

High Numbers Of Activated CD8+ T Cells Targeting HIV Antigens Are Present In Cerebrospinal Fluid In Hyperacute HIV Infection

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ABSTRACT

Infiltration by CD8⁺ T cells into the central nervous system (CNS) is a recognized feature in many neurodegenerative diseases that are associated with neuroinflammation, such as HIV-associated dementia. The frequency of CD8⁺ T cells in cerebrospinal fluid (CSF) is elevated during HIV infection compared to other CNS diseases; however, the role of CD8⁺ T cells in the CNS during acute infection is unknown. We analyzed CD8⁺ T cells in the CSF from a unique cohort, RV254/SEARCH010, in the earliest stages of acute HIV infection. CD8⁺ T cells in the CSF were elevated in HIV individuals during acute infection compared to uninfected. Activated CSF CD8⁺ T cells positively correlated to CSF viral load and to markers of CNS inflammation, neopterin, CD163, and IP-10 whereas activated CSF CD8⁺ T cells in chronic infection were associated with markers of neuronal damage (neurofilament light) and the astroglial activation marker YKL-40. Importantly, we showed that CSF CD8⁺ T cells exhibited a unique restricted TCR Vbeta repertoire and contained HIV-specific CD8⁺ T cells directed to unique HIV epitopes compared to the periphery. Our data suggest that although CSF CD8⁺ T cells in chronic infection are associated with neuropathogenesis, CD8⁺ T cells in hyperacute HIV infection in CSF contain high number of activated cells with increased functional gene expression profile and directed against HIV antigens suggesting that could they could play a potential beneficial role in early acute infection to prevent development HIV-associated neurocognitive disorders if ART is initiated at that stage.

Keywords

HIV, cytotoxic T lymphocytes, HIV-specific CD8⁺ T cells, neuroinflammation, HIV-associated neurocognitive disorders

BACKGROUND

HIV infects the central nervous system (CNS) within days of initial exposure and induces neuroinflammation that includes invasion of infected mononuclear cells and subsequent activation of localized inflammatory cells such as perivascular macrophages, microglia and astrocytes. These activated CNS resident cells release an array of neurotoxins that can be measured in the CSF [1], and additional neuronal injury can occur directly from HIV proteins, such as tat and gp120 [2]. This inflammatory environment in the CNS results in blood brain barrier permeability, neuronal abnormalities and an increased likelihood for the clinical manifestation of HIV-associated neurocognitive disorders (HAND). Antiretroviral therapy (ART) reduces CSF HIV RNA levels and changes the pathogenesis of HIV infection, leading to milder forms of HAND without impacting its overall prevalence [3, 4]. A critically important recent recognition is that tissue damage persists despite ART due to incomplete eradication of HIV reservoirs and sustained CNS inflammation, in part as a result of limitations in CNS drug penetration [5-7]. This ongoing injury likely plays a role in a pervasive low-level encephalopathy presenting as continued mild cognitive impairment [3, 8, 9]. The persistence of HAND in the ART era has revealed the critical importance of understanding the key cellular mediators of HIV neuropathogenesis in HIV-infected individuals. These cellular mechanisms need to be elucidated to develop therapeutic strategies to limit CNS damage and preserve or restore cognitive function in HIV-infected subjects before and after ART initiation.

Infiltration by CD8⁺ T cells into the CNS is a recognized feature of many neurodegenerative diseases associated with neuroinflammation, including multiple sclerosis (MS), Alzheimer's disease, and various encephalitides [10]. CD8⁺ T cells have been shown to decrease replication of rabies and herpes simplex viruses in neurons [11-13]. However, in some virus-induced diseases, CD8⁺ T cells are involved in direct CNS tissue damage [10, 14, 15], and can directly transect neurites *in vitro* [16]. In MS, the number of CD8⁺ T cells in active lesions correlates with severity of neuronal damage [17], and activated CD8⁺ T cells are enriched in the CSF [18]. In the experimental autoimmune encephalomyelitis (EAE) mouse model of MS, CD8⁺ T cells play a role in remission, but also induce tissue damage and brain inflammation in chronic EAE [19-21]. Blocking CD8⁺ T cell infiltration into the CNS of EAE mice completely eradicates the disease [22]. These studies

highlight the dual beneficial and pathogenic role of CD8⁺ T cells in CNS diseases; but in HIV infection, CD8⁺ T cells have been largely associated with neuropathogenesis.

CD8⁺ T cells can cause CNS damage through direct cytotoxicity or release of inflammatory cytokines such as IFN γ or TNF as illustrated by a recent study describing a strong association between CSF CD8⁺ T cells secreting IFN γ and neurocognitive impairment [23]. The frequency of CD8⁺ T cells in lymphocytes in CSF is substantially elevated during HIV infection compared to other CNS diseases [24]. Spudich et al. recently found that CD8⁺ T cells constitute the majority of white blood cells in the CSF of HIV-infected compared to uninfected subjects with a decreased CD4:CD8 ratio [25]. In a dramatic recently-described syndrome, some HIV-infected individuals initiating or maintaining ART develop cognitive impairment and encephalitis in association with an influx of CD8⁺ T cells into the brain [26-29]. This ‘CD8-T cell encephalitis’ may be a form of CNS immune reconstitution inflammatory syndrome, and highlights the potentially deleterious role of CD8⁺ T cells in the CNS. A newly published study reported that neurocognitive decline was associated with ongoing CD8⁺ T cell activation in CSF in HIV-infected individuals on ART, suggesting that CD8⁺ T cells in CSF and brain play a key role in HIV neuropathogenesis [30].

Despite the pathogenic potential of CD8⁺ T cells in the CNS, HIV-specific CD8⁺ T cells can also play a crucial role in mediating antiviral immunity by killing productively infected cells. The critical role of HIV-specific CD8⁺ T cells in controlling viral replication has been demonstrated in the non-human primate SIV model of HIV infection, where CD8⁺ T cell depletion leads to a sharp increase in viremia [31]. In humans, several observations suggest that HIV-specific CD8⁺ T cells are important for the control of HIV replication in the periphery including the generation and maintenance of viral escape mutations in CD8⁺ T cell epitopes, the superior control of viral replication by certain HIV-specific clonotypes restricted by HLA-B57 and B27 [32] or the significant association between HIV-specific CD8⁺ T cell viral inhibition activity *in vitro* and the rate of CD4⁺ T cell loss in early HIV infection and CD4⁺ T cell decline in chronically infected individuals [33]. However, over the course of infection, HIV-specific CD8⁺ T cells become exhausted and lose their ability to control viral replication. High frequencies of SIV-specific CD8⁺ T cells have been detected in CSF of macaques chronically infected with SIV [34, 35]. HIV-specific CD8⁺ T cells have been also detected in CSF of

ART-naïve individuals but their presence has been associated with HIV dementia in chronically-infected subjects, suggesting that these cells are not able to contribute to the control of viral replication and participate in neuronal damage [36-38].

