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Lymphocyte-Dominant Encephalitis and Meningitis in Simian Immunodeficiency Virus—Infected Macaques Receiving Antiretroviral Therapy

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A retrospective neuropathologic review of 30 SIV-infected pigtailed macaques receiving combination antiretroviral therapy (cART) was conducted. Seventeen animals with lymphocyte-dominant inflammation in the brain and/or meninges that clearly was morphologically distinct from prototypic SIV encephalitis and human immunodeficiency virus encephalitis were identified. Central nervous system (CNS) infiltrates in cART-treated macaques primarily comprised $CD20^+$ B cells and $CD3^+$ T cells with fewer $CD68^+$ macrophages. Inflammation was associated with low levels of SIV RNA in the brain as shown by *in situ* hybridization, and generally was observed in animals with episodes of cerebrospinal fluid (CSF) viral rebound or sustained plasma and CSF viremia during treatment. Although the lymphocytic CNS inflammation in these macaques shared morphologic characteristics with uncommon immune-mediated neurologic disorders reported in treated HIV patients, including CNS immune reconstitution inflammatory syndrome and neurosymptomatic CSF escape, the high prevalence of CNS lesions in macaques suggests that persistent adaptive immune responses in the CNS also may develop in neuroasymptomatic or mildly impaired HIV patients yet remain unrecognized given the lack of access to CNS tissue for histopathologic evaluation. Continued investigation into the mechanisms and outcomes of CNS inflammation in cART-treated, SIV-infected macaques will advance our understanding of the consequences of residual CNS HIV replication in patients on cART, including the possible contribution of adaptive immune responses to HIV-associated neurocognitive disorders. *(Am J Pathol 2018, 188: 125—134; https://doi.org/10.1016/j.ajpath.2017.08.035)*

HIV and SIV enter the central nervous system (CNS) early in the course of infection and replicate in resident brain macrophages including microglia.[1—3] In the decades preceding effective combination antiretroviral therapy (cART), high levels of uncontrolled CNS viral replication frequently led to the development of fulminant HIV encephalitis and the most severe form of clinical HIV-associated neurologic disease (HAND), HIV-associated dementia.[4—7] Although widespread access to cART has improved the life expectancy drastically for individuals living with HIV and reduced the incidence of serious complications involving the CNS, the more subtle insidious forms of HAND, known as asymptomatic neurocognitive impairment and mild neurocognitive disorder, continue to occur in up to 50% of cART-treated patients.[8,9] The pathogenesis of asymptomatic neurocognitive impairment and mild neurocognitive disorder in virologically suppressed patients (<50 copies HIV/mL in plasma) is incompletely understood. Based on contemporary clinical and autopsy studies of HAND patients, it appears that high levels of CNS viral replication and associated prototypic HIV encephalitis are no longer likely the major

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neuropathologic processes driving cognitive dysfunction in patients on effective cART regimens.[10] Rather, numerous recent studies have shown compelling relationships between cognitive impairment and biomarkers of chronic sustained immune activation in both plasma and CSF,[11–13] as well as loss of bioenergetic homeostasis in the CNS,[14] glutamate-mediated excitotoxicity,[15,16] and comorbid conditions such as cardiovascular disease.[17]

In addition to mild forms of HAND, HIV patients receiving antiretroviral therapy also are at risk for developing a spectrum of immune-mediated conditions associated with more severe neurologic symptoms. One of the most dangerous and potentially debilitating of these disorders is CNS immune reconstitution inflammatory syndrome (IRIS). CNS IRIS typically occurs in the first weeks to months after cART initiation and is defined by paradoxical worsening of the neurologic condition caused by rapid re-establishment of a robust immune response.[18,19] Classically, this immune response is directed against a pre-existing CNS opportunistic infection, such as *Mycobacterium tuberculosis* or *Cryptococcus neoformans*.[19–22] However, several clinical reports have shown that CNS IRIS also can occur in the absence of opportunistic infection, in which HIV itself, persistent release of viral proteins, or self-antigens, may drive immune responses.[19,20,23,24] Two other neurologic conditions reported in treated HIV patients, neurosymptomatic cerebrospinal fluid (CSF) escape and $CD8^+$ encephalitis, likely exist on a continuum with CNS IRIS and share a number of pathophysiologic features including immune dysregulation and an influx of activated lymphocytes into the CNS, resulting in a primarily lymphocytic encephalitis.[25–30] Although reports of these acutely symptomatic neurologic conditions are uncommon, some studies have suggested that similar CNS immune dysregulation may occur at a subclinical level in treated HIV patients, potentially contributing to asymptomatic neurocognitive impairment and mild neurocognitive disorder.[29,30]

SIV-infected macaques have long served as the closest animal model of HIV-induced CNS damage. In this report, we describe neuropathologic findings in a retrospective cohort of cART-treated, SIV-infected, pigtailed macaques that, similar to many HIV patients, had varying degrees of viral suppression in the plasma and/or CSF. In line with previous studies of untreated SIV-infected macaques,[3,31–33] histomorphologic evaluation of all major brain regions characterizing the composition of cellular infiltrates using immunohistochemistry was performed, and associations between the severity of CNS inflammation and SIV viral loads in the plasma, CSF, and brain tissue at terminal time points was investigated. Because the CNS lesions found in cART-treated macaques shared morphologic similarities to the lymphocyte-dominant encephalitides reported in HIV patients with immune-mediated neurologic disorders, potential associations with known risk factors for these conditions including concurrent opportunistic infections, low CD4 nadir, and CSF viral escape also was evaluated.

