Initiation of HAART during acute simian immunodeficiency virus infection rapidly controls virus replication in the CNS by enhancing immune activity and preserving protective immune responses

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Abstract The CNS remains vulnerable to HIV-induced damage despite highly active antiretroviral therapy (HAART). Using a rigorous simian immunodeficiency virus (SIV) macaque model of HAART that combines three classes of antiretroviral drugs (a protease inhibitor, a reverse transcriptase inhibitor, and an integrase inhibitor), we examined immune responses and virus replication in the plasma and cerebrospinal fluid (CSF) following HAART initiation during acute infection (4 days postinoculation (p. i.)). HAART-treated macaques did not experience the level of acute CD4+ and CD8+ T cell and NK cell count suppression in the peripheral blood normally observed during acute infection. Initiation of HAART produced a rapid four-log decline in viral load in plasma and a slower two-log decline of viral RNA in the CSF over the subsequent 17 days of infection. Despite a dramatic reduction of viral RNA levels in the brain at 21 days p.i., viral DNA levels were not different between the two groups. Expression of most cytokine mRNA in brain of HAART-treated macaques did not significantly differ from untreated controls. Expression of the IFN responsive gene MxA was significantly reduced in the brain of HAART-treated macaques, suggesting control of hyperactive immune responses. Control of virus replication likely was enhanced by significant increases in CD4+ and CD8+ T cell trafficking in the brain of infected animals on HAART therapy and the concomitant increase in levels of IFNγ. Collectively, these data indicate preserved innate and adaptive immune activity in the brain following HAART initiation during acute SIV infection in this macaque model, suggesting profound benefits following acute treatment of SIV.

Keywords SIV · HAART · Acute infection · Macaque · Immune activation · IFN · MxA

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Introduction

Treatment of HIV-infected individuals with HAART has altered the AIDS epidemic globally. In the USA, HAART has greatly decreased the morbidity and mortality associated with HIV infection and reduced the occurrence of HIV dementia in treated individuals (Ances and Clifford 2008; Boisse et al. 2008; Robertson et al. 2007). While HIV dementia has been virtually eliminated, the rate of cognitive impairment continues to increase with over half of HIV-infected individuals on HAART experiencing mild or moderate cognitive impairment (Heaton et al. 2009). Thus, the CNS remains vulnerable to HIV-induced damage despite HAART. In addition, the question of when to initiate HAART is under continued debate; recent studies demonstrate increased morbidity and mortality when HAART is delayed, and there is a direct correlation between preservation of peripheral immune function and timing of administration of HAART (Kitahata et al. 2009; When To Start Consortium 2009). However, the consequences of early preservation of peripheral immune function on the CNS or the comparative effects of early versus delayed HAART on CNS injury and cognitive impairment have not been examined.

Limitations of tissue availability, patient compliance, and differences in HAART regimens of HIV-infected individuals make a simian immunodeficiency virus (SIV) model essential to define the comparative efficacy of early versus late initiation of HAART on control of virus replication in peripheral blood and CNS and the impact on the immune system. The consistent accelerated SIV macaque model of HIV/AIDS is ideal to examine this because of its use in extensive studies of acute events in the periphery and the brain (Barber et al. 2004a, 2006; Clements et al. 2002; Mankowski et al. 2004; Zink and Clements 2002; Zink et al. 1999, 2001). Viral and cellular responses to infection and the pathogenesis of CNS disease have been characterized at length in this model, in which virus is present in the brain at least as early as 4 days after infection (Witwer et al. 2009). Protection against neurological disease is associated with the induction of innate immune responses, and protection is mediated by IFNβ, which controls viral replication (Barber et al. 2006; Clements et al. 2002). Recently, we demonstrated that there is a coordinated regulation of immune responses in the brain from 4 to 10 days after inoculation and that failure of coordination beginning around 21 days after inoculation is associated with progression to neurological disease (Witwer et al. 2009). Thus, innate immune responses must carefully be balanced to allow for antiviral responses while keeping inflammation in check.

We recently developed a rigorous SIV macaque model of HAART therapy in HIV-infected individuals that combines four antiretroviral drugs (saquinavir, atazanavir, tenofovir, and the Merck integrase inhibitor L-870812) on the background of our consistent accelerated model (Dinoso et al. 2009). In this SIV/HAART model, when therapy was initiated after acute infection (day 12 postinoculation), viral load in both the peripheral blood and cerebrospinal fluid (CSF) was reduced to undetectable levels (Dinoso et al. 2009; Zink et al. 2010). Virus replication in brain also was dramatically reduced by HAART. However, viral DNA levels in brain were unchanged as compared with those of the untreated SIV-infected macaques, demonstrating that brain also is a significant viral reservoir (Clements et al. 2002; Zink et al. 2010). Terminally, in the brain of HAART-treated animals (initiated at 12 days), there was a significant reduction in most inflammatory and immune markers in the brain compared with that of the SIV-infected untreated animals, suggesting that HAART treatment returned the brain to close to its normal phenotype.

In the current study, HAART was initiated in SIV-infected macaques at 4 days postinoculation (p.i.), and animals were killed at 21 days p.i. to determine (a) whether early initiation of HAART would be effective in controlling virus replication in the brain and preventing the development of hyperactive immune responses, (b) the impact of HAART therapy on innate immune responses, and (c) the impact of preserving the peripheral immune compartment on viral suppression in the CNS.