These previous studies have been performed in chronically-infected subjects and data on CD8⁺ T cells trafficking to the CNS in acute HIV infection have not been reported. The analysis of CD8⁺ T cells at the earliest stages of HIV infection has been limited due to the difficulty to recruit individuals within days of acquiring HIV. The RV254/SEARCH010 cohort provided a unique opportunity to analyze CD8⁺ T cells in hyperacute infection in the CSF and periphery within the first 20 days of infection. In this cohort, we recently reported that HIV-specific CD8⁺ T cell responses present in the periphery at peak viral load have an enhanced capacity to kill HIV-infected cells and are associated with viral load decline and reduced seeding of the HIV reservoir after ART initiation (RV254 paper in review). Recent studies suggested that early ART initiation would preserve these effective responses in the periphery [39-42]. Preserving potent HIV-specific CD8⁺ T cells in the CNS would be useful in reducing and possibly eliminating the persistent HIV replication in ART treated HIV-infected individuals as it has been proposed in the SIV model [43]. However, the presence of these cells in hyperacute infection in the CNS and the extent to which they have the ability to limit HIV replication in the CNS is unknown. The enrollment of acutely HIV-infected individuals in the RV254/SEARCH010 cohort with the collection of PBMCs and lymphocytes from the CSF at the same time allowed us to address that question. In this study, we analyzed the presence of activated CD8⁺ T cells, their HIV specificity and function in the CSF of subjects in acute infection compared to the periphery.

METHODS

Study participants

All clinical work was completed at the SEARCH clinical office in Bangkok, Thailand. Blood and CSF samples were collected from untreated acute HIV subjects (RV254/SEARCH010), chronic HIV (SEARCH011), and uninfected subjects (RV304/SEARCH013). Subjects from RV254/SEARCH010 were classified based on testing positive by pooled nucleic acid testing and 4th generation (4G) enzyme immunoassay (EIA): stage 1 (4th

gen EIA-, 3rd gen EIA-), stage 2 (4th gen EIA+, 3rd gen EIA-), stage 3 (4th gen EIA+, 3rd gen EIA+, Western blot-/indeterminate) [44]. 4th generation EIA staging was used to estimate time of HIV exposure within the AHI subjects. All subjects signed the study consent forms approved by human subject review boards at Chulalongkorn University (Bangkok, Thailand), the Walter Reed Army Medical Center (Rockville, MD), and the University of California at San Francisco. Study data was collected and managed by REDCap electronic data capture tools hosted at VGTI-Florida [45].

Fluid biomarker analyses in CSF

CSF concentration of the neuroaxonal injury marker neurofilament light (NFL) was measured using the NF-light enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (UmanDiagnostics, Umeå, Sweden). CSF concentration of the astroglial activation marker YKL-40 was measured using an ELISA from R&D systems (Minneapolis, MN). All measurements were performed by board-certified laboratory technicians in one round of experiments using one batch of reagents. Intra-assay coefficients of variation were below 10%.

Primary CD8⁺ T cell expansion for TCR repertoire and HIV specificity analyses

Primary CD8⁺ T cells were isolated from the PBMCs of untreated HIV-infected subjects by fluorescence activated cell sorting. Isolated CD8⁺ T cells were expanded in RPMI supplemented as previously described [46]. Briefly, CD8⁺ T cells were expanded with PHA in 8% human serum culture medium supplemented with both natural and recombinant IL-2 in the presence of feeder cells (irradiated fresh PBMCs from 3 different donors and irradiated B-EBV cells in a 10/1 ratio). CSF pellets were expanded with under the same conditions for two weeks prior to CD8⁺ T cell isolation by cell sorting.

Cell sorting and phenotypic analysis

Thawed PBMCs or CSF were stained for surface makers at 4°C for 20 minutes with the following monoclonal antibodies: α CD27-FITC, α CD8-PE, α PD1-PECy7, α CD14-BV650 (BioLegend), α HLA-DR-PerCP, α CD38-

APC, α CD3-A700, α CD45RA-APCH7 (BD Biosciences), α CD4-BV605 (Life Technologies), and α CD127-V450 (Affymetrix eBioscience). Live/dead stain with Vivid-amcyan was used to exclude dead cells from the sort. Cells were sorted on a BD FACS Aria II (BD Biosciences) and analyzed with FlowJo software (Treestar).

Intracellular staining (ICS) with CD8⁺ T cells

B-EBV lines were generated for each donor by culturing PBMCs in RPMI, 20% fetal bovine serum (FBS), 20nM FK506 (AG Scientific), and Epstein-barr virus (EBV)-containing supernatant from the virus-producing B95.8 marmoset cell line (ATCC) at an MOI of 100. B-EBV cell lines were then plated in the presence of 0.5-5 mg/mL HIV Clade AE peptide pools, 1 μ g/mL staphylococcal enterotoxin B (Sigma-Aldrich) or DMSO overnight. Peptide pools were made with 20 peptides per pool of HIV PTE and HIV Consensus A peptides (gag, pol, nef, env) at 15 amino acids in length. The peptides were obtained through the AIDS Reagent Program, Division of AIDS, NIAID, and NIH. Expanded primary CD8⁺ T cells were added to the loaded B-EBV (2:1) and co-stimulated with 1 μ g/ μ L α CD28/CD49d (BD Biosciences). Stimulated cells were immediately treated with GolgiPlug protein transport inhibitor (BD Biosciences) to prevent secretion of cytokines and incubated at 37°C for 12 hours. After incubation, cells were washed and stained with α CD8-FITC, α CD3-Pacific Blue (BD Biosciences), and α CD20-PECy7 (BioLegend) prior to fixation/permeabilization (2% formaldehyde) and intracellular staining with α IFN γ -APC (BD Biosciences). Live/dead stain with Vivid-amcyan was used to exclude dead cells from the analysis.

TCR Vbeta repertoire analysis

We analyzed the T-cell receptor Vbeta repertoire by flow cytometry using the IOTest[®] Beta mark (Beckman Coulter) in conjunction with α CD8-Pacific Blue (BD Biosciences). Stained CD8⁺ T cells were run on an LSRII flow cytometer using DiVA software (BD Biosciences) and analyzed with FlowJo (Treestar).

Gene expression analysis

CD8⁺ T cells were sorted from the CSF samples into 96 well plates at 100 cells/well to perform the Fluidigm's Biomark Assay. Assays (Primers and Probes) were designed using the Roche Universal Probe Library Assay Design Center (www.universalprobelibrary.com) and were designed to detect multiple transcripts, without respect to isoform prevalence. Cells were sorted directly into PCR plates and immediately frozen. RNA was reverse transcribed and amplified in a single-step RT-STA (Specific Template Amplification) using a pool of all the primer sets and with the Superscript III Platinum One-Step qRT-PCR Kit (without ROX) (Life Technologies, Grand Island, NY). The STA was carried out for 18 cycles of 95°C for 15 seconds and 60°C for 4 minutes. Unincorporated primers and any generated non-specific single-stranded products were then removed by an Exonuclease I (*E. coli*). (NEB, Ipswich, MA) step. High-throughput qPCR on the pre-amplified samples was performed on a 96.96 BioMark™ Dynamic Array (Fluidigm, South San Francisco, CA) [47] for 40 cycles (15 seconds at 95°C, 5 seconds at 70°C, 60 seconds at 60°C). The same primer set used in the RT-STA was used for the probe-based qPCR. Threshold cycle (CT) values were calculated by the Real-Time PCR Analysis Software (Fluidigm) and failed reactions were discarded from the analysis.

