FACTORS RELEASED BY HUMAN MESENCHYMAL STEM CELLS SUPPRESS GLUCOSE-INDUCED INFLAMMATORY RESPONSES OF STABLE RENAL PROXIMAL TUBULAR EPITHELIAL CELL MONOLAYERS

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INTRODUCTION

Although diabetic kidney disease (DKD) is often viewed as being driven by glomerular injury, direct pro-inflammatory and pro-fibrotic effects of prolonged hyperglycaemia on renal proximal tubular epithelial cells (RPTEC) also play an important pathogenic role. Bone marrow derived mesenchymal stem cells (MSC) have shown promise as therapeutic modulators of the inflammatory components of DKD pathogenesis[1].

The aims of this study were to develop an *in vitro* system for modelling inflammatory response of stable human (h)RPTEC monolayers to prolonged high glucose concentration and to investigate the potential for hMSC-associated factors to modulate this response.

METHODOLOGY

Human RPTEC/TERT1 cells were seeded at pre-optimized density to generate stable confluent monolayers during 12-day culture. Media containing "Normal" D-glucose (5 mM), "High" (25mM) D-Glucose or D-Mannitol (osmotic control) was added for a further 5 days (with exchanges on day 3) following which supernatants and cells were collected for analysis by ELISA (for IL-6, IL-8, MCP-1, NGAL and KIM-1) and flow cytometry (for apoptotic/necrotic cell death by Annexin V/PI staining) respectively. Similar experiments were then performed in which High glucose- and Mannitol-exposed RPTEC/TERT1 monolayers were cultured for the final 48 hours in the presence or absence of 10X concentrated 50:50 v/v "total" and "extracellular vesicle (EV) depleted" hMSC conditioned medium (CM). Results were further confirmed by indirect contact of hMSCs (in transwell insert) and RPTEC-TERT1 cells (in 24 well plate). Results were statistically analysed with Graphpad Prism 6.0 ®. Phenotyping of hBM-MSCs from different donors was carried out by flow cytometry using a BD FACS Canto-A and analysed by Flow Jo Software v10.0.

