Effects of CCR5-Δ32 Heterozygosity on HIV-1 Reservoir Size and Immune Activation

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BACKGROUND

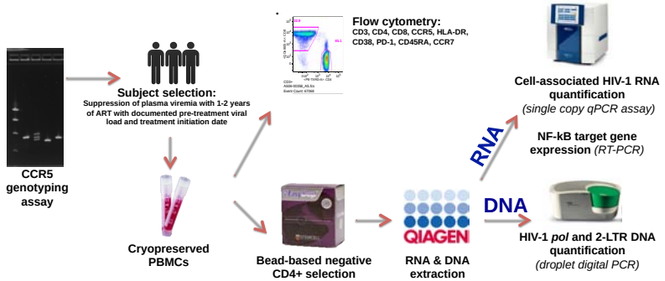
- HIV persists at a steady level during effective antiretroviral therapy and host factors that determine the level of HIV persistence are unknown
- CCR5 Δ32 heterozygosity (wt/Δ32) has been previously linked to long-term non-progression and protection from HIV acquisition[1-3]
- CCR5 wt/Δ32 individuals may have up to a one log reduction in plasma viral load[4-6]
- Limited CCR5 expression on central memory CD4+ T cells helps protect sooty mangabeys from pathogenic infection[7]
- The Berlin Patient was cured and the Boston patients exhibited delayed viral rebound after allogeneic hematopoietic stem cell transplant; all three were CCR5 wt/Δ32 at baseline[8-9]
- Intensification with maraviroc, a CCR5 antagonist, increased CD4+ T cell activation in antiretroviral therapy (ART)-suppressed patients[10]

OBJECTIVE

To determine the impact of CCR5 surface expression on the HIV-1 reservoir

METHODS

We measured cellular HIV-1 *pol* and 2-LTR circle DNA, cell-associated HIV-1 RNA, and immunologic cell surface markers in 18 CCR5 wt/Δ32 and 54 CCR5 wild type individuals on suppressive ART.



Two-tailed Mann-Whitney t-tests, Spearman correlations, and Fisher's exact tests were used to determine statistical significance.

Table 1. Demographic and treatment history for study subjects.

CCR5 genotype		Wild type	CCR5 wt/Δ32	Total
Sample size		54	18	72
Age	Median	39.39	38.85	39.38
	Range	24-57	28-53	24-57
Sex	Male (%)	50 (93%)	18 (100%)	68 (94%)
	Female (%)	4 (7%)	0 (0%)	4 (6%)
Ethnicity	White	40 (74.1%)	16 (88.9%)	56 (77.8%)
	Black	9 (16.7%)	1 (5.6%)	10 (13.9%)
	Latino	5 (9.3%)	0 (0%)	5 (6.9%)
	Mix/other	0 (0%)	1 (5.6%)	1 (1.4%)
Duration of treatment	Median	1.54	1.73	1.59
	Range	1.05-1.98	1.30-1.90	1.05-1.98
CD4 nadir	Median	269.5	320	283.5
Pre-treatment viral load	Median	47,811	45,531	47,810.5

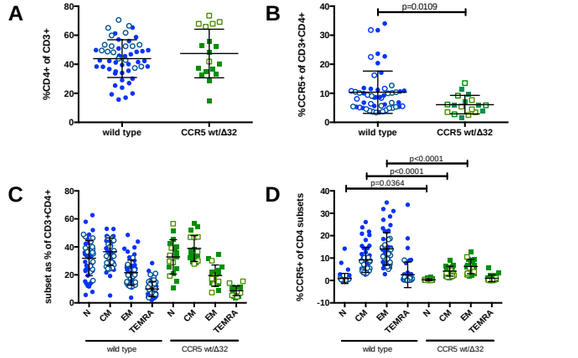


Figure 1. CCR5 cell surface expression is decreased on CD4+ T cells in CCR5 wt/Δ32.
CCR5 wild type and CCR5 wt/Δ32 individuals had similar frequencies of CD4+ T cells (A). The frequency of CCR5+ CD4+ T cells was significantly lower in CCR5 wt/Δ32 (B). These patterns held true for all CD4+ T cell subsets (C – D). Subjects that initiated treatment during early infection and chronic infection are in open and closed circles, respectively.

