Gene expression analysis of T cells subsets from MS patients undergoing AHSC transplant reveals significant changes in immune function

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INTRODUCTION AND AIM

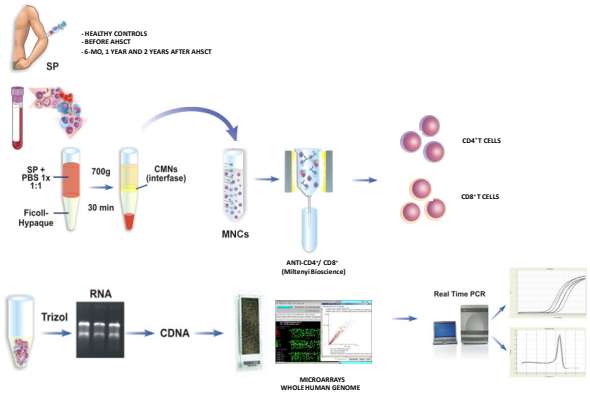
Multiple sclerosis (MS) is an immune mediated disease of the central nervous system characterized by inflammation, demyelination, and neuronal degeneration. High-dose immunosuppression therapy followed by autologous hematopoietic stem cell transplantation (HDI/AHSCT) has emerged in the past few years as a new treatment strategy in patients with severe MS and refractory to conventional treatment. The molecular profile during immune reconstitution of these patients has not been characterized. The aim of this study was to evaluate through a robust and unbiased transcriptomic analysis the global gene expression in CD4⁺ and CD8⁺ T cells from peripheral blood from MS patients who underwent HDI/AHSCT.

SUBJECTS AND METHODS

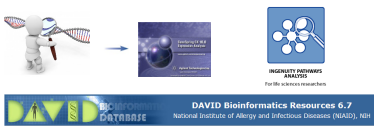
A total of 24 patients on progressive course of multiple sclerosis who had proven refractory to conventional treatment with corticosteroids and IFN-β were selected for the clinical protocol approved by the National Institutional Review Board (CONEP) and autologous CD34 stem cells were mobilized from bone marrow to peripheral blood with cyclophosphamide /G-CSF.

Total RNA of CD4⁺ and CD8⁺ T cells from eight MS patients before transplantation and four patients at 6 months, 1 and 2 years after transplantation was processed for DNA microarray analysis. A comparative gene expression analysis was performed between MS samples from pre-transplantation and from each time point post-transplantation, as well as with 4 samples from healthy controls.. We identified an extensive number of differentially expressed genes (DEG) in both T cell subsets in all periods evaluated after transplantation when compared with samples from pre-transplantation (statistical values, p<0.05 and fold change>2.0). We investigated the molecular and biological function of DEG using bioinformatics tools and selected genes involved with immune response to measure quantitative gene expression by Real-time PCR.

LARGE-SCALE GENE EXPRESSION STUDY



BIOINFORMATIC ANALYSIS



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RESULTS

We carried out a molecular characterization of the differentially expressed genes obtained from comparative analysis of peripheral CD4⁺ and CD8⁺ T cells of MS patients before transplantation and 6 months, 1 year and 2 years after transplantation. In CD8⁺ T cells, we measured the levels of expression of 23 genes modulated after transplantation: nine transcriptional factors (LEF1, FOXD1, CEBPD, JUN, JUNB, RELB, IFI16, AEBP1) including the translational factor PDCD4, the chemokine CCR7 and adhesion molecule L Selectin, three TNF superfamily members (TNFRSF4, TNFRSF19L, LTβ), seven genes involved with molecular signaling (SOCS1, SOCS3, DGKH, CSNK1L1, IKBa, IKBβ, IKBε), and the immune cell receptors CD47 and SIRPG. In CD4⁺ T cells, we evaluated the relative level of expression of STAT3, FcRL3, PDCD1, DGKH, CSNK1L1, L Selectin, CCR7 and PIAS3 that were all modulated after transplantation

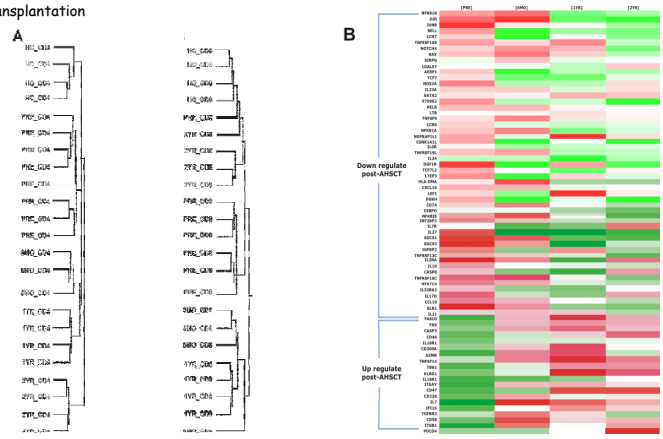

FIGURE 1 (A): Hierarchical clustering of gene expression profiling in CD4⁺ and CD8⁺ T cells from MS patients before (pre) and 6 months (6mo), 1 year (1yr) and 2 years (2yr)after AHSCT. The cluster analysis performed using the numbers of differentially expressed genes (corrected p-values≤ 0.05, one-way ANOVA test as significantly statistical methods) showed different transcription profile in CD4⁺ and CD8⁺ T cells of MS patients followed up transplantation, as well as displayed similarities between CD8⁺ T cells from MS patients 2 years post-transplant and healthy controls (HC) samples.

FIGURE 1 (B): Differentially down- and up regulated genes in CD8⁺ T cells from MS patients at any time point of 6 months (6mo), 1 year (1yr) and 2 years (2yr) after AHSCT as compared with MS before transplantation (pre). The heat map illustration was performed using the numbers of immune-related genes in CD8⁺ T cells analysis previously obtained by statistical unpaired T-test, corrected p-value P≤0.05 and multiple testing correction Benjamini-Hochberg from GeneSpring GX version 10.0.

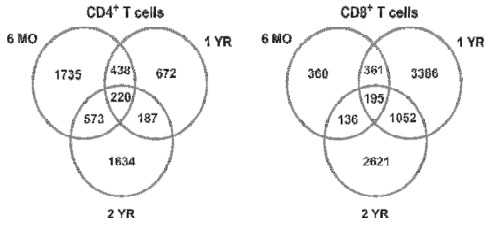

FIGURE 2: Number of significantly expressed genes determined by T-test unpaired with a Benjamin-Hochberg multiple corrections testing yielding p-values <0.05 and Fold change >2.0 as significant. The level of expression obtained from CD4⁺ or CD8⁺ T cells of MS patients group before transplantation was compared with the groups referents at 6 months, 1 year or 2 years after transplantation. Overlapping sections of the Venn diagram demonstrate number of common genes whose expression levels were found among three periods post-transplantation evaluated. The largest numbers of significantly modulated genes were found in CD8⁺ T cells at 1 (yr) and 2 (yr) post-transplant.

CONCLUSION

Our results demonstrate that autologous HSCT induce a significant reprogramming of gene expression in post-transplant peripheral CD4⁺ and CD8⁺ T cells of MS patients. The modulation of genes that are crucially involved in T-cell signaling, activation and differentiation as well as genes that control immune regulatory networks help to explain the observed clinical remission through reestablishment of physiological homeostasis of autoreactive T cells. Further functional immune studies are required to clarify the precise role of some genes as regulators of the immune response in MS.