Enhanced PD-1 Expression by T Cells in Cerebrospinal Fluid Does Not Reflect Functional Exhaustion during Chronic Human Immunodeficiency Virus Type 1 Infection†

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During chronic viral infections, T cells are exhausted due to constant antigen exposure and are associated with enhanced programmed death 1 (PD-1) expression. Deficiencies in the PD-1/programmed death-ligand 1 (PD-L1) pathway are associated with autoimmune diseases, including those of the central nervous system (CNS). To understand the role of PD-1 expression in regulating T-cell immunity in the CNS during chronic infection, we characterized PD-1 expression in cerebrospinal fluid (CSF) and blood of individuals with chronic human immunodeficiency virus type 1 (HIV-1) infection. PD-1 expression was higher on HIV-specific CD8+ T cells than on total CD8+ T cells in both CSF and blood. PD-1 expression on CSF T cells correlated positively with CSF HIV-1 RNA and inversely with blood CD4+ T-cell counts, suggesting that HIV-1 infection drives higher PD-1 expression on CSF T cells. However, in every HIV-positive individual, PD-1 expression was higher on T cells in CSF than on those in blood, despite HIV-1 RNA levels being lower. Among healthy HIV-negative controls, PD-1 expression was higher in CSF than in blood. Furthermore, frequencies of the senescence marker CD57 were lower on CSF T cells than on blood T cells, consistent with our prior observation of enhanced ex vivo functional capacity of CSF T cells. The higher PD-1 expression level on CSF T cells therefore does not reflect cellular exhaustion but may be a mechanism to downregulate immune-mediated tissue damage in the CNS. As inhibition of the PD-1/PD-L1 pathway is pursued as a therapeutic option for viral infections, potential effects of such a blockade on development of autoimmune responses in the CNS should be considered.

Programmed death 1 (PD-1; also called CD279) and its ligands, PD-L1 (also called B7-H1 or CD274) and PD-L2 (also called B7-DC or CD-273), regulate T-cell activation, peripheral tolerance, and autoimmunity (22, 43). PD-1 can be expressed on CD8+ and CD4+ T cells, B cells, natural killer T cells, and activated monocytes. PD-L1 is expressed on various cells, including T and B cells, dendritic cells, macrophages, mast cells, nonhematopoietic cell types (including vascular endothelial cells, pancreatic islet cells, astrocytes, keratinocytes, and microglial cells), and cells in immune privileged sites, including the placenta and the eye (22). PD-L2 expression is inducible and is restricted to dendritic cells, monocytes, macrophages, and mast cells (22). During chronic infections, the PD-1/PD-L1 pathway inhibits antigen-specific T-cell responses (7, 8, 35, 46). In human immunodeficiency virus type 1 (HIV-1)-infected individuals, PD-1 expression on HIV-specific T cells in peripheral blood is upregulated and correlates positively with plasma viremia and inversely with CD4+ T-cell counts (7, 46). PD-1 expression on HIV-specific T cells is also associated with T-cell exhaustion, as defined by a reduced ability to proliferate and produce cytokines (7, 46). Inhibition of the PD-1/PD-L1 pathway augments HIV-specific CD8+ and CD4+ T-cell function, and antiretroviral therapy is associated with a significant reduction of PD-1 expression on HIV-specific T cells in peripheral blood (8).

The PD-1/PD-L1 pathway also limits immune-mediated tissue damage that may be caused by overreactive peripheral T cells, especially in immune privileged sites such as the central nervous system (CNS). In 1999, the importance of PD-1 for peripheral tolerance was first suggested by studies which showed that PD1−/− mice develop lupus-like autoimmune diseases (32). In humans, polymorphisms in the PDCD1 gene, which encodes PD-1, have been associated with autoimmune diseases, including lupus, diabetes, rheumatoid arthritis, and multiple sclerosis (20, 21, 25). Upregulation of PD-L1 in multiple sclerosis lesions from human brain tissue suggests a role for the PD-1/PD-L1 pathway in regulating T-cell activation and controlling immunopathological damage (33).