Materials and Methods

Animal Studies

This study included 30 pigtailed macaques from seven different antiretroviral treatment studies designed to evaluate viral dynamics and host immune responses during and upon release from cART, as well as the efficacy of pharmacologic strategies aimed at promoting viral reactivation and eradication. For all studies, juvenile male pigtailed macaques (*Macaca nemestrina*) were inoculated intravenously with both the neurovirulent clone SIV/17E-Fr and the immunosuppressive swarm DeltaB670. Antiretroviral treatments were initiated at 12 days after inoculation. $CD4^+$ T-cell counts were monitored throughout infection by measuring the percentage of $CD3^+$ $CD4^+$ lymphocytes by flow cytometry and total lymphocyte numbers by complete blood count analysis (IDEXX Laboratories, Westbrook, ME). SIV viral load (VL) in the plasma and CSF were measured at serial time points by quantitative RT-PCR (RT-qPCR) targeting the SIV *gag* gene as previously described.[32,33] Macaques were euthanized between 110 and 630 days after inoculation and whole-body saline-perfused at necropsy. Specific details regarding treatment regimens and study length are provided in Supplemental Table S1. All animal studies were approved by the Johns Hopkins University Institutional Animal Care and Use Committee.

Serologic Testing

A serology panel designed to detect antibodies for several common viruses of nonhuman primates was performed on all study animals (Intuitive Biosciences LLC, Middleton, WI). Included in the panel were simian retrovirus, simian T-lymphotropic virus, SIV, Macacine herpesvirus 1, measles, cytomegalovirus, simian foamy virus, rhesus rhadinovirus, and macaque lymphocryptovirus, a close relative of human Epstein-Barr virus. All animals were negative for antibodies to SIV, simian retrovirus 2 and 5, simian T-lymphotropic virus, and Macacine herpesvirus 1 before SIV inoculation as a requirement for study inclusion.

Histopathology of the CNS

Hematoxylin and eosin–stained sections from all cerebral cortices, the basal ganglia, thalamus, midbrain, pons, and cerebellum were examined independently by two pathologists (L.M.M. and S.E.B.) who were blinded to the animal identification and experimental conditions. Animals were divided into three categories based on the presence and severity of inflammatory infiltrates in the brain parenchyma and/or meninges, as follows: i) none = rare (<3 total inflammatory foci among all examined brain sections) or no detectable lesions in brain or meninges (as typically seen in uninfected macaques); ii) encephalitis = moderate to severe lymphocyte-dominant inflammation (≥3 inflammatory foci) in the brain parenchyma with or without concurrent meningitis; and iii)

Table 1 Primary Antibodies and Epitope Retrieval Methods Used for Immunohistochemistry

Antibody	Type	Company	Clone/product	Dilution	Epitope retrieval
CD20	Mouse monoclonal	Dako (Santa Clara, CA)	L26	1:1000	HIER1 20 minutes
CD3	Rabbit polyclonal	Dako	A0452	1:400	HIER1 20 minutes
CD68	Mouse monoclonal	Dako	KP1	1:4000	HIER1 20 minutes
EBNA2	Mouse monoclonal	Abcam (Cambridge, UK)	PE2	1:2000	HIER1 20 minutes
CD4	Mouse monoclonal	GeneTex (Irvine, CA)	IF6	1:40	HIER2 30 minutes
CD8A	Rabbit polyclonal	Sigma (St. Louis, MO)	HPA037756	1:500	HIER1 30 minutes

EBNA2, Epstein-Barr virus nuclear antigen 2; HIER1, heat-induced epitope retrieval using solution 1; HIER2, heat-induced epitope retrieval using solution 2.

meningitis = moderate to severe meningitis (≥3 inflammatory foci) with rare or no concurrent lesions in the brain parenchyma. For all animals with CNS inflammation, Gram and periodic acid-Schiff (PAS) stains were performed to rule out bacterial, fungal, or parasitic infection.

Immunohistochemistry

For all animals with lymphocyte-rich infiltrates in the CNS, immunostaining for CD3, CD20, CD68, and Epstein-Barr virus nuclear antigen 2 was performed using a Leica Bond RX automated system and polymer-based immunohistochemistry reagents (Leica Biosystems, Nussloch, Germany). A slide from an SIV-infected animal with known, herpes-associated (lymphocryptovirus) CNS B-cell lymphoma was used as a positive control for Epstein-Barr virus nuclear antigen 2. Staining for specific T-cell markers (CD4 and CD8) was performed in a subset of animals. Details regarding the primary antibodies and epitope retrieval protocols are listed in Table 1.

Detection of SIV RNA in Brain Tissue

RT-qPCR of brain tissue to measure levels of SIV RNA and DNA was performed as previously described.[33] SIV RNA was measured in two different areas in the brain (basal ganglia and parietal cortex) and averaged, whereas viral DNA was evaluated in the basal ganglia. *In situ* hybridization (ISH) was performed on the Leica Bond RX automated system using the RNAscope 2.5 LS Assay-Red (Advanced Cell Diagnostics, Newark, CA). SIV RNA was detected using probes against both SIVdeltaB670 (450758; Advanced Cell Diagnostics) and SIVmac239 (416138; Advanced Cell Diagnostics). A negative control probe (dapB, 312038; Advanced Cell Diagnostics) and positive control probe (PolR2A, 310458; Advanced Cell Diagnostics) was run for each animal. The protocol included heat-induced epitope retrieval (95°C for 15 minutes) and protease digestion for 15 minutes.