Initiation of HAART produced a rapid four-log decline in viral load in plasma and a slower two-log decline of viral RNA in the CSF over the subsequent 17 days of infection. Despite a dramatic reduction of viral RNA levels in the brain at 21 days p.i., viral DNA levels were not different between the two groups. HAART-treated macaques did not experience the same level of suppression in CD4+ and CD8+ T cell and NK cell numbers in the peripheral blood usually seen during acute infection. Expression of most cytokine mRNA in brains of untreated and HAART-treated macaques was not significantly different, suggesting that HAART therapy complemented innate immune responses and that viral control under HAART therapy is an active process. Expression of the IFN-responsive gene MxA was significantly reduced in the brain, suggesting that hyperactive immune responses were controlled. Control of virus replication likely was enhanced by significant increases in CD4+ and CD8+ T cell trafficking in the brain of infected animals under HAART therapy and the concomitant increase in levels of IFNγ likely attributable to effector lymphocytes. Collectively, these data demonstrate preservation of innate and adaptive immune response in the brain, indicating a significant advantage of early initiation of HAART.

Materials and methods

Viruses and animals

Twelve juvenile pigtailed macaques (*Macaca nemestrina*) were intravenously inoculated with SIV/DeltaB670 (50 AID50) and SIV/17E-Fr (10,000 AID50). (Zink et al. 1999) Three animals (PBf2, PGh2, and PNg2) were treated with a combination of four antiretroviral drugs, beginning on day 4 p.i. The course of therapy has been previously published, consisting of daily administration of 205 mg/kg saquinavir (SQV) orally, 10 mg/kg of L-870812 orally, 270 mg/kg atazanavir, and 30 mg/kg of 9-*R*-(2-phosphonomethoxypropyl) adenine intramuscularly (Dinoso et al. 2009). Three animals (PGi2, PIh2, and PZf2) were treated in an identical manner but without SQV.

Blood and cerebrospinal fluid (CSF) samples were collected on days 7, 10, 14, and 21 p.i., for hematological analysis, quantitation of viral RNA, and ELISA quantitation of monocyte chemoattractant protein (CCL2), and IL-6 (Zink et al. 1999). Macaques were killed at 21 days p.i. in accordance with federal guidelines and institutional policies. At euthanasia, macaques were perfused with sterile saline to remove blood from the vasculature prior to sampling organs and tissues. Protocols were approved by the Johns Hopkins University Institutional Animal Care and Use Committee and in accordance with the recommendations of the Weatherall Report.

Quantitation of CD4+, CD8+ T lymphocytes, and NK cells

Circulating CD4+, CD8+ T cells, and NK cells were quantitated from whole blood collected at each time point by flow cytometry. Cells were labeled with fluorescently conjugated anti-CD3, CD4, CD8, and CD16 and analyzed on a FACSCalibur instrument (Becton Dickinson).

Quantitation of viral and cellular RNA

Viral RNA was isolated directly from 140 μl of plasma and CSF samples using the QIAamp Viral RNA Mini kit (Qiagen) according to the manufacturer's protocol. Quantification of virion-associated RNA was performed by real-time RT-PCR as previously described (Barber et al. 2004b).

Total RNA was isolated from 50 mg of brain tissue (basal ganglia and parietal cortex) using the RNeasy kit (Qiagen) and treated with two units of Turbo DNAse (Ambion) for 30 min at 37°C. One microgram of purified RNA was analyzed by real-time RT-PCR using specific primers and probes for SIV *gag* (Barber et al. 2004b), CCL2, IL-6, IFNβ, IFNγ, TNFα, and MxA, as previously described (Witwer et al. 2009). PCR reactions were performed in a Chromo4 thermocycler (Biorad) using a Multiplex PCR Mix (Qiagen). Cellular mRNA levels were normalized by 18S ribosomal RNA levels. Quantitation of gene expression was calculated using the ΔΔCt method (Schefe et al. 2006).

Quantification of IL-6 and CCL2 levels in plasma and CSF

CCL2 levels in CSF and plasma, and IL-6 levels in CSF were measured by ELISA (R&D Systems) at each time point, as previously described (Mankowski et al. 2004; Zink et al. 1999, 2001). CCL2 levels were expressed as the ratio of CCL2 in the CSF over that in the plasma.

Quantitative immunohistochemical analysis

CD68, MHC class II, and GFAP levels were quantitated by immunohistochemical staining and digital quantitative analysis of staining in a 2-cm^2 area of basal ganglia, as previously described (Barber et al. 2004b; Zink et al. 1999). Briefly, macrophages were identified by CD68 (KP1; Dako). HLA-DR (Dako) was a marker of macrophage and endothelial cell activation, and GFAP (Dako) was used as a measure of astrocyte activation. CD4+ and CD8+ T cells were stained with anti-CD4 or CD8 (Novocastra and Vector, respectively). NK cells were visualized by dual staining using CD3 (Dako) and TIA-1 (ABCAM).

Statistical analysis

Spearman's rank correlation test was used to test the statistical dependence between two variables. Spearman's is a non-parametric statistical test analogous to the parametric Pearson's estimate. Non-parametric methods are considered to be conservative; therefore, statistically significant results found when using non-parametric methods are assumed to imply a lower bound for the *p* value. All statistical tests were performed as two-sided tests. No statistical differences were obtained between the HAART treated groups with or without saquinavir; therefore, for analysis purposes, the two groups were combined.

