

Comparative Analysis of HIV-related and Post-Autologous Haematopoietic Stem Cell Transplantation (HSCT) T-cell Deficiency

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ABSTRACT

Background: Autologous HSCT recipients, despite a prolonged period of CD4+ T-cell deficiency (almost 9-12 months), present low rates of opportunistic infections compared to HIV-infected patients. We correlated T-cell phenotype and function in HIV-infected patients and autologous HSCT recipients with comparable CD4+ T-cell count.

Methods: We cross-sectionally studied: 5 HIV-infected patients with CD4+ median value of 230 cells/ μ L naive to antiretroviral treatment and free from opportunistic infections; 5 neoplastic patients (2 breast cancer and 3 multiple myeloma) receiving autologous HSCT 9 months before observation with actual CD4 median value of 255 cells/ μ L in absence of opportunistic infections and neoplasia's relapse. T-cell phenotype (CD45RA,CCR7), intracellular cytokine expression and lymphocyte proliferative responses were measured. Mann-Whitney test was used for statistical analysis.

Results: HIV+ patients and autologous HSCT recipients were both characterised by a low percentage of *naive* and *central memory* CD4+ T cells and a compensative expansion of *effector memory* (CD45RA-CCR7-) CD4 T cells. This phenotypic pattern was associated with mainly IFN- γ production and reduced IL-2 expression of CD4+ T-cells in both population. About CD8+ T-cell distribution, autologous HSCT recipients displayed significantly lower levels of *effector memory* cells (p<0.05) and highest levels of *effector* (CD45RA+CCR7+)CD8+ cells (p=0.05), with a *naive* population more represented compared to HIV-infected patients. This translates in higher IFN- γ +CD8+ expression in autologous HSCT patients, while HIV+ subjects showed higher expression of TNF- α CD8+ cells. Analysis of stimulation index revealed low levels of proliferative response for PHA and FLU stimulation in autologous HSCT recipients, comparable to HIV+ patients.

Conclusions: The CD4+ T-cell count deficiency in autologous HSCT recipients is associated with a skewed representation of naive and CM cells and cytokine and proliferative defects, indicating an ineffective T-helper response lasting for almost 9 months after transplantation. The increase in late effector CD8+ T-cells concordant with a more efficient cytokine production, observed in HSCT recipients, suggests a role for these cells in preventing actual infections.

1. INTRODUCTION

HIV-negative recipients of autologous haematopoietic stem cell transplantation (HSCT) suffer from a prolonged post-transplant immune deficiency, most pronounced in T-cell lineage, and cellular immunity regains effective levels of function within 1 year after transplantation. Despite the prolonged immune deficit, the risk of developing infections in HSCT patients is low, with herpetic reactivation as major infectious complications in the course of the first year after transplantation. Differently, in HIV infection the impairment in CD4 T-cell correlates with the risk of developing viral, fungal and protozoan infections, and a CD4+ count less of 200/ μ L is generally associated with major opportunism. We correlated T-cell phenotype and function in HIV-infected patients and autologous HSCT recipients with comparable CD4+ T-cell count.

2. METHODS

Patient population: Five HIV-infected patients naive to antiretroviral treatment and free from opportunistic infections (group A) and 5 neoplastic patients (2 breast cancer and 3 multiple myeloma) receiving autologous HSCT 9 months before observation in absence of opportunistic infections and neoplasia's relapse (group B) were enrolled at the Institute of Infectious and Tropical Diseases, Luigi Sacco Hospital, University of Milan.

The baseline characteristics of patients are summarized below:

Immunologic criteria	Group A (n=5)	Group B (n=5)	P
CD4+ count (cells/ μ L)	<300	<300	
Actual opportunistic infections	0/0	0/0	
Cytotoxic BA/LAKT	0/0	Not Applicable	
Neoplasia's relapse	Not Applicable	0/0	
Baseline characteristics			
Age (years)			
Median	48	43	ns
Range	40-59	30-60	
Actual CD4+ T-cell/ μ L			
Median	255	230	ns
Range	180-300	100-300	
Actual CD8+ T %			
Median	17	16	ns
Range	13-20	10-25	
Actual CD8+ T-cell/ μ L			
Median	458	470	ns
Range	540-823	496-919	
Actual CD8+ T %			
Median	31	24	ns
Range	41-62	40-60	
CD8/CD4 ratio			
Median	0.34	0.29	ns
Range	0.24-0.52	0.10-0.68	

Table 1: Baseline characteristics of HIV-positive patients (group A) and recipients of HSCT (group B).

Immune assays

Whole blood was collected and used within 8 hours to evaluate:

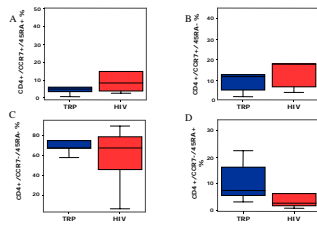
- lymphocyte subsets by flow cytometric analysis using the following antibodies CD4+CCR7/CD45RA, CD8+CCR7/CD45RA;
- *Staphylococcal* b enterotoxin (SEB)-specific, IL-2 and IFN γ expressing CD4+ T cells and IFN γ as well as TNF α expressing CD8 T lymphocytes;
- lymphocyte proliferation tests (3H-thymidine) upon stimulation of PHA and FLU.