Statistical analyses

Neurological testing and neuroninflammatory markers were previously evaluated and available for all CSF specimens studied. P values were calculated using One-Way ANOVA and Kruskal-Wallis with post-hoc Dunn multiple comparison analysis for group comparisons. Statistical analysis for correlations was performed using Spearman rank and the 2-tailed paired or unpaired *t*-test with 95% confidence intervals, correlation and linear regression statistical analysis using the Prism 5 for Macintosh (GraphPad software).

RESULTS

Clinical Composition

We evaluated 57 individuals from uninfected (n=8), acute HIV infection (AHI) stage 1/2 (n=9), AHI stage 3 (n=17) and chronic HIV infection (CHI) (n=23) groups similar in demographic variables. All subjects were ART-naïve and subject demographic details are presented in Table 1. The acutely HIV-infected subjects were

recruited during the earliest stages of acute infection within the first 20 days of acquiring HIV corresponding to pre-peak to peak viremia, as previously described [48]. AHI stages 1 and 2 were grouped together since both stages are before peak viremia and no differences were found between the groups (data not shown) whereas AHI stage 3 correspond to peak viremia. Untreated chronically HIV-infected subjects had acquired HIV more than 2 years prior to enrollment and displayed elevated viral loads in the periphery and CSF. There was no significant difference in age between any of the groups. All subjects were fully characterized by demographic and clinical variables and underwent simultaneous lumbar puncture and blood draw.

Activated CD8+ T cells are elevated in the CSF in acute infection

Viral loads in the plasma and CSF of subjects in acute and chronic stages were measured. Subjects in AHI stage 3 had significantly elevated viral loads in the plasma compared to subjects in AHI stage 1/2 and tended to have also elevated plasma viral load compared to CHI subjects although not significant (Fig. 1A). CSF viral loads were higher in CHI subjects compared to AHI stage 1/2 subjects, but not significantly different than AHI stage 3 subjects. In order to analyze the CD8+ T cell response in early acute HIV infection compared to late stages of infection, cells from CSF were examined by flow cytometry. The number of total CSF CD8+ T cells was highest in the chronic HIV subjects compared to both the acute and uninfected groups but increased numbers of CD8+ T cells were detected already in AHI stage 3 (Fig. 1B). As expected, there were fewer CD4+ T cells than CD8+ T cells in the CSF across all HIV-infected groups (Supp. Fig. 1A). Furthermore, the frequency of activated CD8+ T cells in the CSF characterized by HLA-DR+ CD127- was significantly elevated in CHI subjects compared to all other groups but AHI stage 3 subjects already showed increased activated CD8+ T cell frequencies compared to AHI stage 1/2 subjects and uninfected (Fig. 1C,D). Similarly, the frequency of activated CD4+ T cells was most elevated in CHI subjects but already started to be elevated in AHI stage 3 (Supp. Fig. 1B). Further phenotypic analyses revealed that CD127 expression, the alpha chain of IL-7 receptor as a marker of survival potential, decreased on CD8+ and CD4+ T cells during acute infection and was significantly lower in the chronic stage (Fig. 1E, Supp. Fig. 1C). Full characterization of the CD8+ T cell phenotype revealed no significant difference between the memory CD8+ T cell subsets naïve

(CD45RA+CD27+), TEMRA (terminal differentiated effector memory) (CD45RA+CD27-), TTM (transitional memory) (CD45RA-CD27+), or TEM (effector memory) (CD45RA-CD27-) in the CSF of the different groups (Supp. Fig. 2). Together, these data demonstrate a massive infiltration of activated CD8+ T cells into the CNS as early as AHI stage 3 infection with aggravation of the activation status and loss of survival potential in the chronic phase of HIV infection.

CSF activated CD8+ T cells are associated with CSF viral load and neuroinflammatory markers in acute HIV infection

To investigate whether the activation states of CD8+ T cells in AHI stage 3 and chronic infection were associated with a pathogenic or beneficial effect on viral replication in the CNS, we analyzed the association between the frequency of activated CD8+ T cells in the CSF with viral load and levels of common neuroinflammatory markers measured in the CSF. In AHI stage 3, the frequency of activated CD8+ T cells in the CSF was strongly associated with CSF viral load ($r=0.66$, $p=0.004$) (Fig. 2A), but not with plasma viral load (Supp. Fig. 3A). In contrast, the frequency of activated CD8+ T cells in chronic subjects did not correlate with viral loads in the CSF but did correlate with plasma viral load ($r=0.45$, $p=0.035$) (Fig. 2B, Supp. Fig. 3B). When analyzing the association with neuroinflammatory markers in the CSF, we found that the frequency of activated CD8+ T cells in CSF from AHI stage 3 subjects was positively correlated with CSF levels of neopterin ($r=0.53$, $p=0.005$), interferon-gamma induced protein 10 (IP-10) ($r=0.47$, $p=0.016$), and soluble CD163 ($r=0.75$, $p<0.0001$), all synthesized by activated myeloid cells (Fig. 2C). These correlations were not seen in chronic HIV infection (Supp. Fig. 3C). A similar trend was observed with the frequency of activated CD4+ T cells in the AHI stage 3 CSF and neuroinflammatory markers neopterin and IP-10 and a significant correlation with CD163 (Supp. Fig. 3D), suggesting that the neuroinflammatory markers present in the CNS compartment during acute HIV infection at peak viremia are attracting and/or activating CD8+ T cells and to a lesser extent CD4+ T cells in the CNS. Importantly, CSF levels of neuroinflammatory markers common in later stages of neuroinflammation and neuronal damage, neurofilament light chain (NFL) and YKL-40, were positively correlated with frequency of activated CD8+ T cells in CSF of chronic subjects ($r=0.58$, $p=0.007$, and $r=0.45$,

$p=0.049$ respectively) (Fig. 2D). These neuroinflammatory markers did not correlate with frequency of activated CD8⁺ T in the CSF of acute infection or CD4⁺ T cells in CSF of chronic subjects (Supp. Fig. 4A,B). These data suggest that the presence of high numbers of activated CD8⁺ T cells in the CNS compartment in AHI stage 3 is mainly driven by the presence of HIV antigens in the CNS and the inflammatory response of microglia and myeloid cells. The presence of higher numbers of activated CD8⁺ T cells in CSF during chronic infection is not driven by antigen levels in the CNS or neuroinflammation but rather by plasma HIV antigen levels and are associated with neuronal damage. Therefore, these results suggest that although elevated in both acute and chronic infection, activated CD8⁺ T cells have a pathogenic role in chronic infection in line with previously work, whereas activated CD8⁺ T cells are responding to HIV replication in the CNS in acute HIV infection.

