Altered and Incomplete Immune Reconstitution in HIV+ Stem Cell Transplant Recipients

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Aim

To characterise immune reconstitution in HIV-positive individuals undergoing allogeneic stem cell transplants (ASCT) in different immunological compartments.

Introduction

HIV positive individuals are at increased risk of developing the malignancies that require ASCT. While there is increasing evidence of the safety of ASCT in HIV positive individuals, to date, there has been no description of the immune reconstitution in these individuals post-transplant. We show here that in both PBMCs and lymph nodes immune reconstitution appears to be altered in HIV-positive individuals, compared to uninfected controls.

Method

We retrospectively evaluated HIV and CMV viral loads as well as immune reconstitution (measured by CD4+ and CD8+ peripheral T cell counts) in six HIV-positive individuals on ART who underwent ASCT for haematological malignancies (HIV-pos-ASCT). CD4+ and CD8+ T cell reconstitution were compared with HIV-negative ASCT controls (HIV-neg-ASCT, n=17). In order to compare HIV-pos-ASCT and HIV-neg-ASCT individuals we selected the closest available measurement to 12 months post transplant for HIV-pos-ASCT (prioritising dates measured prior to 12 months as opposed to after) and the 12 month post-transplant measurement for HIV-neg-ASCT subjects.

Four HIV-pos-ASCT subjects also consented to fine-needle biopsy (FNB) of inguinal lymph-nodes pre and up to 4 years post-ASCT. These findings were then compared to non-ASCT HIV-positive (treated n=11, untreated n=10) and HIV-negative (n=10) controls, collected as part of a previous study[1].

Frozen PBMC were available for two subjects and we performed additional, detailed, flow cytometric characterisation of the CD4+ and CD8+ T cell subsets, at time points pre and up to 15 months post-transplant. For the detailed flow cytometric analysis we designed three 14-colour flow cytometry panels - to identify numerous populations of interest post-transplant, including differentiation markers, chemokine receptors and trafficking markers. Markers included CD3, CD19, CD4, CD8, CD45RA, CD45RO, CD31, CD127, CD95, CD25, CD38, CD161, CCR6, CD49d, integrin b7, CD73, CXCR5, CD56, CD45, CD57, CD16, CD14, CD28, CD123, CD11c and HLA-DR

Results

Patient Population

Baseline characteristics for ASCT subjects can be found in Table 1.

Table 1. ASCT Baseline Characteristics

	HIV-pos-ASCT (n=6)	HIV-neg-ASCT (n=17)
Age (Median, Range)	50, 42-60	48 (23-68)
Male	5/6	
Underlying Malignancy:		
Acute Myeloid Leukemia	4/6	7/17
Plasmablastic Non-Hodgkins Lymphoma	1/6	2/17
Multiple Myeloma	1/6	0/17
Chronic Lymphocytic Leukaemia	0/6	2/17
Myelodysplastic Syndrome	0/6	2/17
Other	0/6	4/17
Conditioning:		
Myeloablative	1/6	12/17
Reduced Intensity	5/6	5/17
Suppressed HIV at time of transplant	6/6	n/a
HIV reactivation post-ASCT	0/6	n/a
CMV reactivation post-ASCT	2/5 (one participant had no available CMV data)	Data not available

Peripheral Blood CD4+ and CD8+ T cell reconstitution post-transplant

There were no significant differences in CD4+ T cell counts between HIV-pos-ASCT subjects (median = 367 cells/ul (IQR=188)) and controls (484 cells/ul (444)) at time-points ~6-12 months post-ASCT. In contrast, CD8+ T cells, while not significant, tended to be higher in HIV-pos-ASCT subjects (1693 cells/ul (1556)) vs controls (748 cells/ul (922)) (Figure 1.). In four HIV-pos-ASCT patients, CD8+ T cells , but not CD4+ T cells, continue to rise after 12 months. However, as no HIV- subjects had measurements post 12 months, statistical comparisons were not possible using these time-points.

