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ASSESSMENT OF MESENCHYMAL STEM CELLS EFFECTS ON DENDRITIC CELLS MATURATION

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Abstract
Introduction. Mesenchymal stem cells (MSCs) are adult stem cells (Pittenger et al., 1999, Zhang et al., 2009) able of self-renewal, with low immunogenicity and immunoregulatory property (Jorgensen et al, 2003, English et al., 2008, Zhang et al., 2009). Dendritic cells (DCs) present in the bone marrow play a crucial role in the instruction of adaptive immunity (Nauta et al., 2006, Zhi-Gang et al., 2012) DC have the unique capacity to stimulate naive and memory T cells (Banchereau et al., 2000, Nauta et al., 2006, Wang et al., 2013). The aim of the present study was to assess the effect of MSCs on DC maturation.
Materials and Methods. MSCs were collected from femurs of male Wistar rats. Cells suspension were cultured in DMEM/F12 supplimented with 10% fetal calf serum (FCS), 5% horse serum and 1% antibiotic–antimycotic (Gibco). DCs were prepared from rat bone marrow after red cells lysis and cultured in RPMI 1640 medium (Gibco) supplemented with 10% FCS, 1% antibiotic–antimycotic (Gibco), 10 ng/mL GM-CSF (Sigma), and 5 ng/mL IL-4 (Sigma). MSCs and DCs were cultured for 7d at 37°C. DCs ($5x10^3$) were grown in two different conditions: co-culturing with MSCs and 25 ng/ml TNF-α (I) or without MSCs and 25 ng/ml TNF-α (Sigma) (II) for 48d. Cell phenotype were characterized by flow cytometry (FACSCanto II) using CD11b, CD44, CD86 (Becton Dickinson) antibodies.
Results and Conclusion. After co-culture with MSCs, DC showed a decrease in CD86 expression compared with culture supplemented only with TNF-α which showed an increase in expression of this marker.
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Key Words: *dendritic cells, differentiation, mesenchymal stem cells*

INTRODUCTION

Mesenchymal stem cells (MSCs) are adult stem cells (Pittenger et al., 1999, Zhang et al., 2009) able of self-renewal (Soleymaninejadian et al., 2012), with low immunogenicity and immunoregulatory property (Jorgensen et al, 2003, English et al., 2008, Zhang et al., 2009). MSCs can replicate for a long time while maintaining their multilineage differentiation potential (Eslaminejad et al., 2008, De Miguel., et al., 2012).
Dendritic cells (DCs) the most potent of the antigen presenting cells (Toubai et al., 2014) present in the bone marrow play a crucial role in the instruction of adaptive immunity (Nauta et al., 2006, Zhi-Gang et al., 2012). DCs can be divided into distinct subsets based on differential phenotype and function (Bjorck, 2001, Toubai et al., 2014). DCs have the unique capacity to stimulate naive and memory T cells (Banchereau et al., 2000, Nauta et al., 2006, Wang et al., 2013) and play a major role in development of cell-mediated immunotherapy (Pion et al., 2010). Recent studies indicate that MSCs can inhibit cell proliferation of T cells, B-cells, natural killer cells (NK) and dendritic cells (DC), De Miguel., et al., 2012 and can stop some of the immune cell functions: including the maturation and activation of DCs (De Miguel., et al., 2012)
The aim of the present study was to assess the effect of MSCs on DC maturation.

