Targeting SIV Reservoirs via Autologous Hematopoietic Stem Cell Transplant in Rhesus Macaques

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ABSTRACT

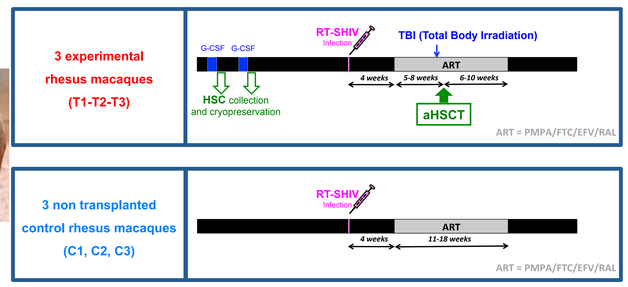
Despite many advances in AIDS research, a cure for HIV infection remains elusive. Here, we performed autologous hematopoietic stem cell transplantation (HSCT) in three Simian/Human Immunodeficiency Virus (SHIV)-infected, antiretroviral therapy (ART)-treated rhesus macaques (RMs) using HSCs collected prior to infection and compared them to three SHIV-infected, ART-treated, untransplanted control animals to assess the effect of conditioning and autologous HSCT on viral persistence. As expected, ART suppressed virus replication below detectable limits in all animals. After a period of prolonged suppression on ART, experimental RMs received myeloablative total body irradiation (1080cGy), which resulted in the depletion of 94-99% of circulating CD4+ T-cells, followed by pre-infection HSC infusion and successful engraftment. ART was interrupted 40-75 days post-transplant, and rapid rebound of plasma viremia was observed in all three untransplanted control animals as well as two out of three transplanted RMs. In the third transplanted animal, plasma SHIV-RNA and SHIV-DNA in PBMCs remained undetectable at week two post-ART interruption; however further analyses conducted post-necropsy revealed detectable SHIV-DNA in sorted circulating CD4+ T-cells, spleen and lymph nodes but not in the gastro-intestinal tract or tonsils. These data show the persistence of virus reservoir despite myeloablation and transplantation of pre-infection HSCs in ART-suppressed, SHIV-infected RMs.

INTRODUCTION

The introduction of antiretroviral therapy (ART) has dramatically reduced the morbidity and mortality associated with HIV infection and AIDS. However, currently available ART requires life long treatment with significant potential side effects and a cost that places an inordinate burden on public health systems. While reduction of HIV viral loads below detectable limits is often achieved in ART-treated individuals, a treatment that can eradicate or functionally cure HIV infection remains elusive. A key obstacle to the development of a cure for HIV is an incomplete understanding of the cellular and anatomic nature of viral reservoirs in the setting of successful ART. To address this gap in our understanding, we developed a model of autologous hematopoietic stem cell transplantation (aHSCT) in SHIV-infected, ART-treated rhesus macaques (RM) to interrogate the origin of the SIV reservoir.

METHODS





	● T1	▲ T2	■ T3	○ C1	△ C2	□ C3	*Mean*
Age (years)	4.3	4.2	4.2	4.2	4.1	4.1	*4.2*
Gender	M[1]	M	M	M	M	M	n/a[2]
Transplant	**+**	**+**	**+**	**-**	**-**	**-**	**n/a**
TNC[3] dose/kg	$4.6x10^8$	$8.9x10^8$	$8.3x10^8$	n/a	n/a	n/a	$7.3x10^8$
$CD34^+$ cell dose/kg	$1.5x10^6$	$5.1x10^6$	$2.0x10^6$	n/a	n/a	n/a	$2.9x10^6$
ART duration (days)	**86**	**78**	**128**	**86**	**78**	**128**	***97.3***
pre transplant	37	38	53	n/a	n/a	n/a	*42.7*
post transplant	49	40	75[4]	n/a	n/a	n/a	*54.7*

[1] *M: male*
[2] *n/a: non applicable*
[3] *TNC: total nucleated cells*
[4] *PMPA was interrupted at day 36 post-transplant*

RESULTS

Autologous HSCT : conditioning and engraftment

TBI induced a massive reset of the lympho-hematopoietic compartment resulting in in the depletion of 94-99% of circulating CD4+ T-cells. Engraftment of the HSC was then successfully achieved in the 3 transplanted RMs as demonstrated by increasing neutrophil and platelet counts unsupported by transfusion.

