

Investigating the Anti-Inflammatory Effects of NestaCell® in an AIRmax Murine Model of Acute Inflammatory Response

Policiquio BO^{1,3,4}; Gonzaga VF^{1,3,4}; Costa, GS³; Ibanez OM²; Ribeiro OG²; Kerkis J¹

1 Genetics Laboratory, Butantan Institute, Sao Paulo, SP, Brazil.

2 Immunogenetics Laboratory, Butantan Institute, Sao Paulo, SP, Brazil.

3 CellAvita Ltd, Sao Paulo, SP, Brazil.

4 Federal University of Sao Paulo, PPGBEF. Sao Paulo, SP, Brazil

Bruna.policiquio@butantan.gov.br

bruna.policiquio@gmail.com

(11)957883965

Introdução: A inflamação é uma resposta biológica a interrupções na homeostase do tecido, frequentemente desencadeada por lesão tecidual de patógenos, agentes físicos ou químicos, reações imunológicas ou necrose. A inflamação aguda é caracterizada por um rápido influxo de leucócitos e proteínas plasmáticas para o local da lesão, acompanhado por aumento da permeabilidade vascular que facilita a migração de células imunes e a liberação de mediadores inflamatórios. As células-tronco mesenquimais (CTM) podem migrar para o local da inflamação por meio de sinais de quimiocina, um processo conhecido como homing. Os fatores parácrinos secretados pelas CTMs criam um microambiente protetor, auxiliando na regeneração do tecido e restaurando a homeostase. Assim, as CTMs não apenas migram para locais inflamatórios, mas também exercem ativamente efeitos anti-inflamatórios, destacando seu potencial como imunomoduladores em aplicações terapêuticas que visam a inflamação. Neste estudo, células-tronco imaturas da polpa dentária humana (CTPDh), um subtipo exclusivo de MSCs derivadas da origem da crista neural, foram utilizadas por suas características distintas e potencial terapêutico.

Objetivos: O objetivo deste estudo é avaliar o potencial imunomodulatório de células-tronco de polpa dentária humana (CTPDh) na quinta passagem, que corresponde ao componente ativo do produto NestaCell® em modelo murino geneticamente selecionado para reatividade inflamatória aguda máxima(AIRmax).

Materiais e métodos: Utilizando um modelo animal para inflamação, modelo murino AIRmax, a inflamação aguda foi induzida usando uma suspensão de 750 µL de Biogel P100. Duas horas após a indução, 100 µL de uma suspensão de células contendo 1×10^6 do produto NestaCell® foram administrados por injeção

no plexo venoso retro-orbital. Após um tratamento de 24 horas com NestaCell®, o exsudato foi coletado e aliquotado para medição subsequente dos níveis de proteína e citocina. (CEUA nº 6597290524)

Resultados e Conclusão: Os resultados indicam que o tratamento com NestaCell® nos camundongos AIRmax pode produzir efeitos anti-inflamatórios significativos. Isso é evidenciado por uma produção reduzida de citocinas no exsudato inflamatório, migração diminuída de leucócitos e produção de espécies reativas de oxigênio, com diferenças estatisticamente significativas entre os grupos de tratamento e controle. Esses resultados destacam o potencial do NestaCell® como um agente terapêutico para modular respostas inflamatórias, necessitando de maior exploração de seus mecanismos de ação e aplicabilidade clínica em condições inflamatórias.

REFERENCES

- [1] DOMINICI, M. et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. **Cytotherapy**, v. 8, n. 4, p. 315–317, 2006.
- [2] RODRÍGUEZ-FUENTES, David E. et al. Mesenchymal stem cells current clinical applications: a systematic review. **Archives of medical research**, v. 52, n. 1, p. 93-101, 2021.
- [3] LIU, Jiaxi et al. Mesenchymal stem cells and their microenvironment. **Stem cell research & therapy**, v. 13, n. 1, p. 429, 2022.
- [4] CUESTA-GOMEZ, N.; GRAHAM, G. J.; CAMPBELL, J. D. M. Chemokines and their receptors: predictors of the therapeutic potential of mesenchymal stromal cells. **Journal of Translational Medicine**, v. 19, n. 1, p. 156, 2021.
- [5] JIANG, Z. et al. Protein profiling identified key chemokines that regulate the maintenance of human pluripotent stem cells. **Scientific Reports**, v. 7, n. 1, p. 14510, 6 dez. 2017.
- [6] HARRELL, C. R.; DJONOV, V.; VOLAREVIC, V. The cross-talk between mesenchymal stem cells and immune cells in tissue repair and regeneration. **International Journal of Molecular Sciences**, v. 22, n. 5, p. 2472, 2021.
- [7] IIDA, Y. et al. Local injection of CCL19-expressing mesenchymal stem cells augments the therapeutic efficacy of anti-PD-L1 antibody by promoting infiltration

