

**Title: Partial CRISPR/Cas9 IL1R1 & IFNGR1 Knock-Down Improves β -cell Survival
And Function Under Cytokine-Induced Inflammation**

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Type 1 diabetes (T1D) is a chronic disease characterized by the autoimmune destruction of pancreatic β cells. This destruction is mediated by lymphocytes T helper and cytotoxic, and by the action of the pro-inflammatory cytokines IL1 β , IFN γ and TNF α inside the islets of Langerhans. We propose a new approach to alleviate islet inflammation by targeting pro-inflammatory cytokine receptors. Our hypothesis is that the downregulation of inflammatory pathways may improve β cell survival in the context of inflammation after T1D onset. We knocked-down IL1R1 or IFNGR1 receptors in the MIN6 β -cell line by using the CRISPR/Cas9 gene editing system. GuideRNA targeting the extracellular domain of the transmembrane receptors IL1R1 and IFNGR1 were designed and cloned into pSpCas9(BB)-2A-GFP plasmid. The knockdown efficiency was evaluated by flow cytometry and qPCR, and ranged from 12 to 40%. MIN6 or CRISPR-MIN6 were treated with IL1 β or IFN γ during 48 h at 5 or 10 ng/mL. Cell viability of CRISPR-IFNGR1 cell lines evaluated by MTT assay was improved after IFN γ exposure compared to naive MIN6 (107 ± 19.3 and 89.7 ± 22.7 vs $76 \pm 22.1\%$; $P = 0.09$ and 0.04) whereas cell viability of CRISPR-IL1R1 cell line tended to be improved after IL1 β treatment (136 ± 33.6 vs $80 \pm 11.0\%$; $P = 0.13$). The assessment of insulin secretion capacities of CRISPR-MIN6 cells showed higher secretion rates (0.61 ± 0.18 vs 0.19 ± 0.06 ng insulin/ μ g protein; $P = 0.04$), as compared to appropriate controls. Gene

expression of the pro-apoptotic receptor *Fas* was decreased inside the CRISPR-MIN6 cell lines and the expression of the pro-inflammatory cytokine *Il6* gene was decreased inside the CRISPR-IL1R1 cell line, as compared to MIN6 control. Similarly, gene expression of ER stress markers *Atf4* and *Chop* decreased inside the CRISPR-IL1R1 and CRISPR-IFNGR1 cell lines, respectively, as compared to controls. Our results show that the targeting of IL1R1 or IFNGR1 could protect pancreatic β cells from the inflammatory attack found in T1D by decreasing apoptosis, inflammation and ER stress. Our results are encouraging and require the development of the three-cytokine receptor (IL1R1, IFNGR1 and TNFR1) knockdown to fully address the potential of this system to be translated into clinical research protocols. The possibility of a translational perspective of our knockdown system is suggested by the ongoing clinical trial using the CRISPR/Cas9 system to evaluate the safety of PD-1 knockout engineered T cells in treating metastatic non-small cell lung cancer (NCT02793856).