OF21.04 | Therapeutic effects of mesenchymal/stromal stem cells and their derived extracellular vesicles in rheumatoid arthritis

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Introduction: Rheumatoid arthritis (RA) is an autoimmune disease characterised by inflammation, progressive damage, and pain in joints. Currently available therapy is inadequate to alleviate the inflammation and reduce the joint damage. While the immune-regulatory effect of MSC-EVs has been tested in many diseases, little is known with respect to their effect on RA. Thus, we aimed to assess the effect of human MSCs and MSC-EVs on T cells and synovial fibroblasts (RASFs) of RA patients. The effect of EVs derived from MSCs primed with interferon beta (IFNb) was also assessed.
Methods: MSC-EVs were collected using a PEG precipitation, followed by ultracentrifugation-based protocol and were characterised via NTA, TEM, imaging flow cytometry (IFCM) and immunoblotting analysis. Immune-regulatory properties of MSCs and MSC-EVs were assessed on CD4+ T cells stimulated with CD3/CD28. Also, the effect of MSC-EVs on RASFs stimulated with TNFa was assessed.
Results: EVs from naïve and IFNb primed MSCs were prepared and all fulfilled MISEV2018 criteria as evaluated by NTA, TEM and immunoblotting. Additionally, IFCM confirmed the recovery of CD9+ and CD63+ small-sized EVs. Applied onto the stimulated CD4+ T cells, EV preparations from IFNb primed MSCs suppressed the expression of more inflammatory cytokines (GM-CSF, IL-2, IL-4 and TNFa; $p < 0.05$, in all cases) associated with the pathogenesis of RA. However, while MSCs suppressed T cell proliferation, all MSC-EVs had a tendency to increase numbers of T regulatory cells. Furthermore, MSC-EVs inhibited ($p < 0.05$) the migration of RASFs and reduced ($p < 0.05$) the expression of the RA surface markers HLA-DR and CD34.
Summary/Conclusion: Both MSCs and MSC-EVs exerted immune-regulatory effects on RA CD4+ T cells and MSC-EVs, but not the MSCs themselves, inhibited RASFs migration. The beneficial effect of MSC-EVs on RA derived T cells and RASFs was further enhanced by priming the MSCs with IFNb.
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OF21.05 | Mesenchymal stromal cell-derived extracellular vesicles attenuate metabolic changes in pre-clinical models of ARDS through mitochondrial transfer

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Introduction: Mesenchymal stromal cells (MSC)-derived extracellular vesicles (EVs) are being investigated as a therapy for acute respiratory distress syndrome (ARDS). Previously, we demonstrated that MSC EVs restore functional activity of the injured cells through mitochondrial transfer. However, the impact of this mechanism on the balance of oxidative phosphorylation and glycolysis is not known. Here we investigated how EV mitochondrial transfer modulates metabolic alterations in the primary human pulmonary cells exposed to LPS or ARDS plasma.
Methods: EVs were isolated from bone-marrow MSCs with normal or dysfunctional mitochondria by ultracentrifugation. Mitochondrial dysfunction in MSCs was induced by Rhodamine6G. EVs were characterized for number, size distribution, tetraspannin expression, and mitochondrial content. Primary human distal lung epithelial and endothelial cells, and monocyte derived macrophages were stimulated with LPS or plasma from ARDS patients and treated with EVs. Mitochondrial respiration and glycolytic flux were assessed by Seahorse metabolic analyser, barrier properties were assessed by xCELLigence, phagocytosis was assessed by flow cytometry. Also, single cell transcriptomic analysis was performed on mouse lungs in the in vivo LPS-induced lung injury model.
Results: Inflammatory stimulation resulted in pronounced reduction of mitochondrial respiration, increase in glycolysis and functional impairment in all cell types. MSC EVs isolated from normal MSCs inhibited glycolytic flux, restored mitochondrial respiration and cell function while mitochondria-depleted EVs were not effective. Analysis of the single cell seq data showed that MSC EVs administration regulates expression of essential genes involved in mitochondrial metabolism in vivo.
Summary/Conclusion: MSC EVs alleviate ARDS-induced metabolic alterations via transfer of healthy mitochondria.
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