TRANSPLANTED BETA CELLS IMMUNE-ESCAPE IN TYPE I DIABETES: A GENE THERAPY APPROACH

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Abstract

Type 1 diabetes is a chronic autoimmune disease caused by the destruction of insuline-producing cells (beta cells) mainly mediated by Tcells CD8+. To date, unfortunately, the only cure for such disease is a lifelong insuline replacement therapy. In the last few years it has been understood that is possible to make a functional beta cell transplant in a NOD mouse, restoring the normal insulin blood level. One of the main issues after the transplant is that these new cells are recognized and killed by the host autoreactive CD8+ (fig.1).

We tried to understand if it's possible, through gene therapy, to force the beta cells to express transmembrane TNF, which leads, thanks to his binding to TNFR2, to down regulation and death of the autoreactive Tcells and promotes survival and proliferation of Tregs. Through this immune escape mechanism, these engineered beta cells have longer life in the NOD model in comparison with normal transplanted beta cells.

iPSC-derived beta-cells

Based on Nair experience (Cell, 2019), we generated iPSCs from NOD mouse pancreas-derived epithelial cells (NPEs); through differentiation we formed clonal colonies of iPSC-derived beta cells (IDBC). We did all the experiments on a control population of 293T cells, then repeated them on our IDBCs.

Transfection

We transfected our cells with a third generation Lentiviral vector (fig.2A) to make them express TNF, generating TNF+ cells (IDBC-TNF+). From now on we worked on two different cell populations: IDBC-TNF+ and IDBC-TNF-, which didn't undergo a process of gene editing, used as control population (CO) (fig.2B). We then conducted different experiments to evaluate TNF expression, TNF-TNFR2 binding and CD8+/Tregs activation.

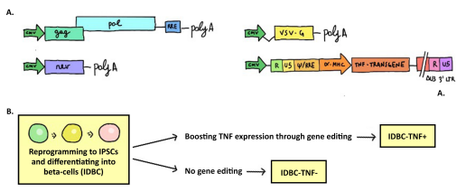

Fig. 2 - A. The third-generation Lentiviral vector we used for transfection. B. Two different cells population after the transfection: IDBC-TNF+ and IDBC-TNF-.

In vivo

We transplanted the IDBC-TNF+ cells in a NOD mouse and checked the outcome in terms of insuline blood level; then we compared the outcome with a IDBC-TNF- transplant. While at 2 months the insuline production is almost the same, at 1 year c-peptide levels are close to 0 for the mouse who has undergone IDBC-TNF- transplant (fig. 6A e 6B). Then we observed the difference between pancreas islets in dissections from IDBC-TNF+ and IDBC-TNF- mouse: the second one showed immune infiltration and necrosys.

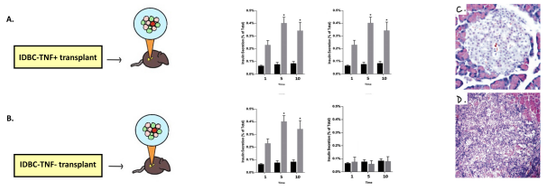

Fig. 6 - C-peptide blood level 2 months and 1 year after transplant of IDBC-TNF+ cells (A) and IDBC-TNF- cells (B). Post dissection analysis of IDBC-TNF+ (C) and IDBC-TNF- (D) pancreas.

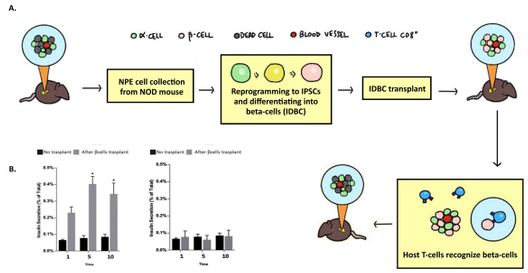

Fig. 1 - A. iPSC-derived beta cells creation and transplant. B. Levels of blood c-peptide 2 months and 1 year after transplant, after the transplant recognition by host CD8+.

Is TNF expressed?

We compared the TNF expression of cells that have undergone gene editing with cells that didn't (fig.3). Both WB (a), qPCR (b), FACS(c) and IF (d) showed an increased TNF expression in the genetically engineered cells.

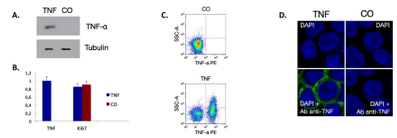

Fig. 3 - A. Western Blot. B. RT-PCR. C. ELISA. D. Immunofluorescence

Is TNF binding to TNFR2?

