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EARLY ART IN ACUTE HIV LIMITS DETRIMENTAL CX3CR1 MONOCYTES LINKED TO CNS DYSFUNCTION

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Background

- Despite suppressive combination antiretroviral therapy (cART), cognitive dysfunction continues to affect 30-50% of individuals with chronic HIV infection (CHI)[1].
- Transmigration of HIV-infected monocytes into the central nervous system (CNS) is hypothesized to contribute to HIV seeding in the brain leading to resident neuronal cell injury and, in concert with persistent neuro inflammation[2-5], may lead to cognitive decline.
- The CX3C chemokine receptor 1 (CX3CR1) on monocytes, drives tissue homing[6] and results from an ACTG A5248 cohort study found that in CHI, inflammatory and patrolling monocytes express reduced CX3CR1 density compared to HIV uninfected controls. In addition, CX3CR1 on microglia has been reported to have neuromodulatory effects in the brain.
- The relationship between CX3CR1 expressing monocytes in acute HIV infection and with cognitive impairment remains unknown.
- We investigated the dynamics of CX3CR1 density on monocyte subpopulations in individuals who initiated (cART) during AHI and assessed the relationship with central nervous system outcomes.

Methods

Cohort Descriptions: High-risk participants were screened for acute HIV-infection (AHI) at The Thai Red Cross Anonymous Clinic. Participants were categorized into Fiebig I, Fiebig II, Fiebig III or Fiebig IV and immediately began cART. Controls included individuals during CHI (SEARCH 011, NCT00782808) and age-matched HIV-uninfected individuals (SEARCH013) from Thailand.

NCI Assessment by Neuropsychological (NP) testing: NP tests included Trail Making A, Color Trails 1&2 and Grooved Pegboard to compute a summary NPZ-4. All raw scores were transformed to standard z-scores using Thai normative data.

Cell surface CX3CR1 expression by flow cytometry: Cryopreserved peripheral blood mononuclear cells were stained with viability dye (Live/Dead® Fixable Dead Cell Stain) and antibodies against CD3, CD56, CD19, CD20 to exclude lymphocytes, natural killer and B cells. HLA-DR+ monocytes were subset by CD14 and CD16 levels, classical (CD14++CD16-), intermediate (CD14++CD16+) and non-classical (CD14+CD16+) monocytes, and assessed for CX3CR1. FlowJo was used for compensation and gating.

Statistical analysis: Wilcoxon Rank Sum and Wilcoxon Signed Rank tests were compared within and between groups, respectively. Associations analyses were made by Spearman correlations coefficients. Generalized Estimating Equation (GEE) modelling used. Statistical analyses were performed with R v3.2.2.

1. Study participants and demographics

	HIV-Uninfected	HIV-Infected			P-value
		Acute (Fiebig I/II)	Acute (Fiebig III)	Chronic	
	n = 12	n =10	n =8	n = 27	
Gender (% male)	8 (67%)	8 (80%)	8 (100%)	15 (56%)	0.040
Age (years)	31 (27, 35)	36 (27, 41)	27 (23, 30)	33 (29, 37)	0.093
Education (years)	11 (6, 14)	6 (6, 6)	6 (5, 6)	12 (8, 16)	<0.001
NPZ global score	0.23 (-0.16, 0.40)	-0.11 (-0.59, 0.10)	-0.35 (-0.70, -0.13)	0.10 (-0.43, 0.38)	0.211
Log_{10} Plasma Viral Load (copies/ml)	-	4.3 (3.4, 5.3)	5.6 (5.5, 6.3)	5.0 (4.4, 5.4)	0.012
CD4 count (cells/μL)	-	551 (390, 647)	401 (380, 573)	264 (191, 357)	<0.001
CD8 count (cells/μL)	-	405 (239, 550)	864 (568, 1085)		0.034

Data are shown as median (interquartile range), except for gender.
P-values calculated by Chi-squared test for categorical and Kruskal-Wallis test for continuous variables

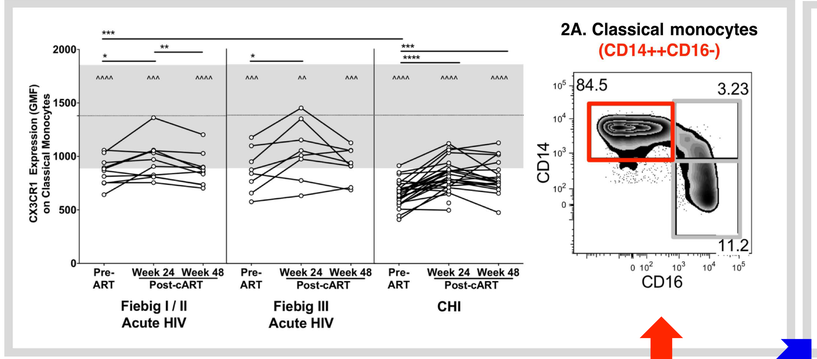
3. Lower CX3CR1 on intermediate monocytes correlates with a reduction in neuropsychological scores in CHI

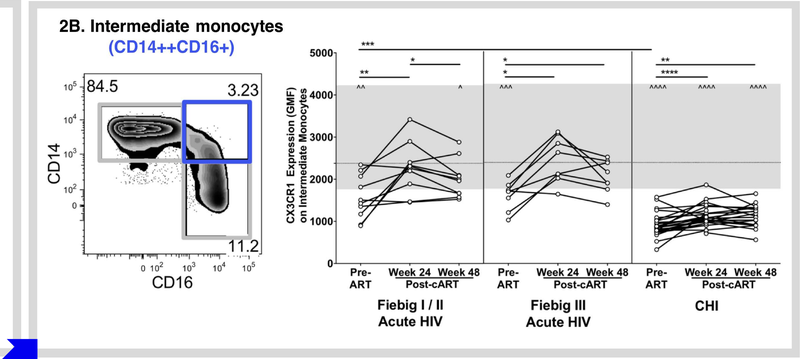
Correlations between CX3CR1 density (geometric mean fluorescence intensity (GMFI)) and neuropsychological-4 Z scores 24 weeks (left) and 48 weeks (right) post-cART.