The CNS is involved by HIV-1 early during primary infection (6, 13), and approximately 40% of patients who develop advanced AIDS without receiving antiretroviral therapy develop cognitive impairment (6, 13, 38). While HIV-1 proteins gp120 (3, 16) and Tat (30) are directly neurotoxic and may contribute to HIV-associated dementia, detrimental neuropathogenic effects have also been postulated for inflammatory and innate immune cells, especially monocytes/macrophages and T cells (11, 19, 49, 50). Immune responses cause neuropathogenesis during other viral infections, and cytotoxic T lymphocytes can worsen the disease through direct cytotoxicity or release of inflammatory cytokines such as gamma interferon.
(IFN-γ) (14). However, we recently described higher frequencies of functional HIV-specific CD8+ T cells in cerebrospinal fluid (CSF) than in blood among asymptomatic HIV-positive individuals with little or no HIV-1 RNA in CSF, suggesting that HIV-1-specific CD8+ T cells help to control intrathecal viral replication (40).

To understand the role of the PD-1/PD-L1 pathway in regulating T-cell responses during viral infection of the CNS, we characterized PD-1 expression on T cells in CSF and peripheral blood among asymptomatic HIV-positive individuals. We hypothesized that T-cell PD1 expression would be lower in CSF than in blood, since HIV-1 RNA concentrations are lower in CSF than in plasma and the magnitude and breadth of IFN-γ-secreting HIV-specific T cells are greater in CSF than in blood (40). We show that, in CSF, HIV-1 RNA correlates directly with PD-1 expression on CD4+, CD8+, and HIV-specific CD8+ T cells. Unexpectedly, PD-1 expression on all T cells is higher in CSF than in blood in HIV-positive patients and healthy HIV-negative controls. In contrast, expression of the senescence marker CD57 is lower in CSF than in blood. These data suggest that higher PD-1 expression on T cells in CSF may be a mechanism to regulate T-cell immunity in the CNS, rather than indicating T-cell exhaustion, and that this regulation is increased by HIV-1 replication.

MATERIALS AND METHODS

Study participants. Individuals with chronic HIV-1 infection were recruited for study participation from the Vanderbilt University-affiliated Comprehensive Care Center (Nashville, TN). Of the 15 participants in the present study, 4 were included in a previous report (40). Participants were antiretroviral naïve. Additional eligibility criteria included at least 100,000 platelets/mm3 within 1 week prior to lumbar puncture, no history of significant CNS abnormality, such as trauma, congenital malformation, or genetic disorder, and no physical examination or magnetic resonance imaging (MRI) evidence of a CNS mass lesion.

Flow cytometry. Mononuclear cells from CSF were pelleted by centrifugation at 350 x g for 15 min at 4°C. Approximately 100 ml of peripheral blood was obtained immediately before lumbar puncture and processed in parallel. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation.

Flow cytometry. Mononuclear cells from CSF and blood were labeled ex vivo with a panel of fluorochrome-labeled antibodies. Freshly isolated cells were incubated sequentially with HIV-specific HLA class I tetramers (labeled with phycoerythrin or allophycocyanin; Beckman Coulter or NIH Tetramer Facility) at room temperature for 10 min, anti-PD-1 (Biologend) at room temperature for 20 min, and goat anti-mouse immunoglobulin G (Pacific Blue; Invitrogen) (secondary antibody for PD-1 antibody) for 30 min on ice, followed by incubation with a cocktail of antibodies to CD3, CD8, CD4, CD14, CD19, CD56, and 7-aminoactinomycin D as described above.

Cytokine assays. A cytokine bead array assay (BD Biosciences) was used to quantitate IL-2 and IL-7 in CSF following the manufacturer's instructions. The theoretical lower limit of detection for the assay was 11.2 pg/ml for IL-2 and 0.5 pg/ml for IL-7.