Statistical analysis

Procedures were based on non-parametric analyses (Mann-Whitney); comparisons between the different groups were made using a two-tailed T-test. Statistical analysis was performed using the SPSS statistical package (SPSS Inc. Chicago, Illinois, USA).

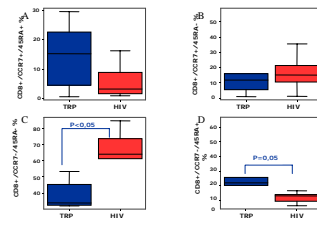
3. RESULTS

Figure 1: CD4+ SUBPOPULATIONS: NAIVE AND MEMORY CELLS



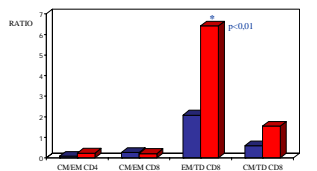
No significant differences in CD4+ subpopulations distribution were observed in the groups. Both HIV+ patients and HSCT recipients (9 months after transplantation) displayed: low levels of CD4+ naive cells (panel A) and a memory pattern characterized by increased levels of effector memory (EM) cells (panel C) and low percentages of central memory (CM) (panel B) and effector, terminally differentiated, cells (TD) (panel D).

Figure 2: CD8+ SUBPOPULATIONS: NAIVE AND MEMORY CELLS



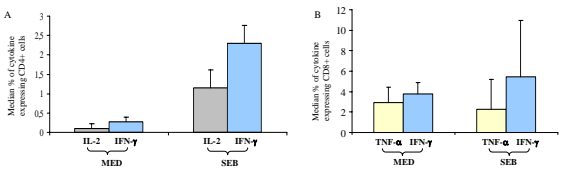
A trend in increased levels of naive CD8+ cells was observed in HSCT recipients evaluated 9 months after transplantation compared to HIV-positive patients (panel A). Memory CD8+ cells showed different patterns in the two groups: in HSCT recipients TD late effector cells were significantly augmented (p<0.05) (panel D) than in the HIV-positive group; effector memory CD8+ cells (pre-terminally differentiated) were rather significantly over-represented in HIV patients (panel C). No significant differences were observed in central memory CD8+ cells (panel B).

Figure 3: CD4+ and CD8+ NAIVE AND MEMORY CELLS



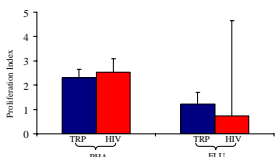
Ratios between the various subsets of memory and naive CD4 and CD8 cells were evaluated for HSCT recipients (9 months after transplantation) and HIV patients. The EM/TD ratio for CD8 T lymphocytes was significantly higher in HIV-positive patients compared to HSCT recipients; this reflects the over-representation of pre-terminally differentiated (EM) CD8+ cells and the contemporary low levels of TD CD8+ cells in HIV-positive patients.

Figure 4: CYTOKINE EXPRESSION ANALYSIS



The analysis of cytokine expression in HSCT recipients after 9 months from transplantation showed a skewed expression of cytokine for CD4+ and CD8+ T-cells populations. The pattern of cytokine expression for CD4+ T-cells was more consistent in IFN- γ secreting lymphocytes rather than in IL-2 secreting cells, thus mimicking the behaviour of HIV-infected CD4+ cells (panel A). The analysis of cytokine expression for CD8+ T-cells showed in HSCT recipients a more represented IFN- γ secreting lymphocytes population compared to TNF- α secreting cells, thus indicating a functional adequate cytokine response (panel B).

Figure 5: LYMPHOCYTE PROLIFERATION ANALYSIS



The analysis of lymphocyte proliferation in HSCT recipients after 9 months from transplantation and in HIV-infected patients did not show significant differences. In particular, both population presented low levels of lymphocyte proliferation in response to recall antigens, thus confirming the persistence of a predominantly CD4+ related functional defect even 9 months after transplantation in HSCT recipients.

4. CONCLUSIONS

HSCT recipients 9 months after transplantation showed:

- low levels of naive CD4+ T-cells and a pattern of memory CD4+ T-cells characterized by CM deficit and over-expression of EM cells
 - reduced expression of IL-secreting CD4+ T-cells
 - reduced lymphocyte proliferation in response to recall antigen
 - adequate naive and memory CD8+ distribution
 - adequate expression of IFN- γ secreting CD8+ T-cells
- INEFFECTIVE T-HELPER RESPONSE**

ADEQUATE CD8+ EFFECTOR REPRESENTATION AND FUNCTION

HIV-positive patients with comparable CD4+ and CD8+ T-cell count showed:

- low levels of naive CD4+ T-cells and a pattern of memory CD4+ T-cells characterized by CM deficit and over-expression of EM cells
 - reduced expression of IL-secreting CD4+ T-cells
 - reduced lymphocyte proliferation in response to recall antigen
 - low levels of naive CD8+ T-cells and a pattern of memory CD8+ T-cells characterized by over-expression of pre-TD and deficit of TD cells
- INEFFECTIVE T-HELPER RESPONSE**
- INEFFECTIVE MATURATION TO EFFECTOR CD8+ T CELLS**

The CD4+ T-cell count deficiency in autologous HSCT recipients is associated with a skewed representation of naive and CM cells and cytokine and proliferative defects, indicating an ineffective T-helper response lasting for almost 9 months after transplantation. The increase in late effector CD8+ T-cells concordant with a more efficient cytokine production, observed in HSCT recipients, suggests a role for these cells in preventing actual infections.