of immune cells. **Journal for ImmunoTherapy of Cancer**, v. 8, n. 2, p. e000582, 16 jul. 2020.

[8] HUANG, Yutong; WU, Qiang; TAM, Paul Kwong Hang. Immunomodulatory mechanisms of mesenchymal stem cells and their potential clinical applications. *International Journal of Molecular Sciences*, v. 23, n. 17, p. 10023, 2022.

[9] MÁZLÓ, A. et al. MSC-like cells increase ability of monocyte-derived dendritic cells to polarize IL-17-/IL-10-producing T cells via CTLA-4. **iScience**, v. 24, n. 4, p. 102312, 2021.

[10] GE, Yining et al. Mechanisms of the Immunomodulation Effects of Bone Marrow-Derived Mesenchymal Stem Cells on Facial Nerve Injury in Sprague–Dawley Rats. **Stem Cells and Development**, v. 28, n. 7, p. 489-496, 2019.

[11] HERRERO, C.; PÉREZ-SIMÓN, J. A. Immunomodulatory effect of mesenchymal stem cells. **Brazilian Journal of Medical and Biological Research**, v. 43, p. 425-430, 2010.

[12] MARKOV, Alexander et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. **Stem cell research & therapy**, v. 12, p. 1-30, 2021.

[13] GAO, F. et al. Mesenchymal stem cells and immunomodulation: current status and future prospects. **Cell death & disease**, v. 7, n. 1, p. e2062-e2062, 2016.

[14] SALDAÑA, L. et al. Immunoregulatory potential of mesenchymal stem cells following activation by macrophage-derived soluble factors. **Stem Cell Research and Therapy**, v. 10, n. 1, p. 1–15, 2019.

[15] YAGI, Hiroshi et al. Mesenchymal stem cells: mechanisms of immunomodulation and homing. **Cell transplantation**, v. 19, n. 6-7, p. 667-679, 2010.

[16] BEEKEN, Lydia J.; TING, Darren SJ; SIDNEY, Laura E. Potential of mesenchymal stem cells as topical immunomodulatory cell therapies for ocular surface inflammatory disorders. **Stem Cells Translational Medicine**, v. 10, n. 1, p. 39-49, 2021.

[17] HA, Dae Hyun et al. Mesenchymal stem/stromal cell-derived exosomes for immunomodulatory therapeutics and skin regeneration. **Cells**, v. 9, n. 5, p. 1157, 2020.