Neurological examinations and neuroimaging. Prior to lumbar puncture, each participant underwent a complete neurological examination, as well as standard structural MRI on a 3.0 T Achieva MR scanner (Philips Medical Systems) with an eight-channel Sense (sensitivity-encoding) head coil to rule out significant structural brain abnormalities. Structural MRIs consisted of standard T1-weighted, T2-weighted, and fluid-attenuated inversion recovery (FLAIR) sequences. Regional structural brain abnormalities were characterized using a volumetric region of interest analysis approach to quantify total white matter volume. The average structural MRI on a 3.0 T Achieva MR scanner was used to identify regions of the brain with significant structural abnormalities. MRI findings were correlated with clinical and demographic data.

RESULTS

Comparisons of viral loads and T-cell numbers in peripheral blood and CSF. We compared CSF and blood of 15 HIV-infected adults. The duration of known HIV positivity ranged from 1 to 17 years (median = 8 years). All participants were asymptomatic, had normal neurological examinations, and had no structural abnormalities on neuroimaging. Basic parameters from CSF and peripheral blood are listed in Table 1. Concentrations of HIV-1 RNA in CSF correlated with those in blood (r = 0.8634; P < 0.0001) but were lower in CSF (median = 3.4 log10 copies/ml) than in blood (median = 4.0 log10 copies/ml). Ratios of CD4+ to CD8+ T cells were similar in CSF (median = 0.6) and blood (median = 0.9) (P > 0.05). All participants had CSF total protein levels of <40 mg/dl. Differential counting of CSF cells identified neither neutrophils nor evidence of erythrocyte contamination for any individual, indicating that CSF lymphocytes were not contaminated by peripheral blood lymphocytes introduced during

<table>
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<th>Subject ID</th>
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a The value <1.7 indicates that the level was below the assay limit of quantification (50 HIV-1 RNA copies/ml).
lumbar puncture. The median number of mononuclear cells isolated from CSF was 35,000 (range, 20,000 to 300,000).

Enhanced expression of PD-1 on T cells in CSF compared to that in blood. At least 5,000 freshly isolated CSF cells from each HIV-positive participant were evaluated, in parallel with PBMCs, for PD-1 expression on CD4+ and CD8+, and HIV-specific or cytomegalovirus (CMV)-specific CD8+ T cells (Fig. 1A to C). HIV-specific or CMV-specific CD8+ T cells were detected with HLA class I tetramers chosen based on earlier screening with peripheral blood. Depending on the total yield of CSF cells, we assayed for as many of these tetramer responses as was feasible, using paired CSF and blood samples. Blood and CSF were analyzed with 10 HIV-specific and 4 CMV-specific tetramers, based on the HLA types of the participants, with a total of 24 individual HIV-specific and 6 individual CMV-specific tetramer responses (see Table S1 in the supplemental material). Frequencies of HIV-specific CD8+ T cells in CSF (median = 2.6%) were higher than those in blood (median = 1.2%) (P = 0.0002), as observed previously in a cohort of HIV controllers (40). In contrast, frequencies of CMV-specific CD8+ T cells tended to be lower in CSF (median = 0.7%) than in blood (median = 2.4%) (P = 0.06) (see Fig. S1 in the supplemental material).

The mean fluorescence intensities (MFIs) of PD-1 expression on CD4+, CD8+, and HIV- and CMV-specific CD8+ T cells were higher in CSF than in blood (Fig. 1C and 2). PD-1hi T cells were gated on CD8+ T cells, and that gate was applied to CD4+ and HIV- and CMV-specific CD8+ T cells (Fig. 1C). Frequencies of PD-1hi T cells were also greater among CD4+, CD8+, and CMV-specific CD8+ T cells (Fig. 2). Among HIV-specific CD8+ T cells in CSF and blood, there was no significant difference in frequency for the PD-1hi subset, apparently because HIV-specific CD8+ T cells in blood were already enriched (>80%) for the PD-1hi subset. To determine whether enhanced PD-1 expression on T cells in CSF was a consequence of HIV infection, we studied five healthy, HIV-negative controls. Among these controls, MFIs of PD-1 expression and frequencies of PD-1hi subsets on total CD4+ and CD8+ cells were also higher in CSF than in blood (Fig. 3), indicating that enhanced PD-1 expression on T cells in CSF is an aspect of normal cellular physiology, rather than solely a consequence of HIV infection.

