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Immunomodulatory effect of UC MSCs: Flowcytometry versus B-rdu ELISA

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Background: Umbilical cord blood (UCB) gained high interest as a source of stem cells for use in cellular therapies. The immunomodulatory effect of mesenchymal stem cells (MSCs) from bone marrow, adipose tissue and amniotic membrane was proved.

Aim: In this study we aimed at isolation, culture & characterization of MSCs from UCB, and evaluation of their immunomodulatory effect on peripheral blood lymphocyte proliferation by using two different techniques; Brdu ELISA and CFSE flowcytometric technique.

Methods and Results: The immunosuppressive effect of MSCs on peripheral blood lymphocytes was examined by co-culturing mitomycin c treated UCB MSCs passage 4 with PHA stimulated lymphocytes for 72 hours. Using carboxyfluorescein diacetate succinimidyl ester (CFSE) method, the mean SI of lymphocytes was 5.025±3 and of co-cultured PB lymphocytes with MSCs was 1.9±0.49 (lymphocytes proliferation was reduced to 37% in the coculture). The thymidine analog BrdU [5-bromo-2-deoxyuridine] incorporated into the newly synthesized DNA of replicating cells was quantitated by ELISA. The proliferation of stimulated human PB lymphocytes was reduced to 56% of the control; the mean SI of PHA stimulated lymphocytes was 3.7±1.6 and of co cultured PHA stimulated PB lymphocytes with MSCs was 2.16±1.1. Levels of cytokines [IFN-γ, TGFβ, IL10] in cell culture supernatants were studied by ELISA. IFNγ secretion by human PB lymphocytes was 1243.625±1097pg/ml. It decreased significantly (mean 680.05±635 pg/ml) in the supernatants of co-culture of lymphocytes with UCB MSCs (p=0.000) thus the immunomodulatory effect of MSCs may be due to inhibition of IFNγ secretion from activated lymphocytes. The level of TGFβ and IL10 increased.

Conclusion: UCB MSCs suppresses the proliferation of mitogen stimulated lymphocytes. CFSE dilution assay allowed tracing the kinetics of suppression of lymphocytes proliferation, thus it has superior and more informative results.