EXTRACELLULAR VESICLES DERIVED FROM MESENCHYMAL STEM CELLS INHIBIT TUBULAR INJURY, T CELL PROLIFERATION AND PROMOTE REGULATORY T CELL DIFFERENTIATION THROUGH TRANSFER OF SPECIFIC RNAs: PROTECTIVE ROLE IN T-CELL-MEDIATED KIDNEY GRAFT REJECTION

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BACKGROUND

Mesenchymal stem cells (MSCs) are known to exert regenerative and immunomodulatory effects by releasing paracrine mediators including extracellular vesicles (EVs), small particles involved in cell-to-cell communication through transfer of proteins and genetic information.
MSCs may have a protective role in T-cell mediated rejection (TCMR) through the release of immunomodulatory factors including EVs.

METHODS

MSCs were isolated from bone marrow and EVs were characterized for size, protein and RNA content. The biological effects of EVs were studied on T cells isolated from peripheral blood of kidney transplant recipients co-cultured with B cells purified by the spleen of matched deceased donors or on human kidney-derived tubular epithelial cells cultured in an inflammatory midkine (IM) typical of TCMR.

OBJECTIVES

The aim of this study was to evaluate the protective role of MSC-derived EVs in the mechanisms of T-cell mediated rejection (TCMR) in kidney transplantation

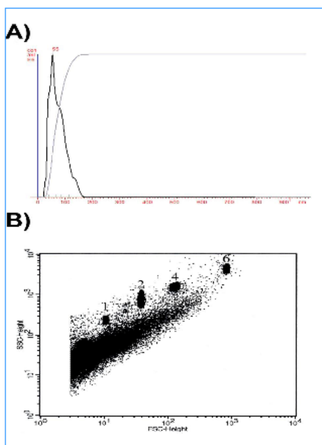

Figure 1: (A) Nanosight analysis of EV size; (B) FACS analysis of purified EVs.

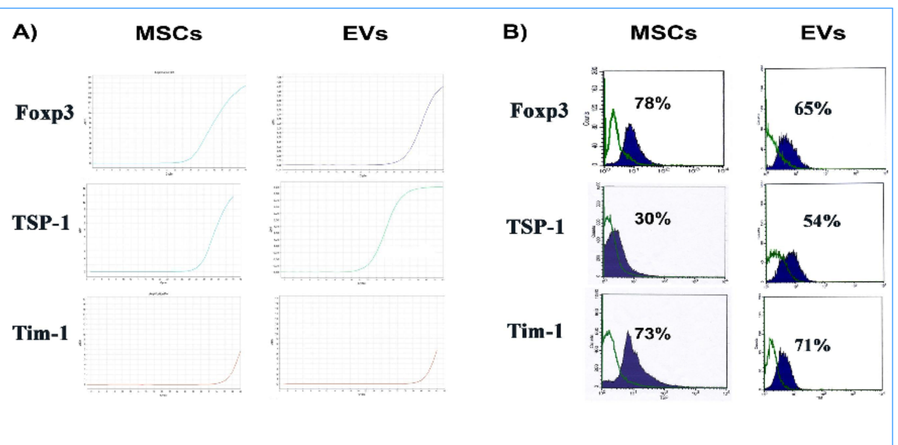

Figure 2: Representative RT-PCR curve of Foxp3, Tim-1 and TSP-1 mRNA in MSC and EVs derived from MSCs (A). FACS analysis of Foxp3, TIM-1 and TSP-1 protein expression in MSC and EVs derived from MSCs (B).

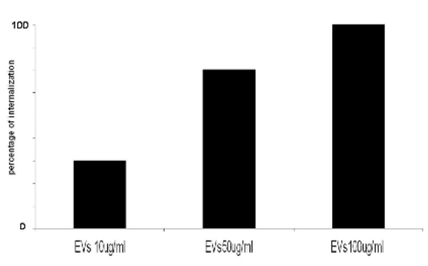

Figure 3: FACS analysis of dose-resposne EV internalization in T cells.