- [18] DE WOLF, Charlotte; VAN DE BOVENKAMP, Marja; HOEFNAGEL, Marcel. Regulatory perspective on in vitro potency assays for human mesenchymal stromal cells used in immunotherapy. **Cytotherapy**, v. 19, n. 7, p. 784-797, 2017.
- [19] ANDRUKHOV, Oleh et al. Immunomodulatory properties of dental tissue-derived mesenchymal stem cells: implication in disease and tissue regeneration. **World journal of stem cells**, v. 11, n. 9, p. 604, 2019.
- [20] KERKIS, I. et al. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. **Cells Tissues Organs**, v. 184, n. 3–4, p. 105–116, 2006.
- [21] IBANEZ, Olga M. et al. Genetics of nonspecific immunity: I. Bidirectional selective breeding of lines of mice endowed with maximal or minimal inflammatory responsiveness. **European Journal of Immunology**, v. 22, n. 10, p. 2555-2563, 1992.
- [22] VORRARO, Francisca et al. Genetic control of IL-1 β production and inflammatory response by the mouse Irm1 locus. **The Journal of Immunology**, v. 185, n. 3, p. 1616-1621, 2010.
- [23] STIFFEL, C. et al. Genetics of acute inflammation: inflammatory reactions in inbred lines of mice and in their interline crosses. **Experimental and clinical immunogenetics**, v. 7, n. 4, p. 221-233, 1990.
- [24] SONG, Na; SCHOLTEMEIJER, Martijn; SHAH, Khalid. Mesenchymal stem cell immunomodulation: mechanisms and therapeutic potential. **Trends in pharmacological sciences**, v. 41, n. 9, p. 653-664, 2020.
- [25] WEISS, Andreas Robert Rudolf; DAHLKE, Marc Hendrik. Immunomodulation by mesenchymal stem cells (MSCs): mechanisms of action of living, apoptotic, and dead MSCs. **Frontiers in immunology**, v. 10, p. 1191, 2019.
- [26] MARGRAF, Andreas; LOWELL, Clifford A.; ZARBOCK, Alexander. Neutrophils in acute inflammation: current concepts and translational implications. **Blood, The Journal of the American Society of Hematology**, v. 139, n. 14, p. 2130-2144, 2022.
- [27] THEOFILIS, Panagiotis et al. Inflammatory mechanisms contributing to endothelial dysfunction. **Biomedicines**, v. 9, n. 7, p. 781, 2021.

- [28] SINGH, Varinder et al. ICAM-1 and VCAM-1: Gatekeepers in various inflammatory and cardiovascular disorders. **Clinica Chimica Acta**, p. 117487, 2023.
- [29] MARCUZZI, Annalisa et al. Autoinflammatory diseases and cytokine storms—imbalances of innate and adaptative immunity. **International Journal of Molecular Sciences**, v. 22, n. 20, p. 11241, 2021.
- [30] JANG, Dan-in et al. The role of tumor necrosis factor alpha (TNF- α) in autoimmune disease and current TNF- α inhibitors in therapeutics. **International journal of molecular sciences**, v. 22, n. 5, p. 2719, 2021.
- [31] ZELOVÁ, Hana; HOŠEK, Jan. TNF- α signalling and inflammation: interactions between old acquaintances. **Inflammation research**, v. 62, p. 641-651, 2013.
- [32] WANG, Jinglin et al. Interleukin-10 secreted by mesenchymal stem cells attenuates acute liver failure through inhibiting pyroptosis. **Hepatology Research**, v. 48, n. 3, p. E194-E202, 2018
- [33] SHEPHARD, Matthew T.; MERKHAN, Marwan M.; FORSYTH, Nicholas R. Human mesenchymal stem cell secretome driven T cell immunomodulation is IL-10 dependent. **International Journal of Molecular Sciences**, v. 23, n. 21, p. 13596, 2022..
- [34] IYER, Smita S.; ROJAS, Mauricio. Anti-inflammatory effects of mesenchymal stem cells: novel concept for future therapies. **Expert opinion on biological therapy**, v. 8, n. 5, p. 569-581, 2008
- [35] KHUBUTIYA, Mogeli S. et al. Paracrine mechanisms of proliferative, anti-apoptotic and anti-inflammatory effects of mesenchymal stromal cells in models of acute organ injury. **Cytotherapy**, v. 16, n. 5, p. 579-585, 2014.
- [36] WANG, Lu et al. Regulation of inflammatory cytokine storms by mesenchymal stem cells. **Frontiers in immunology**, v. 12, p. 726909, 2021.
- [37] JUNG, Kyong Jin et al. Mesenchymal stem cells decrease oxidative stress in the bowels of interleukin-10 knockout mice. **Gut and Liver**, v. 14, n. 1, p. 100, 2019.
- [38] BLASER, Heiko et al. TNF and ROS crosstalk in inflammation. **Trends in cell biology**, v. 26, n. 4, p. 249-261, 2016.

[39] KIM, Yongsun et al. Antioxidant and anti-inflammatory effects of intravenously injected adipose derived mesenchymal stem cells in dogs with acute spinal cord injury. **Stem cell research & therapy**, v. 6, p. 1-10, 2015.