Results

CNS Inflammation in cART-Treated Macaques Is Morphologically Distinct from SIV Encephalitis

Seventeen of the 30 cART-treated, SIV-infected, pigtailed macaques included in this study had lymphocyte-dominant lesions in the CNS. Twelve animals had moderate to severe lesions affecting the brain parenchyma with or without concurrent meningitis (encephalitis group), whereas five animals had moderate to severe meningitis without significant parenchymal lesions (meningitis group). Thirteen animals had minimal or no inflammation in the brain sections examined (none group). Animals with lymphocyte-dominant encephalitis were identified in all of the seven treatment groups included in this study. Pathology scores for individual animals are included in Table 2.

Parenchymal lesions were present most commonly in white matter, with occasional cortical and subcortical gray matter involvement. In contrast to the perivascular accumulations of macrophages and multinucleated giant cells seen in untreated pigtailed macaques with classic SIV encephalitis (SIVE) and patients with HIV encephalitis,[31] lesions in cART-treated animals consisted of dense perivascular cuffs of lymphocytic cells, often with large, irregularly round to indented nuclei, as well as variable numbers of mature plasma cells (Figure 1A). In animals with more severe lesions, infiltrates often extended into the surrounding neuroparenchyma (Figure 1B). Meningeal lesions were characterized by nodular perivascular aggregates of similar mononuclear cells (Figure 1C). Although glial nodules were present in some animals with lymphocyte-dominant encephalitis, multinucleated giant cells—a hallmark of SIVE—were not observed in the brain or meninges of any of the cART-treated macaques included in this study.

Immunophenotyping of CNS Infiltrates in cART-Treated Macaques

Perivascular infiltrates in the brain and meninges of cART-treated animals comprised primarily $CD20^+$ B cells and $CD3^+$ T cells, both of which also were present in neuroparenchyma adjacent to perivascular lesions (Figure 1, D and E). Immunostaining for CD68 highlighted lower numbers of perivascular macrophages and activated microglia (Figure 1F). Immunostaining for T-cell subtypes was performed in a subset of animals and showed a mixture of $CD4^+$ and $CD8^+$ T cells, both within the perivascular cuffs and surrounding parenchyma (Figure 1, G and H).

Lack of CNS Opportunistic Infections

In HIV patients, opportunistic infections of the CNS are major risk factors for the development of CNS IRIS.[19] In our study, there was no evidence of bacterial, fungal, or parasitic infections

Table 2 Summary of Results from SIV/cART Treatment Studies

ID	Positive serologic tests	CD4 nadir, cells/μL; days after inoculation	SIV VL during cART (plasma and CSF)	Brain pathology score
1	CMV, LCV, RRV	470; 7	Rebound in plasma and CSF	Encephalitis
2	CMV, RRV	458; 7	Sustained viremia in plasma and CSF	Meningitis
3	CMV, RRV	739; 28	Rebound in plasma, suppressed in CSF	None
4	CMV, LCV, RRV	106; 7	Rebound in plasma, suppressed in CSF	None
5	CMV, LCV, RRV, SFV	304; 7	Suppressed in plasma, rebound in CSF	None
6	CMV, LCV, RRV, SFV	148; 7	Rebound in plasma and CSF	Encephalitis
7	CMV, LCV, RRV	587; 7	Rebound in plasma, suppressed in CSF	Encephalitis
8	CMV, LCV, RRV, SFV	449; 7	Rebound in plasma and CSF	Meningitis
9	CMV, LCV, RRV	269; 7	Rebound in plasma and CSF	Encephalitis
10	CMV, LCV, RRV, SFV	356; 7	Rebound in plasma, suppressed in CSF	None
11	CMV, RRV, SFV	209; 7	Suppressed in plasma and CSF	None
12	CMV, LCV, RRV	582; 7	Rebound in plasma and CSF	Meningitis
13	CMV, LCV, RRV, SFV	308; 7	Suppressed in plasma and CSF	None
14	CMV, RRV	245; 10	Rebound in plasma and CSF	Encephalitis
15	CMV, LCV, RRV, SFV	136; 7	Rebound in plasma and CSF	Encephalitis
16	CMV, LCV, RRV	239; 10	Rebound in plasma, suppressed in CSF	None
17	CMV, LCV, RRV	258; 10	Rebound in plasma, suppressed in CSF	None
18	CMV, LCV, RRV	382; 7	Sustained viremia in plasma, rebound in CSF	Encephalitis
19	CMV, LCV, RRV	163; 7	Suppressed in plasma and CSF	Encephalitis
20	CMV, LCV, RRV, SFV	326; 7	Suppressed in plasma, rebound in CSF	None
21	CMV, LCV, RRV	604; 28	Sustained viremia in plasma, suppressed in CSF	Encephalitis
22	CMV, LCV, RRV	354; 10	Rebound in plasma and CSF	Encephalitis
23	CMV, LCV, RRV	410; 70	Sustained viremia in plasma and CSF	Meningitis
24	CMV, LCV, RRV, SFV	378; 7	Sustained viremia in plasma, rebound in CSF	None
25	CMV, LCV, RRV	256; 7	Rebound in plasma and CSF	Encephalitis
26	CMV, LCV, RRV	61; 14	Sustained viremia in plasma and CSF	Meningitis
27	CMV, LCV, RRV, SFV	422; 7	Suppressed in plasma and CSF	None
28	CMV, LCV, RRV	294; 154	Suppressed in plasma and CSF	None
29	CMV, LCV, RRV	322; 10	Suppressed in plasma and CSF	None
30	CMV, LCV, RRV	612; 7	Suppressed in plasma, rebound in CSF	Encephalitis

Details regarding treatment protocols are provided in Supplemental Table S1.