MATERIALS AND METHODS

MSCs were collected from femurs of male Wistar rats. Cells suspension were cultured in 25-cm^2 tissue culture flasks at a concentration of 10^6 cells/mL in DMEM/F12 supplimented with 10% fetal calf serum (FCS), 5% horse serum and 1% antibiotic–antimycotic (Gibco). After 72h incubation at 37°C in a 5% CO_2 humidified atmosphere (60%), nonadherent cells were removed and the adherent fraction were cultured in fresh medium basal medium. The MSCs were used only after 5 passages in order to eliminate monocytes.
DCs were prepared from rat bone marrow. After red cells lysis mononuclear cells were separated by Hypaque 1077 density gradient centrifugation (Sigma). Then cells were washed, counted, and

plated at 1× 10^5 onto T25 tissue culture plates in RPMI 1640 medium (Gibco) supplemented with 10% FCS, 1% antibiotic–antimycotic (Gibco), 10 ng/mL GM-CSF (Sigma), and 5 ng/mL IL-4 (Sigma). Culture medium were replaced, and the nonadherent cells were after 48h hours of initial culture and every 4 days thereafter.
MSCs and DCs were cultured for 7d at 37°C. DCs ($5x10^5$) were grown in two different conditions: co-culturing with MSCs and 25 ng/ml TNF-α (I) or without MSCs and 25 ng/ml TNF-α (Sigma) (II) for 48d. Cell phenotype were characterized by flow cytometry (FACSCanto II) using CD11b, CD44, CD86 (Becton Dickinson) antibodies. Quantified data are presented as the mean ±SD. Significance were accorded at $p< 0.05$.

RESULTS AND DISCUSSIONS

Microscopic examination of the bone marrow derivated cells after 72 h revealed cellular heterogeneity. A high percentage of cells were elongated or oval/round shape with smooth borders and a part of the cells were in suspension. After a few days were observed an intense proliferation of bipolar fibroblastoid cells with a significant reduction of the cells with round morphology (fig.1).

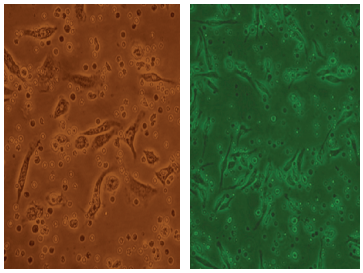

Fig. 1 – Mesenchymal- like cells derived from rat bone marrow

The MSCs were expanded for 5 passages in normal culture medium and were analyzed for the expression of cell surface molecules by flow cytometry (fig 2).

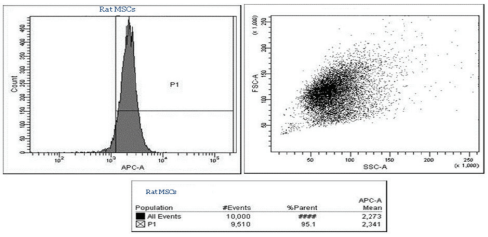

Fig. 2 -Immunophenotypic profile of rat MSCs (CD44 positive cells)

Freshly isolated mononuclear cells were cultured overnight at 37 °C and 5% CO_2 in standard media. Microscopic analysis revealed cells with irregularly shaped eccentric nuclei and abundant cytoplasmic extensions (fig.3).

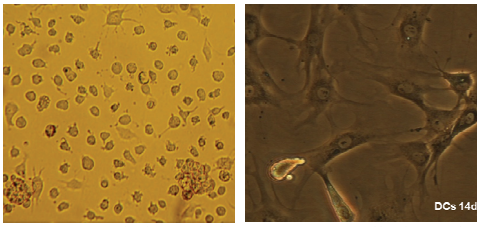

Fig. 3 - Morphological characteristic of cells derived from rat bone marrow

After co-culture of DCs cells with MSCs, were observed a flattening and widening of dendrites with a reduction in their length and a slight rounding of the cells. Also, DCs cells showed a decrease in CD86 expression compared with culture supplemented only with TNF-α which showed an increase in expression of this marker (fig.4).

