CXCR3 and CCR5 expression are associated with 2-LTR circles in patients on antiretroviral therapy

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Abstract

Background: Identifying a subset of T-cells that are enriched for HIV infection in patients on suppressive antiretroviral therapy (ART) will be critical to developing new curative strategies. This study aimed to assess how the expression of inflammatory (CCR5, CXCR3, CCR6, CXCR5) and homeostatic (CCR7) chemokine receptors (CKR) and their ligands correlate with the size of the latent reservoir in patients receiving suppressive ART.

Methodology: 48 subjects on ART for >3 years with HIV viral load <50 copies/ml were recruited in San Francisco. Using flow cytometry, we quantified the expression of the CKR CCR5, CXCR3, CCR6, CXCR5 and CCR7 on CD4+ T-cells. The corresponding ligands for these chemokine receptors were quantified in plasma (Luminex). Total, integrated and 2LTR circle HIV DNA and cell associated unspliced (CA-US) HIV RNA was quantified in CD4+ T-cells. Spearman correlations and negative binomial regression models were performed to test associations between markers of virus persistence and CKR expression on CD4+ T-cells adjusting for current and nadir CD4 T-cell counts.

Results: Subjects were predominantly male (96%) with a median CD4= 684 (IQR=533-858) cells/μl. There were statistically significant negative correlations between current CD4 count and expression of the inflammatory CKR CCR5 (r=-0.50, p<0.001), CCR6 (r=-0.42, p<0.01) and CXCR3 (r=-0.57, p<0.0001) and a positive correlation with the homeostatic CKR CCR7 (r=0.59, p<0.0001). There was a statistically significant negative correlation between integrated HIV DNA and current CD4 (r=-0.52, p=0.0001) but not nadir CD4. CCR5 was the only CKR that substantially correlated positively with integrated HIV DNA (r=0.29, p=0.045) but this largely disappeared when controlled for current or nadir CD4 count in a negative binomial regression model. 2-LTR circles were negatively correlated with nadir CD4 (r=-0.45, p=0.002) but not current CD4. In a negative binomial regression model, 2LTR circles were positively correlated with CXCR3 expression (p<0.001) and CCR5 expression (p=0.032) and both remained statistically significant after adjusting for nadir or current CD4 count. There was no significant relationship between total HIV DNA, CA-US RNA and CKR expression. There was no statistically significant relationship between virus persistence and any of the chemokines measured in plasma, except for a positive correlation between 2LTR circles and the ligand for CCR7, CCL21 (r=0.30, p=0.045).

Conclusions: The association of 2LTR circles with nadir CD4 and CXCR3 and CCR5 expression on CD4 T-cells raises the possibility of ongoing replication in cells that express these specific inflammatory CKR, such as effector memory T-cells, particularly in individuals who initiate ART at lower CD4 counts.

Background

Latently infected CD4+ T-cells are a major barrier to the eradication of HIV. Central memory, transitional memory and naïve CD4+ T-cells are the main source of HIV-infected T-cells that persist during long-term suppressive cART (Wightman, JID, 2010; Chomont, Nat Med, 2009; Finzi, Science, 1999; Chun, Nature, 1997). In vitro, chemokines which bind to chemokine receptors CCR7, CCR6 and CXCR3 are able to induce post-integration latency in resting CD4+ T-cells (Saleh, Blood, 2007; Cameron, PNAS, 2010). Chemokine receptors are important for the homing of CD4+ T-cells to the tissue during an immune response and also to niches that are essential for CD4+ T-cell homeostasis.

Hypothesis and Aims

Hypothesis: CD4+ T-cells expressing chemokine receptors CCR6 and CXCR3 are preferential targets of HIV infection and are an important reservoir of HIV during suppressive cART.

Aim: To assess how the expression of chemokine receptors and their ligands correlate with the size of the latent reservoir in patients receiving suppressive cART.

Cohort Demographics

Parameter	n=48	n=20
Gender, n (%)		
Male	46 (96%)	20 (100%)
Female	1 (2%)	0 (0%)
Transgender	1 (2%)	0 (0%)
Age, years	57 (51-62)	58 (50-62)
Nadir CD4 count, cells/μl	216 (133-385)	136 (78-376)
Current CD4 count, cells/μl	684 (533-858)	639 (496-733)
Current CD4 percentage	32 (26-41)	30 (23-35)
ART, years	8.53 (5.02-12.37)	11 (5.33-13.13)
Viral load (copies/ml)	<50	<50

Table 1: Patient demographics. 48 patients receiving suppressive cART were recruited into the study at the University of California, San Francisco. Values are median (interquartile range), unless stated otherwise..

