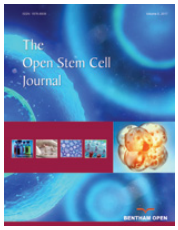

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Supplementary Material

The Secretome of Mesenchymal Stem Cells Prevents Islet Beta Cell Apoptosis *via* an IL-10-Dependent Mechanism

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Abstract:

Background:

Type 1 Diabetes Mellitus (T1DM) is partly driven by autoimmune destruction of the pancreatic beta cell, facilitated by the release of inflammatory cytokines, including IFN-γ, TNF-α and IL-1β by cells of the innate immune system. Mesenchymal stem cells (MSCs) have been used to counteract autoimmunity in a range of therapeutic settings due to their secretion of trophic and immunomodulatory factors that ameliorate disease independently of the cells themselves.

Objective:

The aim of this study was to assess the effect of the secretome of human bone-marrow derived MSCs on cytokine-driven beta cell apoptosis.

Methods:

All experiments were conducted in two insulin-secreting islet cell lines (BRIN-BD11 and βTC1.6) with selected experiments confirmed in primary islets. MSC secretome was generated by conditioning serum-free media (MSC-CM) for 24 hours on sub-confluent MSC populations. The media was then removed and filtered in readiness for use.

Results:

Exposure to IFN-γ, TNF-α and IL-1β induced apoptosis in cell lines and primary islets. The addition of MSC-CM to cell lines and primary islets partially reversed cytokine-driven apoptosis. MSC-CM also restored glucose-stimulated insulin secretion in cytokine-treated cell lines, which was linked to improved cell viability following from cytokine challenge. Characterization of MSC-CM revealed significant concentrations of IL-4, IL-10, PIGF and VEGF. Of these, IL-10 alone prevented cytokine-driven apoptosis. Furthermore, the inhibition of IL-10 through the addition of a blocking antibody reversed the anti-apoptotic effects of MSC-CM.

Conclusion:

Overall, the protective effects of MSC-CM on islet beta cell survival appear to be largely IL-10-dependent.

Keywords: Apoptosis, Beta-cell, Islet, IL-10, MSCs, Mesenchymal Stromal Cells, Mesenchymal Stem Cells, Secretome.