Higher proportions of memory CD8+ and CD4+ T cells in the CNS have been observed under both normal and pathological conditions (31, 48). To ensure that the apparent enhanced PD-1 expression on T cells in CSF was not simply due to an absence of naïve T cells, CSF and blood cells from five HIV-positive individuals were also stained for CD62L and...
CD45RO in an expanded panel and analyzed on an LSRII flow cytometer. With naïve T cells (CD45RO<sup>−</sup> /H11002<sup>−</sup>CD62L<sup>−</sup> /H11001<sup>−</sup>) excluded from the analysis, PD-1 expression on the remaining memory T cells was still higher in CSF (CD8<sup>+</sup>/H11001<sup>−</sup>T-cell PD-1 MFI median = 833; CD4<sup>+</sup>/H11001<sup>−</sup>T-cell PD-1 MFI median = 813) than in blood (CD8<sup>+</sup>/H11001<sup>−</sup>T-cell PD-1 MFI median = 460 [<i>P</i> = 0.06]; CD4<sup>+</sup>/H11001<sup>−</sup>T-cell PD-1 MFI median = 424 [<i>P</i> = 0.06]). Failure to achieve statistical significance likely reflects the small sample size for this subanalysis. Among all 15 HIV-positive patients, PD-1 expression on CD45RO<sup>+</sup> memory T cells was also significantly higher in CSF (CD4<sup>+</sup> T-cell MFI median = 1,838; CD8<sup>+</sup> T-cell MFI median = 2,584) than in blood (CD4<sup>+</sup> T-cell MFI median = 1,038 [<i>P</i> < 0.0001]; CD8<sup>+</sup> T-cell MFI median = 1,517 [<i>P</i> < 0.0001]). Among five healthy controls, PD-1 expression on CD45RO<sup>+</sup> memory T cells tended to be higher in CSF (CD4<sup>+</sup> T-cell MFI median = 1,268; CD8<sup>+</sup> T-cell MFI median = 2,680) than in blood (CD4<sup>+</sup> T-cell MFI median = 935 [<i>P</i> = 0.06]; CD8<sup>+</sup> T-cell MFI median = 1,290 [<i>P</i> = 0.06]).

**Increased PD-1 expression on HIV-specific T cells in CSF and blood.** In peripheral blood, PD-1 expression has been shown to be higher on HIV-specific T cells than on total CD8<sup>+</sup> and CMV-specific CD8<sup>+</sup> T cells (7, 35, 46). To test whether HIV-specific T cells in CSF also have higher expression levels ofPD-1, we compared PD-1 expression on different T-cell subsets in CSF and blood from HIV-positive individuals (Fig. 4). Consistent with previous reports, PD-1 expression on T cells in peripheral blood was significantly higher on HIV-specific CD8<sup>+</sup> T cells (MFI median = 1,693) than on total CD8<sup>+</sup>
T cells (MFI median = 822; \( P = 0.002 \)) or CMV-specific CD8\(^+\) T cells (MFI median = 476; \( P = 0.01 \)) (Fig. 4A). Similar to the case of blood, PD-1 expression on HIV-specific CD8\(^+\) T cells in CSF (MFI median = 3,703) was significantly higher than that on total CD8\(^+\) T cells in CSF (MFI median = 2,429; \( P = 0.025 \)) and somewhat higher than that on CMV-specific CD8\(^+\) T cells in CSF (MFI median = 1,156; \( P = 0.08 \)) (Fig. 4B).