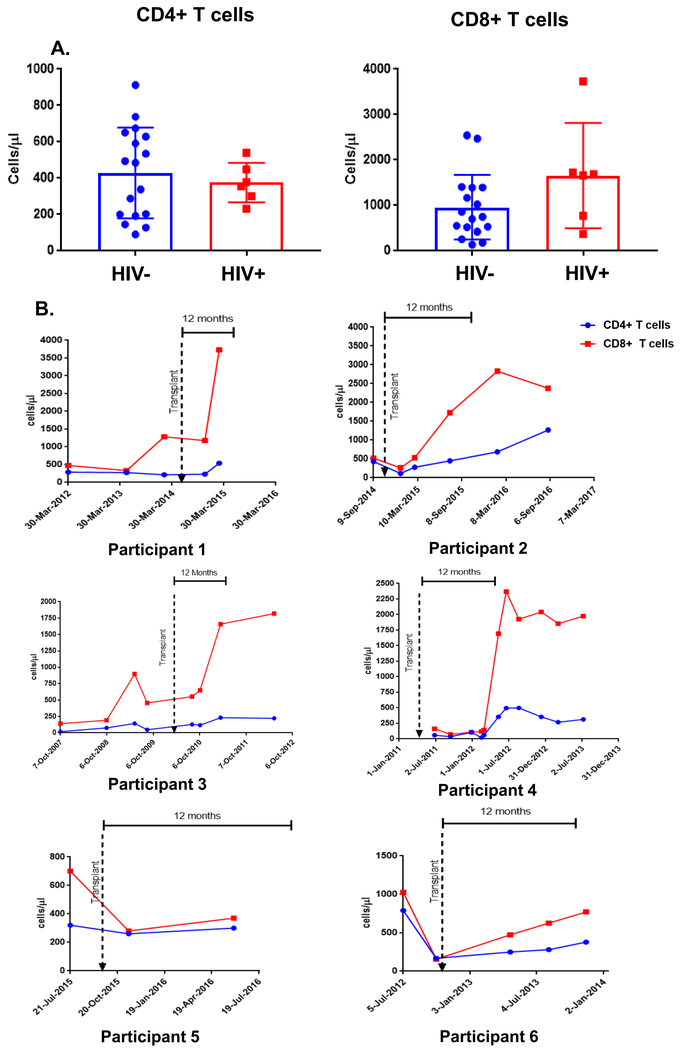


Figure 1. (A) Comparison of CD4+ T cells and CD8+ T cells between HIV-pos-ASCT and HIV-neg-ASCT individuals at approximately 12 months post-transplant . (B) CD4+ T cell and CD8+ T cell counts at all available time-points (HIV-pos-ASCT participants only).

Lymph Node CD4+ T cell Reconstitution Post-Transplant

Comparisons of FNB T cells showed much lower CD4+ T cells in HIV-pos-ASCT (median=3.1x10^4 cells (IQR=1.5x10^5), compared to HIV-negative non-ASCT controls (8.9x10^5 cells (9.8 x10^5)) and HIV+ non-ASCT subjects, both treated (2.3x10^5 cells (1.9x10^5)) and untreated (5.9x10^5 cells (1.1x10^6)) (Figure 2.).

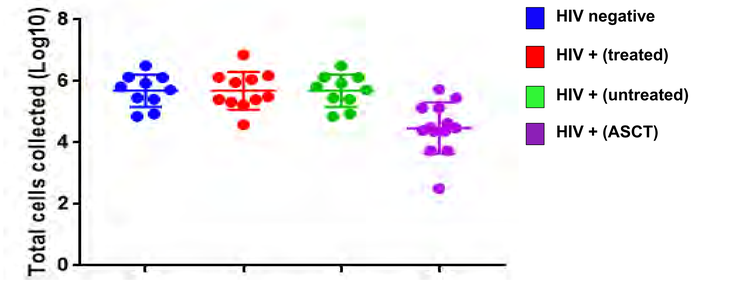


Figure 2. Comparison of total CD4 + T cells isolated from FNBs of HIV-pos-ASCT patients compared to non-ASCT HIV-positive (treated n=11, untreated n=10) and HIV-negative (n=10) patients. Total CD4+ T cells isolated from FNB's were Log transformed and plotted.