RESULTS

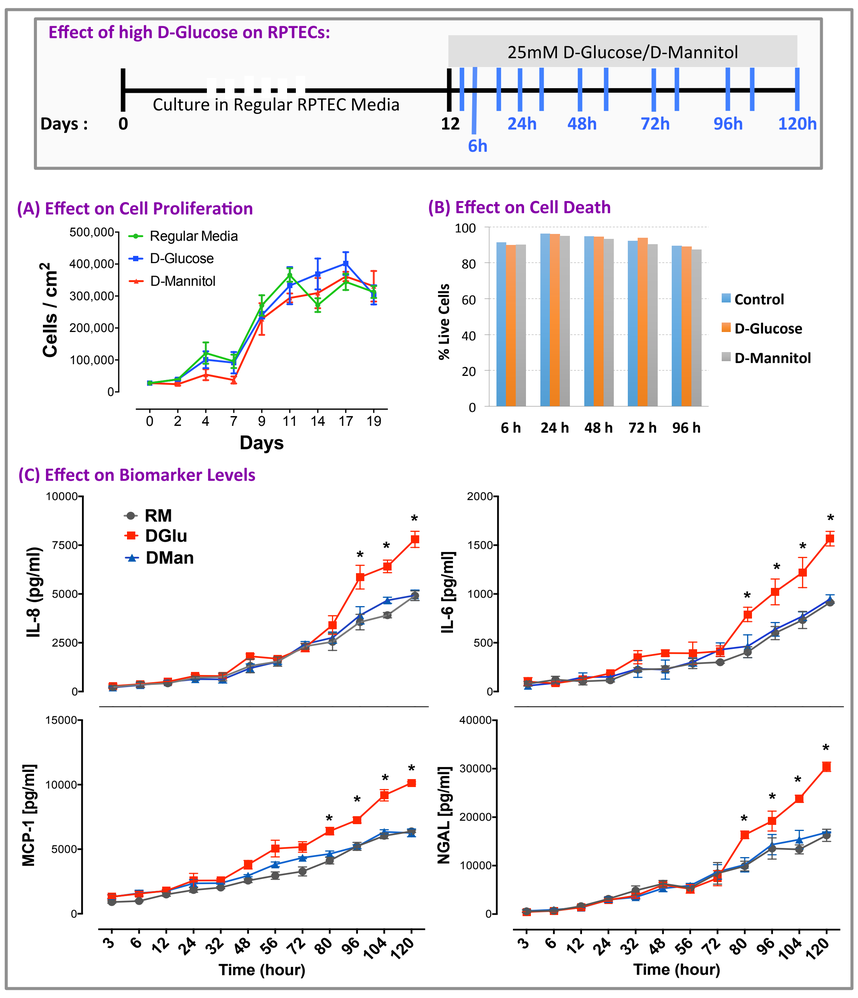

Figure 1: Effect of High D-Glucose on RPTEC/TERT1 Cells. Five-day exposure to high glucose caused significant increases in RPTEC/TERT1 cell secretion of IL-6 (~1.5 fold), IL-8 (~1.7 fold), MCP-1 (~2 fold) and NGAL (~2 fold) compared to normal glucose and mannitol without any increase in apoptotic or necrotic cell death. (A) Cell Count per square centimetre; (B) Flow cytometry based cell death analyses of Annexin-PI stained RPTEC-TERT1 cells; (C) Effect of high D-Glucose on cytokine production by RPTEC-TERT1 cells. Significant elevations in IL-8, IL-6, MCP-1 and NGAL levels. Here, p=<0.05.

CONCLUSION

- High glucose (± albumin/inflammatory cytokine stimuli) induces secretion of pro-inflammatory mediators by hRPTEC stable monolayers.
- Factors released by hMSC suppress high glucose-induced hRPTEC secretion of inflammatory cytokines but not NGAL.
- Indirect contact of hBM-MSCs with RPTEC/TERT1 cells result in more potent anti-inflammatory effects.

FUTURE WORK

- Investigation of the mechanisms by which MSC mediate anti-inflammatory effects on RPTEC under diabetic conditions.

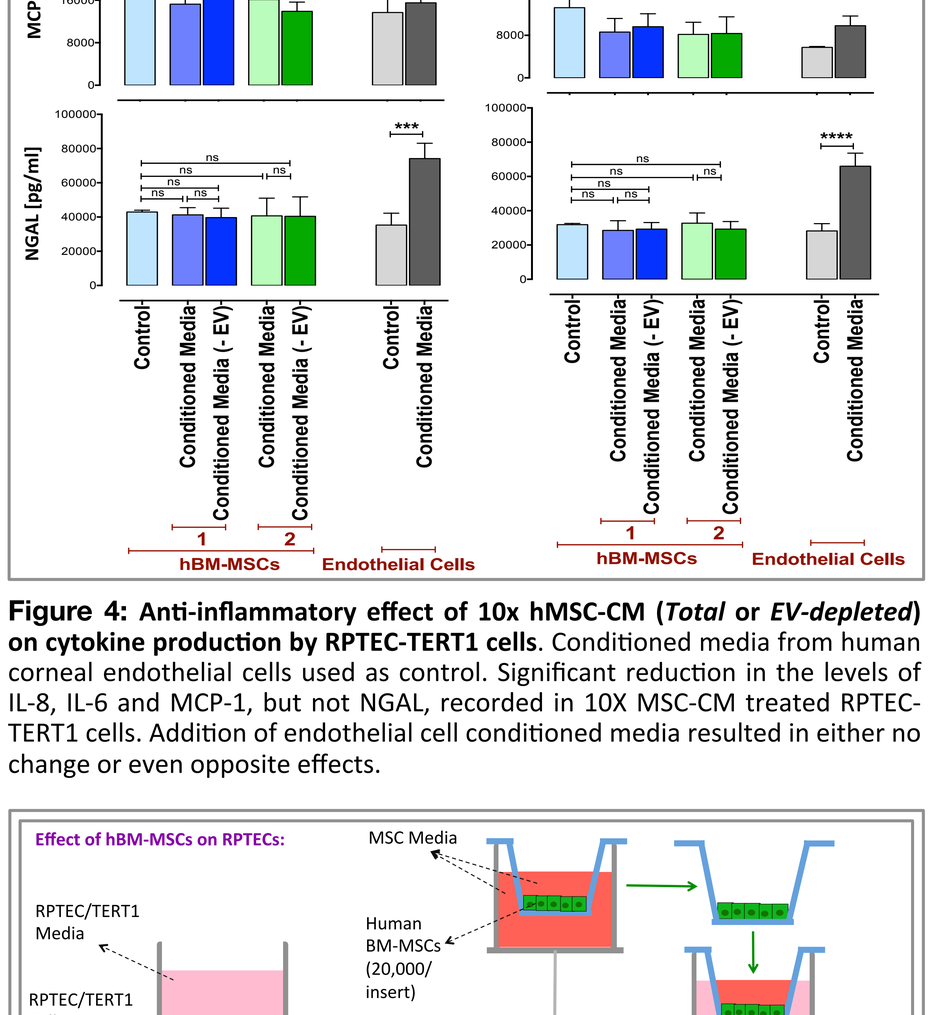

Figure 4: Anti-inflammatory effect of 10x hMSC-CM (*Total* or *EV-depleted*) on cytokine production by RPTEC-TERT1 cells. Conditioned media from human corneal endothelial cells used as control. Significant reduction in the levels of IL-8, IL-6 and MCP-1, but not NGAL, recorded in 10X MSC-CM treated RPTEC-TERT1 cells. Addition of endothelial cell conditioned media resulted in either no change or even opposite effects.

