Reconstitution of naïve and regulatory T cell populations in type 1 diabetes after autologous hematopoietic stem cell transplantation

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INTRODUCTION AND OBJECTIVE
High dose immunosuppression (HDI) followed by autologous hematopoietic stem cell transplantation (AHSCT) has emerged in last past years as a therapeutic alternative for newly diagnosed type 1 diabetes mellitus (T1D) patients. After transplantation, C-peptide levels increased significantly and the majority of patients achieved insulin independence with good glycemic control. To address the immune mechanisms by which HDI/AHSCT induces remission in T1D patients, we evaluated the reconstitution of the naïve and regulatory T cell populations in T1D patients treated with HDI/AHSCT. The thymic production of naïve T cells can be evaluated by quantification of the TCR rearrangement excision circles (TRECs) that are formed during rearrangement of the T-cell receptor gene and are not duplicated during mitosis.

RESULTS
Significant increase of TRECs levels were found in T1D patients at days +360 (p<0.05), +540 (p<0.001), +720 (p<0.001), +900 (p<0.0001) and +1080 (p<0.05) when compared to the day +100 after transplantation (Figure 1). Furthermore, significant increase of TREC levels were found in T1D patients at pre-transplantation and at various time points after transplantation versus healthy individuals (control line). (*) p < 0.05. Pre-mod and pre-cond = pre-transplant period.

SUBJECTS AND METHODS
Peripheral blood was collected from healthy controls (N=16) and TID patients (N=16) at pre-transplantation and at various time points after transplantation. Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque and used for immunophenotyping by flow cytometry analysis and for DNA extraction. The quantification of TRECs levels was performed by real-time PCR. A standard curve was established with known copies of plasmids containing TRECs fragments and the results were represented as molecules/100g DNA.

Peripheral blood mononuclear cells (PBMC) isolation by Ficoll-Hypaque
DNA extraction by D Azerbai (Qiagen)
Absolute quantification by Real time PCR

FIGURE 1: Recovery of recent-thymic emigrants CD4+CD8αα+CD31+ TID post-AHSCT
(A) Levels of CD4+CD8αα+CD31+ cells in patients at pre-transplantation and at various time points after transplantation versus healthy individuals (control line). (*) p < 0.05. Pre-mod and pre-cond = pre-transplant period. (B) Absolute TREC quantification at F sing PBMC isolated from healthy individuals and TID patients at pre-transplantation period. (C) Absolute TREC quantification at F sing PBMC isolated from TID patients at pre-transplantation and at days +100, +360, +540, +720, +900, +1080 and +1080 post-transplantation periods. The results were represented in range (mean ± standard deviation) (’’’p < 0.0001, ’’p < 0.001, ‘’p < 0.05). Pre-Tx = pre-transplant.

CONCLUSIONS
Our results suggest that the mechanisms of action of the HDI/AHSCT involve an improvement of the peripheral immunoregulatory mechanisms after HDI/AHSCT, which may contribute to reestablishment of self-tolerance and control of autoreactivity in the TID patients. Furthermore, the increase in TRECs levels in these patients indicates an active thymic production of naïve T cells during immune reconstitution and consequently a generation of a diverse T cell repertoire after transplantation.

REFERENCES

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