Results

HAART treatment initiated at 4 days p.i. reduced viral load in the peripheral blood and CSF

Previous studies examining HAART treatment using the SIV model have elected to initiate therapy during asymptomatic or chronic infection to best model treatment in human disease. Given recent studies suggesting considerable benefit to earlier treatment, there is considerable debate

on when therapy should be initiated. In our SIV macaque model, the brain is infected by 4 days p.i., and the peak of viral RNA in plasma occurs in untreated animals at 7 days p.i. Thus, treatment at 4 days represents a critical period in which the brain is actively being seeded, and immune responses in the periphery and CNS have not yet managed to suppress virus replication. The 21-day p.i. time point was chosen to allow for a direct insight into the brain parenchyma to determine the impact of HAART on the pathophysiology in the brain at a time when animals either coordinately regulate immune responses and avoid neurological disease or fail in their coordination and subsequently develop encephalitis.

Plasma viral load was significantly reduced in the SIV-infected HAART-treated macaques as compared with that of the untreated SIV-infected macaques at both 7 ($p=0.002$) and 10 days ($p=0.002$) p.i. (Fig. 1a, b). Thus, HAART treatment was effective in reducing viral load in plasma within 3 days. Plasma viral load continued to decline in the HAART-treated macaques at 14 and 21 days p.i., with a three-log reduction in plasma viral load by 14 days p.i. Peak viral RNA levels in both plasma and CSF of HAART-treated macaques were one-log lower than in untreated animals, indicating that the antiretrovirals were able to affect very early and increasing levels of viral replication.

CSF viral load was significantly lower in the SIV-infected, HAART-treated macaques at both 7 ($p=0.002$) and 10 days ($p=0.002$) p.i. (Fig. 1c, d) and continued to decline, albeit with a broader range of variation, in the treated animals compared with that of the untreated. The rate of viral RNA decline in plasma was more rapid than in the CSF (1,726,000 versus 38,400 copy eq./day; slope ranges, −0.36 to −0.92 and −0.17 to −0.73, respectively). At least two factors may be responsible for the difference in the rate of decline. First, viral RNA in plasma at its peak (7 days p.i.) was 100-fold higher in plasma than in CSF. As a result, there would likely be more infected cells and thus exponentially greater cell-to-cell spread that would be impacted by HAART therapy. Second, the HAART regimen used had minimal CNS penetration (Letendre et al. 2008; Zink et al. 2010), so viral decline might be more dependent on immune control of virus replication as compared with direct HAART effects in the CNS. Despite the more rapid decline of viral load in plasma, there was a significant effect of HAART treatment on CSF viral load only 3 days after initiation of HAART.

Immune cells in the peripheral blood and CNS
of HAART-treated and untreated SIV-infected macaques

Given the extreme downward pressure of HAART on viral load in the periphery at a time when adaptive immune responses were developing, we examined whether HAART

Fig. 1 HAART reduced viral load in plasma and CSF and reduced immune cell decline in peripheral blood. Plasma viral load was significantly reduced in the SIV-infected HAART-treated macaques (a) as compared with that of the untreated SIV-infected macaques (b) at both 7 and 10 days p.i. ($p=0.002$ each). CSF viral load was significantly lower in the SIV-infected, HAART-treated macaques (c) at both 7 ($p=0.002$) and 10 days ($p=0.002$) p.i. than in untreated macaques (d). Median CD4+ T cell count (e), CD8+ T cell count (f), and NK cell count (g). The normal decline of CD4+ and CD8+T cells as well as NK cells in the peripheral blood of SIV-infected macaques during acute infection was substantially lessened ($p=0.002$) in HAART-treated macaques compared with that in untreated macaques

could normalize CD4+ T cell, CD8+ T cell, and NK cell levels in peripheral blood. The more rapid decline of CD4+ and CD8+T cells as well as NK cells in the peripheral blood of SIV-infected macaques during acute infection was substantially lessened in HAART-treated macaques compared with that in untreated macaques (Fig. 1e–g). Thus, HAART treatment during acute infection rapidly altered the decline of immune cells in peripheral blood, suggesting that immune activation and loss of CD4+ lymphocytes was positively impacted by early initiation of HAART.

HAART increased T lymphocyte numbers in the brain

Since the decline of immune cells in the periphery was stemmed in HAART-treated macaques, we reasoned that there may be more cells available to traffic to the brain. We examined the effect of HAART on immune cells in the brain of the HAART-treated macaques at a time when adaptive immune responses should be near their peak. CD4+ and CD8+ T lymphocytes and NK cells were enumerated in the basal ganglia of SIV-infected HAART-treated and untreated macaques at 21 days p.i. There were significantly higher numbers of CD4+ and CD8+ T cells in the brain of the HAART-treated macaques, with cell numbers doubling for CD4s and nearly doubling for CD8s as compared with those of the untreated animals ($p=0.009$ and 0.041, respectively; Fig. 2a, b). No significant increase in the number of NK cells trafficking in the brain was observed (Fig. 2c).