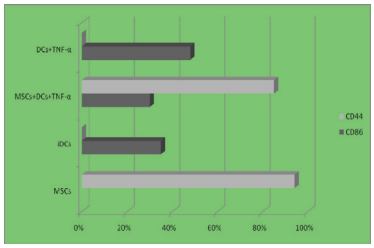

Fig. 4 - Immunophenotypic changes according to different cultural conditions

Mesenchymal stem cells are plastic adherent multipotent cells characterized by their expression of surface markers, capable of differentiating into various lineages, (Pittenger et al., 1999, Wang et al., 2006, Bydlowski et al., 2009, Szade et al., 2011, de Vasconcellos Machado et al., 2013, Majumdar et al., 2013, Wen-hua et al., 2013). MSCs have the ability to potently suppress immunological activity. This capacity is possible by activating on various cells of the immune system (Wen-hua et al., 2013). MSCs are capable of interfering in the differentiation, maturation and function of dendritic cells (de Vasconcellos Machado et al., 2013). Many studies show that the inhibitory effect of MSCs is dose-dependent; a high concentration of stem cells is more pronounced inhibitory effects (Krampera et al., 2003, Le Blanc et al., 2003, de Vasconcellos Machado et al., 2013). The inhibitory effect of

MSCs may also affect the expression of DCs surface markers and cytokine secretion profile and obviously the development of DCs function (English et al., 2008, Wen-hua et al., 2013). After co-culture with MSCs, DCs showed a decrease in CD86 expression compared with culture supplemented only with TNF-α which showed an increase in expression of this marker. These aspects are very important and need further studies to reveal all the involved mechanisms. This study demonstrates that MSCs interfered with the DCs maturation

CONCLUSION

Mesenchymal stem cells are characterized by stable undifferentiated phenotype under normal culture conditions; these cells have the capacity to prevent upregulation of DCs maturation markers. DCs cultured with MSC showed significantly reduced of CD86 ($p < 0.05$), but after maturation by TNF- resulted in increased surface expression of these markers. Our study demonstrates that MSC have an influence in DCs maturation through cell-to-cell contact, and secretion of specific cytokines.

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REFERENCES

Banchereau J., Briere F., Caux C., Davoust J., Lebecque S., Liu Y.J., Pulendran B., Palucka K. 2000. Immunobiology of dendritic cells. Annu. Rev. Immunol, 18: 767–811.

Bjorck Pia, 2001. Isolation and characterization of plasmacytoid dendritic cells from Flt3 ligand andgranulocyte-macrophage colony-stimulating factor–treated mice. Blood, 98, 13.

Bydlowski S.P., Debes A.A., Maselli L.M., Janz F.L., 2009. Características biológicas das células-tronco mesenquimais. Rev Bras Hematol Hemoter., 31(Suppl 1):25-35.

De Miguel M.P., Fuentes-Julian S., Blazquez-Martinez A., Pascual C.Y., Aller M.A., Arias J., Arnalich-Montiel F., 2012. Immunosuppressive Properties of Mesenchymal Stem Cells: Advances and Applications. Current Molecular Medicine, 12(5):574-91.

de Vasconcellos Machado C., Paloma Dias da Silva Telles, Ivana Lucia Oliveira Nascimento, 2013. Immunological characteristics of mesenchymal stem cells. Rev Bras Hematol Hemoter. 35(1):62-7.

English K., Frank P.B., Bernard P.M., 2008.Murine mesenchymal stem cells suppress dendritic cell migration, maturation and antigen presentation. Immunology Letters 115, 50–58.

Eslaminejad M.B., Nazarian H., Taghiyar L., 2008. Mesenchymal Stem Cell Isolation from the Removed Medium of Rat's Bone Marrow Primary Culture and their Differentiation into Skeletal Cell Lineages. Yakhteh Medical Journal, 10,1,65-72.

Jorgensen C., Djouad F., Apparailly F., Noel D., 2003. Engineering mesenchymal stem cells for immunotherapy, Gene Ther.10:928-931

Krampera M., Glennie S., Dyson J., Scott D., Laylor R., Simpson E., Dazzi F., 2003.Bone marrow mesenchymal stem cells inhibit the response of naïve and memory antigen-specific T cells to their cognate peptide, Blood, 101(9):3722-9.

Le Blanc K., Tammik L., Sundberg B., Haynesworth S.E., Ringdén O., 2003.Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex, Scand J Immunol., 57 (1):11-20.