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SUPPLEMENTRY FIGURES

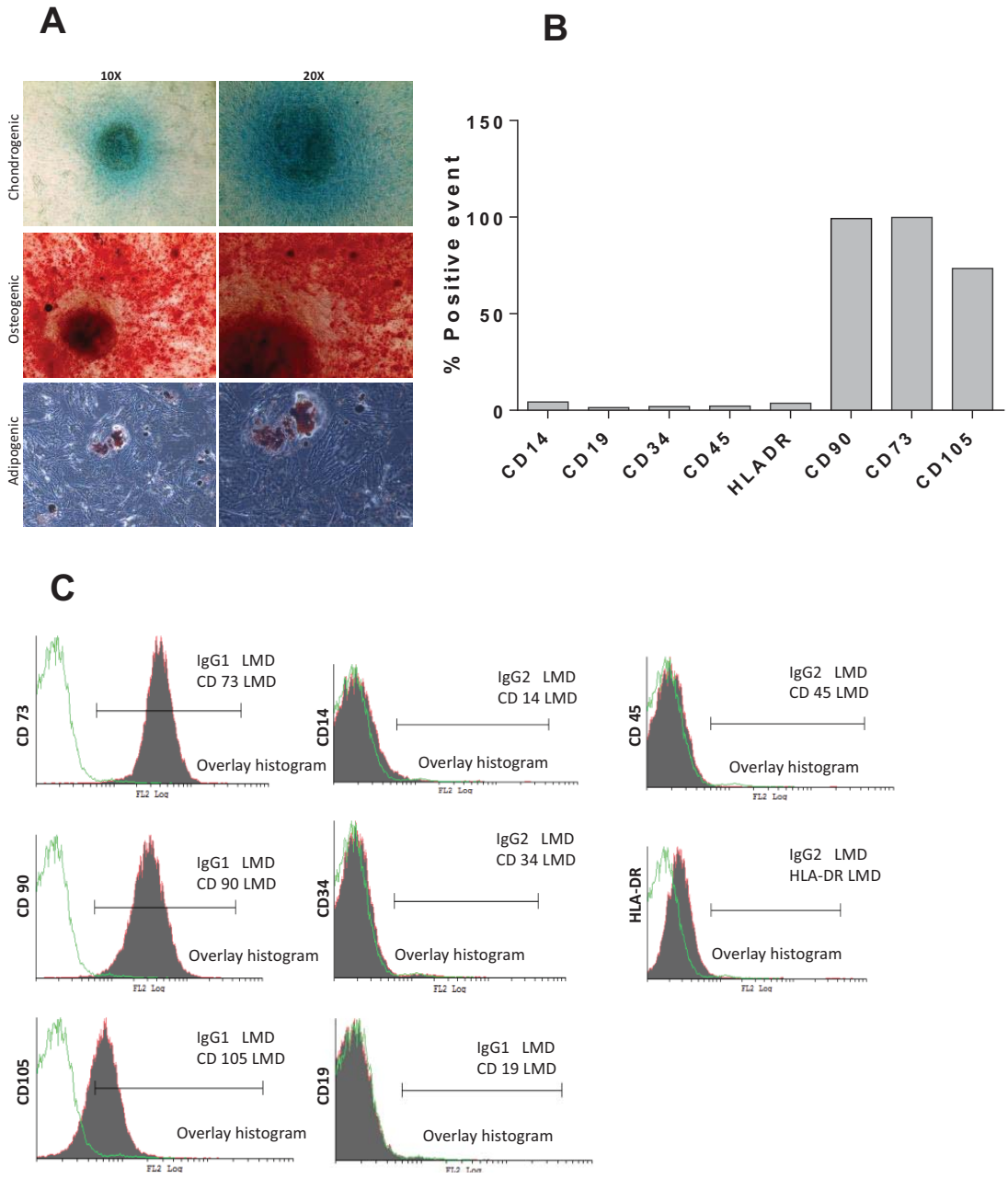

Fig. S1. Characterisation of MSCs

(**A**) Classical Trilineage differentiation of hMSC. (**B**) The percentage positive event was quantified relative to relevant isotype control marker IgG1 and IgG2. (**C**) Expression of typically negative (CD14, CD19, CD37, CD45, HLA-DR) and typically positive (CD90, CD73 and CD105) MSC markers in mononuclear mesenchymal stem cells used in this study.

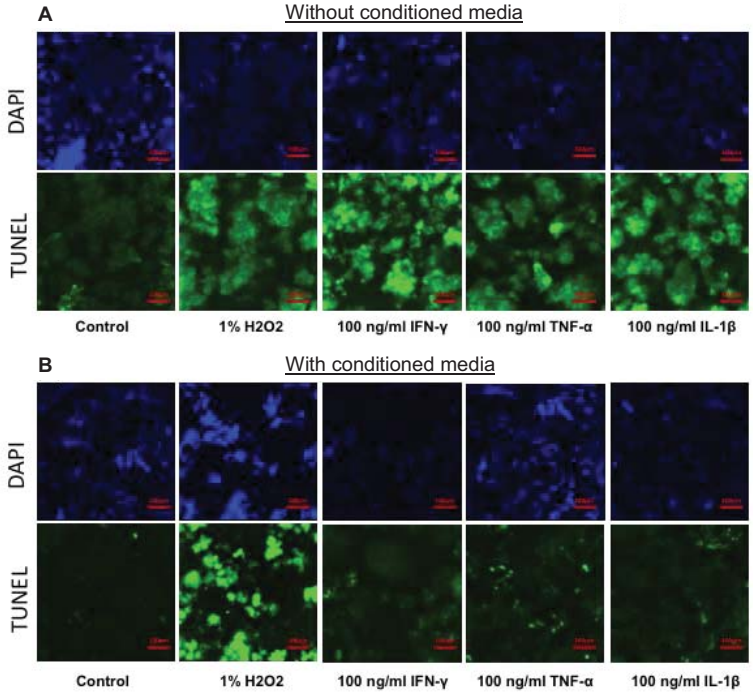

Fig. S2. MSC-CM confers protection against against cytokine-driven apoptosis in primary mouse islets
Isolated islets were exposed to 100 ng/ml IFN-γ, 100 ng/ml TNFα, and 100 ng/ml IL- 1β for 24h in the absence (**A**) or absence (**B**) of MSC-CM and the induction of apoptosis assessed by TUNEL assay. 1% H2O2 acted as a positive control in these experiments. Blue staining represents DAPI staining of the nuclei while green staining indicates TUNEL positive cells The scale bar in all images equals 100 µm. MSC-CM, Mesenchymal Stem Cell Condition Media; IFNγ, Interferon gamma; TNF- α, tumour necrosis factor alpha; IL-1β, Interleukin 1 beta.

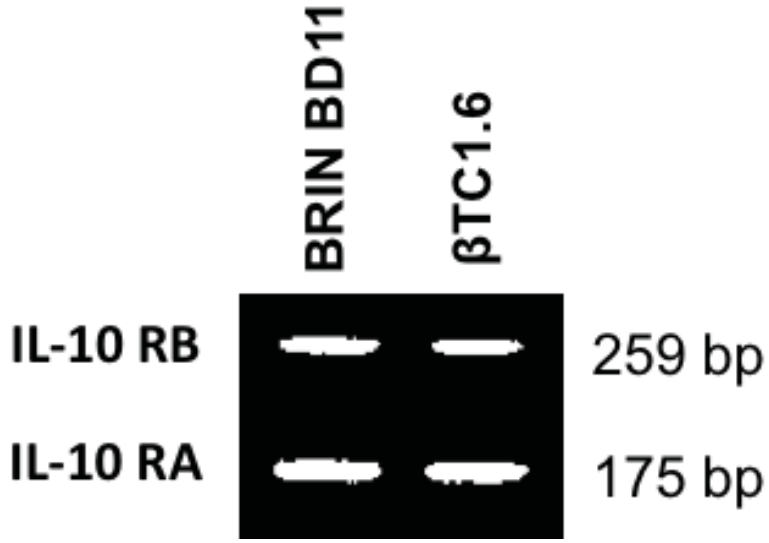

Fig. S3. IL-10 receptors are expressed in BRIN-BD11 and βTC1.6 cells
RT-PCR confirmed mRNA expression of IL-10RA and IL-10RB in both cell lines. Images representative of three independent experiments are shown.