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UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”

FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

Priming com INF-ϒ e TNF-α aumenta potencial imunomodulador de células tronco mesenquimais caninas *in vitro*

NATIELLY DIAS CHIMENES

BOTUCATU – SÃO PAULO

MARÇO – 2023

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Dissertação apresentada ao curso de Pós-graduação em Medicina Veterinária, como requisito para elaboração da dissertação para obtenção do título de Mestre.

Orientador: Prof. Associado Rogério Martins Amorim

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TÍTULO: *Priming* com INF-Ɣ e TNF-α aumenta potencial imunomodulador de células tronco mesenquimais caninas *in vitro*

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LISTA DE ABREVIAÇÕES

Ad-MSCs- Células Tronco Mesenquimais derivadas do tecido adiposo

BDNF- fator neurotrófico derivado do cérebro

Breg- célula B reguladora

cAMP- AMP cíclico

C- grupo experimental controle

cAd-MSCs- Células Tronco Mesenquimais derivadas do tecido adiposo canina

CCL2- ligante de quimiocina 2

CD- células dendríticas

C-LCR- grupo experimental com líquido cefalorraquidiano de cão crontrole

COX 1- ciclo-oxigenase 1

COX 2- ciclo-oxigenase 2

C-S- grupo experimental com soro de cão controle

CXCL8- interleucina 8

cDNA- ácido desoxirribonucleico complementar

DLA classe II- dog leucocyte antingen classe II

DMEM- Dulbecco's Modified Eagle's Medium

DMSO- dimetilsufóxido

DPBS- Dubecco’s Phosphate Buffered Saline

EAE- encefalite experimental autoimune

EM- esclerose múltipla

GCN2- controle geral não depressível 2

GDNF-fator neurotrófico derivado das células da glia

GM-CSF- fator estimulador de colônias de granulócitos e macrófagos

GVHD- doença do enxerto contra o hospedeiro

HGF- fator de crescimento de hepatócitos

HLA-DR- molécula do complexo de histocompatibilidade de classe II

HLA-G- antígeno leucocitário humano G

HO-1- heme oxigenase 1

HPRT- hipoxantina-guanina fosforribosiltransferase

IDO- indoleamina -2,3- dioxigenase

IF2- fator de iniciação da tradução eucariótica

IFN-Ɣ- interferon gama

IL-1- interleucina 1

IL-1α- interleucina 1 alfa

IL-1ß- interleucina 1 beta

IL1RA- antagonista de receptor de interleucina 1

IL-4- interleucina 4

IL-5- interleucina 5

IL-6- interleucina 6

IL-8- interleucina 8

IL-10- interleucina 10

IL-12- interleucina 12

IL-13- interleucina 13

IL-17- interleucina 17

IL-23- interleucina 23

IL-35- interleucina 35

iNOS- óxido nítrico sintase induzida

LCR-líquido cefalorraquidiano

LEN- leucoencefalite necrosante

LPS- lipopolissacarídeo

M1- fenótipo da micróglia pró-inflamatório

M2- fenótipo da micróglia anti-inflamatório

MCP-1- proteína quimioatraente de monócitos 1

MEG- meningoencefalomielite granulomatosa

MEN- meningoencefalite necrosante

MHC II- molécula do complexo de histocompatibilidade de classe II

mRNA- ácido ribonucleico mensageiro

MSCs- células tronco mesenquimais

MUO- meningoencefalite de origem desconhecida

MUO-LCR- grupo experimental com liquido cefalorraquidiano de cão com MUO

MUO-S- grupo experimental com soro de cão com MUO

MTT- (3-(4,5-dimetiltiazol-2yl)-2,5-difenil brometo de tetrazolina)

NF-kβ- fator nuclear kappa beta

NGF- fator de crescimento neural

NK- natural killer

NO- óxido nítrico

NOS- óxido nítrico sintase

P0- passagem celular zero

P1- passagem celular 1

P2- passagem celular 2

P3- passagem celular 3

P4- passagem celular 4

PD-L1- ligante de morte 1 programado

PD-L2- ligante de morte 2 programado

PGE2- prostaglandina E 2

PTGES2- prostaglandina E sintetase 2

qPCR- reação em cadeia de polimerase quantitativa em tempo real

RNA- ácido ribonucleico

RM- ressonância magnética

ROS- espécies reativas de oxigênio

RPS5- proteína ribossômica S5

RPS19- proteína ribossômica S19

SFB- soro fetal bovino

SNC- sistema nervoso central

TGF-ß- fator de crescimento transformador beta

Th- linfócito T helper

TNF-α- fator de necrose tumoral beta

Treg- linfócitos T reguladores

TRLs- receptor tipo toll like

TSG-6- proteína do gene 6 induzida

SUMÁRIO

Página

CHIMENES, N.C. ***Priming* com INF-Ɣ e TNF-α aumenta potencial imunomodulador de células tronco mesenquimais caninas *in vitro.*** Botucatu, 2023. 103 p. Defesa (Mestrado) – Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, Universidade Estadual Paulista.

