

Marked Increase of Regulatory T Cells and HIV Viral Load in the Cerebrospinal Fluid Is Associated with NeuroAIDS Opportunistic Infections

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Abstract

OBJECTIVE: To investigate the expression of regulatory T cells (Tregs) [CD3+ CD4+ CD25+ FoxP3+] during opportunistic infections (OIs) of the central nervous system (CNS) of HIV-infected patients.

METHODS: The frequency of Tregs cells in cerebrospinal fluid (CSF) was investigated by flow cytometric analysis (FACS) in 56 patients with AIDS with and without CNS-OIs. Infiltration of the CNS by Tregs was also studied by immunostaining of brain tissues from autopsy cases. The relationship between the frequency of Tregs in the CSF, CSF HIV viral load, and CSF cytokine profiles were analyzed.

RESULTS: A significantly higher frequency of Tregs was found in the CSF of patients with cryptococcus meningitis (CM), tuberculous meningitis (TBM) and toxoplasma encephalitis (TE) as compared with the non CNS-OIs control group. The frequency of Tregs was lower in the paired blood samples and no significant differences were found in blood Tregs in OIs vs. non-OIs. Furthermore, FoxP3+CD3+ cell infiltration was demonstrated in the brain of patients deceased after neuroAIDS OIs. The frequency of Tregs in CSF showed a positive relationship with CSF HIV viral loads and IL-10 levels, a Treg-associated anti-inflammatory cytokine.

CONCLUSION: This study provides new evidence of active trafficking of regulatory T cells of CD4+CD25+FoxP3+ phenotype in patients with neuroAIDS OIs. These observations support the view that Tregs may contribute to an immunosuppressive environment that facilitates HIV replication within the CNS.

Introduction

Opportunistic infections (OIs) in the central nervous system (CNS) are responsible for a high mortality in patients with AIDS in developing countries. In Colombia, the estimated prevalence of HIV infection is 0.9%. The presence of OIs in HIV infected patients has been associated with accelerated disease progression¹. OIs in the CNS may impact HIV-mediated neuropathogenesis by increasing the HIV burden and the trafficking of infected cells into the brain^{2,3}. However, the immune regulatory mechanisms that control brain inflammation may also alter HIV replication and viral load within the CNS compartment. Regulatory T cells, CD4+ CD25+ FoxP3+, also known as Tregs, are a subset of CD4+ T cells with immune suppressive properties that prevent autoimmunity (natural Treg) or blunt protective immunity against infections (adaptive Treg)^{4,5}. Tregs have gained importance in the context of HIV pathogenesis but it remains controversial whether they play a protective or pathological role in HIV disease. The expansion of adaptive Tregs in the central nervous system during chronic HIV infection may mediate neuropathogenesis. The suppressive activity of Tregs may impair specific T cell responses, favoring HIV reactivation and pathogenic processes. Alternatively, Tregs may have a protective role by limiting CNS immunopathogenesis. The main goals of this study were to determine whether increases in regulatory T cells (Tregs) in the CSF are associated with neuroAIDS OIs, and to correlate their presence with the magnitude of HIV viral load or a particular cytokine/chemokine profile in the CNS.

Study Design and Patients

A descriptive study was conducted at the Universidad del Valle-University Hospital (UV-UH) in Cali-Colombia between April 2007 and April 2008. The study was approved by the UV-UH IRB and written informed consent was obtained from all patients or closest relatives. The study enrolled 56 new diagnosed HIV adult patients, 45 patients with well characterized neurological OIs and 11 HIV/AIDS controls without CNS-OIs (Table 1). Diagnosis was established following the CDC and American Academy of Neurology AIDS Task Force criteria. The mortality rate at 3 months of follow up was 44%. (Figure 1).