A subset of 20 patients consented to additional procedures including leukaphoresis and tissue collection. Large volume bloods were used in CD4+ T-cell subset sorting experiments.

Methods

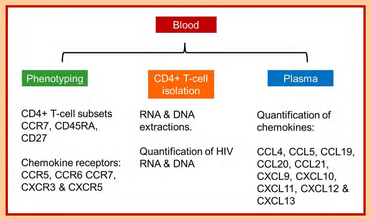


Figure 1: Total and CD4+ T-cell subsets were phenotyped for chemokine receptor expression. Total CD4+ T-cells were isolated using negative depletion, DNA and RNA were extracted for quantification of the reservoir. Plasma chemokine levels were quantified using multiplex Luminex bead arrays.

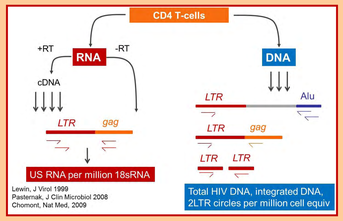
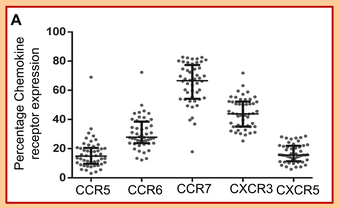


Figure 2: Quantification of the HIV reservoir in sorted CD4+ T-cells from PBMC isolated from patients receiving suppressive cART. Cell-associated unspliced HIV RNA was quantified using semi-nested QPCR and HIV DNA species also with QPCR..

Results

(1) Current CD4+ T-cell count and expression of homeostatic and inflammatory chemokine receptors are strongly associated.



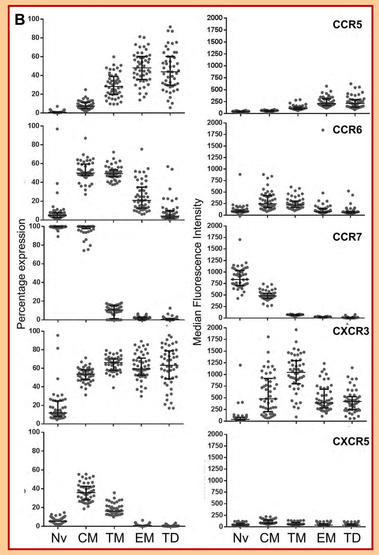


Figure 3: The expression of chemokine receptors on total and CD4+ T-cell subsets. Inflammatory chemokine receptors CCR5, CCR6, CXCR3 and CXCR5, plus the homeostatic chemokine CCR7 were measured on total CD4+ T-cells., expressed as a percentage (A). CD4+ T-cell subsets, (B), percentage (left panel) and MFI (right panel). Naïve (Nv); Central Memory (CM); Transitional Memory (TM); Effector Memory (EM); Terminally Differentiated (TD).

Percentage		CD4+ T-cell count cells/μl
CCR7+CD4+	r	0.561
	pvalue	**<0.0001**
CCR6+CD4+	r	-0.421
	pvalue	**<0.01**
CXCR3+CD4+	r	-0.551
	pvalue	**<0.0001**
CCR5+CD4+	r	-0.493
	pvalue	**<0.001**
CXCR5+CD4+	r	-0.148
	pvalue	0.317

Table 2: Correlations between chemokine receptor expression and current CD4+ T-cell count. Spearman Rho correlations revealed strong statistically significant positive relationships between CCR7 expression and current CD4+ T-cell count and inverse relationships with CCR5, CCR6, and CXCR3,

(2) Current and nadir CD4+ T-cell counts are inversely correlated with integrated DNA and 2-LTR circles

Table 3: Correlations between HIV reservoir markers and current and nadir CD4+ T-cell count. Statistically significant inverse relationships were seen between current CD4+ T-cell count and integrated HIV DNA, and nadir CD4+ T-cell count and 2-LTR circles. p value <0.05 are statistically significant and in red bold

HIV Reservoir		Current CD4+ T-cell count	Nadir CD4+ T-cell count
Integrated HIV DNA	r	-0.523	-0.097
	p value	**<0.0001**	0.512
Total HIV DNA	r	-0.174	-0.066
	p value	0.247	0.661
2-LTR HIV DNA	r	-0.060	-0.454
	p value	0.697	**0.002**
Cell-associated Unspliced HIV RNA	r	-0.126	-0.095
	p value	0.415	0.539

(3) CCR5 expression is associated with integrated HIV DNA.