**PD-1 expression in CSF correlates with status of HIV disease.** PD-1 expression on CD4\(^+\), CD8\(^+\), and HIV-specific CD8\(^+\) T cells in peripheral blood from HIV patients has been shown to correlate with disease progression (i.e., with higher plasma HIV-1 RNA concentrations and lower CD4\(^+\) T-cell counts) (7, 35, 46). We similarly found a direct correlation between PD-1 MFIs on CD4\(^+\), CD8\(^+\), and HIV-specific CD8\(^+\) T cells in blood and the HIV-1 RNA concentration in plasma (\( r = 0.83 \) for CD4\(^+\) T cells, \( r = 0.84 \) for CD8\(^+\) T cells, and \( r = 0.74 \) for HIV-specific CD8\(^+\) T cells) and an inverse correlation with peripheral blood CD4\(^+\) T-cell counts (\( r = -0.68 \) for CD4\(^+\) T cells, \( r = -0.6 \) for CD8\(^+\) T cells, and \( r = -0.4 \) for HIV-specific CD8\(^+\) T cells). To evaluate determinants of PD-1 expression on T cells in the CNS, we characterized relationships between the MFI of PD-1 expression on T cells in CSF,
HIV-1 RNA in CSF, and peripheral blood CD4+ T-cell counts (Fig. 5). As in blood, MFIs of PD-1 expression on CD4+, CD8+, and HIV-specific CD8+ T cells in CSF correlated positively with HIV-1 RNA in CSF (\(r\) 0.61 for CD4+ T cells, \(r\) 0.51 for CD8+ cells, and \(r\) 0.32 for HIV-specific CD8+ T cells) and inversely with blood CD4+ T-cell counts (\(r\) = −0.56 for CD4+ T cells and \(r\) = −0.59 for CD8+ T cells).

**Lower frequencies of CD57hi T cells in CSF than in blood.** In addition to PD-1, we evaluated expression of CD57, a marker for T-cell senescence (4), on T cells in CSF. CD57+ T cells have low proliferation and expansion potential, although they can secrete the cytokines IFN-\(\gamma\) and tumor necrosis factor alpha (TNF-\(\alpha\)) in response to cognate antigen (4, 26). We have previously shown that HIV-specific T cells in CSF have the ability to expand readily ex vivo and that among CSF T cells expanded with phytohemagglutinin, HIV-specific IFN-\(\gamma\) responses are broader and of higher magnitude among cells from CSF than among those from blood (40). With anti-CD57 staining, we identified CD57hi, CD57int, and CD57lo populations (Fig. 6A). Gates drawn on CD8+ T cells were applied to CD4+ and HIV- and CMV-specific CD8+ T-cell populations. Frequencies of CD57hi subsets on CD4+, CD8+, and HIV- and CMV-specific CD8+ T cells were lower in CSF (CD4+ median, 1.5%; CD8+ median, 13.7%; HIV-specific CD8+ median, 20.8%; CMV-specific CD8+ median, 11.2%) than in blood (CD4+ median, 3.2%; CD8+ median, 27.5%; HIV-specific CD8+ median, 24.8%; CMV-specific CD8+ median, 53.8%) (Fig. 6B to E). When CD57 expression levels were analyzed based on any level of positivity (i.e., combining the CD57hi and CD57int subsets), there was no significant difference between frequencies of these subsets in CSF and blood among CD4+, CD8+, and HIV-specific CD8+ T cells. T cells in CSF had increased levels of CD57hi populations, while T cells in blood had increased levels of CD57int populations. To ensure that differences in CD57 expression levels on CSF and blood T cells were not due to differences in frequencies of memory T cells, we analyzed CD57 expression levels on memory T cells in CSF and blood. Frequencies of CD57hi subsets were lower in CSF (CD4+ median, 1.5%; CD8+ median, 16.7%) than in blood (CD4+ median, 4.3% \([P = 0.0007]\); CD8+ median, 21.8% \([P = 0.02]\) among CD45RO+ CD4+ and CD8+ T cells. For five HIV-positive patients analyzed additionally for CD62L, frequencies of CD57hi subsets tended to be lower in CSF (CD4+ median, 1%; CD8+ median, 7.1%) than in blood (CD4+ median, 2.6% \([P = 0.06]\); CD8+ median, 22.2% \([P = 0.06]\) among memory (based on exclusion of CD45RO+ CD62L+ naïve T cells) CD4+ and CD8+ T cells. Thus, T cells in CSF, although expressing higher levels of PD-1, may retain substantial proliferative potential, as indicated by lower frequencies of CD57hi T cells. This is consistent with our prior study in which we observed good expansion potential of HIV-specific T cells from CSF as well as better functional T-cell responses in terms of breadth and magnitude of...
IFN-γ responses to HIV-specific peptide stimulation than those in cells from blood (40). Frequencies of CD57hi T-cell subsets on CD8+ and HIV-specific CD8+ T cells in CSF did not correlate with the MFI of PD-1 expression (see Fig. S2 in the supplemental material). For CD4+ and CMV-specific CD8+ T cells, there was a trend between frequencies of CD57hi T-cell subsets and the MFI of PD-1 expression (see Fig. S2 in the supplemental material). Among CD45RO+ memory T cells, frequencies of CD57hi T-cell subsets on CD8+ T cells did not correlate with the MFI of PD-1 expression (r = 0.18; P was not significant), but there was a correlation among CD4+ CD45RO+ memory T cells (r = 0.55; P = 0.03). Lower CD57 expression levels on CD8+ T cells in CSF and the lack of correlation with PD-1 expression may explain the maintenance of proliferative potential of CSF T cells, despite relatively high levels of PD-1 expression.