HAART initiated at 4 days p.i. reduced viral RNA
but not viral DNA in brain

Since HAART prevented the typical depletion of immune cells, and increased adaptive immune surveillance of the brain, we wished to examine the impact of HAART on viral RNA and DNA levels in the brain. We therefore quantitated SIV RNA in both basal ganglia and parietal cortex of HAART-treated and untreated macaques at 21 days p.i. to directly examine virus replication in brain. There was no significant difference in viral RNA between basal ganglia and parietal cortex. SIV RNA was significantly reduced in
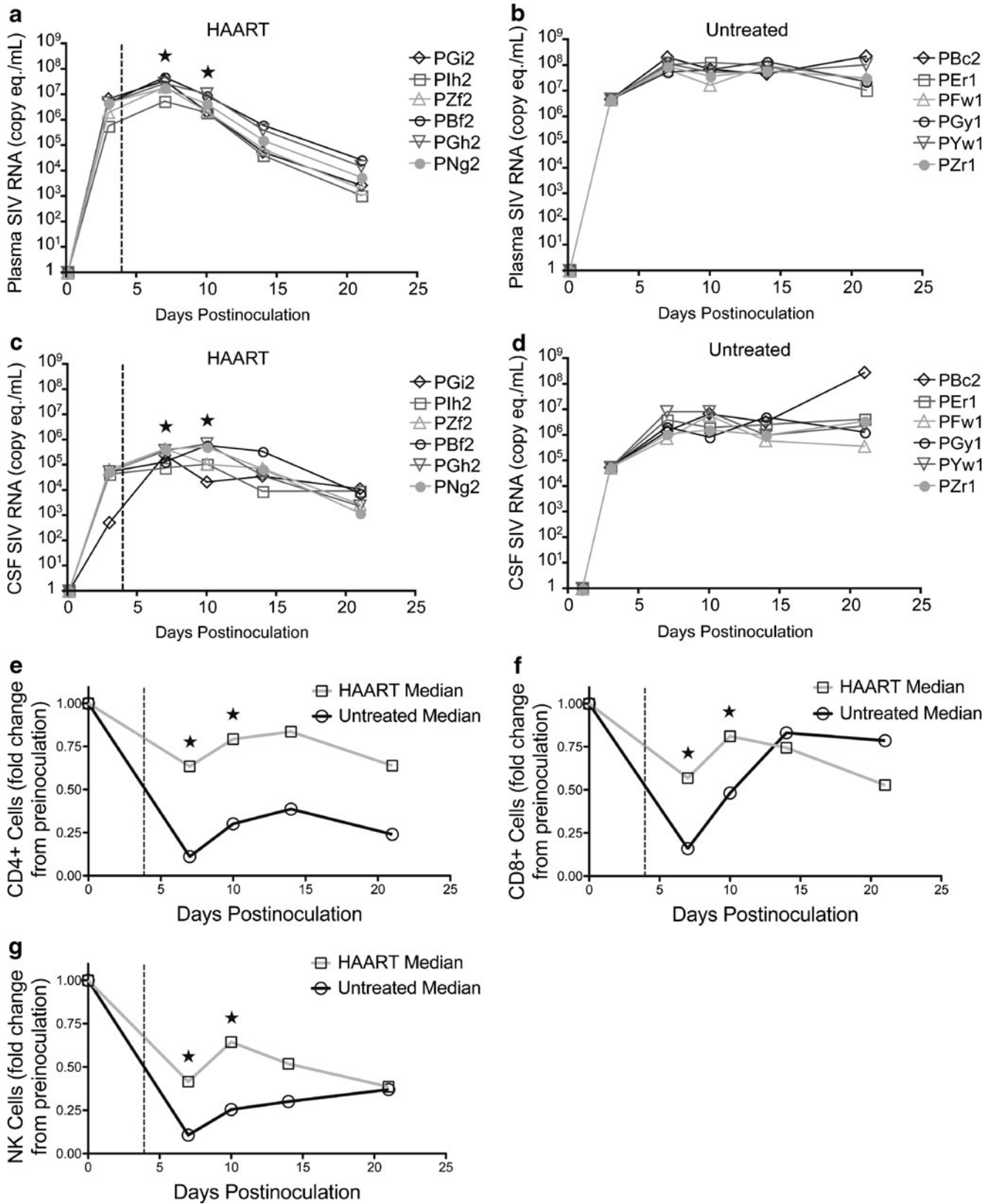
Fig. 2 HAART treatment increased T lymphocyte numbers and suppressed viral RNA, but did not decrease viral DNA in the brain. CD4+ T cell count (**a**), CD8+ T cell count (**b**), and NK cell