CSF CD8⁺ T cell associated genes are upregulated in acute HIV infection

To assess the potential beneficial role of CD8⁺ T cells in CSF during acute HIV infection, we measured the gene expression profile of CD8⁺ T cells in CSF from acute and chronic subjects using a high-throughput nanofluidic qPCR system. The system allowed us to detect 48 genes simultaneously from the very few CD8⁺ T cells present in the CSF. Gene expression was normalized to a reference gene and fold change in gene expression in CD8⁺ T cells CSF of HIV-infected individuals were calculated compared to CD8⁺ T cells in CSF of uninfected controls. Genes associated with CD8⁺ T cytolytic effector function, cell cycle, T cell receptor (TCR) signaling, and transcription factors were significantly elevated in AHI stage 3 CSF CD8⁺ T cells compared to CSF CD8⁺ T cells from AHI stage 1/2 and to chronic infection (Fig. 3). Increased expression of these genes could be seen as early as AHI stage 1/2, however, by chronic stage the expression of these pathways was downregulated compared to uninfected controls suggesting CD8⁺ T cell exhaustion. These results demonstrate a very distinct gene expression profile of CD8⁺ T cells present in the CSF in acute and chronic infection supporting the distinct role of these cells suggested by the associated with different neuroinflammatory markers in the CSF described above. These data suggest that overall activation and upregulation of critical CD8⁺ T cell associated genes begins as early as AHI stage 1/2 and peaks at AHI stage 3, suggesting that CD8⁺

T cells in CSF during acute HIV infection have increased proliferation, effector functions and have been TCR triggered and are likely to respond to HIV antigen presence to control viral replication in the CNS. In contrast, their counterparts in chronic infection displayed a very distinct blunted gene expression profile suggesting a dysfunctional state unable to contribute to local viral replication.

CSF CD8+ T cells express a unique TCR Vbeta repertoire compared to activated CD8+ T cells in the periphery

To determine whether the presence of activated CD8+ T cells in the CSF is unique to the CNS compartment and is not only a passive migration of peripheral activated CD8+ T cells, we analyzed the TCR Vbeta repertoire of CD8+ T cells from AHI stage 3 subjects in CSF, and activated and non-activated CD8+ T cells in the periphery. The results from the 8 AHI stage 3 subjects analyzed showed that CD8+ T cells in CSF expressed a unique TCR repertoire profile compared to the periphery (Fig. 4A). The TCR Vbeta repertoire diversity was significantly lower in the CD8+ T cells in CSF compared to activated and non-activated CD8+ T cells in peripheral blood mononuclear cells (PBMCs) (Fig. 4B). Importantly, CSF CD8+ T cells showed up to 40% unique Vbeta usage compared to their matched activated CD8+ T cells from the periphery (Fig. 4C). Despite CSF CD8+ T cells expressing lower TCR repertoire diversity compared to the periphery, they displayed a unique TCR Vbeta repertoire in the CSF during acute infection reflecting differences in compartmentalization.

HIV-specific CD8+ T cells are present in the CSF in acute infection

The presence of HIV-specific CD8+ T cells in CNS is expected to have a beneficial effect on neuronal damage by killing HIV infected cells and decreasing HIV replication in the brain. In order to determine the presence and specificity of CD8+ T cells in CSF, we generated CD8+ T cell lines and autologous B-EBV cell lines as previously described [46] from 10 AHI stage 3 subjects. B-EBV cells are used as antigen presenting cells and loaded with peptide pools for *gag*, *pol*, *env*, and *nef*. These pools were selected based on potential T cell epitopes common in HIV clade AE, which is dominant in Thailand. HIV peptide loaded B-EBV cells were then co-cultured with donor matched CD8+ T cells from either the CSF or periphery (activated and non-activated CD8+ T cells) and IFN-gamma production was measured. HIV-specific CD8+ T cells were present in high

frequency in the activated CD8⁺ T cell population in the CSF and PBMCs, but not in the non-activated CD8⁺ T cell population in the PBMCs (Fig. 5A). When assessing the specific peptide recognized by CD8⁺ T cells from CSF and PBMCs in the same subjects, we found that CD8⁺ T cells were directed against shared, but also unique HIV epitopes in CSF compared to periphery (Fig. 5B). These results show that a high proportion of HIV-specific CD8⁺ T cells are expanding in acute HIV infection and are present in the CNS compartment. These results also demonstrate that the CD8⁺ T cell specificity differs between the two compartments in acute infection and provide evidence for the presence of HIV-specific CD8⁺ T cells able to control viral replication in the CNS. Altogether, these results provide evidence for a distinct role of CD8 T cells in acute and chronic infection in the CNS compartment, with activated CD8⁺ T cells associated with neuropathogenesis in chronic infection whereas in acute infection, activated CD8⁺ T cells in the CSF exhibit enhanced functional gene expression, a unique repertoire and contain HIV-specific CD8⁺ T cells, suggesting that these cells could play a beneficial role of controlling HIV replication and preventing CNS damage if ART was initiated at that stage.

DISCUSSION

Despite the large presence of CD8⁺ T cells in the CNS during early HIV infection, their role in neuropathogenesis remains unknown. In this study, we provide new information on (i) the phenotype and activation state of CD8⁺ T cells present in the CSF in the earliest stages of acute HIV infection, (ii) determine how the CD8⁺ T cells change throughout infection from acute to chronic infection in ART naïve subjects, (iii) how the activated CD8⁺ T cells relate to neuroinflammatory markers in the different stages of infection, (iv) and elucidate on the HIV specificity and potential beneficial role of CD8⁺ T cells in acute infection. Previously, Valcour et. al. detected HIV RNA in the CSF of subjects from this hyperacute infection cohort as early as 8 days after estimated HIV infection and identified elevated neuroinflammatory markers in the CSF in these acutely-infected subjects [49]. Here, we found positive correlations with the frequency of activated CD8⁺ T cells in the CSF to early biomarkers of immune activation in the CNS (CSF neopterin, CSF IP-10, and CSF CD163) as well as CSF viral load at peak viremia in acute infection. These early markers of CNS inflammation did not correlate with activated CD8⁺ T cells in chronically-infected subjects, where levels of NFL and YKL-

40, biomarkers of neuroaxonal injury and astroglial activation in chronic CNS inflammation and neurodegeneration correlated with the frequency of activated CD8⁺ T cells in the CSF. These results depict a very distinct immune response and inflammatory environment between acute and chronic infection and suggest that activated CD8⁺ T cells are recruited early in acute HIV infection responding to early neuroinflammatory markers in the CNS and might play a beneficial role in preventing neuronal damage if viral replication is halted at that stage by ART by killing HIV infected cells. Indeed, we recently demonstrated that activated CD8⁺ T cells in the periphery were fully differentiated into effector cells at peak viremia and were able to decrease viral production and reduce HIV reservoir seeding after ART initiation at that stage of acute HIV infection (paper submitted).