CMV, cytomegalovirus; CSF, cerebrospinal fluid; ID, pigtailed macaque identification; LCV, macaque lymphocryptovirus; RRV, rhesus rhadinovirus; SFV, simian foamy virus; Suppressed, viral load consistently below limit of detection; Sustained viremia, viral load consistently detectable with no time points suppressed below the limit of detection; Rebound, viral load detectable at a time point after previous suppression below the limit of detection; VL, viral load.

by examining hematoxylin and eosin, Gram, and PAS-stained brain sections of cART-treated macaques with CNS inflammation. In addition, although all 30 study animals had detectable antibodies to cytomegalovirus and 26 animals had antibodies to lymphocryptovirus, we did not find any histologic changes consistent with active γ-herpesviral infection, such as viral inclusion bodies or karyomegalic cells. Positive serologic tests for individual animals are listed in Table 2. Furthermore, immunostaining for Epstein-Barr virus nuclear antigen 2, which cross-reacts with macaque lymphocryptovirus, was negative in all animals with lymphocyte-rich CNS inflammation, making herpesvirus-associated CNS lymphoma unlikely (Supplemental Figure S1).

CNS Inflammation in cART-Treated Animals Is Not Associated with Low CD4$^+$ T-Cell Nadir

In HIV patients, a low CD4$^+$ T-cell nadir (<50 cells/μL) is associated with an increased risk of developing IRIS, symptomatic CSF escape, and HAND.[29,30,34] Among cART-treated macaques, there were no significant differences in CD4 nadir between animals that developed lymphocyte-rich inflammation in the brain and meninges versus those with no lesions (Supplemental Figure S2). Overall, the CD4 nadirs among the 30 study animals occurred between 7 and 154 days after inoculation and ranged from 61 to 739 cells/μL, with an average of 342 cells/μL. The CD4 nadir values for individual animals are included in Table 2.

Longitudinal SIV Viral Load Analysis

SIV viral loads in the plasma and CSF of all cART-treated macaques were determined at longitudinal time points throughout infection. Graphs in Figure 2 show the median values for each group in this study (encephalitis, meningitis, and none) for up to 100 days after inoculation, along with data from a group of untreated animals that developed prototypic SIVE (previously reported by Beck et al[35]). Time points after inoculation in which VL data were available from only one animal were excluded from longitudinal group analyses. Although the