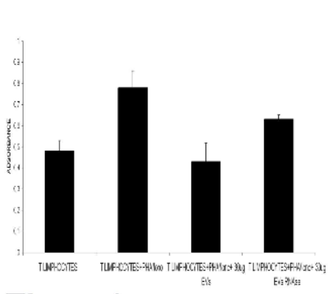

Figure 4: Proliferation of T cells (BrdU) stimulated with PHA/Ionomycin alone or with 30ug of EVs or RNase-treated EVs.

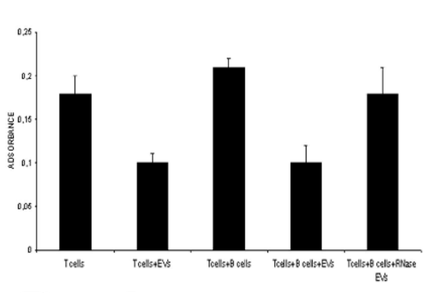

Figure 5: BrdU assay evaluating kidney transplant recipient T cell proliferation in presence of donor-related B cells and 30 ug EVs or RNase-treated EVs.

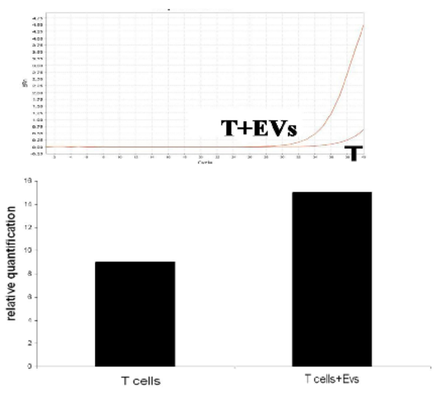

Figure 6: Representative RT-PCR of Foxp3 mRNA and relative quantification in T cells alone or stimulated with EVs.

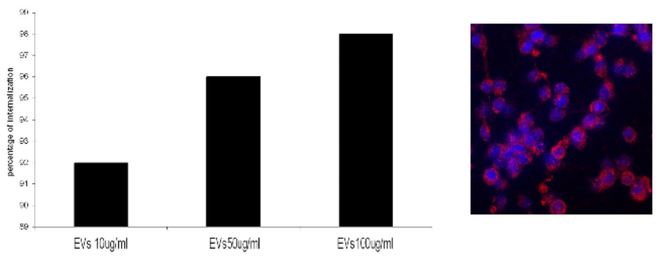

Figure 7: EV dose-response internalization in human tubular epithelial cells (TECs) (left panel). Confocal microscopy analysis of red labelled EV internalization in TECs (right panel).

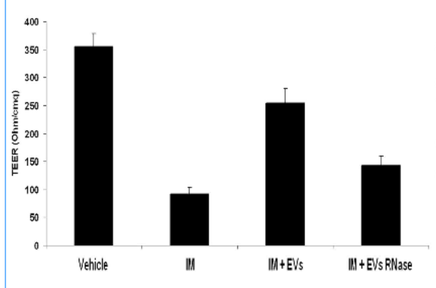

Figure 8: Analysis of tubular cell polarity evaluated by TEER (trans-epithelial resistance) in different experimental conditions (IM: Inflam-matory Midkine).

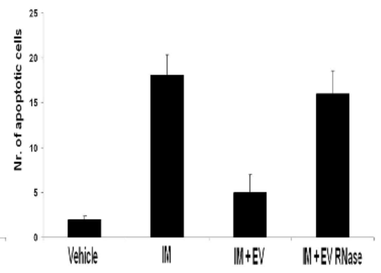

Figure 9: Analysis of tubular cell apoptosis evaluated by TUNEL assay in different experimental con-ditions (IM: Inflammatory Midkine).