Majumdar M.K., Keane-Moore M., Buyaner D., Hardy W.B., Moorman M.A., McIntosh K.R., Mosca J.D., 2003. Characterization and functionality of cell surface molecules on human mesenchymal stem cells, J Biomed Sci ,10(2): 228–241.

Nauta Alma .J, Kruisselbrink A.B., Lurvink Ellie, Willemze R., Fibbe W. E., 2006. Mesenchymal Stem Cells Inhibit Generation Monocyte-Derived Dendritic Cells -Derived and+and Function of Both CD34 J Immunol , 177:2080-2087.

Pion M., Serramia M.J., Diaz L., Bryszewska M., Gallart T., Garcia F., Gómez R., de la Mata F.J., Muñoz-Fernandez M.Á., 2010. Phenotype and functional analysis of human monocytes-derived dendritic cells loaded with a carbosilane dendrimer, Biomaterials ,31, 8749–8758.

Pittenger M.F., Mackay A.M., Beck S.C., Jaiswal R.K., Douglas R., Mosca J.D., Moorman M.A., Simonetti D.W., Craig S., Marshak D.R., 1999. Multilineage potential of adult human mesenchymal stem cells. Science,.284:143-147

Soleymaninejadian E., Pramanik K., Samadian E., 2012. Immunomodulatory properties of mesenchymal stem cells: cytokines and factors, Am J Reprod Immunol, Jan; 67(1):1-8.

Szade K., Zuba-Surma E., Rutkowski A.J., Jozkowicz A., Dulak J., 2011. CD45–CD14 +CD34 + murine bone marrow low-adherent mesenchymal primitive cells preserve multilineage differentiation potential in long-term in vitro culture,Mol Cells, 31(6): 497–507.

Toubai T., Mathewson N., Reddy P., The Role of Dendritic Cells in Graft-Versus-Tumor Effect, Front Immunol., 2014 Feb 21;5:66. eCollection 2014.

Wang X., Hisha H., Taketani S., Adachi Y., Li Q., Cui Y., Wang J., Song C., Mizokami T., Okazaki S., Li Q., Fan T., Fan H., Lian Z., Gershwin M.E., Ikehara S., 2006. Characterization of mesenchymal stem cells isolated from mouse fetal bone marrow, Stem Cells, 4(3): 482–493.

Wang Z., Xiaoqiong T., Wen Xu, Zeng C., Li Sun, Weiming Li, Qiubai Li, Zhigang Z, Ping Z.,2013. The Different Immunoregulatory Functions on Dendritic Cells between Mesenchymal Stem Cells Derived from Bone Marrow of Patients with Low-

Risk or High-Risk Myelodysplastic Syndromes, Plos One, Volume 8, Issue 3, e57470- e57470

Wen-hua Liu, Jing-jin Liu, Jian Wu, Lu-lu Zhang, Fang Liu, Li Yin, Mao-mao Zhang,Bo Yu, 2013. Novel Mechanism of Inhibition of Dendritic Cells Maturation by Mesenchymal Stem Cells via Interleukin10 and the JAK1/STAT3 Signaling Pathway, PLOS ONE, 8 Issue 1, e55487.

Zhang B., Lianming L., Rui L., Dan S., Zenke M., Xingxia L., Yuan C., Robert C. H. Zhao Xiaowei D., Xishan Z., Chunhua Lu, Wei Liang, 2009. Mesenchymal stem cells induce mature dendritic cells into a novel Jagged-2–dependent regulatory dendritic cell population, Blood, 113: 46-57.

Zhi-Gang Z., Wen X., Li S., Wei-Ming L., Qiu-Bai L., Ping Z., 2012. The characteristics and immunoregulatory functions of regulatory dendritic cells induced by mesenchymal stem cells derived from bone marrow of patient with chronic myeloid leukaemia, European Journal of Cancer Volume 48, Issue 12 , Pages 1884-1895.