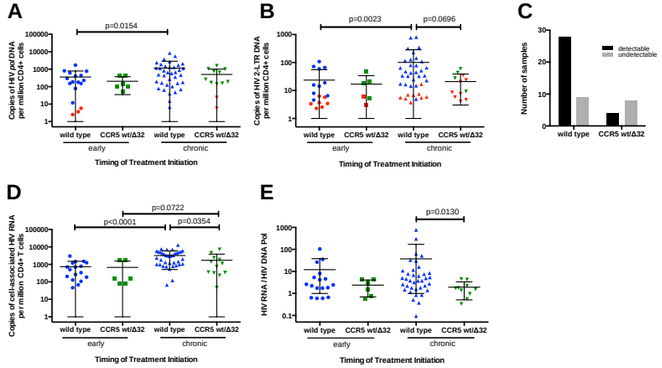


Figure 2. Markers of ongoing viral replication in CD4+ T cells are reduced in CCR5 wt/Δ32.
The HIV-1 reservoir size was evaluated by copies of HIV-1 *pol* DNA (A), copies of 2-LTR circle DNA (B), proportion of detectable 2-LTR circle DNA in subjects who initiated treatment during chronic infection (C), and copies of cell-associated HIV-1 RNA (D). HIV-1 transcription was measured as HIV-1 RNA:DNA ratio (E). Subjects with undetectable HIV-1 DNA by droplet digital PCR are indicated in red, reported as the limit of detection.

RESULTS

- CCR5 wt/Δ32 have lowered CCR5 cell surface expression in all CD4+ subsets
- No difference was observed in HIV-1 *pol* DNA levels between CCR5 genotypes
- Cell-associated HIV-1 RNA (p=0.035), HIV-1 RNA:DNA ratios (p=0.013), and frequency of detectable 2-LTR circle DNA (p=0.013) were significantly lower in CCR5 wt/Δ32
- Cell-associated HIV-1 RNA was significantly correlated with CCR5 cell surface expression on CD4+ T cells (r^2=0.136, p=0.002)
- No difference was observed in %HLA-DR+CD38+ and %PD-1+ CD4+ T cells and in CD4+ T cell subsets between the two CCR5 genotypes (data not shown)
- NF-κB pathway (IκBa, IKK) and target genes (IL-6, TNF-a, IFNg) were expressed at similar levels between genotype groups (data not shown)

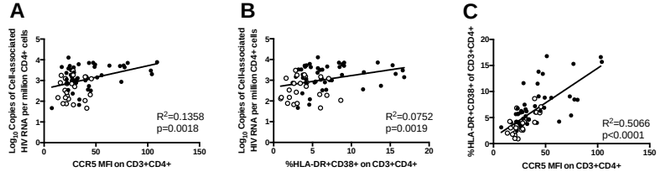


Figure 3. Cell-associated HIV-1 RNA is significantly associated with CCR5 cell surface expression.
Cell-associated HIV-1 RNA was significantly correlated with CCR5 MFI on CD4+ T cells (A) and with frequency of activated CD4+ T cells (B). Frequency of activated CD4+ T cells was correlated with CCR5 MFI (C). Subjects that initiated treatment during early infection are in open circles and chronic infection in closed circles.

CONCLUSIONS

- The gene dosage effect of CCR5 Δ32 is seen equally in all CD4 subsets
- Limited CCR5 surface expression decreases HIV-1 transcription
- Limited CCR5 surface expression may suppress ongoing viral replication
- CCR5 surface expression does not impact NF-kB transactivation potential
- Decreased CCR5 surface expression has different consequences than masking of CCR5 by maraviroc

FUTURE DIRECTIONS

- Characterize the effects of CCR5 Δ32 on HIV-1 population genetics in ART-suppressed individuals
- Determine if CCR5 surface expression modulates HIV-1 residual replication using in vitro models
- Examine the effects of CCR5 surface expression on the HIV-1 reservoir in lymphoid tissues

Acknowledgements:

This research was supported by amfAR grant 108545 with support from FAIR, and NIH grants R21 AI108503, U19 AI096109, and P30 AI027763.

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