count (**c**) in the brain at day 21 p.i. There were significantly higher numbers of CD4+ and CD8+ T cells in the brain of the HAART-treated macaques, with cell numbers doubling for CD4s and nearly doubling for CD8s as compared with untreated animals. NK cells numbers, while significantly higher than in uninfected animals, were not reduced by HAART. SIV RNA (**d**), but not DNA (**e**), was significantly reduced in HAART-treated macaques compared with those of the untreated controls
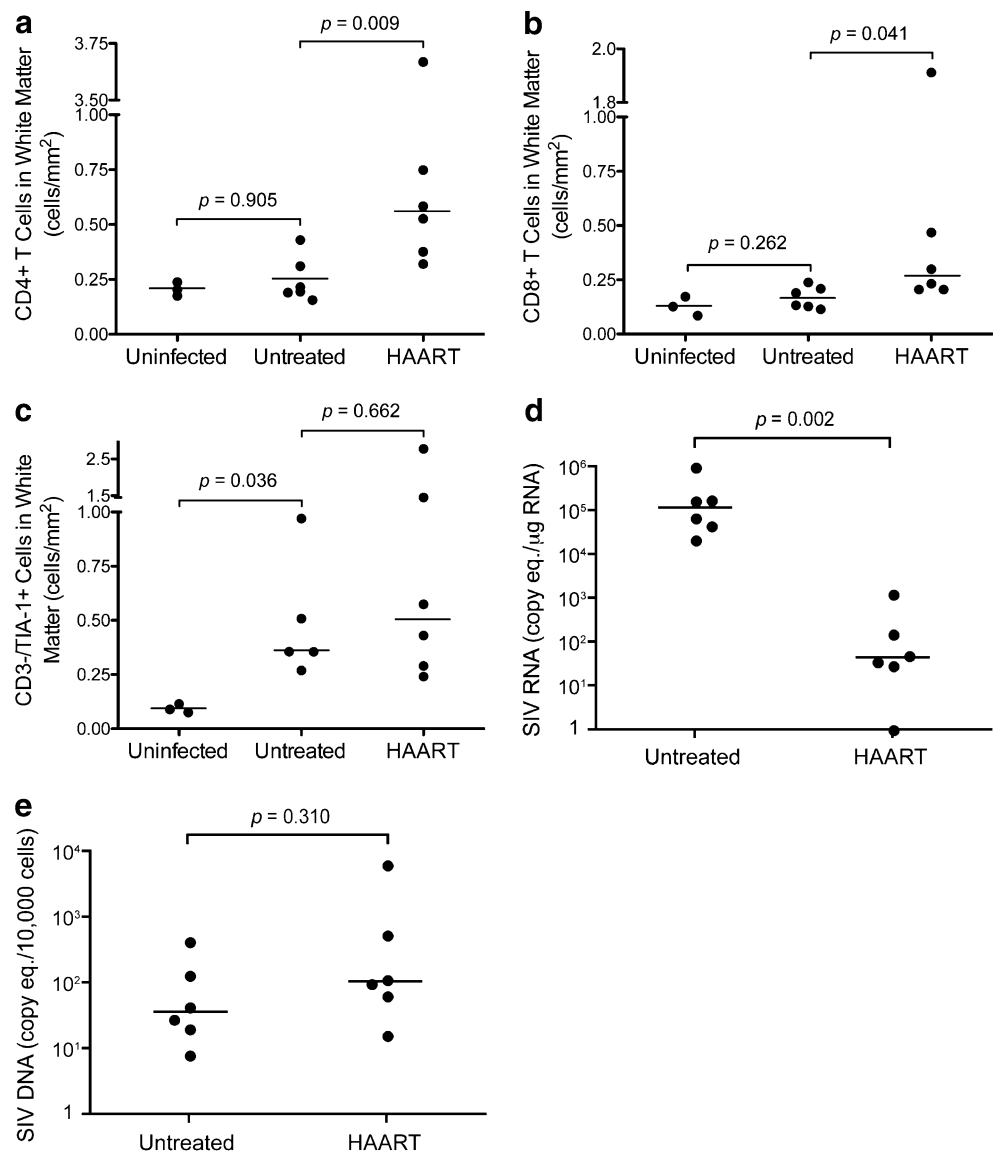
the brain of the HAART-treated macaques compared with viral RNA levels in SIV-infected, untreated macaques (10,000-fold decrease; $p=0.002$, Fig. 2d). Thus, the reduction of virus detected in the CSF of the HAART-treated macaques reflected reduced viral replication in the brain parenchyma.