RESUMO

A meningoencefalite de origem desconhecida (MUO) é uma enfermidade neuroimune em cães, com grande potencial de se tornar um modelo animal natural para a esclerose múltipla. O *priming in vitro* das células tronco mesenquimais derivadas de tecido adiposo canino (cAd-MSCs) é uma estratégia para potencializar a ação imunomodulatória e imunossupressora das cAd-MSCs. O objetivo deste trabalho foi avaliar o *priming* com IFN-Ɣ e TNF-α, LCR e soro de cães com MUO nas cAd-MSCs. Para tanto, foi realizado o *priming in vitro* por 72 horas das cAd-MSCs (P3-P4) em seis grupos: controle (C), com IFN-Ɣ em associação com TNF-α (IFN-Ɣ+ TNF-α), soro controle (C-S), soro de cães com MUO (MUO-S), LCR controle (C-LCR) e LCR de cães com MUO (MUO-LCR) e avaliada a expressão gênica para BDNF, GNDF, HGF, IDO, PTGE2, IL-10, IFN-Ɣ e TNF-α por qPCR e quantificação proteica de GM-CSF, IL-10, IL-2, IL-8 e MCP-1 por ensaio multiplexado. Comparando todos os grupos com o grupo C, o IFN-Ɣ+ TNF-α apresentou aumento de HGF, IDO e TNF-α e IL-8 (p= <0,001), nos grupos C-LCR e MUO-LCR houve aumento de IFN-Ɣ e TNF-α e redução de BDNF e GNDF (p= <0,001), os grupos C-S e MUO-S apresentaram redução na quantificação proteica de IL-10 (p= <0,001), e o grupo C-S teve redução de BDNF e GNDF (p= <0,001) e o grupo MUO-S teve redução de HGF (p= <0,001). Diante dos resultados, a técnica priming IFN-Ɣ + TNF-α demonstrou maior potencial de perfil imunomodulatório com tendência anti-inflamatória em comparação com os demais grupos.

Palavras-chaves: neuroimunes, citocinas, meningoencefalite, esclerose múltipla.

CHIMENES, N.C. **Priming INF-α and TNF-α enhances the immunomodulatory potential of canine mesenchymal stem cells in vitro.** Botucatu, 2023. 103 p. Defesa (Mestrado) – Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, Universidade Estadual Paulista.

Abstract

Meningoencephalitis of unknown origin (MUO) is a neuroimmune disease affects dogs, with great potential to become a natural experimental model for multiple sclerosis. In vitro priming of mesenchymal stem cells derived from canine adipose tissue (cAd-MSCs) is a strategy to enhances the immunomodulatory and immunosuppressive action of cAd-MSCs. The aim of this work is to evaluate the priming with IFN-γ and TNF-α, CSF and serum from dogs with MUO on cAd-MSCs. Therefore, in vitro priming of cAd-MSCs was performed in six groups: control (C), with IFN-α in association with TNF-α (IFN-α + TNF-α), control serum (C-P), serum from dogs with MUO (MUO-P), CSF control (C-CSF) and CSF from dogs with MUO (MUO-CSF) and evaluated gene expression for BDNF, GNDF, HGF, IDO, PTGE2, IL-10, IFN- Ɣ and TNF-α by qPCR and protein expression of GM-CSF, IL-10, IL-2, IL-8 and MCP-1 by multiplex assay. In comparison with group C, the group with IFN-γ+ TNF-α showed an increase in HGF, IDO and TNF-α and IL-8 (p= <0.001), in the C-CSF and MUO-CSF groups there was an increase in IFN -Ɣ and TNF-α and a reduction in BDNF and GNDF (p= <0.001), the C-S and MUO-S groups showed a reduction in IL-10 (p= <0.001), and the C-S group had a reduction in BDNF and GNDF (p= <0.001) and the MUO-S group had reduced HGF (p= <0.001). In view of the results, the IFN-γ + TNF-α priming technique demonstrated a greater potencial for immunomodulatory profile with an anti-inflmmatory tendency compared to the other groups.

Keywords: neuroimmune, cytokines, meningoencephalitis, multiple sclerosis.

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Declarations

Ethical approval and consent to participate

All experimental procedures involving animals were approved by the Ethics Committee of the Faculty of Veterinary Medicine and Zootechnics, UNESP-Botucatu (protocol No. 0171/2021). Blood collection and CSF of dogs naturally affected by MUO treated at Veterinary Neurology and collection of canine adipose tissue were performed according to the free and informed consent of the guardians of the animals.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its information files complementary.

Competitive interests

"The authors declare that they have no conflicting interests" in this section.

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3 CONCLUSÃO GERAL

O priming celular é uma técnica nova que tem por objetivo melhorar a capacidade imunomoduladora das MSCs, e o uso de fluidos biológicos é algo inédito demonstrando a importância deste estudo ser o primeiro estudo com uso de LCR e soro sanguíneo em Ad-MSCs na espécie canina. Embora o LCR e o soro de cães com MUO não tenham produzido o efeito imunomodulador significativo nas Ad-MSCs novos estudos com concentrações e tempo de estimulação diferentes são fundamentais para melhor conhecimento do perfil dessas células submetidas a um ambiente com LCR e soro de cães com MUO.

A técnica *priming* das cAd-MSCs com o uso de INF-Ɣ+TNF-α se mostrou com um perfil com maior endência imunomodulatória e imunossupressora em comparação com o grupo controle e entre os grupos. A viabilidade das cAd-MSCs não sofreu alteração nos grupos avaliados após o *priming* e sua taxa metabólica não foi prejudicada quando submetidas a diferentes concentrações de LCR e Soro.

O modelo experimental proposto é de suma importância para o estudo *in vitro* do comportamento das cAd-MSCs em ambientes que mimetizam as doenças neuroimunes como a esclerose múltipla, contudo apresentando limitações como a difuculdade de padronização do LCR e soro inflamatório. Como perspectivas futuras novos estudos com deferentes concentrações de LCR e soro podem se demonstrar resultados mais favoráveis a essas técnicas *priming*.