The secretome of periodontal ligament stem cells from MS patients protects against EAE

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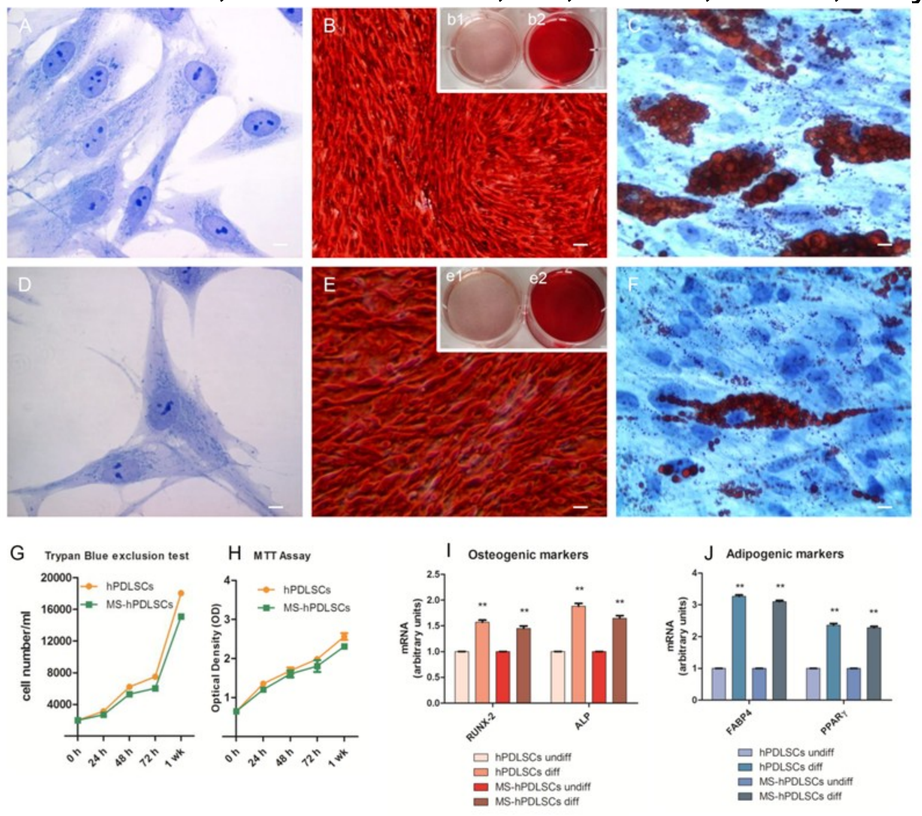
OBJECTIVES: In the present study, we evaluated the regenerative and immunomodulatory properties of hPDLSCs-conditioned whole culture medium (hPDLSCs-CM) and purified exosomes/microvescicles (hPDLSCs-EMV) obtained from Relapsing Remitting Multiple sclerosis (RR-MS) patients and compared them with hPDLSC-CM and hPDLSC-EMV obtained from healthy donors. To this end, we reported the characterization of the RR-MS-hPDLSCs in terms of expression of stemness markers, morphological features, proliferation rate and capability to differentiate into osteogenic and adipogenic lineages in comparison with hPDLSCs derived from healthy donors. Furthermore, we studied, *in vivo,* the clinical score and body weight, myelin regeneration and dendritic parameters, modulation of the anti-inflammatory immune responses, and regulation of apoptosis in spinal cord and/or spleen of experimental autoimmune encephalomyelitis (EAE) mice model systemically administered with RR-MS patients or donors hPDLSCs-CM and purified EMVs.

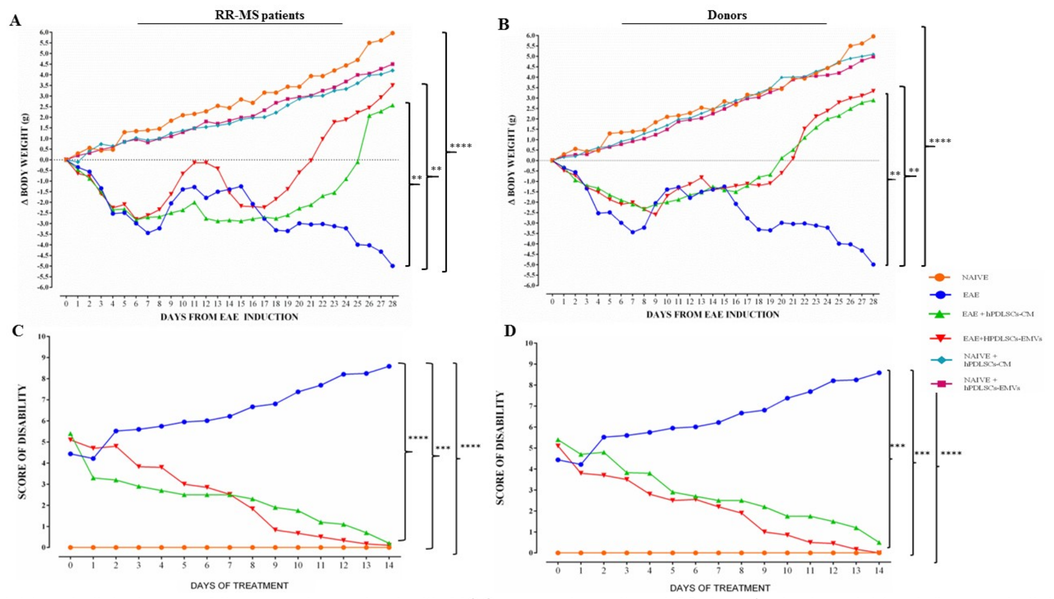
MATERIAL AND METHODS: EAE was induced by immunization with myelin oligodendroglial glycoprotein peptide (MOG)35–55 in C57BL/6 mice. After immunization, mice were observed every 48 hours for signs of EAE and weight loss. At the onset of disease, approximately 14 days after immunization, EAE mice were subjected to a single intravenous injection of hPDLSC-CM and hPDLSC-EMV obtained from RR-MS patients and healthy donors into the tail vein.