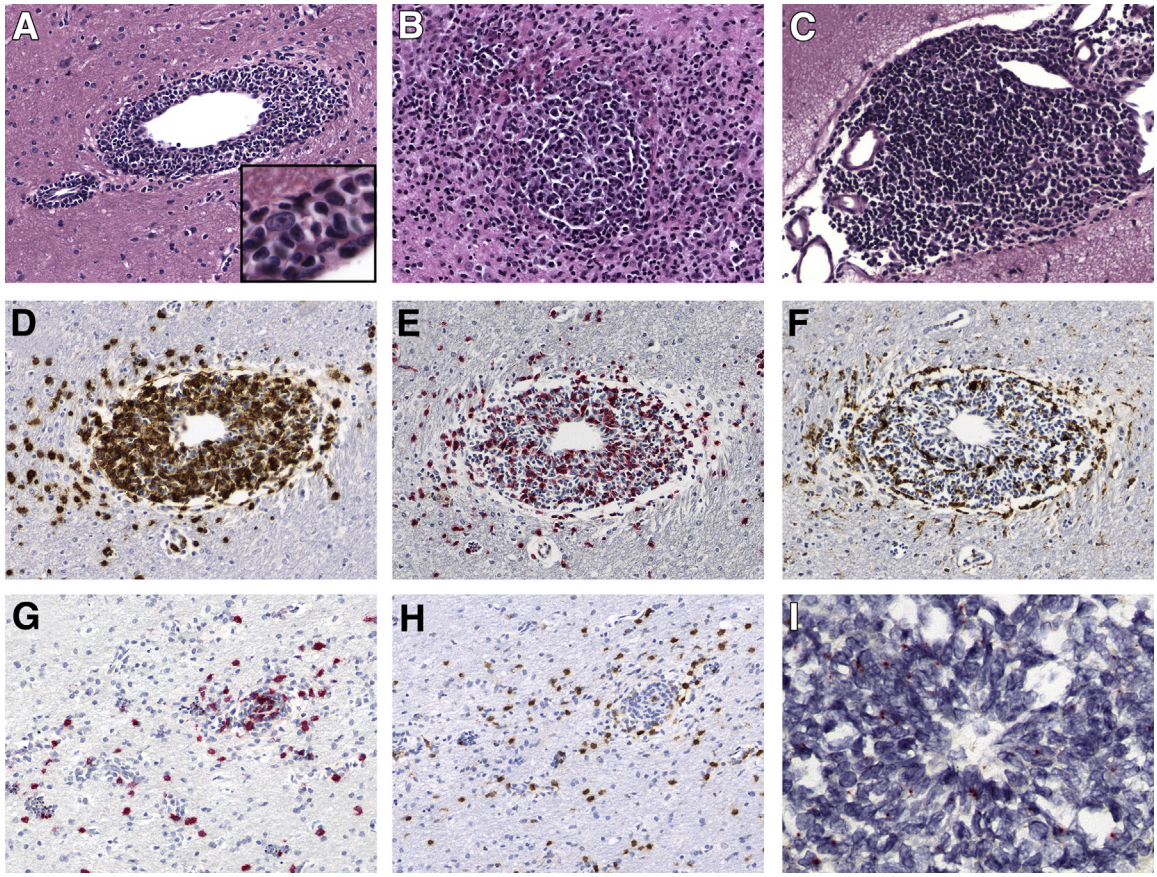

Figure 1 Histopathologic, immunohistochemical, and *in situ* hybridization findings in the central nervous system (CNS) of combination antiretroviral therapy (cART)-treated SIV-infected macaques. **A:** Representative image of a perivascular cuff in the white matter of a macaque with lymphocyte-dominant encephalitis. The perivascular spaces are expanded by numerous infiltrating mononuclear cells (H&E). **Inset:** Higher magnification of perivascular infiltrates shows a mixture of small lymphocytes, mature plasma cells with eccentric nuclei, and larger cells with ovoid nuclei, open chromatin, and prominent nucleoli (H&E). **B:** In more severe lesions, perivascular infiltrates often extended into surrounding neuroparenchyma (H&E). **C:** In animals with meningitis, the leptomeninges are expanded multifocally by nodular perivascular infiltrates of mononuclear cells similar to those seen in parenchymal lesions (H&E). **D**–**F:** Immunostaining of CNS lesions shows that perivascular infiltrates are composed of abundant $CD20^+$ B cells (**D**) and $CD3^+$ T cells (**E**), along with lower numbers of $CD68^+$ macrophages (**F**). CD68 staining also shows activated microglia in the surrounding neuropil. **G** and **H:** Staining for T-cell subsets shows that infiltrates include a mixture of $CD4^+$ (**G**) and $CD8^+$ (**H**) T cells (immunostaining with brown or red chromogen and hematoxylin counterstain). **I:** RNAscope (Advanced Cell Diagnostics) SIV *in situ* hybridization shows low levels of punctate staining in association with foci of inflammation in the brain and meninges of cART-treated macaques (RNAscope *in situ* hybridization with red chromogen and hematoxylin counterstain). Original magnification: ×200 (**A**–**C**); ×1000 (**A**, **inset**); ×200 (**D**–**H**); ×400 (**I**). H&E, hematoxylin and eosin.

median SIV VL of cART-treated groups were consistently three to four logs lower in plasma and one to two logs lower in CSF (Figure 2) compared with untreated animals by 25 days after inoculation, these analyses showed that, similar to cohorts of HIV patients receiving long-term cART, the macaques in this study showed varying degrees of viral suppression in the plasma and CSF.

Longitudinal VL data from individual cART-treated animals were evaluated to determine whether episodes of viral rebound or sustained detectable viremia over the course of treatment were associated with the presence of lymphocyte-dominant CNS inflammation on neuropathologic examination. For this purpose, viral rebound was defined as at least one time point with detectable SIV RNA after a previous time point when SIV RNA was suppressed below the assay limit of detection (420 copies/mL) (Figure 2). In total, 15 animals had viral rebound in the plasma, whereas 14 animals developed rebound in the CSF. When comparing animals that developed encephalitis with those without parenchymal lesions (including both the none and meningitis groups), viral rebound in the plasma was not associated with the development of encephalitis (Fisher exact test, $P = 0.71$; odds ratio, 1.75). However, animals that experienced viral rebound in the CSF were more likely to have lymphocytic encephalitis at terminal time points (Fisher exact test, $P = 0.02$; OR, 7.80). Therefore, CSF rebound that preceded terminal time points best predicted the development of lymphocyte-rich encephalitis.

For five macaques in this cohort, viral load in the CSF and/or plasma remained above the limit of detection throughout the entire treatment study (Figure 2), which was defined as sustained