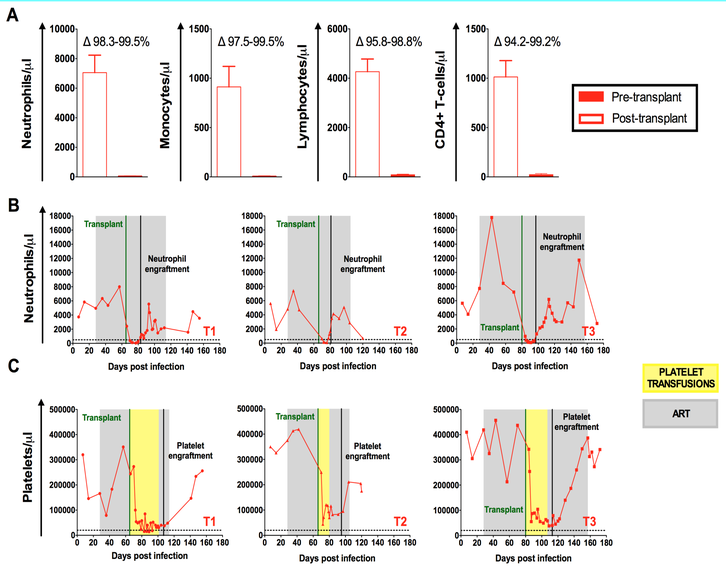


Figure 1. Autologous hematopoietic stem cell transplant. (A) Comparison of the mean pre- and post-transplant absolute numbers of circulating neutrophils, monocytes, lymphocytes and CD4+ T-cells. Pre-transplant time point is the final assessment prior to TBI. Post-transplant time point represents the nadir cell count observed during the eleven days following TBI. (B) Longitudinal assessment of the absolute numbers of circulating neutrophils and (C) platelets. The dotted lines indicate the minimum level of neutrophils or platelets used to define engraftment.

Effect of autologous HSCT on CD4+ T-cells

Following transplantation and engraftment, we observed a slow reconstitution of the circulating CD4+ T-cells. Immunophenotypic analyses revealed a significant increase in the proportion of memory CD4+ T-cells (including memory stem cells, central memory, and effector memory) following transplantation.