Evaluating T cells in CSF is typically limited by the small amount of cells that can be extracted from the CSF and is not equivalent to analyzing brain tissue, but it is the best available cells that we have access to assess the immunologic status of the brain in these acutely-infected individuals. We developed novel techniques that allowed for the comprehensive characterization of CD8⁺ T cells on a very small number of cells detected in the CSF. Our data demonstrates major differences in CD8⁺ T cells present in the periphery compared to the CSF and highlight the importance of analyzing CSF cells to investigate their role in CNS rather than PBMCs. Additionally, the frequency of activated CD8⁺ T cells in the CSF of acute stage 3 subjects positively correlated with the CSF viral load, but not the plasma viral load, indicating differences between CSF and plasma markers and the potentially inaccuracy of using plasma markers as accurate reflections of CNS activities. Our group previously demonstrated phenotypic, gene expression and functional differences in CD8⁺ T cell profiles in the periphery of acutely and chronically HIV-infected subjects [50]. In the current study, we found that activated CD8⁺ T cells exhibited distinct gene expression pathways associated with CD8⁺ T cells activation such as cell cycle, TCR signaling, effector function in the CSF during acute infection compared to chronic infection. This elevated activation state was not as apparent in CSF CD4⁺ T cells during acute infection.

The IL-7 cytokine plays an important role in the activation of CD8⁺ T cells in response to viral infection as it promotes T cells survival and potentiates cytolytic activity and perforin accumulation intracellularly [51-

53]. It has been well documented that expression of the IL-7 receptor alpha chain (CD127) is reduced on CD8⁺ T cells in HIV-infected individuals and partial recovery is observed in subjects that initiate ART [54]. Report have also shown in chronically-infected individuals that CD8⁺ T cells with lower CD127 expression were unable to become activated lymphoblasts and upregulate CD25 [55]. Previously, we demonstrated that the expression of CD127 on HIV-specific CD8⁺ T cells in the periphery in acute HIV infection was associated with a lower viral set point in untreated chronic infection. Here we observed a significant decrease in CD127 expression on activated CD8⁺ T cells in the CSF of chronic subjects. Interestingly, this decrease in CD127 expression appears as early as AHI stage 1/2 eventhough the frequency of activated CD8⁺ T cells is not elevated yet and could be an early sign of impaired CD8⁺ T cell response. We also noticed a slight increase in PD-1 expression, a known marker of T cell activation and exhaustion (data not shown). Together, these data show that the profile of CD8⁺ T cells are being altered very early on in acute infection that could explain the loss of virus-specific CD8⁺ T cell response that is seen in chronic infection as viral replication persists. Therefore, these observations suggest the very early initiation of ART may prevent these permanent changes in CD8⁺ T profile and preserve CD8⁺ T cell antiviral response in the CNS.

We have previously shown that chronic inflammation results in a dramatic clonal focusing of HCMV-specific CD8⁺ T cells in the synovial fluid compared to periphery in rheumatoid arthritis individuals suggesting that inflammation in tissues is reflected by T cell selection more significantly than in the PBMCs [46]. In ART-naïve individuals, activated HIV-specific CD8⁺ T cells are maintained at high frequency due to constant HIV antigen stimulation. Here we show that as early as in acute infection, T cell clonotypes are expanded as CD8⁺ T cells in CSF exhibited unique Vbeta usage compared to their matched activated CD8⁺ T cells from the periphery. These data suggest that CD8⁺ T cells in CSF exhibit a restricted unique TCR repertoire that allows for the longitudinal follow up of these clones over time and provide the rationale to assess whether persisting clonotypes in CSF under ART associate with residual neuroinflammation. Future studies will determine if CD8⁺ T cell clonotypes that persist after ART in CSF are associated with persistent CNS neuroinflammation injury.

The presence of HIV-specific CD8⁺ T cells in CNS is expected to have a beneficial effect on neuronal damage by killing HIV infected cells and decreasing HIV replication in the brain. Little is known about the frequency of CD8⁺ T cells recognizing HIV antigens over the course of HIV infection especially in the CNS. A recent study suggested that inflammatory cytokines present during untreated chronic HIV infection triggers proliferation and expression of activation markers in CD8⁺ T cells in the periphery independent of antigen specificity [56]. These bystander cells might be unable to eliminate HIV-infected cells. However, we demonstrated here that HIV-specific CD8⁺ T cells are present in acute HIV infection already at peak viremia in the CSF and ART initiation in acute infection could lead to the preservation of these effector HIV-specific CD8⁺ T cells and could be critical for reducing the residual HIV replication in the CNS. Here, we demonstrated not only that HIV-specific CD8⁺ T cells are present in the CSF, but that they respond to unique HIV epitopes in the CSF compared to the CD8⁺ T cells in the periphery. This data along with the specific TCR Vbeta repertoire suggest very early infiltration and local expansion of HIV-specific CD8⁺ T cells in the CNS that may be interacting with unique HIV epitopes that are present in the CNS and not in the periphery. Our data demonstrate trafficking of highly activated and HIV-specific CD8⁺ T cells to the CNS within the first weeks after HIV exposure and provide the rationale to analyze CSF cell populations rather than PBMCs to understand immune responses in the CNS. Determining whether CD8⁺ T cells can play a beneficial role in the CNS of HIV-infected subjects will be determined by future longitudinal studies in this unique acute infection cohort initiating ARVs in the earliest stage of infection as it will determine if early ART initiation will preserve beneficial HIV-specific CD8⁺ T cells in the brain, that have the potential to control viral replication. These findings will be critical in targeting the HIV reservoirs that persists in the CNS despite ART.

Footnotes

Acknowledgements

The authors would like to thank the research participants in the SEARCH cohorts. We would also like to thank Collin Adams and Derek Ochi for administrative support and Zhong He for his flow cytometry expertise and

sorting. The study team is grateful to the contribution of the staff at the Thai Red Cross AIDS Research Centre and the Department of Retrovirology, U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences (AFRIMS).

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Financial Support

This work was supported by the National Institutes of Health grants [R01AI10843] (LT), [R21MH086341], (VV), [R01MH095613] (VV & SS), and a cooperative agreement (W81XWH-07-2-0067) between the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., and the U.S. Department of Defense (DoD). Thai Pharmaceutical Organization, Merck, ViiV Healthcare and Gilead.

Potential Conflicts of Interest

J. Ananworanich has received honorarium from ViiV Healthcare and Gilead. S. Spudich has received travel support and an honorarium from AbbVie. Other authors declare no conflicts of interest.

Previous Presentations

The results of this study were presented as a poster at the 2015 Keystone Symposium in Boston, Massachusetts and as an oral report at the International NeuroHIV Cure Consortium in Silver Springs, Maryland in 2015.

Figure and Table Captions

Table 1. Demographic and clinical parameters of study participants. CD4 T cell count is cells/mm³ in plasma. Abbreviations: AHI - acute HIV infection, CHI – Chronic HIV infection. Acute HIV-infected subjects were classified based on testing positive by pooled nucleic acid testing and 4th generation (4G) enzyme immunoassay (EIA): stage 1 (4th gen EIA-, 3rd gen EIA-), stage 2 (4th gen EIA+, 3rd gen EIA-), stage 3 (4th gen EIA+, 3rd gen EIA+, Western blot-/indeterminate) *Days of infection is estimated time of exposure and enrollment. If a range of dates was provided, the mean was used.