Since viral RNA levels represent potentially infectious virus, but not cells that have integrated provirus (viral reservoirs), we measured SIV DNA in brain at 21 days p.i. SIV DNA was not significantly reduced in the HAART-treated macaques as compared with that of the untreated animals ($p=0.310$) (Fig. 2e). There were no significant differences in SIV DNA between basal ganglia and parietal cortex, similar to our previous findings in untreated animals (Clements et al. 2002). These data suggest that HAART therapy does not affect the archiving of virus in the brain on infected animals at 21 days p.i. However, since we have previously shown

that CD4+ T cell levels are increased in the brains of HAART treated animals, it is unclear how many of these cells harbor proviral DNA and the proportion of signal contributed by CD4s versus other cell types in the brain.

Cytokine and chemokine levels in CSF during acute and post-acute infection

The proinflammatory markers IL-6 and CCL2 are elevated during acute infection in CSF (Witwer et al. 2009). At 10 days p.i., levels of IL-6 were elevated equally in the CSF of both untreated and HAART-treated macaques ($p=0.818$ and 0.662, respectively; Fig. 3a). CSF of untreated and HAART-treated macaques at 10 and 21 days p.i. all had significantly higher levels of IL-6 than uninfected macaques (10 days, $p=0.001$, 0.001, respectively; 21 days, $p=0.051$ and 0.044, respectively).
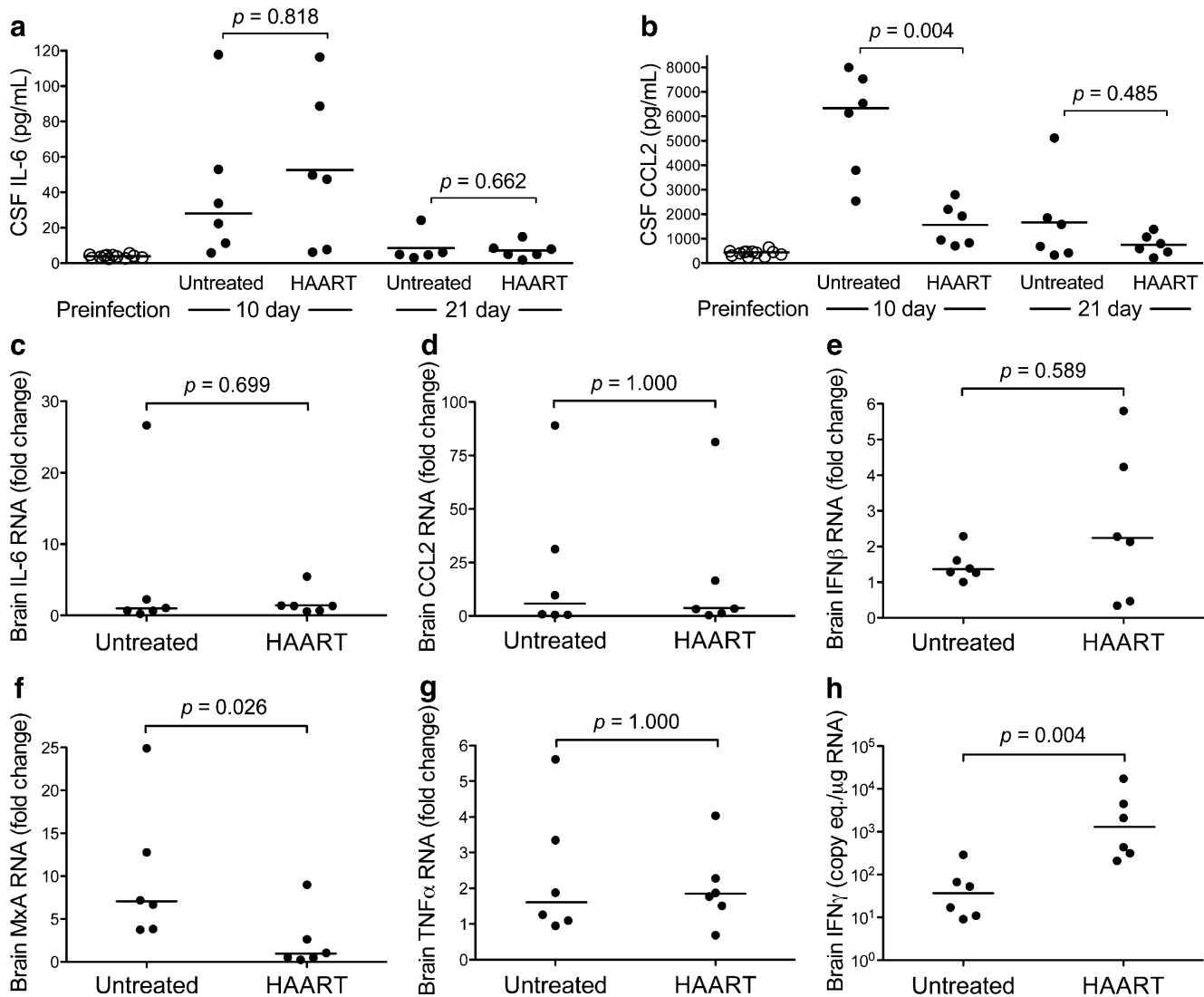
Fig. 3 Cytokine and chemokine levels in CSF during acute and post-acute infection. There were no significant differences in ELISA measures of CSF IL-6 (**a**) levels at days 10 or 21 p.i., whereas CSF CCL2 (**b**) levels were significantly lower in HAART-treated macaques compared with those of the untreated controls at day 10 p.i., but not day 21 p.i. Brain IL-6 (**c**), CCL2 (**d**), IFNβ (**e**), MxA (**f**), and TNFα (**g**) mRNA fold changes were assessed by real-time RT-PCR for treated and untreated macaques. There were no significant differences in CSF cytokine or chemokine RNA levels at day 21 p.i., except for the IFN responsive gene MxA. Brain IFNγ mRNA levels (**h**) were significantly higher in the HAART-treated macaques

At 10 days p.i., CCL2 was increased in the CSF of both HAART-treated macaques and untreated macaques as compared with that of the uninfected macaques ($p=$ 0.001). In addition, levels of CCL2 in the CSF of untreated macaques were significantly higher than in HAART-treated macaques ($p=0.004$; Fig. 3b). Elevated CSF CCL2 at 10 days p.i. would recruit large numbers of monocytes/macrophages to the brain. This likely represents a hyperactive immune response to virus, since both groups of animals were exposed to virus sufficient to induce appropriate adaptive immune responses. At 21 days p.i., levels of CCL2 also were elevated in the CSF of both groups of SIV-infected macaques as compared with those of the uninfected animals, but the differences did not reach statistical significance ($p=0.068$ and 0.122, respectively), and there was no significant difference between treated and untreated ($p=0.485$).

Cytokine and chemokine mRNA expression in brain at 21 days p.i.

We previously have reported that even at >150 days of HAART treatment, there were some proinflammatory responses in the brain parenchyma as indicated by increased CD68 and TNF alpha (Zink et al. 2010), suggesting the continual activation of virus in the brain

and the need for ongoing immune control. Given the lessened destruction of CD4+ T cells, CD8+ T cells, and NK cells that occurred with earlier initiation of HAART, we wished to determine the status of immune responses in the brains of treated animals at 21 days. We quantitated the expression of several cytokine and chemokine mRNAs by real-time RT-PCR directly in the brain (basal ganglia) of all SIV-infected macaques. There was no significant difference in expression of IL-6 or CCL2 mRNA in the brains of SIV-infected, HAART-treated versus untreated macaques (Fig. 3c, d).

