situ hybridization (Table 2).

Viral RNA was not detected in CNS sections by in situ hybridization in any of the five macaques inoculated with SIV/17E-CI. PCR amplification of DNA extracted from the basal ganglia and thalamus was performed on four of the five macaques (all except L471), and three of the four (M693, M862, and M458) were positive (Table 2). All animals inoculated with SIV/17E-CI were strongly positive for viral RNA in the spleen and in lymph nodes by in situ hybridization.

Immune status and antigenemia. The absolute CD4⁺ cell counts of macaques inoculated with either virus varied considerably. Terminal CD4⁺ cell counts of macaques inoculated with SIV/17E-Fr averaged $1,183 \pm 841$ cells/ μ l, and those of macaques inoculated with SIV/17E-CI averaged $1,079 \pm 535$ cells/ μ l, a difference that was not significant. Both animals with opportunistic infections, N868 (inoculated with SIV/17E-Fr) and L471 (inoculated with SIV/17E-CI), had the lowest absolute CD4⁺ lymphocyte counts at necropsy, 149 and 309 CD4⁺ cells/ μ l, respectively. Two of the macaques inoculated with SIV/17E-Fr which developed neurological lesions (H178 and L859) had six or fewer measurements of CD4⁺ cell counts throughout the observation period (Table 4). Therefore, the estimated changes in CD4⁺ cell counts from these animals were omitted from summarizations. Of the four remaining macaques inoculated with SIV/17E-Fr that developed neurological lesions, only macaque M819 (i.e., 25% of this group) exhibited a decreasing CD4⁺ cell count (decline of 99 CD4⁺ cells/month; $P = 0.03$). Of the three animals inoculated with SIV/17E-Fr that did not develop CNS lesions, one (N868) (33%) was estimated to have a significantly declining CD4⁺ cell count (-82 CD4⁺ cells/month; $P < 0.01$). Lastly, one (20%) of the five macaques inoculated with SIV/17E-CI, macaque L471, showed a significant decline in CD4⁺ cell counts postinoculation (-128 CD4⁺ cells/month; $P < 0.01$). There was detectable plasma antigenemia, ranging from 53 to 4416 pg/ml, during the second week after inoculation in seven of the nine macaques inoculated with SIV/17E-Fr (Table 4). Only one of the five macaques inoculated with SIV/17E-CI had detectable plasma antigenemia at the same time postinoculation.

TABLE 3. CNS lesions in macaques inoculated with SIV/17E-Fr

Macaque	CNS histopathological findings	Severity
H178	Multifocal mononuclear perivascular cuffs and glial nodules in basal ganglia, thalamus, and cerebrum; multifocal meningitis	Mild
L859	Multifocal mononuclear perivascular cuffs and glial nodules in basal ganglia, thalamus, and cerebrum; multifocal choroiditis and meningitis; myelitis of cervical spinal cord	Moderate
L839	Multifocal mononuclear perivascular cuffs in basal ganglia, thalamus, and cerebrum; multifocal meningitis; radiculoneuritis	Mild
M069	Multifocal mononuclear perivascular cuffs and gliosis; meningitis	Mild
G060	Multifocal mononuclear perivascular cuffs in basal ganglia, thalamus, and cerebrum; multifocal meningitis; ganglioneuritis and radiculoneuritis	Moderate
M819	Multifocal mononuclear perivascular cuffs with multifocal gliosis in basal ganglia, thalamus, and cerebrum; meningitis; choroiditis; radiculoneuritis	Moderate
N907	Focal meningitis	None
N868	Focal meningitis	None
L745	Multifocal meningitis	None

FIG. 1. Characteristic lesions of SIV encephalitis in macaques inoculated with SIV/17E-Fr include focal gliotic nodules (a) and perivascular mononuclear infiltrates (b). Similar lesions were not detected in macaques inoculated with SIV/17E-Cl. Magnifications, $\times 100$ (a) and $\times 200$ (b); hematoxylin-and-eosin staining.

DISCUSSION

This study, using two recombinant viruses that were congenic except for the transmembrane portions of *env* and *nef*, demonstrated that these portions contain the molecular determinants of neurovirulence. Macaques inoculated with a recombinant clone, SIV/17E-Cl, that contained the transmembrane portion of *env*, *nef*, and the 3' LTR of the nonneurovirulent virus, SIVmac239, did not develop neurological lesions during a 2-year period of infection. In contrast, six of nine macaques inoculated with SIV/17E-Fr, a recombinant clone that contained the transmembrane portion of *env*, *nef*, and the 3' LTR from a neurovirulent parent virus, SIV/17E-Br, developed neurological lesions, some as early as 2 to 4 months postinoculation. Since the surface portion of the envelope is the viral component that interacts with the CD4 molecule to initiate the process of viral entry, this suggests that neurovirulence may not be just a function of virus entry into the appropriate cell(s) in the CNS but that the terminal portions of the virus play a role in the development of neurological disease. The component of the virus which interacts with coreceptors has not yet been determined. If the transmembrane portion of the envelope is involved in the virus-receptor-coreceptor interaction, this portion of the envelope gene may contribute to neurovirulence by enabling the virus to replicate in only CNS cells that express certain coreceptors.