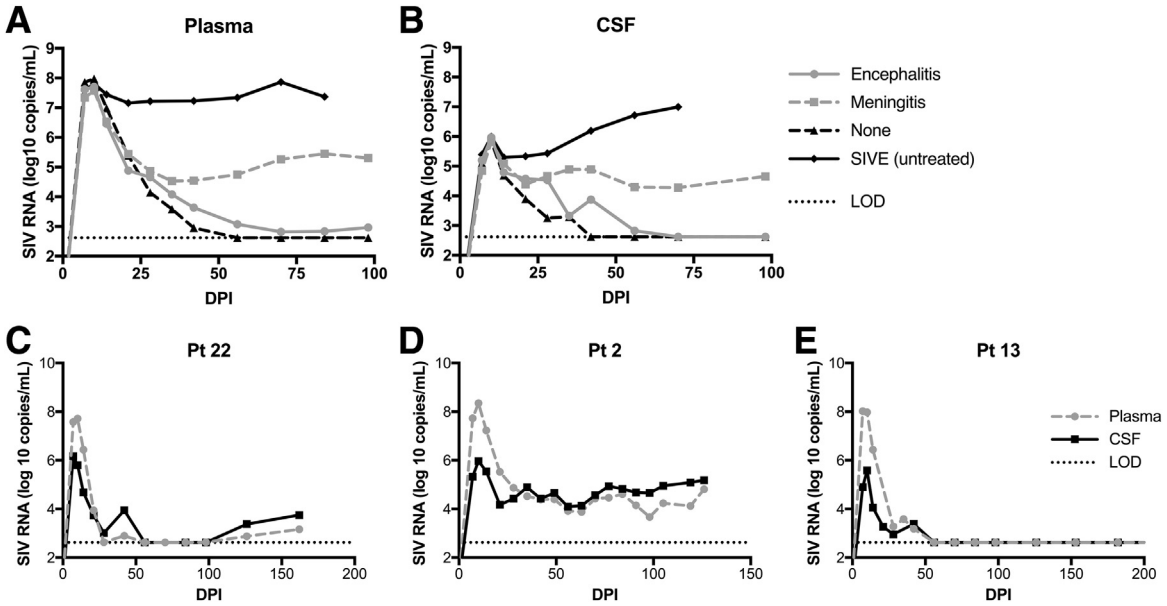

Figure 2 Longitudinal viral load and contingency analyses. **A** and **B:** Median longitudinal SIV-RNA levels in the plasma and cerebrospinal fluid (CSF) are shown for groups of combination antiretroviral therapy (cART)-treated, SIV-infected animals with lymphocyte-dominant encephalitis (Encephalitis), meningitis only (Meningitis), and no significant central nervous system inflammation (None), as well a group of untreated SIV-infected macaques with prototypic SIV encephalitis (SIVE). After cART initiation at 12 days after inoculation, the median plasma and CSF viral loads in all cART-treated groups were consistently lower than those of untreated animals with SIVE, which were euthanized at 84 days after inoculation. LOD was 420 copies/mL. **C**–**E:** Individual cART-treated animals showed varying degrees of viral suppression in the plasma and CSF over the course of SIV infection. **C**–**E:** Graphs show longitudinal SIV-RNA levels for representative animals that experienced viral rebound (**C**) and sustained viremia (**D**), compared with an animal with consistent suppression below the LOD in both the plasma and CSF (**E**). DPI, days after infection; LOD, limit of detection; Pt, pigtailed macaque.

viremia. Animals with sustained viremia were more likely to be included in the meningitis group based on neuropathologic examination compared with encephalitis and none. This relationship was similar for both plasma and CSF ($P = 0.04$; OR, 11.00; and $P = 0.009$; OR, 43.40, respectively; Fisher exact test), and corresponded with the findings in the longitudinal group analyses in which the median VL for the meningitis group was consistently four to five logs above the limit of detection in both plasma and CSF. Results of all contingency analyses are summarized in Table 3.

Terminal Plasma, CSF, and Brain SIV Viral Loads Are Highest in Animals with Meningitis Only

Comparison of plasma and CSF viral loads at terminal time points did not show significant differences between cART-treated animals that developed lymphocyte-dominant encephalitis and those without CNS lesions (Figure 3, A and B). Similarly, no difference in the amount of SIV RNA in the brain tissue of animals with lymphocyte-dominant encephalitis was found compared with animals without lesions (Figure 3C). However, in animals that developed meningitis only, significantly higher amounts of SIV RNA were observed in both the plasma and the CSF at terminal time points compared with animals with or without lymphocytic encephalitis (Figure 3, A and B). Animals with only meningitis also had significantly higher levels of SIV RNA in the brain tissue than those without lesions (Figure 3C). In contrast, there were no differences in the amount of SIV DNA in brain tissue among any of the three groups (Figure 3D).

Table 3 Contingency Analysis

Viral rebound	Encephalitis	Meningitis	None
Plasma rebound			
Present	7	2	6
Absent	5	3	7
CSF rebound			
Present	9*	2	3
Absent	3	3	10
Sustained plasma VL			
Present	2	3*	1
Absent	10	2	12
Sustained CSF VL			
Present	0	3*	0
Absent	12	2	13

Contingency analyses were performed to determine whether viral rebound or sustained viremia in the plasma or CSF was associated with the development of lymphocyte-dominant central nervous system inflammation. Plasma and CSF were analyzed independently. cART-treated animals with at least one episode of CSF rebound were significantly more likely to develop encephalitis ($P = 0.02$; OR, 7.80), whereas viral rebound in the plasma was not associated with the development of encephalitis ($P = 0.71$; OR, 1.75). Animals with sustained viremia in plasma ($P = 0.04$; OR, 11.00) or CSF ($P = 0.009$; OR, 43.40) were significantly more likely to develop meningitis only. All P values were obtained by Fisher exact contingency analyses.

*$P < 0.05$.

cART, combination antiretroviral therapy; CSF, cerebrospinal fluid; OR, odds ratio; VL, viral load.