IFNβ is critical for control of viral replication in the brain and is a sensitive indicator of ongoing immune responses (Barber et al. 2004a, 2006). Therefore, IFNβ mRNA in brain was examined to assess innate immune responses. There was no significant difference between IFNβ mRNA levels in the HAART-treated compared with those of the untreated macaques ($p=0.589$; Fig. 3e), suggesting that ongoing innate immune responses continued in the brain during HAART therapy. However, since there can be a discordance between mRNA expression and protein expression, we measured levels of MxA mRNA in HAART-treated macaques. We found that MxA was significantly lower in treated than in untreated macaques ($p=0.026$; Fig. 3f) at 21 days p.i. MxA mRNA expression is upregulated by IFNβ protein, and induction of MxA is greatly amplified compared with the levels of IFNβ

mRNA or protein. Thus, there must have been decreased IFNβ protein expression in the HAART-treated macaques that was not reflected in mRNA levels. There also were no differences in the levels of TNFα mRNA levels in the brains of HAART-treated and untreated macaques (Fig. 3g).

Levels of IFNγ are heightened in the brain of HAART-treated animals

Collectively, these data suggest that HAART has managed to reduce the mechanisms that likely lead to neurological disease (CCL2 and IFN-responsive genes) despite continued inflammation. It was our hypothesis that the reduction of viral burden during the acute phase allowed a more cooperative sharing of the burdens of immune defense between the adaptive and innate immune systems, as suggested by the increased trafficking of CD4+ and CD8+ T cells. An alternative hypothesis is that the increased number of immune cells in the periphery means an increased number in the brain, and these cells are not functional. Therefore, we measured the level of IFNγ in the brains of untreated and HAART-treated SIV-infected macaques, a cytokine specific to effector lymphocytes. IFNγ mRNA levels were significantly higher in the HAART-treated macaques than in untreated animals ($p=$

Fig. 4 Inflammatory responses in CNS at 21 days postinoculation. Levels of MHC class II (**a**), GFAP (**b**), and CD68 (**c**) expression were quantitated by immunohistochemical analysis in a 2-cm² area of basal ganglia of uninfected, untreated, and HAART-treated macaques at day 21 p.i. There was no statistically significant difference in the expression of MHC class II or GFAP in the treatment groups. There was a significant increase in CD68 expression in the SIV-infected, untreated macaques as compared with that of the uninfected macaques, and expression of CD68 was substantially down-regulated in the HAART-treated animals
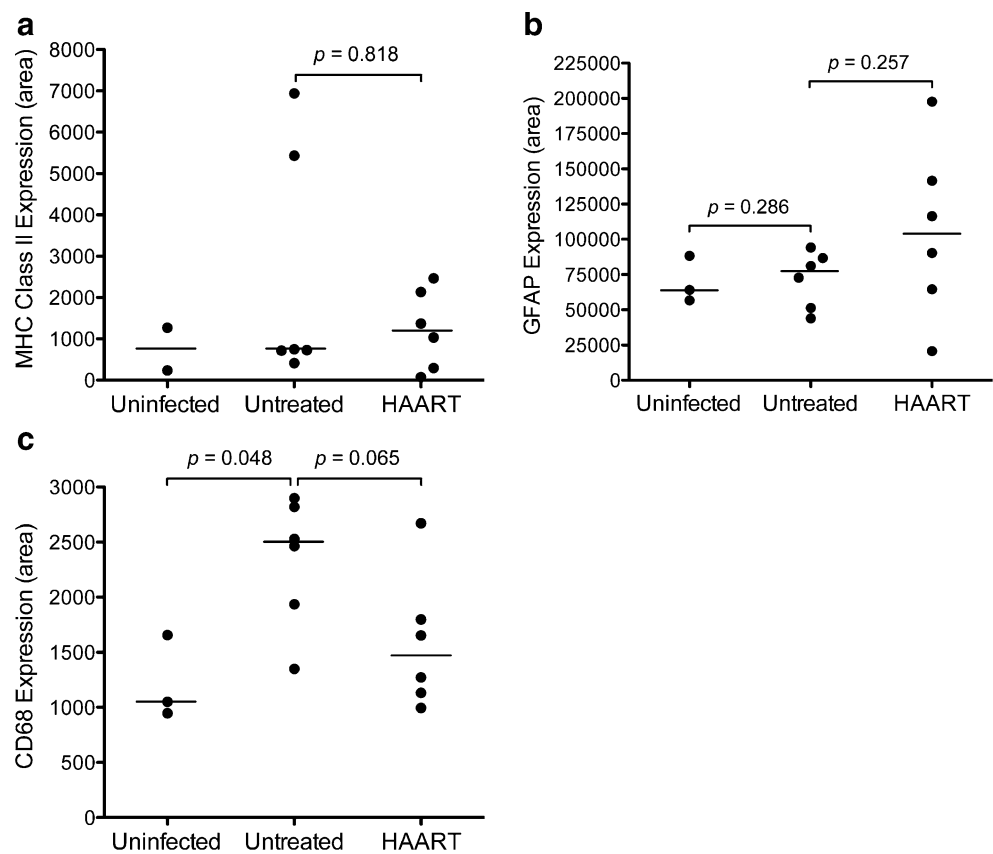
0.004; Fig. 3h). This suggested that T cells in the brain truly were effector T cells and that HAART treatment allowed the development of the adaptive immune responses that participate in controlling virus infection.