Flow Cytometric Characterisation of elevated Peripheral Blood T cells

In two subjects, both with elevated CD8+ T cell counts, further flow cytometry characterisation of PBMCs showed that one individual had elevated activated non-gut homing terminally differentiated CD8+ T cells, while the other predominantly had activated gut homing CD8+ T cells (Figure 3.).

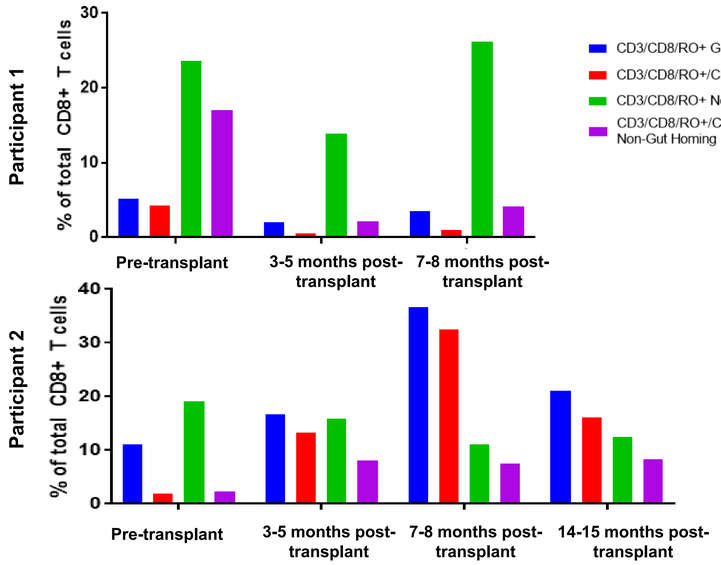


Figure 3. Subsets of CD45RO+ CD8 T cells, including integrin alpha4 (CD49d)+integrin ß7+ gut homing cells and integrin alpha4 (CD49d)+integrin ß7- non-gut homing CD8+ T cell subsets. Participant 1 predominantly had an elevation of non-gut homing CD8+ T cells post transplant. Participant 2 had an elevation of activated (CD38+) gut-homing CD8+ T cells, particularly at 7-8 months post-transplant. T cell subsets are presented as percentages of total CD8+ T cells

Conclusion

- Compared to HIV-neg-ASCT controls, HIV-pos-ASCT individuals showed comparable peripheral blood recovery of CD4+ T-cells, but increased numbers of CD8+ T-cells post-ASCT – observations that warrant further investigation in a larger, well controlled cohort
- FNB data suggests that, compared with non-ASCT controls, peripheral CD4+ T cell recovery is impaired in HIV-pos-ASCT individuals, even several years post-transplant.
- Detailed flow cytometry suggests that the subsets that make up the observed elevated PBMC CD8+ T cells vary between individuals
- While subject numbers are limited, the differences seen here suggest that HIV associated factors, such as co-infections and pre-existing immune impairment, impact immune reconstitution in HIV+ individuals undergoing ASCT
- Future work will assess the role of herpesvirus reactivation on the levels of CD8+ T cells post-transplant and how the altered immune reconstitution impacts HIV-reservoir.

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[1]Hey-Nguyen, W.J., et al., *Quantification of Residual Germinal Center Activity and HIV-1 DNA and RNA Levels Using Fine Needle Biopsies of Lymph Nodes During Antiretroviral Therapy.* AIDS Res Hum Retroviruses, 2017. 33(7): p. 648-657.