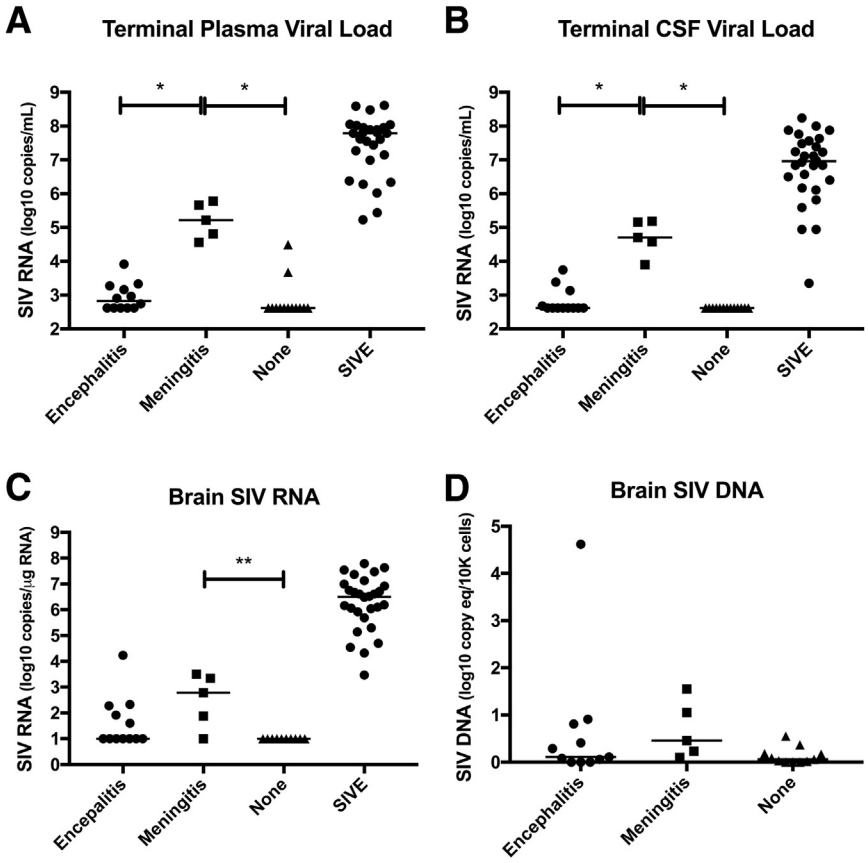

Figure 3 Terminal SIV levels in the plasma, cerebrospinal fluid (CSF), and brain. **A** and **B:** At terminal time points, combination antiretroviral therapy (cART)-treated animals with lymphocytic meningitis only (Meningitis) had significantly higher SIV-RNA levels in the plasma and CSF compared with animals with lymphocyte-dominant encephalitis (Encephalitis) and those with no significant central nervous system (CNS) inflammation (None). Terminal plasma and CSF viral loads were not significantly different between the encephalitis group and the none group. **C:** Levels of SIV RNA in brain tissue also were significantly higher in cART-treated animals in the meningitis group compared with the none group, but were not different between the encephalitis and meningitis groups or encephalitis compared with none. **D:** Levels of integrated SIV DNA in brain tissue were not significantly different among or between groups. Bars represent group medians. Data from a group of untreated SIV-infected macaques with prototypic SIV encephalitis (SIVE) were included in the graphs for visual comparison with cART-treated groups, but were not included in statistical analyses (**A**–**C**). $^{*}P < 0.05$, $^{**}P < 0.01$ (Kruskal-Wallis test with the Dunn post-test for multiple comparisons). copy eq, copy equivalents.

Lymphocyte-Dominant CNS Lesions Contain Low Numbers of SIV-Positive Cells by ISH

By using RNAscope ISH, rare SIV-positive cells in association with lymphocyte-dominant inflammation were identified in the brain parenchyma and meninges of 15 of the 16 affected macaques examined by this method. Positive cells typically contained a single punctate focus of staining that corresponds to a single copy of viral RNA using the sensitive RNAscope method (Figure 1I).[36] Only one animal evaluated by ISH in this study (pigtailed macaque 1) was negative for SIV by ISH; however, this macaque's brain tissue was fixed in Streck's tissue fixative (Streck, Omaha, NE) rather than 10% neutral buffered formalin, which is not optimal for RNAscope ISH. For another animal (pigtailed macaque 6), fixed, paraffin-embedded brain tissue was not available for RNAscope analysis, but positive SIV ISH results for this macaque were reported in a previous study.[37]

Discussion

The pathogenesis of persistent neurocognitive impairment in HIV patients receiving effective cART remains incompletely understood. Because studies of HIV patients are limited by difficulty in directly sampling the CNS, animal models are critical tools for understanding the underlying neuropathologic and immunologic mechanisms of HAND. This large-scale report of neuropathology in cART-treated SIV-infected macaques showed that 17 of 30 SIV-infected pigtailed macaques on cART developed lymphocyte-dominant perivascular infiltrates in the brain or meninges that comprised numerous $CD20^{+}$ B cells and $CD3^{+}$ T cells admixed with fewer $CD68^{+}$ macrophages. This inflammatory phenotype was clearly distinct from prototypic SIVE that, similar to HIV encephalitis, is characterized by microglial nodules, macrophage-rich perivascular infiltrates, and frequent multinucleated giant cells.[38] Although the development of SIVE has been associated strongly with high viral loads in the CSF,[38] lymphocyte-rich encephalitis in cART-treated, SIV-infected macaque animals was associated with episodes of CSF SIV RNA rebound that preceded terminal time points rather than higher terminal CSF viral loads versus animals without CNS lesions. Also, although untreated animals with fulminant SIVE have high levels of SIV RNA in brain tissue at necropsy by both RT-qPCR and ISH,[31,39] SIV RNA was below the limit of detection by RT-qPCR in CNS of most treated macaques with lymphocyte-rich encephalitis, and sensitive RNAscope ISH correspondingly showed infrequent SIV-RNA–positive cells within inflammatory lesions. Collectively, these findings suggest that lymphocyte-dominant encephalitis in cART-treated, SIV-infected macaques is associated with residual or re-emergent low-level SIV replication in the CNS despite cART.

Another unique finding in cART-treated macaques was that animals in the meningitis group had higher levels of SIV RNA in the plasma, CSF, and the brain terminally than animals with lymphocytic encephalitis, and were significantly more likely to have persistently detectable SIV RNA (sustained viremia) in the plasma and CSF compared with animals with either lymphocytic encephalitis or no lesions. Higher sustained SIV viral loads in the plasma and CSF may contribute to dampened adaptive immune responses to SIV or other antigens in the brain parenchyma of these animals. Detailed investigation of functional immune responses in the periphery and CNS of cART-treated animals may reveal the mechanism behind this finding.