RESULTS: We show that hPDLSCs-CM and hPDLSCs-EMV reduce proinflammatory cytokines IL-17, IFN-γ, IL-1β, IL-6, TNF-α, and induce anti-inflammatory IL-10. In addition, apoptosis related STAT1, p53, Caspase 3, and Bax expressions were attenuated.

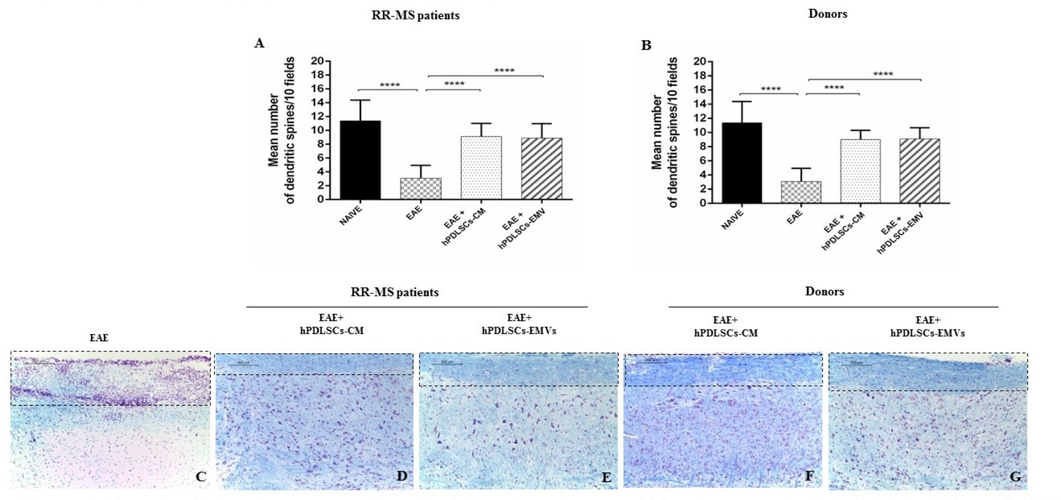
CONCLUSION: We speculate that the multiple beneficial effects produced by conditioned medium might be attributed to its EMVs fractions, adding further support in the context of personalized regenerative stem-cell free therapy for MS. Stem-cell free therapy approach has been emerged as potentially safer and cost-effective alternatives for a wide range of diseases (1-3). Our findings unravel the immunosuppressive effects of hPDLSCs-CM and hPDLSCs-EMVs in EAE mice, and suggest simple alternative autologous source for patient-customized cell-free targeting treatment in MS patients.



Toluidine blue staining of primary cultures of hPDLSCs observed by light microscopy, the cells display a fibroblast-like appearance in both hPDLSCs [**A**] and RR-MS-hPDLSCs [**D**] samples. Cell proliferation and viability are assessed by Trypan blue exclusion test [**G**] and MTT assay [**H**]. hPDLSCs, and RR-MS-hPDLSCs induced to osteogenic differentiation were stained with Alizarin Red S, after 3 weeks of culture [**B** and **E**, respectively]. Insets display uninduced hPDLSCs from healthy donors [**b1**] and RR-MS patients [**e1**]; **b2** and **e2** show differentiated hPDLSCs and RR-MS-hPDLSCs, respectively. The bar graph shows mRNA levels of osteo-related genes, i.e., alkaline phosphatase (*ALP*) and Runt-related transcription factor-2 (*RUNX2*) at 7 days of culture **[I]**. Adipogenic differentiation has been evaluated by the appearance of oil-red O-positive lipid vacuoles [**C** and **F**]**.** The adipo-related genes, i.e., fatty acid binding protein 4 (*FABP4*) and peroxisome proliferator-activated receptor γ (*PPARγ*), analyzed by real-time PCR are shown [**J**]. Scale bars = 10μm.



Body weight and clinical score. Mice were immunized with MOG $_{35–55}$ and monitored 28 days for body weight gain/loss and clinical disease score. **A** and **B** The variation of body weight has been expressed compared to day of EAE induction (day 0) for each experimental group. **C** and **D** Naive mice did not display motor deficit. EAE mice displayed a grading of disease, while significant reduction in the clinical scores was observed in EAE mice treated with RR-MS patients hPDLSCs-CM or hPDLSCs-EMVs and in EAE mice treated with donors hPDLSCs-CM or hPDLSCs-EMVs.



Golgi staining and LFB. Dendritic spines were reduced in EAE mice, whereas EAE mice treated with hPDLSCs-CM or hPDLSCs-EMVs obtained from RR-MS patients [**A**] and donors [**B**] showed significant augmentation in spine density. In addition, EAE mice without treatment exhibited remarkably reduced myelin in the spinal cord [**C**; area showed in dotted square], whereas treatment with hPDLSCs-CM or hPDLSCs-EMVs obtained from RR-MS patients [**D** and **E**] and donors [**F** and **G**] reduced demyelination and axonal loss in EAE mice with an intense LFB positive staining.

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