Figure 1. Activated CD8+ T cells are elevated in the CSF during acute HIV infection. A. CSF viral load in acute HIV infection Stage1/2, acute HIV infection Stage 3 and chronic HIV infection (CHI) samples. Limit of detection 100 viral copies/ml. B. Number of CD8+ T cells per mL of CSF in the different groups of donors. C. Representative dot plots of activated CD8+ T cells defined by CD38+ CD127- in the CSF of a representative subject in the different groups. D. Frequency of activated CD8+ T cells (CD38+, CD127-) within the CD8+ T cells in CSF in the different groups. E. Frequency of CD127+ CD8+ T cells within CD8+ T cells in CSF in the different groups. Uninfected subjects (white), AHI Stage 1/2 subjects (light grey), AHI Stage 3 subjects (dark grey) and CHI subjects (black). Asterisks denote different P values: *P< 0.05; **P < 0.005; *** < 0.0001.

Fig 2. Activated CD8+ T cells in CSF from AHI subjects positively correlates with CSF viral load and CSF markers of neuroinflammation. A. Correlation of activated CD8+ T cells with viral load in the CSF in AHI Stage 3 subjects. B. Correlation of activated CD8+ T cells with plasma viral load in CHI subjects plasma viral load in chronic subjects. C. Frequency of CSF activated CD8+ T cells in AHI Stage 1/2 and 3 correlates with CSF neuroinflammatory markers neopterin, IP-10, and CD163. D. Frequency of activated CD8+ T cells in the CSF of CHI subjects correlates with CSF neurofilament and YKL-40.

Fig 3. Gene expression of CD8+ T cells in the CSF of acute and chronic HIV infected subjects. Targeted gene expression profile of 48 genes by multiplex qPCR involved in cell cycle, transcription factors, CD8

effector functions, and TCR signaling pathways for the CD8⁺ T cells in the CSF of AHI stage 1/2, AHI stage 3, and CHI subjects compared to uninfected subjects. Scale represents fold change in gene expression of 100 CD8 T cells in the analyzed group compared to uninfected subjects.

Fig 4. TCR Vbeta repertoire of CD8⁺ T cells in CSF and PBMCs from 8 AHI stage 3 subjects. *A.* Frequency of Vbeta family usage of CD8⁺ T cells per AHI stage 3 subject (n=8) from the CSF, activated CD8⁺ T cells in PBMCs (PBMC ACT), and non-activated CD8⁺ T cells in PBMCs (PBMC nonACT). *B.* Number of Vbeta families out of the 24 Vbeta families tested that were expressed on CD8⁺ T cells from CSF, activated and non-activated CD8⁺ T cells in PBMCs per subject in each compartment, * $p < 0.05$, *** $p < 0.0005$. *C.* Frequency of Vbeta families unique to the CSF compared to the PBMCs for each AHI stage 3 subject analyzed.

Figure 5. Frequency of HIV-specific CD8⁺ T cells in the CSF and PBMCs in acute HIV infection. *A.* Frequency of activated CD8⁺ T cells from AHI stage 3 subjects (n=10) expressing IFN γ in response to stimulation with Gag, Pol, Env, and Nef in activated CD8⁺ T cells in the CSF (CSF ACT), activated CD8⁺ T cells in the periphery (PBMC ACT), and non-activated CD8⁺ T cells in the periphery (PBMC non-ACT), * $p < 0.05$, ** $p < 0.005$. *B.* Frequency of HIV-specific CD8⁺ T cells that responded to peptide pool stimulation (4-5 pools per HIV protein) in the CSF and activated PBMCs (ACT) by measuring IFN γ production. Grey bar color represents HIV-specific CD8⁺ T cell responses that were unique to either the CSF or PBMCs and black represents responses that were shared by both compartments.

Supplementary Fig 1. Frequency of activated CD4⁺ T cells in the CSF. *A.* Number of CD4⁺ T cells in the CSF of uninfected subjects (white), AHI Stage 1/2 subjects (light grey), AHI Stage 3 subjects (dark grey) and CHI subjects (black). *B.* Frequency of activated CD4⁺ T cells within the CD4⁺ T cells in CSF in the different groups. *C.* Frequency of CD127⁺ CD4⁺ T cells within CD4⁺ T cells in CSF in the different groups. Asterisks denote different P values: * $P < 0.05$; ** $P < 0.005$; *** < 0.0001 .

Supplementary Fig 2. Distribution of CD8+ T cell memory subsets in CSF. Proportions of naïve, TEMRA=terminal differentiated effector memory, TTM=Transitional memory, TEM=effector memory CD8+ T cells in CSF in the different groups: uninfected subjects (white), AHI Stage 1/2 subjects (light grey), AHI Stage 3 subjects (dark grey) and CHI subjects (black).

Supplementary Fig 3. Correlation of activated CD8+ and CD4+ T cells in the CSF with viral load and neuroinflammatory marker. *A.* Frequency of activated CD8+ T cells in the CSF does not correlate with plasma viral load in acute HIV infection. *B.* Frequency of activated CD8+ T cells in the CSF does not correlate with CSF viral load in chronic infection. *C.* Frequency of activated CD8+ T cells in CHI subjects showed no correlations to common early neuroinflammatory markers (IP-10, neopterin) in the CSF. *D.* Frequency of activated CD4+ T cells in the CSF does not significantly correlate with neuroinflammatory markers neopterin or IP-10 in acute subjects (AHI Stage 1/2 and 3), but does correlate with CD163.

Supplementary Fig 4. Correlation of activated CD8+ and CD4+ T cells in the CSF with viral load and neuroinflammatory marker. *A.* Frequency of activated CD8+ T cells in the CSF does not correlate with NFL, a marker of neuroaxonal injury or YKL-40, a marker of astroglial activation in acute HIV infection. *B.* Frequency of activated CD4+ T cells does not correlate with NFL or YKL-40 levels in CHI subjects.

References

1. Kaul M, Garden GA, Lipton SA: **Pathways to neuronal injury and apoptosis in HIV-associated dementia.** *Nature* 2001, **410**(6831):988-994.
2. Nath A: **Human immunodeficiency virus (HIV) proteins in neuropathogenesis of HIV dementia.** *J Infect Dis* 2002, **186 Suppl 2**:S193-198.
3. McArthur JC, Steiner J, Sacktor N, Nath A: **Human immunodeficiency virus-associated neurocognitive disorders: Mind the gap.** *Ann Neurol* 2010, **67**(6):699-714.