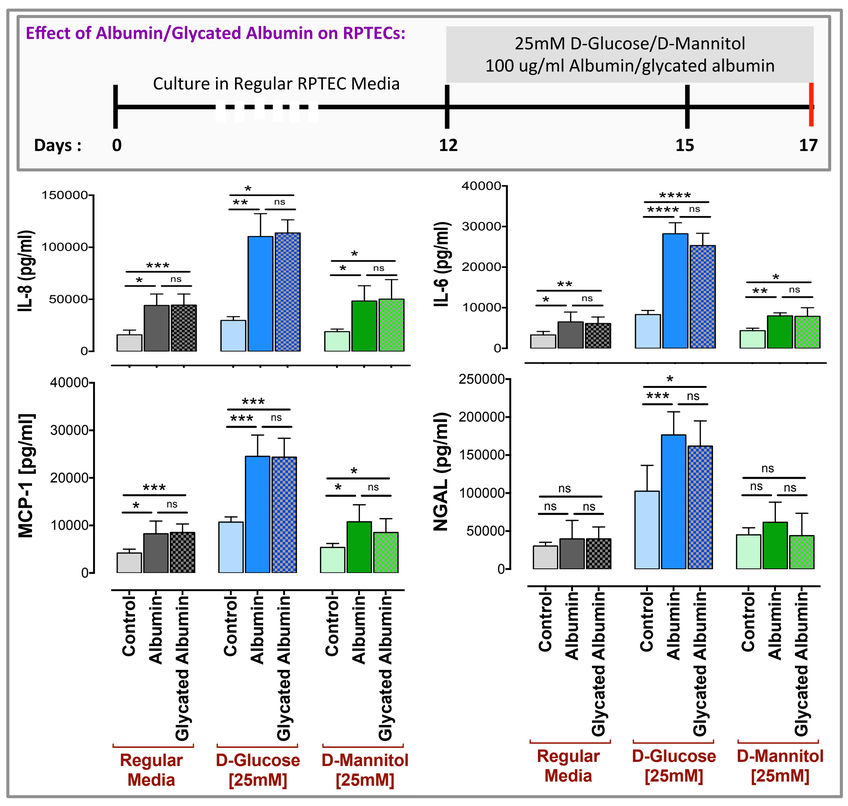

Figure 2: Combined effect of high D-Glucose and human albumin/ glycated-albumin (advanced glycation end product) on cytokine production by RPTECs. Significant elevations in IL-8, IL-6, MCP-1 and NGAL found when RPTEC-TERT1 cells were treated with either albumin or glycated albumin (AGE) at 100 µg/ml. No difference recorded between the effects of albumin and glycated albumin.

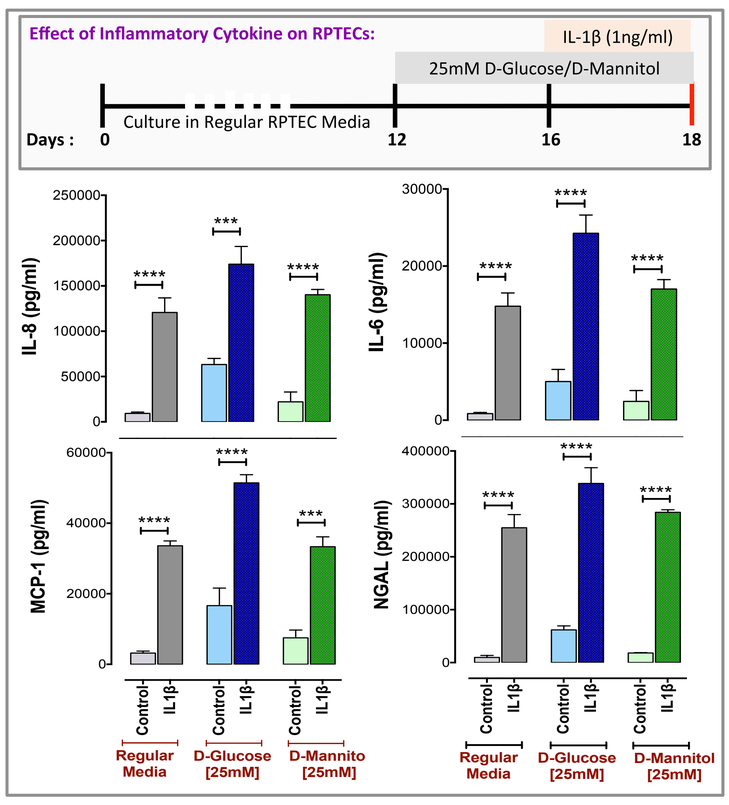

Figure 3: Combined effect of high D-Glucose and IL-1β on cytokine production by RPTEC-TERT1 cells. Significant elevations found in IL-8, IL-6, MCP-1 and NGAL levels when RPTEC cells were treated with 1 ng/ml IL-1β. Similar but less potent effects also recorded by inflammatory stimuli TNF-α.