Inflammatory responses in CNS at 21 days p.i.

We assessed activation of macrophages and astrocytes in the brain. Sections of basal ganglia from HAART-treated and untreated, SIV-infected macaques were immunohisto-chemically stained with antibody to MHC class II, CD68, and GFAP (Fig. 4a–c). There was no significant difference in expression of MHC class II or GFAP in SIV-infected, untreated macaques as compared with that of the uninfected controls, and HAART did not decrease expression. There was a significant increase in CD68 expression in the SIV-infected, untreated macaques as compared with uninfected macaques, and expression of CD68 was substantially down-regulated in the HAART-treated animals (p=0.065).

Since virus replication occurs in macrophages in brain, the significant reduction of the CD68, an activation marker for these cells, likely reflects the reduction of virus replication in the brain corroborated by the substantially lower levels of viral RNA and further supports our hypothesis that early initiation of HAART therapy is able to preserve adaptive immune responses that work cooperatively with innate immune responses to control SIV infection in the brain.

Discussion

Our previous studies have shown a tremendous effect of the innate immune system in controlling SIV infection in the brain of infected macaques. Specifically, an early robust response driven by IFNβ is protective against neurological disease. IFNβ drives antiviral responses and also helps in the coordinated regulation of other inflammatory and cytokine responses as mediated by C/EBPβ (Barber et al. 2004a, b, 2006; Descombes and Schibler 1991; Dudaronek et al. 2007; Henderson and Calame 1997; Henderson et al. 1995, 1996; Honda et al. 1998; Hoshino et al. 2002; Li et al. 2007; Tanaka et al. 2005; Weiden et al. 2000). Failure of this coordinated regulation results in the development of neurological disease. This burden likely is due to the marked systemic immunosuppression that occurs during acute infection and is a hallmark of our model, since neurological disease is accelerated by dual inoculation with an immunosuppressive virus SIV/Delta B670 and a neuro-virulent virus SIV/17E-FR. These findings demonstrate HAART suppression of viral replication even when administered beginning at 4 days p.i. As suggested by advocates of a "test-and-treat" HIV treatment strategy, we observed a dramatic impact on viral load both in the periphery and CNS, and a protection of the cells most susceptible to depletion (principally CD4+ T cells, but also CD8+ T cells and NK cells). This potentially preserves the immune repertoire allowing for the development of effective adaptive immune responses to SIV infection and is evidenced by the increased number of putative CD4+ and CD8+ effector T cells trafficking into the brain of infected animals. The increased presence of effector cells in the brain in combination with decreased viral load may be responsible for the observed reductions in CCL2 levels and the IFN responsive gene MxA in the brain. Thus, we hypothesize that the foot has likely been removed from the accelerator by HAART therapy, and the adaptive immune system is working cooperatively with innate immune responses to control viral infection and avoid encephalitis.

A truly remarkable finding is the general lack of suppression of inflammatory responses in the brains of HAART-treated animals. We interpret these findings in light of the burden that the brain must be able to bear with regard to inflammation. Regardless of whether the brain is undergoing active virus replication, or adaptive immune responses, it must cap inflammatory responses, since inflammation quickly alters brain function. It is clear that the immune system (innate and adaptive) does not clear proviral DNA. Thus, as in the periphery, the innate immune system in the CNS must sound the alarm and fight to control virus. In the case of HAART, the CNS can at least rely on an adaptive immune response to assist in returning to the brain to homeostasis.

Control of SIV infection in the brain under HAART still needs to be elucidated. For example, although enhanced immune surveillance clearly is occurring in the brains of HAART treated animals, are these effector cells themselves infected? How long do cells loaded with antiretroviral drugs produce non-infectious virus? Is the non-infectious virus produced from cells undergoing HAART therapy equally stimulating to innate immune responses and thus contributing to a pro-inflammatory state, or in fact promoting protection in the brain by initiating IFNβ dependent responses like C/EBPβ? Some hints to these answers may be found by examining the kinetics of viral decay in the brain versus the periphery, where there is a dramatic difference in the slope of viral decay. Possible explanations for this phenomena are that viral levels in the brain are driven by virus replication in the periphery, that virus replication in the brain is a slower process, or that macrophages/microglia, the primary target for viral replication in the brain, do not replicate virus to the same high levels as lymphocytes. Clearly, multiple time points in the brain must be examined in order to fully understand what the rates of decay of cells in the brain harboring virus are depending on the timing of HAART intervention. However,

taken collectively with our study examining initiation of HAART at 12 days p.i., it is likely that the 4-day HAART-treated animals at minimum have more resources to bear toward clearing viral infection.

These data suggest that we must carefully consider the test-and-treat philosophy in the context of HIV neurological disease, given that we are now observing approximately 50% of individuals in the HAART era afflicted by HIV cognitive disorder. Not only does there appear to be a boost in adaptive immune responses that actively participate in control of viral replication in the brain but there also is the problem of the CNS viral DNA reservoir that must be addressed from the earliest stages of infection if we hope to be able to clear HIV infection through treatment.

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