Lymphocyte-dominant CNS inflammation resembling our findings in cART-treated macaques has been documented in rare neurologic conditions in HIV patients receiving cART including neurosymptomatic CSF escape and CNS IRIS. In neurosymptomatic CSF escape, patients on long-term cART develop subacute neurologic symptoms corresponding with an episode of CSF viral escape, defined as detectable CSF HIV RNA despite undetectable plasma viral load (<50 copies/mL) or HIV-RNA levels more than one log higher in CSF compared with plasma.[25,40] Clinical findings in these patients typically include CSF pleocytosis and/or increased protein levels consistent with meningeal inflammation, along with evidence of multifocal white matter inflammation on magnetic resonance imaging. In a case series by Peluso et al,[25] brain biopsies from two patients showed dense perivascular infiltrates composed of a mixture of mature and immature B cells and predominantly $CD8^+$ T lymphocytes. The investigators postulated that the inflammation in these patients represented an equilibrium between antigen and immune responses within the CNS in the setting of relative immune preservation rather than a direct sequela of immune reconstitution, as in classic IRIS. Although the limit of detection for our SIV RT-qPCR assay for CSF and plasma was not low enough to replicate the exact definition of CSF escape used for these HIV patients, a significant association was found between the presence of lymphocyte-rich inflammation in the brain parenchyma at necropsy and an episode of CSF rebound, which was defined as at least one time point with detectable SIV RNA after viral load suppression below the limit of detection of the assay used in this study.

Similar to the lesions we observed in cART-treated macaques, neuropathologic findings in CNS IRIS without concurrent opportunistic infections consist of widespread perivascular and parenchymal infiltration of lymphocytes primarily affecting white matter.[22,23,27,28,41] Meningeal involvement also has been reported.[42,43] Unlike CNS IRIS, no significant relationship was found between low $CD4^+$ T-cell nadir and the development of lymphocyte-dominant CNS inflammation among the cART-treated macaques. Furthermore, in contrast to our immunophenotyping results in the macaques, the lymphocytic infiltrates in HIV patients with CNS IRIS are composed predominantly of $CD8^+$ T cells, with few to no appreciable B cells or $CD4^+$ T cells.[26,27,29,42] One potential explanation for this difference between CNS IRIS in HIV patients and our findings in macaques is the very early initiation of antiretroviral therapy in the macaque studies. In HIV patients, early cART initiation has been associated with enhanced $CD4^+$ T-cell recovery and preservation of B-cell function,[44,45] which may influence immune responses to viral replication in the CNS.

The high prevalence of B-cell–rich lymphocytic infiltrates in the brain parenchyma and meninges of these macaques suggests that the role of humoral immune responses in the CNS may be underappreciated in HIV patients on cART. Multiple studies have shown that a large proportion of untreated HIV patients have increased levels of intrathecal Ig production that often persists for up to 2 years after cART initiation.[46–49] Additional analyses by Bonnan et al[47] showed that the proportion of intrathecal Ig that was specific to HIV in a cohort of cART-naive patients was small (4.3% ± 3.7%), and that the number of antibody-secreting cells present in the CSF itself may not account for the high levels of intrathecal antibody production, suggesting that the bulk of intrathecal Ig originates from antibody-secreting cells in the meninges or perivascular areas of the CNS. In an autopsy study, asymptomatic HIV patients were found to have higher numbers of B cells in perivascular and parenchymal compartments of the brain compared with uninfected individuals.[50] Together, these findings suggest that B cells traffic into the CNS at increased rates during HIV infection and produce antibodies to a wide range of antigens. The potential importance of self-reacting CNS antibodies was shown in a clinical study by Lackner et al,[51] which showed that patients with active HAND had significantly higher titers of myelin oligodendrocyte glycoprotein antibodies in the serum and CSF compared with HIV patients without neurologic symptoms. Findings in this report indicate that SIV-infected macaques may serve as a highly translational model to evaluate the dynamics of B-cell–mediated immunity in the CNS during long-term antiretroviral therapy, as well as the potential impact of anti-CD20 immunotherapies, such as those used to treat multiple sclerosis. Furthermore, although we suspect that the neuroinflammation observed in cART-treated macaques is being driven by low-level SIV replication, the possibility of an underlying autoimmune mechanism has not been ruled out.

In conclusion, our study showed that cART-treated, SIV-infected macaques developed lymphocyte-dominant CNS inflammation that was distinct from prototypic SIVE and was not associated with opportunistic agents in the CNS, other viral infections, or specific treatment regimens. Although the CNS lesions in cART-treated macaques were similar morphologically to those described in HIV patients with neurosymptomatic CSF escape and CNS IRIS, no significant relationship was found between lymphocytic CNS inflammation and low $CD4^+$ T-cell nadir and these macaques did not develop progressive neurologic signs. Rather, the high prevalence of lesions and lack of overt neurologic symptoms among these macaques support the premise that robust or dysregulated adaptive immune responses in the CNS may impact treated HIV patients at a subclinical level and may contribute to the continued high prevalence of HAND.[29,30] Further investigation into the pathogenesis of persistent neuroinflammation in SIV-infected macaques on cART,

including a more detailed functional characterization of peripheral and CNS lymphocyte populations, may accelerate the development of new adjunctive immunomodulatory therapies for neurocognitive impairment in HIV patients.

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Supplemental Data

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