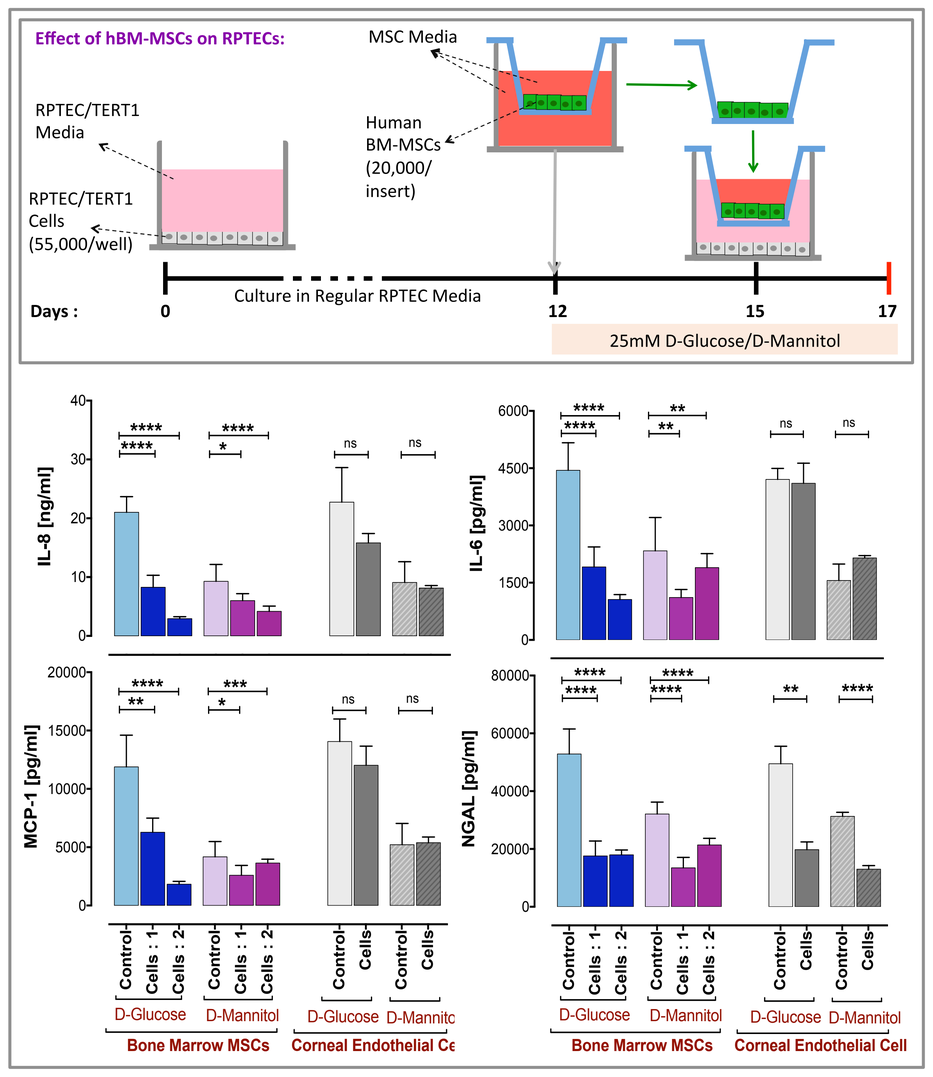

Figure 5: Anti-inflammatory effect of indirect contact of hBM-MSC (1:10) on cytokine production by RPTEC-TERT1 cells. Significant reduction in the levels of IL-8, IL-6 and MCP-1 recorded by indirect contact of hMSCs (from different donors) with RPTEC-TERT1 cells. Indirect contact of human corneal endothelial cells, used as control, did not show any changes in those biomarkers. However, NGAL showed significant reduction by all these cells.

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REFERENCES:

1. Griffin et al. The Promise of Mesenchymal Stem Cell Therapy for Diabetic Kidney Disease. Curr Diab Rep. 2016.

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Md Nahidul Islam
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