DISEASE	n	CD4 ⁺ /ml	CD4/CD8 Ratio	Plasma VL (Log ₁₀)
CNS Opportunistic Infection				
Toxoplasma Encephalitis (TE)	12	65.2 ± 36.5	0.13 ± 0.08	5.26 ± 0.69
Cryptococcus Meningitis (CM)	14	69.8 ± 34	0.22 ± 0.14	5.52 ± 0.78
Tuberculous Meningitis (TBM)	6	119.4 ± 90.8	0.41 ± 0.28	5.23 ± 0.62
Lymphocytic Meningitis	5	172.3 ± 123.1	0.24 ± 0.14	5.52 ± 0.96
Tumors	3	56.6 ± 24.9	0.20 ± 0.23	4.78 ± 0.92
Neurospilitis	3	255 ± 461.5	0.29 ± 0.12	8.53 ± 0.67
HCMV Encephalitis	2	39.3 ± 9.3	0.13 ± 0.06	4.53 ± 0.39
HIV/AIDS Non CNS OIs (Control group) **				
	11	61.8 ± 57.5	0.28 ± 0.21	4.85 ± 1.16

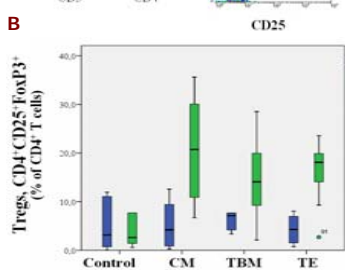
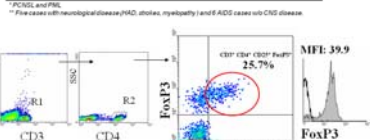


Figure 2. Enrichment of Tregs in the CSF. FACS analysis of CSF Tregs from a CM case (A). Comparison of the mean percentages of Tregs from OI and non-OI groups (B). Wilcoxon control vs. OI: CM P=0.0004; TE P=0.033; TBM P=0.039. Wilcoxon CSF vs. blood: CM P=0.0001; TE P=0.0002; TBM P=0.14

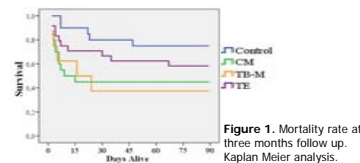


Figure 1. Mortality rate at three months follow up. Kaplan Meier analysis.

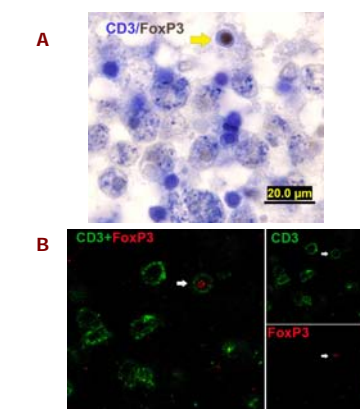


Figure 3. Treg cell infiltration (arrow) into the CNS as revealed by double immunostaining for FoxP3 (brown) and CD3 (blue) (A). Confocal laser microscopy in brain tissue from a patient with toxoplasmosis encephalitis (B) demonstrating the nuclear localization of FoxP3 (red) in CD3 cells (green).

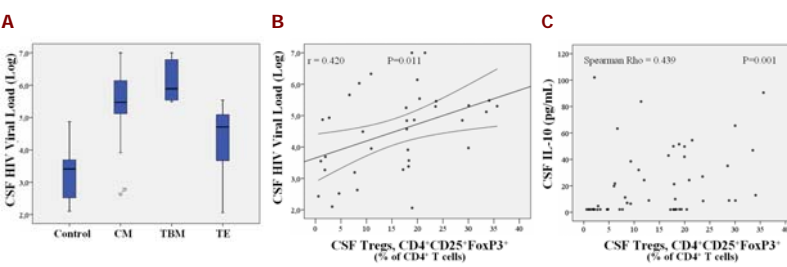


Figure 4. Tregs correlate with HIV viral load in the CSF. Viral load in NeuroAIDS-OIs (A); Pearson's correlation of Tregs and HIV viral load (B) and the relationship between Tregs and IL-10 (C) in CSF.