4. Heaton RK, Clifford DB, Franklin DR, Jr., Woods SP, Ake C, Vaida F, Ellis RJ, Letendre SL, Marcotte TD, Atkinson JH *et al*: **HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study.** *Neurology* 2010, **75**(23):2087-2096.
5. Eden A, Price RW, Spudich S, Fuchs D, Hagberg L, Gisslen M: **Immune activation of the central nervous system is still present after >4 years of effective highly active antiretroviral therapy.** *J Infect Dis* 2007, **196**(12):1779-1783.
6. Yilmaz A, Price RW, Spudich S, Fuchs D, Hagberg L, Gisslen M: **Persistent intrathecal immune activation in HIV-1-infected individuals on antiretroviral therapy.** *J Acquir Immune Defic Syndr* 2008, **47**(2):168-173.
7. Harezlak J, Buchthal S, Taylor M, Schifitto G, Zhong J, Daar E, Alger J, Singer E, Campbell T, Yiannoutsos C *et al*: **Persistence of HIV-associated cognitive impairment, inflammation, and neuronal injury in era of highly active antiretroviral treatment.** *AIDS* 2011, **25**(5):625-633.
8. Langford TD, Letendre SL, Larrea GJ, Masliah E: **Changing patterns in the neuropathogenesis of HIV during the HAART era.** *Brain pathology* 2003, **13**(2):195-210.
9. Letendre SL, Ellis RJ, Everall I, Ances B, Bharti A, McCutchan JA: **Neurologic complications of HIV disease and their treatment.** *Topics in HIV medicine : a publication of the International AIDS Society, USA* 2009, **17**(2):46-56.
10. Neumann H, Medana IM, Bauer J, Lassmann H: **Cytotoxic T lymphocytes in autoimmune and degenerative CNS diseases.** *Trends in neurosciences* 2002, **25**(6):313-319.
11. Hooper DC, Morimoto K, Bette M, Weihe E, Koprowski H, Dietzschold B: **Collaboration of antibody and inflammation in clearance of rabies virus from the central nervous system.** *J Virol* 1998, **72**(5):3711-3719.
12. Liu T, Khanna KM, Carriere BN, Hendricks RL: **Gamma interferon can prevent herpes simplex virus type 1 reactivation from latency in sensory neurons.** *J Virol* 2001, **75**(22):11178-11184.
13. Griffin DE: **Immune responses to RNA-virus infections of the CNS.** *Nat Rev Immunol* 2003, **3**(6):493-502.
14. Bilzer T, Stitz L: **Immune-mediated brain atrophy. CD8+ T cells contribute to tissue destruction during borna disease.** *J Immunol* 1994, **153**(2):818-823.
15. Murray PD, Pavelko KD, Leibowitz J, Lin X, Rodriguez M: **CD4(+) and CD8(+) T cells make discrete contributions to demyelination and neurologic disease in a viral model of multiple sclerosis.** *J Virol* 1998, **72**(9):7320-7329.
16. Medana I, Martinic MA, Wekerle H, Neumann H: **Transection of major histocompatibility complex class I-induced neurites by cytotoxic T lymphocytes.** *The American journal of pathology* 2001, **159**(3):809-815.
17. Bitsch A, Schuchardt J, Bunkowski S, Kuhlmann T, Bruck W: **Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation.** *Brain : a journal of neurology* 2000, **123 (Pt 6)**:1174-1183.
18. Junker A, Ivanidze J, Malotka J, Eiglmeier I, Lassmann H, Wekerle H, Meinl E, Hohlfeld R, Dornmair K: **Multiple sclerosis: T-cell receptor expression in distinct brain regions.** *Brain* 2007, **130**(Pt 11):2789-2799.
19. Jiang H, Zhang SI, Pernis B: **Role of CD8+ T cells in murine experimental allergic encephalomyelitis.** *Science* 1992, **256**(5060):1213-1215.
20. Sun D, Whitaker JN, Huang Z, Liu D, Coleclough C, Wekerle H, Raine CS: **Myelin antigen-specific CD8+ T cells are encephalitogenic and produce severe disease in C57BL/6 mice.** *J Immunol* 2001, **166**(12):7579-7587.
21. Huseby ES, Liggitt D, Brabb T, Schnabel B, Ohlen C, Goverman J: **A pathogenic role for myelin-specific CD8(+) T cells in a model for multiple sclerosis.** *The Journal of experimental medicine* 2001, **194**(5):669-676.
22. Luo Q, Sun Y, Gong FY, Liu W, Zheng W, Shen Y, Hua ZC, Xu Q: **Blocking initial infiltration of pioneer CD8(+) T-cells into the CNS via inhibition of SHP-2 ameliorates experimental autoimmune encephalomyelitis in mice.** *Br J Pharmacol* 2014, **171**(7):1706-1721.

23. Schrier RD, Hong S, Crescini M, Ellis R, Perez-Santiago J, Spina C, Letendre S: **Cerebrospinal fluid (CSF) CD8+ T-cells that express interferon-gamma contribute to HIV associated neurocognitive disorders (HAND)**. *PLoS One* 2015, **10**(2):e0116526.
24. Kowarik MC, Grummel V, Wemlinger S, Buck D, Weber MS, Berthele A, Hemmer B: **Immune cell subtyping in the cerebrospinal fluid of patients with neurological diseases**. *J Neurol* 2014, **261**(1):130-143.
25. Ho EL, Ronquillo R, Altmeppen H, Spudich SS, Price RW, Sinclair E: **Cellular Composition of Cerebrospinal Fluid in HIV-1 Infected and Uninfected Subjects**. *PLoS ONE* 2013, **8**(6):e66188.
26. Miller RF, Isaacson PG, Hall-Craggs M, Lucas S, Gray F, Scaravilli F, An SF: **Cerebral CD8+ lymphocytosis in HIV-1 infected patients with immune restoration induced by HAART**. *Acta Neuropathol* 2004, **108**(1):17-23.
27. Lescure FX, Moulignier A, Savatovsky J, Amiel C, Carcelain G, Molina JM, Gallien S, Pacanovski J, Pialoux G, Adle-Biassette H *et al*: **CD8 encephalitis in HIV-infected patients receiving cART: a treatable entity**. *Clin Infect Dis* 2013, **57**(1):101-108.
28. Gray F, Lescure FX, Adle-Biassette H, Polivka M, Gallien S, Pialoux G, Moulignier A: **Encephalitis with infiltration by CD8+ lymphocytes in HIV patients receiving combination antiretroviral treatment**. *Brain Pathol* 2013, **23**(5):525-533.
29. Moulignier A, Lescure FX, Savatovsky J, Campa P: **CD8 transverse myelitis in a patient with HIV-1 infection**. *BMJ case reports* 2014, **2014**.
30. Grauer OM, Reichelt D, Gruneberg U, Lohmann H, Schneider-Hohendorf T, Schulte-Mecklenbeck A, Gross CC, Meuth SG, Wiendl H, Husstedt IW: **Neurocognitive decline in HIV patients is associated with ongoing T-cell activation in the cerebrospinal fluid**. *Ann Clin Transl Neurol* 2015, **2**(9):906-919.
31. Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, Racz P, Tenner-Racz K, Dalesandro M, Scallon BJ *et al*: **Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes**. *Science* 1999, **283**(5403):857-860.
32. Chen H, Ndhlovu ZM, Liu D, Porter LC, Fang JW, Darko S, Brockman MA, Miura T, Brumme ZL, Schneidewind A *et al*: **TCR clonotypes modulate the protective effect of HLA class I molecules in HIV-1 infection**. *Nature immunology* 2012, **13**(7):691-700.
33. Shan L, Deng K, Shroff NS, Durand CM, Rabi SA, Yang HC, Zhang H, Margolick JB, Blankson JN, Siliciano RF: **Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation**. *Immunity* 2012, **36**(3):491-501.
34. von Herrath M, Oldstone MB, Fox HS: **Simian immunodeficiency virus (SIV)-specific CTL in cerebrospinal fluid and brains of SIV-infected rhesus macaques**. *J Immunol* 1995, **154**(10):5582-5589.
35. Moniuszko M, Brown C, Pal R, Trynieszewska E, Tsai WP, Hirsch VM, Franchini G: **High frequency of virus-specific CD8+ T cells in the central nervous system of macaques chronically infected with simian immunodeficiency virus SIVmac251**. *J Virol* 2003, **77**(22):12346-12351.
36. Jassoy C, Johnson RP, Navia BA, Worth J, Walker BD: **Detection of a vigorous HIV-1-specific cytotoxic T lymphocyte response in cerebrospinal fluid from infected persons with AIDS dementia complex**. *J Immunol* 1992, **149**(9):3113-3119.
37. Petit C, Torres-Muñoz J, Zielger F, McCarthy M: **Brain CD8+ and cytotoxic T lymphocytes are associated with, and may be specific for, human immunodeficiency virus type 1 encephalitis in patients with acquired immunodeficiency syndrome**. *J Neurovirol* 2006, **12**(4):272-283.
38. Sadagopal S, Lorey SL, Barnett L, Basham R, Lebo L, Erdem H, Haman K, Avison M, Waddell K, Haas DW *et al*: **Enhancement of human immunodeficiency virus (HIV)-specific CD8+ T cells in cerebrospinal fluid compared to those in blood among antiretroviral therapy-naive HIV-positive subjects**. *J Virol* 2008, **82**(21):10418-10428.
39. Oxenius A, Price DA, Easterbrook PJ, O'Callaghan CA, Kelleher AD, Whelan JA, Sontag G, Sewell AK, Phillips RE: **Early highly active antiretroviral therapy for acute HIV-1 infection preserves immune function of CD8+ and CD4+ T lymphocytes**. *Proc Natl Acad Sci U S A* 2000, **97**(7):3382-3387.