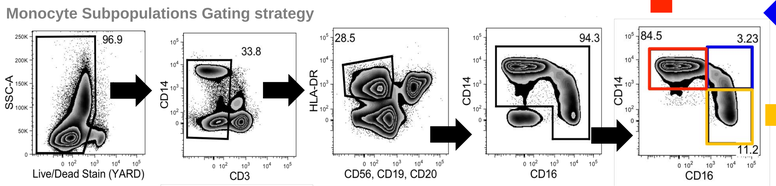
Rho and p values were generated by Spearman's correlations.

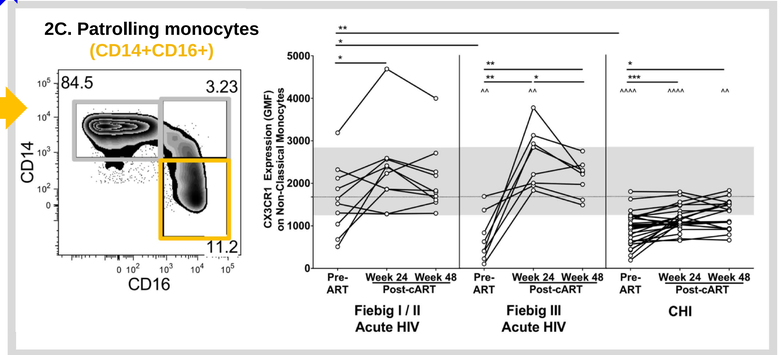
		Neuropsychological Global Z-score							
		HIV-Uninfected		HIV-Infected					
				Acute (Fiebig I/II)		Acute (Fiebig III)		Chronic	
		n = 12		n =10		n =8		n = 27	
GMFI CX3CR1+ on Monocyte Subpopulations	Subset	Time	rho	p value	rho	p value	rho	p value	rho	p value
	Classical Monocytes	Week 0	-0.767	0.021	0.233	0.552	-0.200	0.714	-0.068	0.737
		Week 24			-0.517	0.162	0.429	0.299	-0.047	0.804
		Week 48							-0.107	0.663
	Intermediate Monocytes	Week 0	-0.728	0.026	0.450	0.230	0.029	1.000	0.106	0.598
		Week 24			-0.100	0.810	0.383	0.349	-0.380	**0.038**
		Week 48							-0.486	**0.035**
	Non-Classical Monocytes	Week 0	-0.350	0.359	0.450	0.230	0.200	0.714	0.301	0.127
		Week 24			0.200	0.613	0.381	0.360	-0.018	0.923
		Week 48							-0.119	0.627

2. Differential CX3CR1 density on circulating monocyte subpopulations from acute and chronic HIV infected individuals as compared with uninfected HIV controls









Monocytes, positive for HLA-DR, were subset based on CD14 and CD16 expression into **(2A) classical (CD14++CD16-)**, **(2B) intermediate/inflammatory (CD14++CD16+)** and **(2C) non-classical/patrolling (CD14+CD16+) monocytes**. Geometric mean fluorescence intensity (GMF) of CX3CR1 in acute HIV infection, stratified by Fiebig I/II and Fiebig III stages, and chronic HIV infection (CHI), before and after (24 and/or 48 weeks) cART initiation. Connected data points represent longitudinal data for one participant. Gray boxes indicate range of HIV uninfected control values while dashed lines indicate median HIV uninfected CX3CR1 expression. P-values calculated by Wilcoxon rank sum tests to compare between groups and Wilcoxon signed rank tests to compare within groups, without multiple comparison adjustment.

* p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001 **between the HIV groups** and

^ p<0.05; ^^ p<0.01; ^^^ p<0.001; ^^^^ p<0.0001 **between HIV infected and HIV uninfected controls**

Conclusion

- Reduced CX3CR1 density on monocytes occurs in CHI and *remains low* despite 48 weeks of cART when compared to HIV uninfected controls.
- We found that, post ART in CHI, *lower* circulating CX3CR1 expressing monocytes associates with *a reduction in* neuropsychological scores.
- Our data demonstrate that unlike in treated CHI, early cART instituted in AHI restores perturbed CX3CR1 density on inflammatory monocytes and these residual monocytes *are not* associated with any aberrant cognitive measures.
- These data reveal *benefits* of initiation of cART early during acute HIV infection. Early cART *may halt a detrimental cascade* initiated by monocyte trafficking to the CNS, weakly associated with a reduction in cognitive decline, consistent with the need to examine additional cases to validate these findings.

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Disclaimers: The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70–25. *The views expressed are those of the authors and should not be construed to represent the positions of the U.S. Army or the Department of Defense.*