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

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INTRODUCTION AND OBJETIVE

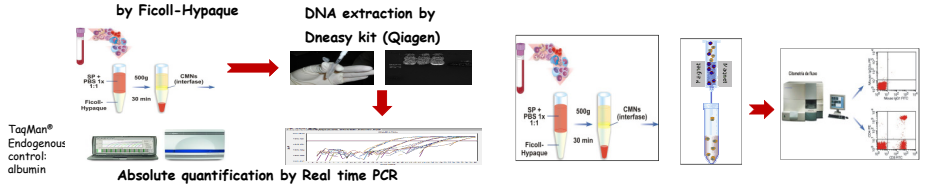
High dose immunossuppression (HDI) followed by autologous hematopoietic stem cell transplantation (AHSCT) has emerged in last past years as a therapeutic alternative for newly diagnosed type 1 diabetes mellitus (T1D) patients[1]. After transplantation, C-peptide levels increased significantly and the 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 naive 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[2].

SUBJECTS AND METHODS

Peripheral blood was collected from healthy controls (N=16) and T1D 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/100ng DNA.

Peripheral blood mononuclear cells (PBMC) isolation by Ficoll-Hypaque

DNA extraction by Dneasy kit (Qiagen)



RESULTS

Significant increase of TREC 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, there was a significant increase in levels of $CD4^+CD45RA^+CD31^+$ after transplantation when compared to pre-transplantation period (Figure 2) . We found significant increased numbers of $CD8^+CD28^-$ regulatory/suppressor T cells at days +60 (319,2±171,8 p<0.05), +100 (357,1±320,4 p<0.05), +180 (349,8±249,8 p<0.05), +270 (332,9±366,9 p<0.05) and +540 (361,1±281,4 p<0.05) post-AHSCT when compared with pre-transplantation period (172,9±95,7) (Figure 3). We found no significant increase of $CD4^+CD25^{hi}$ regulatory T cell numbers after transplantation, however their reconstitution kinetics was faster than of total $CD4^+$ T cells (Figure 4).

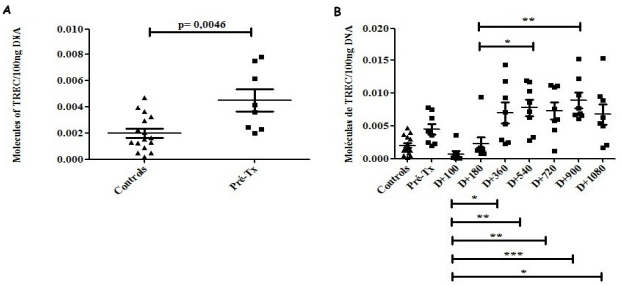


FIGURE 1: Absolute TREC quantification on PBMCs isolated from healthy individuals and T1D patients. (A) Absolute TREC quantification (molecules/100ng DNA) on PBMCs isolated from healthy individuals and T1D patients at pre-transplantation period. (B) Absolute TREC quantification (molecules/100ng DNA) on PBMCs isolated from T1D patients at pre-transplantation and at days D+100, D+ 180, D+ 360, D+540, D+ 720, D+900 and D+1080 post-transplantation periods. The results were represented as mean ± std deviation. (*) p < 0,05; (**) p < 0,001; (***) p < 0,0001. Pre-Tx = pre-transplant

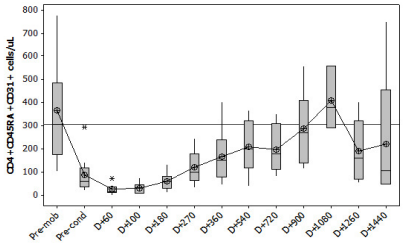


FIGURE 2: Recovery of recent-thymic emigrants $CD4^+CD45RA^+CD31^+$ in T1D post-AHSCT. Levels of $CD4^+CD45RA^+CD31^+$ cells in patients at pre-transplantation and at various time points after transplantation versus healthy individuals (control line). (*) p < 0,05. Pre-mod and pre-cond = pre-transplant

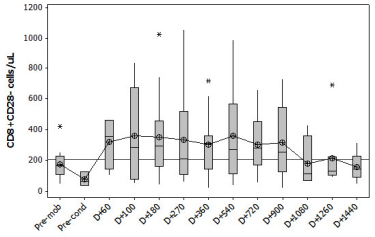


FIGURE 3: Numbers of $CD8^+CD28^-$ regulatory/supressor T cells on PBMCs from healthy individuals and T1D patients after AHSCT. Levels of $CD8^+CD28^-$ cells in patients at pre-transplantation and at various time points after transplantation versus healthy individuals (control line). (*) p < 0,05 . Pre-mod and pre-cond = pre-transplant

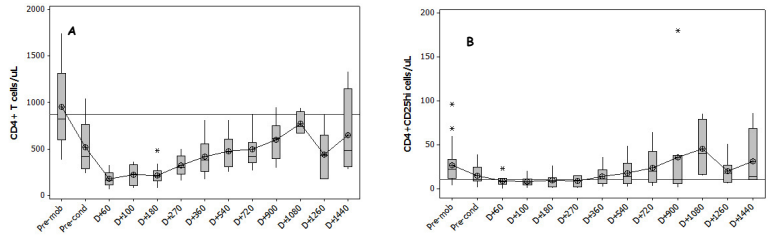


FIGURE 4: Numbers of $CD4^+$ T cell and regulatory T cell on PBMCs from healthy individuals and T1D patients after AHSCT. (A) Levels of $CD4^+$ cells in patients at pre-transplantation and at various time points after transplantation versus healthy individuals (control line). (B) Levels of $CD4^+$ $CD25^{hi}$ cells in patients at pre-transplantation and at various time points transplantation versus healthy individuals (control line). (*) p < 0,05 . Pre-mod and pre-cond = pre-transplant period

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 autoimmunity in the T1D patients. Furthermore, the increase in TRECs levels in these patients indicates an active thymic production of naive T cells during immune reconstitution and consequently a generation of a diverse T cell repertoire after transplantation.

REFERENCES

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