**Incubating PBMCs in CSF does not induce PD-1 expression.** The common γ-chain cytokines IL-2, IL-7, IL-15, and IL-21 play an important role in peripheral T-cell expansion and survival and upregulate PD-1 expression on T cells in vitro in an antigen-independent manner. To determine whether enhanced PD-1 expression on CSF T cells is a consequence of CSF exposure, perhaps due to cytokines/chemokines in CSF, PBMCs from six HIV-positive individuals and two HIV-negative healthy controls were cultured for 7 days in autologous CSF alone, R-10 culture medium alone, or R-10 with 100 U of IL-2 (see Fig. S3 in the supplemental material). Culture in CSF or R-10 alone did not enhance the MFI of PD-1 expression or the frequency of PD-1hi CD8+ or CD4+ T cells compared to direct staining for PD-1 on PBMCs without ex vivo culture. In contrast, culture with IL-2 significantly enhanced the MFI of PD-1 expression on CD8+ and CD4+ T cells (P = 0.02) (see Fig. S3 in the supplemental material). Neither IL-2 nor IL-7 was detectable in CSF of the 15 HIV-positive individuals in this study (the assay’s lower limit of detection was 11.2 pg/ml for IL-2 and 0.5 pg/ml for IL-7). These results indicate that enhanced PD-1 expression is not caused by γ-chain cytokines in

![FIG. 6. Lower frequencies of CD57hi T cells in CSF than in blood of HIV-positive patients.](image-url)
DISCUSSION

Immune control of viral infections in the CNS requires suppression or elimination of virus while minimizing immune-mediated injury to vital cells. T-cell responses in the CNS must therefore be highly regulated. PD-1 is a negative immunoregulatory factor that has also been implicated in exhaustion of T cells during chronic infection. In the present study, we examine the role of PD-1 expression on CSF T cells during chronic asymptomatic HIV-1 infection. We show that PD-1 expression on T cells is upregulated in CSF compared to that in blood. Data from lymphocytic choriomeningitis virus models as well as previous studies of HIV-positive patients suggest that high levels of PD-1 expression on virus-specific T cells are indicative of T-cell exhaustion due to persistent antigen exposure (2, 7, 10, 35, 46). Our study of PD-1 expression on T cells in CSF and blood provides a unique opportunity to relate HIV-1 replication and immune responses within two distinct compartments in the same individual. We show that PD-1 expression on T cells in CSF is enhanced above that on blood T cells, despite lower HIV-1 RNA concentrations in CSF than in plasma. We also show that compared to blood T cells, CSF T cells downregulate the senescence marker CD57. We also previously reported that T cells have broader and higher magnitudes of HIV-specific IFN-γ responses in CSF than those in blood (40). Furthermore, among healthy, HIV-negative controls, we also found higher PD-1 expression on T cells in CSF than on those in blood. These findings indicate that the higher PD-1 expression on CSF T cells may not necessarily reflect exhaustion of T cells, but rather may be a mechanism to control T-cell activation and prevent immune-mediated damage in the CNS.