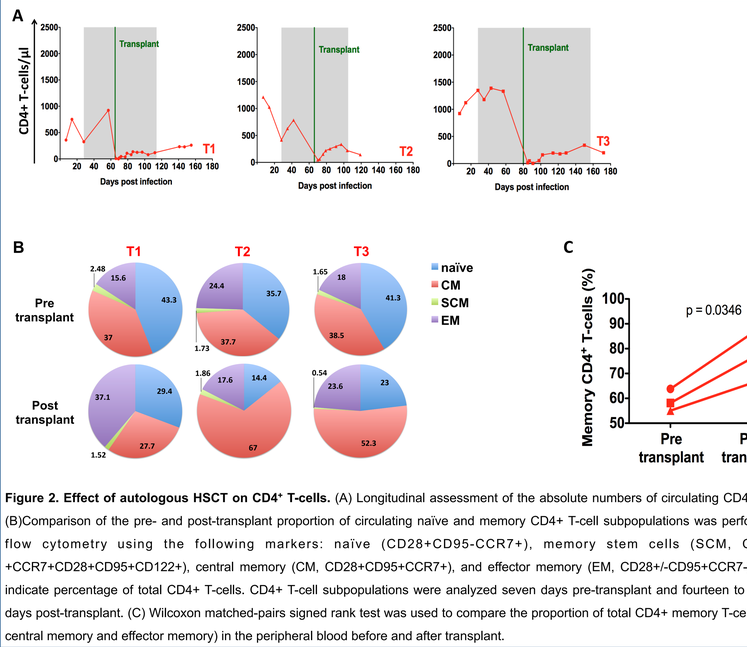


Figure 2. Effect of autologous HSCT on $CD4^+$ T-cells. (A) Longitudinal assessment of the absolute numbers of circulating $CD4^+$ T-cells. (B)Comparison of the pre- and post-transplant proportion of circulating naïve and memory CD4+ T-cell subpopulations was performed by flow cytometry using the following markers: naïve (CD28+CD95-CCR7+), memory stem cells (SCM, $CD45RA^+$CCR7+CD28+CD95+CD122+), central memory (CM, CD28+CD95+CCR7+), and effector memory (EM, CD28+/-CD95+CCR7-). Labels indicate percentage of total CD4+ T-cells. CD4+ T-cell subpopulations were analyzed seven days pre-transplant and fourteen to nineteen days post-transplant. (C) Wilcoxon matched-pairs signed rank test was used to compare the proportion of total CD4+ memory T-cells (SCM, central memory and effector memory) in the peripheral blood before and after transplant.

Effect of autologous HSCT on viral loads

RMs receiving autologous HSCT maintained undetectable SHIV-RNA viremia under ART with the exception of a few minor blips and exhibited a prompt clearance of SHIV-DNA from PBMCs. However, after ART interruption, 2 of the 3 transplanted RMs showed a very rapid rebound of viremia. The third transplanted RM, who was sacrificed for clinical reasons at day 14 post ART interruption, had no detectable virus in plasma, PBMCs, tonsils, and GI tract, but detectable levels of SHIV-DNA in sorted peripheral CD4+ T-cells, lymph nodes, and spleen.

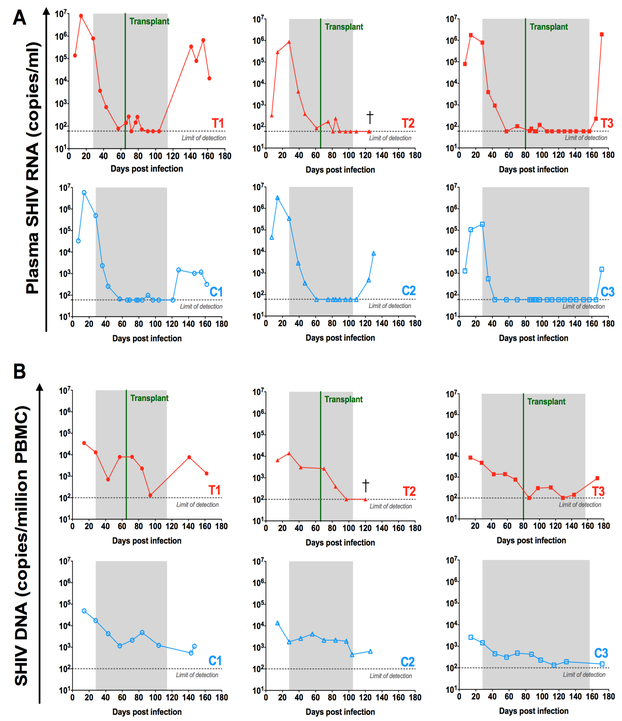


Figure 3. Plasma SHIV-RNA level and SHIV-DNA level in blood and tissues. Longitudinal assessment of the level of (A) SHIV-RNA, expressed as copies/ml of plasma and (B) SHIV-DNA, expressed as copies/million PBMCs. (C) SHIV-DNA levels expressed as copies/million cells, at necropsy, in sorted peripheral CD4+ T-cells. Lines are drawn at the geometric mean. Mann Whitney U test was used to determine significance. (D) SHIV-DNA levels expressed as copies/million cells obtained at necropsy from the ileum, jejunum, colon, rectum, superficial and mesenteric lymph nodes, and tonsils are shown for each individual animal.

CONCLUSION

In conclusion, we have conducted the first test-of-concept study of autologous HSCT in ART-treated SHIV-infected RMs. This experiment demonstrated that autologous HSCT is a feasible intervention that can lead to a marked reduction of the virus reservoir in blood and tissues but is not sufficient to eradicate the virus. This study provides a strong rationale for testing further interventions in this newly-established combined SHIV/HSCT model aimed at curing HIV infection in humans.