Materials and Methods

Flow cytometric analysis (FACS) of Regulatory T cells
 CSF cells were obtained immediately after lumbar puncture. CSF cells and peripheral blood mononuclear cells (PBMC) from paired blood samples were stained for analysis of Tregs, (CD3⁺CD4⁺CD25⁺FoxP3⁺) by FACS using the human Treg cell staining kit (eBioscience, San Diego, CA, USA) and anti-CD3 antibody (BD Biosciences). Cells were analyzed on a FACSCalibur flow cytometer (Becton Dickinson®) using CellQuest Pro software. Forward and side scatter signals were used to gate live lymphocytes. 5 x 10⁴ - 1 x 10⁵ events were acquired per sample. Treg cells were expressed as a percentage of total CD4⁺ T cells.

Immunohistochemistry of Tregs in brain tissue
 Brain tissue from patients deceased after neuroAIDS OIs, cryptococcus meningitis and toxoplasma encephalitis cases, from the Johns Hopkins NeuroAIDS brain bank were used for identification of Tregs by immunostaining with anti-FoxP3 (eBioscience) and anti-CD3 (DAKO®) antibodies.
Cytokine/Chemokine detection by multiplexed bead array techniques
 A multiplexed immunocytometric assay was used for cytokine measurement in CSF/serum samples (Beadlyte® Human 22-plex, Millipore®) in a Luminex® 200™ IS Total System.

HIV-1 RNA Load
 HIV-1 RNA was quantified in CSF and plasma samples by COBAS® Ampliprep/COBAS TaqMan HIV-1 assay (Roche Diagnostics) with a sensitivity of 50 copies/ml.
Data Analysis
 Non-parametric Wilcoxon rank test was used to evaluate differences between the mean values of Tregs or HIV RNA titers from OIs and non OIs groups. The correlation between Tregs values and cytokine levels was estimated by Spearman's rank correlation. The relationship between percentage of Tregs and HIV viral load in CSF was evaluated by Pearson's correlation. All tests were 2-sided and significance was considered when P≤0.05.

Results

Patients with OIs had higher frequency of CSF Treg cells (18.4% vs. 5.8%; P=0.033) as compared with HIV/AIDS controls without CNS OIs (Figure 2). The mean percentage of Tregs was markedly higher in the CSF than in the blood of all OIs. Treg cell frequency was significantly higher in cryptococcus meningitis (CM) and toxoplasma encephalitis (TE) cases (P=0.0001 and P=0.0002) as compared with HIV+ without OIs. Similarly, the expression level (MFI) of FoxP3 molecule, a key regulator of Treg function, was higher in the CSF cells than in their paired blood counterparts (p<0.001). These findings indicate that OIs drive Treg cell accumulation at the CNS. Similarly, Treg cells were detected in the brain parenchyma and perivascular spaces in tissue from CM and TE cases, confirming the CNS infiltration by regulatory T cells after OI infection (Figure 3). Mean CSF viral load was higher in the OIs group as compared with controls (5.0 log vs. 2.9 log; P=0.002). Furthermore, CSF viral load distinguished CM or TBM from TE. (Figure 4A). Patients with meningitis had more viral copies in the CSF than TE (5.6 log vs. 4.4 log; P=0.013). No differences were found in HIV-1 viral load, Treg cells or CD4 T cell counts in the blood between the OI and controls without CNS OIs. HIV viral titers in CSF correlated positively with the percentages of Tregs (r=0.439 P=0.011), suggesting Tregs may provide a suppressive environment that impair the viral control in the brain (Figure 4B). A preliminary analysis of the cytokine/chemokine profiles in CSF showed a positive relationship between the Treg cells and the levels of IL-10 (Spearman's Rho=0.4 P=0.0009), an anti-inflammatory cytokine that has been related to the suppressive function of Tregs (Figure 4C).

Conclusions

The increased accumulation of regulatory T cells and HIV viral load in the cerebrospinal fluid of patients with NeuroAIDS opportunistic infections supports the view that Treg cells contribute to an immune suppressive environment. This environment facilitates HIV replication within the CNS. These findings provide new evidence that HIV/opportunistic co-infection may alter HIV neuropathogenesis by influencing the balance between effector and regulatory immune mechanisms.

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