The senescence marker CD57 was present at a lower frequency on T cells in CSF than on those in blood among these asymptomatic HIV-positive patients. CD57+ T cells have undergone more cell divisions, have shorter telomeres, have limited ability to proliferate, and are more prone to activation-induced cell death (4). CD8+CD57+ T cells are antigen-driven effector cells still capable of producing IFN-γ and TNF-α and having very high effector cytotoxic capabilities (26). Smaller amounts of HIV-1 antigens in CSF than in blood may, at least in part, explain the relatively lower frequencies of CD57+ T cells in CSF. CD8+CD57+ T cells were found to have higher expression levels of CX3CR1 than CD8+CD57− T cells (26), and CX3CL1, a ligand for CX3CR1, is upregulated in astrocytes of patients with HIV-associated dementia (34, 45). It will be important to determine whether patients with HIV-associated dementia have higher expression levels of CD57 on T cells in CSF and whether these are also CX3CR1+.

In HIV-positive patients, the infection appears to drive higher expression of PD-1 on T cells in CSF. PD-1 expression on HIV-specific CD8+ T cells is higher than on total CD8+, CD4+, and CMV-specific CD8+ T cells, and PD-1 expression on CD8+ and CD4+ T cells in CSF correlates directly with HIV-1 RNA in CSF and inversely with blood CD4+ T-cell counts. Several studies have shown that PD-1 is upregulated on HIV-specific CD8+ T cells in blood and that PD-1 expression correlates with higher plasma viral loads and lower CD4 counts, predictors of disease progression (7, 35, 46). To our knowledge, this is the first human study to show enhanced PD-1 expression on HIV-specific CD8+ T cells in CSF and to correlate PD-1 expression with HIV-1 RNA in another anatomic compartment. In another chronic viral disease, hepatitis C virus infection, enhanced PD-1 expression on T cells from blood and liver is associated with immune dysfunction, with decreased proliferation and IFN-γ and IL-2 secretion (12, 37). In vitro blockade of PD-1 by anti-PD-L1 and anti-PD-L2 antibodies restores functional competence of these cells. In contrast to intrathecal HIV-specific PD-1hi T cells, intrahepatic hepatitis C virus-specific PD-1hi T cells express high levels of CD57 (12). It is possible that although expansion potential and IFN-γ production were not impaired for CSF T cells (40), there may be defects in secretion of other cytokines, such as TNF-α and/or IL-2. The limited number of lymphocytes obtained from CSF limited our ability to assess the polyfunctionality of HIV-specific CD8+ T cells.

This study has potential implications for understanding immune-mediated neuropathogenesis. Normal uninfected brain has multiple intrinsic mechanisms to maintain immune quiescence. These include tight junctions and low levels of adhesion molecule expression by capillary endothelial cells, neuronal expression of CD200, which reduces microglial cell activation, local production of neurotrophins, and transforming growth factor beta secretion by astrocytes and meningeal cells (14). The PD-1/PD-L1 pathway also plays an important role in controlling inflammation in the CNS. Human and mouse microglial cells from the CNS constitutively express PD-L1, and PD-L1 expression is upregulated on microglia under both in vitro (external cytokine stimulation) and in vivo (experimental autoimmune encephalitis and multiple sclerosis) inflammatory conditions (27, 33, 41). In mice, expression levels of PD-L1 were substantially higher on microglial cells than on splenocytes, upon stimulation (27). Thus, PD-L1 expression helps to minimize inflammatory damage by activated immune cells in the CNS. Our finding that PD-1 levels are higher on T cells in CSF than on those in blood, not only in subjects with HIV-1 infection but also in healthy HIV-negative individuals without inflammation, suggests that upregulation of PD-1 on CSF T cells may be a general mechanism which, in combination with upregulation of PD-L1 during inflammation, limits T-cell-mediated damage in the CNS. The present study focused on antiretroviral-naive HIV-infected individuals without neurological symptoms. In patients with neuro-AIDS, exhaustion of HIV-specific CD8+ T cells in the CNS under conditions of chronic antigen exposure may lead to eventual loss of viral control in the CNS and to the development of neurocognitive symptoms. Characterization of T cells in HIV-positive individuals with advanced AIDS, particularly those with neuropsychological impairment, will further our understanding of the role of PD-1 expression on CNS T cells in the control of HIV replication and immune-mediated neuropathogenesis.