Of the macaques without encephalitis, viral DNA was detected in the brain parenchyma of three of four animals inoculated with SIV/17E-Cl (M693, M862, and M458) and one of three inoculated with SIV/17E-Fr (N868). This was unlikely to be due to virus in contaminating peripheral blood leukocytes, since all the animals were perfused with sterile saline prior to necropsy. It is possible that the source of the viral DNA was trafficking cells, although the expected paucity of these cells in nonencephalitic brains makes it unlikely that they would have been sampled consistently for PCR. Furthermore, viral RNA was not detected in the CNS of any of these PCR-positive macaques without encephalitis. Together, these data suggest that virus may be present in cells within the brain parenchyma without replicating significantly or resulting in neurological lesions. That viral DNA was detected in the CNS of three of the four SIV/17E-Cl-inoculated macaques tested but none of these animals had neurological lesions provides further evidence of the importance of sequences in the transmembrane portion of *env* and *nef* in the development of neurological disease.

Both recombinant clones used in this study replicate productively in primary blood-derived macrophages, a phenotype which has been shown by us and others to be encoded by the surface portion of the envelope gene (1, 5, 15). Because macaques inoculated with these two recombinant clones had different outcomes with respect to the development of neurological disease, this study showed that the viral determinants of macrophage tropism and neurovirulence are separate. Thus, although macrophage tropism appears to be necessary for neurovirulence (4, 8, 22), it is not sufficient.

The neurological lesions in the macaques inoculated with SIV/17E-Fr were typical of SIV encephalitis (10, 19, 20) but were only mild to moderate in severity. It is possible that the lesions would have been more severe had the macaques been significantly immunosuppressed. In HIV infection, clinical signs of neurological disease occur most often in immunocompromised individuals (14, 17, 18), and a recent study of macaques demonstrated a correlation between the rapidity of the decline in CD4⁺ cell counts and the severity of encephalitis (24). Although virus probably enters the CNS early during infection, an early antiviral immune response in the periphery can be transferred to the CNS via the trafficking of antigen-sensitized lymphocytes which may recognize and lyse virus-

FIG. 2. Tissue section of basal ganglia from a macaque inoculated with SIV/17E-Fr, demonstrating perivascular accumulations of activated brain macrophages as denoted by lectin histochemical staining with RCA-I. Magnification, $\times 400$; hematoxylin counterstain.

FIG. 3. Viral RNA was detected by in situ hybridization in cells in and around blood vessels (arrowheads) in macaques inoculated with SIV/17E-Fr. Magnification, $\times 200$; hematoxylin counterstain.

infected cells in the brain parenchyma, thus down regulating virus replication (7). When the systemic immune response declines in the terminal stages of infection, surveillance of the CNS may also decline, allowing for increased replication and spread of virus in the brain and subsequent development of encephalitis. With the exception of two macaques inoculated with SIV/17E-Fr (M819 and N868) and one inoculated with SIV/17E-CI (L471), none of the macaques in this study developed profound immunosuppression as indicated by declining CD4⁺ cell counts. This suggests that neither SIV/17E-CI nor SIV/17E-Fr has a strong immunosuppressive phenotype and may be one reason why none of the animals developed severe neurological lesions.

Evidence of virus replication as measured by p27 levels in plasma during the second week after inoculation was significantly greater in macaques inoculated with SIV/17E-Fr than in those inoculated with SIV/17E-CI. This suggests that SIV/17E-Fr is able to establish a higher virus load, at least early in infection. An increased virus load in the periphery may result

in higher virus load in the CNS and thus may contribute to the development of neurological lesions. However, virus was detected in the peripheral blood mononuclear cells of all macaques throughout infection, indicating that both virus strains had access to the CNS via trafficking cells. Furthermore, viral RNA levels in both the lymph nodes and spleen were similar in the two groups of animals at necropsy. These data suggest that the relative ability of a virus to replicate in the periphery may contribute to but is not the sole determinant of neurovirulence.

Meningitis was detected in SIV/17E-CI-inoculated and SIV/17E-Fr-inoculated macaques that did not have encephalitis. The cerebrospinal fluid (CSF), which percolates through the meninges, is partly partitioned from the CNS parenchyma because it is produced not only by drainage of interstitial fluid from the brain parenchyma via the perivascular spaces but also by filtration of plasma through the choroid plexus. The CSF itself does not percolate through the brain parenchyma but is partly formed by fluid from the parenchyma (3, 21). This partitioning of the brain and CSF has been confirmed by studies demonstrating that virus strains in the CSF and brain parenchyma of HIV-1-infected individuals may differ not only in their genotypes but also even in cell tropism (9). The virus strains present in the CSF frequently reflect those that predominate in the blood. Therefore, lesions in the meninges may occur as a result of replication of viruses such as SIV/17E-CI that are nonneurovirulent (i.e., that do not produce lesions in the brain parenchyma).

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TABLE 4. Changes in CD4⁺ cell counts

Macaque and SIV strain	No. of observations	Estimated initial CD4 ⁺ cell count (cells/ μ l)	Estimated change in CD4 ⁺ cell count (cells/ μ l)/mo	p27 Antigenemia at week 2 postinfection (pg/ml)
SIV/17E-Fr				
H178	4	837	–75	2,804
L859	6	1,888	–139	0
L839	8	1,972	+66	170
M069	8	2,043	+76	352
G060	8	866	+7	1,672
M819	9	1,345	–99 ^a	4,416
N907	9	1,510	–53	0
N868	9	1,092	–82 ^a	3,720
L745	21	1,197	+14	53
SIV/17E-CI				
L471	23	3,166	–128 ^a	0
M693	11	1,306	+24	0
M862	11	1,151	–6	151
M684	19	1,022	+1	0
M458	17	906	+3	0

^a Indicates data with $P < 0.05$. H_{0a} change = 0; H_{1a} change < 0.

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