40. Streeck H, Jessen H, Alter G, Teigen N, Waring MT, Jessen A, Stahmer I, van Lunzen J, Lichtenfeld M, Gao X *et al*: **Immunological and virological impact of highly active antiretroviral therapy initiated during acute HIV-1 infection.** *J Infect Dis* 2006, **194**(6):734-739.
41. Cellerai C, Harari A, Stauss H, Yerly S, Geretti AM, Carroll A, Yee T, Ainsworth J, Williams I, Sweeney J *et al*: **Early and prolonged antiretroviral therapy is associated with an HIV-1-specific T-cell profile comparable to that of long-term non-progressors.** *PLoS one* 2011, **6**(4):e18164.
42. Freel SA, Picking RA, Ferrari G, Ding H, Ochsenbauer C, Kappes JC, Kirchherr JL, Soderberg KA, Weinhold KJ, Cunningham CK *et al*: **Initial HIV-1 antigen-specific CD8+ T cells in acute HIV-1 infection inhibit transmitted/founder virus replication.** *J Virol* 2012, **86**(12):6835-6846.
43. Graham DR, Gama L, Queen SE, Li M, Brice AK, Kelly KM, Mankowski JL, Clements JE, Zink MC: **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.** *Journal of neurovirology* 2011, **17**(1):120-130.
44. Ananworanich J, Fletcher JL, Pinyakorn S, van Griensven F, Vandergeeten C, Schuetz A, Pankam T, Trichavaroj R, Akapirat S, Chomchey N *et al*: **A novel acute HIV infection staging system based on 4th generation immunoassay.** *Retrovirology* 2013, **10**:56.
45. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG: **Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support.** *J Biomed Inform* 2009, **42**(2):377-381.
46. Trautmann L, Rimbart M, Echasserieau K, Saulquin X, Neveu B, Dechanet J, Cerundolo V, Bonneville M: **Selection of T Cell Clones Expressing High-Affinity Public TCRs within Human Cytomegalovirus-Specific CD8 T Cell Responses.** *The Journal of Immunology* 2005, **175**(9):6123-6132.
47. Spurgeon SL, Jones RC, Ramakrishnan R: **High throughput gene expression measurement with real time PCR in a microfluidic dynamic array.** *PLoS One* 2008, **3**(2):e1662.
48. Ananworanich J, Phanuphak N, de Souza M, Paris R, Arroyo M, Trichavaroj R, Sirivichayakul S, Shikuma C, Phanuphak P, Kim JH *et al*: **Incidence and characterization of acute HIV-1 infection in a high-risk Thai population.** *J Acquir Immune Defic Syndr* 2008, **49**(2):151-155.
49. Valcour V, Chalermchai T, Sailasuta N, Marovich M, Lerdlum S, Suttichom D, Suwanwela NC, Jagodzinski L, Michael N, Spudich S *et al*: **Central nervous system viral invasion and inflammation during acute HIV infection.** *J Infect Dis* 2012, **206**(2):275-282.
50. Trautmann L, Mbitikon-Kobo FM, Goulet JP, Peretz Y, Shi Y, Van Grevenynghe J, Procopio FA, Boulassel MR, Routy JP, Chomont N *et al*: **Profound metabolic, functional, and cytolytic differences characterize HIV-specific CD8 T cells in primary and chronic HIV infection.** *Blood* 2012, **120**(17):3466-3477.
51. Tan JT, Dudl E, LeRoy E, Murray R, Sprent J, Weinberg KI, Surh CD: **IL-7 is critical for homeostatic proliferation and survival of naive T cells.** *Proc Natl Acad Sci U S A* 2001, **98**(15):8732-8737.
52. Schluns KS, Kieper WC, Jameson SC, Lefrancois L: **Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo.** *Nat Immunol* 2000, **1**(5):426-432.
53. Smyth MJ, Norihisa Y, Gerard JR, Young HA, Ortaldo JR: **IL-7 regulation of cytotoxic lymphocytes: pore-forming protein gene expression, interferon-gamma production, and cytotoxicity of human peripheral blood lymphocytes subsets.** *Cell Immunol* 1991, **138**(2):390-403.
54. Colle JH, Moreau JL, Fontanet A, Lambotte O, Joussemet M, Delfraissy JF, Theze J: **CD127 expression and regulation are altered in the memory CD8 T cells of HIV-infected patients--reversal by highly active anti-retroviral therapy (HAART).** *Clin Exp Immunol* 2006, **143**(3):398-403.
55. Vingerhoets J, Bisalinkumi E, Penne G, Colebunders R, Bosmans E, Kestens L, Vanham G: **Altered receptor expression and decreased sensitivity of T-cells to the stimulatory cytokines IL-2, IL-7 and IL-12 in HIV infection.** *Immunol Lett* 1998, **61**(1):53-61.
56. Bastidas S, Graw F, Smith MZ, Kuster H, Gunthard HF, Oxenius A: **CD8+ T cells are activated in an antigen-independent manner in HIV-infected individuals.** *J Immunol* 2014, **192**(4):1732-1744.