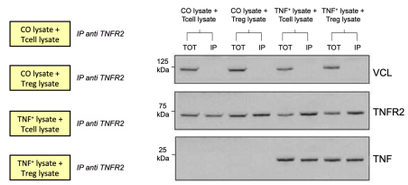

Fig. 4 - Coimmunoprecipitation between lysate from TNF+/CO cells and Tcells/Tregs. Western blot shows the presence of TNF in the precipitated only from IDBC-TNF+ cells.

Is TNFR2 activated?

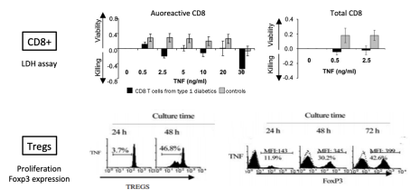

Fig. 5 - Effect of the TNF-TNFR2 binding on Tcells CD8+ and Tregs: TNF selectively kills autoreactive diabetic CD8 Tcells but not control CD8 Tcells and helps Tregs survival. Foxp3 is essential for specifying the Treg cell lineage and for Tregs function.

Discussion

We understood that, in vitro, it is possible to create a population of IDBC-TNF+ capable of interacting with the Tcells, strenghtening the Tregs response and decreasing the autoreactive Tcells activity. We also concluded that there is a possibility of a IDBC-TNF+ functional transplant, which has a better outcome in terms of durability compared to IDBC-TNF- transplant.

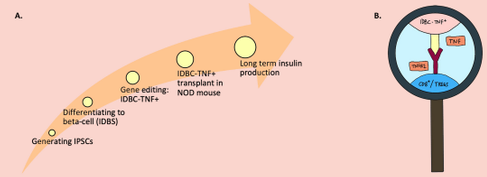

Fig. 7 - A. Main project's steps. B. Schematization of the TNF-TNFR2 interaction.

Pitfalls and future perspective

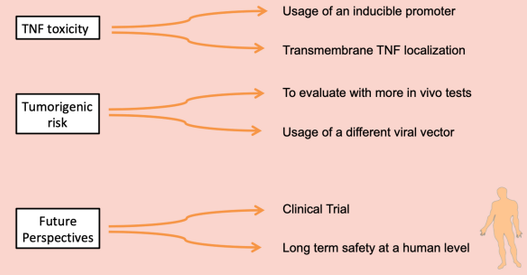

Fig. 8 - Main pitfalls and future perspectives of the project.

Project cost and time

PRODUCT	COST
Nod mice (x40)	1.550€ (Jackson Laboratory)
Stabulation for mice	~ 500€/month
293T cell line	~ 300€ (Gen Hunter)
IPs reprogramming Kit	~ 3.000€
TNF Lentiviral Vector	500€ each (Abm inc.)
Western blot kit	~ 400€ each (Thermo Fisher)
RT-PCR kit	~ 200€ each (Sigma Aldrich)
ELISA kit	~ 450€ each (NovusBio)
Immunofluorescence kit	~ 500€ each (Thermo Fisher)
Immunopretipitation kit	~ 360€ each (Abcam)
LDH Assay kit	~ 500€ each (Abcam)

1st year
2nd year
3rd year
4th year
Results

PhD Project (4 years)

~ 50 000 €

Fig. 9 - Specific project costs, non including non evaluable routine lab experiments costs. The expected duration of the project is 4 year long.

REFERENCES: Incani M, Baroni MG. La patogenesi autoimmune del diabete di tipo 1. 2016; Pearson JA et al. The importance of the Non Obese Diabetic (NOD) mouse model in autoimmune diabetes; Rajaei B et al. Glucose-Responsiveness of Pancreatic β-Like (GRP β-L) Cells Generated from Human Pluripotent Stem Cells. 2019; Anazawa T et al. Current state and future evolution of pancreatic islet transplantation. 2018; Johannesson B et al. Toward beta cell replacement for diabetes. 2015; Beihack A et al. A Selective TNFR2 Agonist Expands Host Treg Cells in Vivo to Protect from Acute Graft-Versus-Host Disease. 2014; Jeon K et al. Differentiation and transplantation of functional pancreatic beta cells generated from induced pluripotent stem cells derived from a type 1 diabetes mouse model. 2012; https://www.abmgood.com/TNF-Lentivirus-System-LV470974.html; Salomon B et al. Tumor Necrosis Factor α and Regulatory T Cells in Oncoimmunology. 2018; Li x, Zheng Y. Regulatory T cell identity: formation and maintenance. 2015; Ban L et al. Selective death of autoreactive T cells in human diabetes by TNF or TNFR2 agonism. 2008