

**MULTIPOTENT MESENCHYMAL STROMAL CELLS ENDOVENOUSLY ADMINISTERED CONTRIBUTES TO NKT CELLS REPOPULATION IN THE LIVER AND PREVENTS NON-ALCOHOLIC STEATOHEPATITIS IN OBESE MICE WITH METABOLIC SYNDROME.**

**Conget Paulette, PhD.** Instituto de Ciencias, Facultad de Medicina Clínica Alemana-Universidad del Desarrollo. Av. Las Condes 12438, Santiago, Chile. Tel: 562 3279157, Fax: 562 2999306, pconget@udd.cl

**Ezquer Marcelo, PhD; Ezquer Fernando, PhD; Ricca Micaela; Simon Valeska and Conget Paulette, PhD.**  
Santiago, Chile.

The hepatic manifestation of metabolic syndrome is non-alcoholic fatty liver disease (NAFLD). A subset of patients with NAFLD progress to non-alcoholic steatohepatitis (NASH). The clinical relevance of these conditions is due to the high and increasing prevalence of metabolic syndrome in general population, and the potential of NASH to evolve towards end-stage liver disease, including cirrhosis and hepatocellular carcinoma. Recently, we have demonstrated that bone marrow-derived multipotent mesenchymal stromal cells (MSC) endovenously administered into animals with NAFLD, prevents NASH onset. This beneficial effect of MSC transplantation is not related to a reversion of metabolic syndrome, since MSC-treated mice kept hyperglycemic, hiperinsulinemic, insulin resistant and hypercholesterolemic.

Depletion of hepatic natural killer T (NKT) cells has been associated with a shift from anti-inflammatory (Th2) to pro-inflammatory (Th1) cytokines expression, and the later in critical for NASH development. Hence, we evaluated if MSC hepatoprotection observed in obese mice with metabolic syndrome relates to hepatic NKT cells recovery. For this, C57BL6 mice were exclusively fed with a high-fat diet (HFD) containing 60% saturated fat and 33 weeks latter, separated in two groups. One group received two times  $0.5 \times 10^6$  MSC (MSC-treated). The other group received vehicle (untreated). Both groups continued to eat HFD all along the study period (50 weeks).

The frequency of NKT cells [CD3+, NK1.1+, CD4+] and the gene expression level of anti-inflammatory [IL-4, IL-10] and pro-inflammatory [IL-1beta, TNFalpha] cytokines were assessed -by flow cytometry and real time RT-PCR, respectively- in the liver of normal, untreated and MSC-treated. Compared to age- and sex-matched normal mice, the percentage of NKT cells was selectively reduced in the liver of obese mice with metabolic syndrome. In contrast, 2 and 17 weeks post-MSC administration the frequency of NKT cells was at baseline. Compared to untreated obese mice, MSC-treated mice expressed higher levels of IL-4 and IL-10 and lower levels of IL-1beta and TNFalpha. Compared to normal mice, MSC-treated mice overexpressed IL-10.

The presence of donor cells was assessed -by flow cytometry- in blood, heart, lung, kidney, pancreas, bone marrow, spleen and liver, 2 and 17 weeks after MSC<sup>GFP</sup> administration. Donor cells were found into heart, kidney, bone marrow and liver of obese mice with metabolic syndrome. While in most of the tissues were MSC<sup>GFP</sup> home, they abundance maintain constant or tend to diminish, en the liver they increased over the time. Our results show that endovenously administered MSC home into the liver of obese mice with metabolic syndrome, contribute to hepatic NKT cells repopulation and reestablish the balance between anti- and pro-inflammatory molecules, thus preventing NASH development.

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