Percentage		Integrated HIV DNA copies/million
CCR7+CD4+	r	-0.196
	pvalue	0.182
CCR6+CD4+	r	0.247
	pvalue	0.091
CXCR3+CD4+	r	0.265
	pvalue	0.069
CCR5+CD4+	r	0.290
	pvalue	**0.045**
CXCR5+CD4+	r	0.135
	pvalue	0.362

Table 4: Correlations between chemokine receptor expression and the reservoir. Spearman Rho correlations were performed and CCR5 expression was moderately statistically significantly associated with integrated HIV DNA. There were no other significant relationships between other markers of the reservoir – total or 2- LTRHIV DNA and US-RNA. There were no satistically significant correlations between chemokine concentration in the plasma and the reservoir.

(4) In multivariate models, adjusting for current and nadir CD4+ T-cell counts, CXCR3 and CCR5 are associated with 2-LTR circles.

Table 5. Negative binomial regressions models: Four models were run (1) Single predictor which compared integrated HIV DNA to the percentage chemokine receptor expression on total CD4+ T-cells. (2) Adjusted for the effect of current CD4+ T-cell count. (3) Adjusted for the effect of nadir CD4+ T-cell count. (4) Adjusted for the effects of both current and nadir CD4+ T-cell count. Percentage CCR5+ CD4+ T-cells values were Log2 transformed; p value <0.05 are statistically significant and in bold. CCR5-integrated HIV DNA model data represented here is post-sensitivity testing where two outliers were removed.

Predictor	Integrated HIV/10^6 CD4+ T-cells Fold change	95% CI	P value	2-LTR circles/10^6 CD4+ T-cells Fold change	95% CI	P value
1 Single predictor						
% CCR5*	1.386	1.042-1.843	**0.025**	0.613	0.392-0.958	**0.032**
% CXCR3	1.028	0.998-1.059	0.069	0.947	0.918-0.977	**0.001**
2 Concurrent CD4+ T-cell count						
% CCR5*	1.060	0.841-1.335	0.624	0.575	0.367-0.900	**0.015**
% CXCR3	1.009	0.978-1.042	0.558	0.944	0.903-0.988	**0.012**
3 Nadir CD4+ T-cell count						
% CCR5*	1.358	1.053-1.750	**0.018**	0.610	0.432-0.862	**0.005**
% CXCR3	1.028	0.999-1.057	0.056	0.943	0.919-0.966	**<0.0001**
4 Both concurrent & nadir CD4+ T-cell count						
% CCR5*	1.079	0.855-1.361	0.523	0.727	0.463-1.142	0.167
% CXCR3	1.010	0.979-1.041	0.537	0.955	0.913-0.999	**0.043**

(5) Integrated HIV DNA is enriched in central memory CD4+ T-cells that express CCR6 and CXCR3

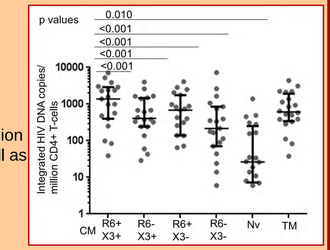


Figure 4: In a subset of 20 patients who underwent leukaphoresis CD4+ T-cell were sorted into Naïve (Nv), Transitional Memory (TM) and Central Memory (CM) subsets expressing both (R6+X3+), individually (R6-X3+ or R6+X3-) or negative (R6-X3-) for chemokine receptors CCR6 and CXCR3.

(6) Summary

1. There is a strong association between current CD4 and expression of both inflammatory and homeostatic chemokine receptors as well as the size of the reservoir as measured by int DNA

2. There is a strong association between nadir CD4 and 2-LTR circles suggests that persistent virus replication may be greatest in those who initiate ART late.

3. Multivariate analysis adjusting for current and nadir CD4, showed that CXCR3 and CCR5 were associated with 2-LTR expression

4. CM cells that express CXCR3 and CCR6 were enriched for integrated DNA

Conclusions

1. The association of 2LTR circles with nadir CD4 and CXCR3 and CCR5 expression on CD4 T-cells raises the possibility of ongoing replication in cells that express these specific inflammatory CKR, such as effector memory T-cells, particularly in individuals who initiate ART at lower CD4 counts.

2. CXCR3+CCR6+ CM have the highest frequency of integrated DNA and may identify a subset of cells that have increased susceptibility to latent infection.

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