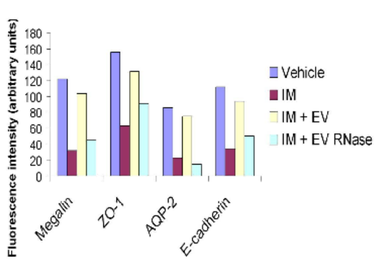

Figure 10: FACS analysis of tubular cell expression of Megalin, ZO-1, Aquaporin-2 (AQP-2) and E-cadherin in different experimental conditions (IM: Inflammatory Midkine).

Name	Other Names	Solutes/Molecules Trasported	Localization
SLC12a3	NCC; NCCT	Na/Cl	Distal Convoluted
SLC17a1	NAPI1/NPT1	Na/PO4	Proximal Convoluted
SLC34a1	NPT2	Tr Na/PO4 type 2	Proximal Convoluted
SLC5a2	SGLT2;GLT2	Na/Glucose	Proximal Convoluted
SLC6a19	B0AT1; HND	Neutral amino-acid/ Na	Proximal Convoluted
SLC12a2	NKCC	Na/K/Cl	thick ascending loop of henle

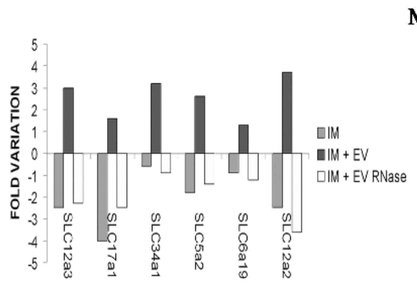

Figure 11: RT-PCR analysis of mRNA coding for different solut carriers (SLCs) expressed by tubular cells in different conditions (IM: Inflammatory Midkine).

RESULTS

As detected by Nanosight analysis (Fig. 1A), MSC-derived EVs sized 60-150 nm. FACS analysis (Fig. 1B) confirmed on MSC-derived EV surface the presence of molecules of the integrin family essential for their internalization within target cells (not shown). EVs carried different microRNAs and mRNAs including the immunoregulatory Foxp3, Tim-1 and thrombospondin-1 similarly to cells from which they originated (Fig. 2A). The same immunoregulatory molecules were also found at protein level as detected by FACS analysis (Fig. 2B). EVs were internalized in T cells isolated from kidney transplant recipients in a dose-response manner (Fig. 3), inhibiting their proliferation induced by phytohemagglutinin + ionomycine (Fig. 4) or by co-culture with donor spleen-derived B cells used as antigen presenting cells (Fig. 5).
Of interest, EVs horizontally transferred to T cells Foxp3 mRNA, inducing a Treg phenotype (RT-PCR results for Foxp3 mRNA in Fig. 6).
EVs were internalized in a dose-response manner also in human tubular epithelial cells (TECs) (Fig. 7). EVs inhibited functional alterations (loss of polarity evaluated by trans-epithelial electrical resistance in Fig. 8) and apoptosis (TUNEL assay in Fig. 9) induced by an inflammatory midkine (IM: cytokines, Fas-Ligand, perforin and granzyme-B) similar to that observed in TCMR. EVs preserved the expression of molecules typical of fully differentiated TECs (FACS analysis of megalin, ZO-1, AQP-2, E-cadherin in Fig. 10) and of different solute carriers (SLCs), (RT-PCR analysis in Fig. 11) that are all down-regulated by the inflammatory microenvironment. All the biological effects exerted by EVs were decreased after their treatment with 1U/ml RNase, the enzyme able to degrade all RNA subsets present in EVs (Fig. 4-5 and Fig. 8-11). These results suggest a key role for RNAs carried by MSC-derived EVs in their immunomodulatory and anti-inflammatory effects.

CONCLUSIONS

MSC-derived EVs may have a protective role in TCMR by inhibiting T cell proliferation, by inducing T cell to acquire a Treg phenotype and by decreasing apoptosis and preserving functional integrity of tubular cells exposed to an inflammatory microenvironment.
These protective effects are mediated by the horizontal transfer of specific RNAs from EVs to target cells. In particular, the transfer of Foxp3 mRNA from EVs to activated T cells may induce a Treg phenotype and immune tolerance.