

CO-TRANSPLANTATION OF BONE MARROW DERIVED MSCS MODULATES CYTOKINE PROFILE AND DECREASES THE INCIDENCE OF GVHD IN MOUSE EXPERIMENTAL MODEL OF ALLOGENEIC HSCT

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Introduction aHSCT is widely used to treat a series of hematologic diseases but its success is hampered by the appearance of GVHD. MSCs possess immunomodulatory properties and have emerged as a promising therapeutic modality for GVHD prevention and treatment. The purpose of this study was to investigate whether co-transplantation with MSCs could prevent acute GVHD in murine aHSCT focusing clinical condition, histological findings and *in vivo* cytokine production.

Methods C57BL/6 mice (H2b) were used as donors of aHSCT, splenocytes (Sple) and MSCs; and FVB GFP+ (H2q) mice as receptors. Recipients were distributed in following groups: **1-** normal control (n=9); **2-** irradiated control (n=10); **3-** syngeneic HSCT (n=4); **4-** aHSCT (n=10); **5-** aHSCT+Sple (n=10); **6-** aHSCT+MSCs (n=10) and **7-** aHSCT+Sple+MSCs (n=10). MSCs ($0,5 \times 10^6$) and/or Sple ($0,2 \times 10^6$) were co-infused with aHSCT (5×10^6) in allogeneic models on the day of transplantation. To assess the degree of GVHD, clinical condition scoring (Cooke *et al.*, 1996), histological findings scoring (spleen, liver and small bowel) and *in vivo* serum cytokine analyses were performed on day 5 after transplantation.

Results Clinical analyses performed 5 days after transplantation showed the following scores in groups: **1-** 0,0; **2-** 1,4 ($\pm 1,0$); **3-** 0,5 ($\pm 0,5$); **4-** 1,9 ($\pm 0,5$); **5-** 3,9 ($\pm 0,7$); **6-** 1,4 ($\pm 0,9$) and **7-** 2,9 ($\pm 0,9$). Histopathological findings such as portal and lobular infiltrates, hepatocytes apoptosis and endothelialitis in centrilobular vessels in the liver and mucosal atrophy, inflammatory infiltrates, and cell debris and apoptosis in the small bowel were more conspicuous in groups **4** and **5** in comparison with groups **6** and **7**, respectively. Our own standardized scoring system showed the following scores for spleen, liver and small bowel (respectively) in groups: **2-** 4,1 ($\pm 0,6$), 1,2 ($\pm 1,2$), 2,0 ($\pm 1,0$); **3-** 3,5 ($\pm 1,0$), 1,0 ($\pm 0,8$), 1,5 ($\pm 1,7$); **4-** 5,1 ($\pm 0,8$), 3,2 ($\pm 1,1$), 3,2 ($\pm 0,6$); **5-** 4,7 ($\pm 1,6$), 5,6 ($\pm 2,0$), 3,6 ($\pm 1,4$); **6-** 3,2 ($\pm 1,0$), 2,9 ($\pm 1,1$), 2,6 ($\pm 1,0$) and **7-** 4,3 ($\pm 1,3$), 3,4 ($\pm 1,0$), 3,3 ($\pm 1,4$). Cytokine analyses showed that group **5** presented average concentrations (pg/ml) to cytokines IL-2 ($7,5 \pm 6,0$), IL-6 ($316,1 \pm 342,7$), MCP-1 ($992,1 \pm 661,9$), TNF- α ($40,0 \pm 21,4$) and IFN- γ ($90,3 \pm 85,5$) significantly higher in comparison with those observed on groups **4**, **6** and **7**. On the other hand, group **6** was the one which presented the least dysregulated profile of the pro-inflammatory cytokines IL-6 ($7,4 \pm 8,0$), MCP-1 ($121,7 \pm 30,2$), IFN- γ ($6,2 \pm 7,4$) and TNF- α ($11,8 \pm 5,2$), and of the cytokines IL-2 ($1,8 \pm 2,6$), IL-12p70 ($2,8 \pm 3,6$) in comparison with the others groups which received allogeneic transplantation. The average concentration of IL-10 cytokine did not show significant differences between the groups which were submitted to aHSCT (**4**, **5**, **6** and **7**).

Conclusion In our study, MSCs co-infusion with aHSCT decreased GVHD incidence on studied groups (**6** and **7**) while Sple co-infusion increased the incidence of the disease. In the groups which received MSCs, clinical and histopathological less conspicuous findings were found in comparison with groups that did not receive MSCs (**4** and **5**). These results are directly associated with a less dysregulated pro-inflammatory and T cells sub-type (Th1 e Tc1) cytokine profile in groups which received MSCs, indicating that the process of GVHD prevention by MSCs on allogeneic transplantation is firstly related to a lower production of soluble factors that favors the proliferation and migration of these kinds of effector cells to the target tissues of GVHD. MSCs are been used to treat and prevent GVHD and the utilization of therapeutic protocols that opt for doses, timing, and number of cells to be applied can diminish GVHD effects, thus increasing aHSCTs efficiency.