Higher PD-1 expression on T cells in CSF could be explained by either preferential migration of PD-1hi T cells into the CNS, selective retention of PD-1hi T cells in the CNS, or upregulation of PD-1 after T cells enter the CNS. Recent studies indicated that PD-1 expression may also be enhanced during activation and differentiation of T cells (42). HIV-
Epstein-Barr virus- and influenza virus-specific CD8 T cells are mostly PD-1hi, whereas CMV-specific T cells are less frequently PD-1hi (7, 15, 35). In macaques, PD-1 expression increases following DNA vaccination and during persistent lentiviral infection (18). Earlier induction of PD-1 in simian immunodeficiency virus (SIV)-infected sooty mangabeys, with subsequent reduction in immune activation, distinguishes non-immunodeficiency virus (SIV)-infected sooty mangabeys from pathogenic infection in macaques (9). Thus, not all PD-1hi T cells may be exhausted but may indicate a balanced reaction to immune-activating stimuli. In macaques, activated T cells preferentially enter the CNS and increase in frequency early after acute SIV infection (23, 29). In humans, studies showing activated T cells in CSF among both HIV-infected and HIV-uninfected individuals (29, 31, 39) support homing of activated T cells to the CNS. Other groups have noted selective recruitment of memory T cells to the brain and higher proportions of CD8+ memory T cells in the brains of both HIV-infected and uninfected individuals under both normal and neuropathologic conditions (31, 44, 48). Although enhanced PD-1 expression on CSF T cells could be due to an increase frequency of activated memory T cells in CSF, we found PD-1 expression to be higher in CSF even in analyses restricted to memory T-cell subpopulations. The common gamma-chain (γc) cytokines IL-2, IL-7, IL-15, and IL-21 play an important role in peripheral T-cell expansion and survival. These cytokines upregulate PD-1, and all but IL-21 upregulate PD-L1 on T cells (24). γc cytokine-induced PD-1 does not interfere with cytokine-driven peripheral T-cell expansion/survival but may suppress certain effector functions upon T-cell receptor engagement, thereby minimizing immune-mediated damage to the host. Various cytokines, including IL-2, IL-12, IL-23, IFN-γ, and TNF-α, are produced in the CNS by different neural cells under pathological as well as physiological conditions (5). SIV infection in rhesus macaques leads to enhanced brain levels of IL-15, which has been implicated in enrichment and persistence of SIV-specific T cells in the CNS (28). In the HIV-positive individuals in the present study, IL-2 and IL-7 were undetectable in CSF, and culturing PBMCs ex vivo in autologous CSF did not enhance PD-1 expression on these cells. It is possible that there are cytokines/chemokines produced in the CNS, but undetectable in CSF, that could upregulate PD-1 expression on T cells in the CNS even under normal conditions.

In animal models of virus infection (lymphocytic choriomeningitis virus-infected mice and SIV-infected macaques), in vivo inhibition of the PD-1/PD-L1 pathway by use of monoclonal antibodies has been shown to enhance expansion, cytokine production, and cytotoxicity of virus-specific T cells and to reduce viral replication (2, 47). Humanized anti-PD-1 antibody has been approved for testing in clinical trials for cancer and infectious diseases. Our study suggests that as PD-1 inhibition is pursued as a therapeutic strategy, consideration should be given to potential effects on PD-1/PD-L1-induced T-cell tolerance in the CNS. In this regard, inhibiting PD-L1 during experimental autoimmune encephalitis or diabetes exacerbates disease (1, 41). Therapeutic options for autoimmune diseases currently being tested in animal models include enhancement of PD-L1 expression, leading to enhanced PD-1/PD-L1 signaling (17, 36).

In summary, among asymptomatic HIV-infected individuals, expression of PD-1 is enhanced on CD8+, CD4+, and HIV-specific CD8+ T cells in CSF. This enhanced PD-1 expression in CSF, however, is not associated with T-cell senescence. Enhanced PD-1 expression in the CNS may be a physiologic mechanism to prevent immune-mediated neuronal damage.

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