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.
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 cytokine-driven apoptosis in primary mouse islets
Isolated islets were exposed to 100 ng/ml IFN-γ, 100 ng/ml TNF-α, 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.