Conditioned medium of human periodontal stem cells from Multiple Sclerosis patients reduces the expression and release of inflammatory cytokines in human monocytic cell line (THP-1) challenged with Lipopolysaccharide (LPS)

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Periodontal diseases(PD) are among the most common chronic infections in humans. PD affect the teeth supporting structures, but have been reported to be associated with increased levels of pro-inflammatory cytokines [e.g. tumor necrosis factor alpha (TNF)α and interleukin (IL)-1β]. These pro-inflammatory molecules have been proposed to act as a link between PD and the progression of neuroinflammatory/neurodegenerative disorders(1). Moreover, recently, in ApoEnull mice infected with Porphyromonas gingivalis (PG) it has been reported the presence of the bacterial genomic DNA in the brain(2). In the pre-symptomatic phase of the disease in experimental autoimmune encephalomyelitis (EAE) mice, the most widely used animal model of Multiple Sclerosis (MS), a brain blood barrier breakdown has been shown (3), thus plasma components as immune cells and inflammatory agents can enter the CNS and trigger, in genetically susceptible subjects, an autoimmune response. Different pro-inflammatory molecules and bacterial endotoxins can modulate neuroinflammation through the activation of their own receptors expressed in neuronal cells including microglia (4). The administration of multipotent mesenchymal stem cells (MSCs) for the treatment of neuroinflammatory/neurodegenerative diseases is considered an interesting strategy. We have previously isolated pluripotent stem cells from adult human periodontal ligament (hPDLSCs) with high self-renewal capability and multipotency feature and we have recently shown that their administration to EAE mice exerts beneficial effects on the disease symptoms and progression (5). The present research aimed to explore in vitro the possible role of MSC conditioned medium (CM) in their therapeutic actions. Thus, we studied the effect of CM derived from hPDLSCs collected from healthy donors (hPDLSCs-CM) and from Relapsing Remitting Multiple Sclerosis patients (RR-MS hPDLSCs-CM) on the expression and production of inflammatory cytokines by THP-1 cells, as an in vitro model of microglia, in response to PG-lipopolysaccharide (LPS-PG). The sample tissues were obtained from premolar teeth during root scaling and were subsequently cultured. The effect of hPDLSCs-CM and RR-MS hPDLSCs-CM on THP-1 cell viability was measured using a 3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide (MTT) assay. The expression of ha TNFα, IL-1β and IL-6 was evaluated by Reverse-Transcriptase Polymerase Chain Reaction and enzyme-linked immunosorbent assay (ELISA). The expression level of the Toll-like Receptor (TLR)4 was evaluated by Western blot analysis. LPS-PG significantly increased TNFα, IL-1β and IL-6 mRNA expression and protein levels in THP-1 cells. Treatment with hPDLSCs-CM or with RR-MS hPDLSCs-CM significantly attenuated the LPS-PG-induced expression and production of these pro-inflammatory cytokines. The CM from both healthy donors and RR-MS patients also reduced the LPS-G stimulated protein levels of TLR4 in THP-1 cells. On the whole our data add new evidence on the anti-inflammatory effects of these peculiar stem cells even when derived from RR-MS patients. Furthermore it is to be underlined that hPDLSCs do not rise ethical concerns and derive from an easily accessible site

during a routine dental hygiene practice, features that make them promising candidates worth to be tested for the potential use of cell-free based novel therapeutic approach to neuroinflammatory/neurodegenerative pathological conditions. Further experiments, including proteomic and lipidomic ones are needed to clarify the beneficial mechanisms of their secretome.

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