

Article - Human & Animal Health

# Mesenchymal Stromal Cells Modulate PAF-stimulated Equine Alveolar Macrophages

**Pedro Vicente Michelotto**<sup>1\*</sup>

<https://orcid.org/0000-0003-0893-1654>

**Fernanda Zettel Bastos**<sup>1</sup>

<https://orcid.org/0000-0003-1238-4104>

**Fernanda Cristina Mendes Barussi**<sup>1</sup>

<https://orcid.org/0000-0003-3134-8096>

**Alexandra Cristina Senegaglia**<sup>2</sup>

<https://orcid.org/0000-0002-8052-6357>

**Anita Nishiyama**<sup>3</sup>

<https://orcid.org/0000-0001-6902-0948>

<sup>1</sup> Pontifical Catholic University, School of Life Sciences, Department of Animal Science, Curitiba, Paraná, Brazil. <sup>2</sup> Pontifical Catholic University, School of Medicine, Laboratory for Experimental Cell Culture, Curitiba, Paraná, Brazil. <sup>3</sup> Federal University of Paraná, Department of Physiology, Curitiba, Paraná, Brazil.

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\*Correspondence: p.michelotto@pucpr.br; Tel.: +55-41992349218 (P.V.M.)

## HIGHLIGHTS

- BMMNCs used in this study were viable after cryopreserved for 45 months.
- PAF stimulated alveolar macrophages' actions.
- controlled by BMMNCs and BMMSCs.
- BMMSCs effects were significantly better than BMMNCs.

**Abstract:** Platelet-activating factor (PAF) is a potent proinflammatory mediator that is produced in increased amounts in the lungs of asthmatic humans and horses. The present pilot study, shows that mesenchymal stromal cells can modulate alveolar macrophage function in asthma, interfering in the activity of PAF, being another potential pathway for mesenchymal stromal cells benefits in asthma.

**Keywords:** Asthma; Horses; Lung; Platelet-activating factor; Pulmonary inflammation.

## INTRODUCTION

Platelet-activating factor (PAF) is a potent proinflammatory mediator that is produced in increased amounts in the lungs of asthmatic humans [1,2], horses [3,4], and laboratory animals [5]. During pulmonary inflammation, PAF can be responsible for microvascular leakage [6], bronchoconstriction, increased airway responsiveness and eosinophilic chemotaxis [7], and increased mucus secretion [5]. Moreover, PAF can provoke a pulmonary influx of neutrophils mediated by leukotriene B<sub>4</sub> [1], and increased phagocytic activity of neutrophils, which result in interleukin (IL)-8 secretion, leading to exacerbated neutrophil chemotaxis to the lungs [8].

PAF has also been shown to be involved in the pathophysiology of equine asthma, causing pulmonary influx of eosinophils and neutrophils, and increased adhesion and activation of neutrophils, thus impairing pulmonary function [3,9]. Alveolar macrophages (AM) are one of the sources of PAF. Meanwhile, PAF can interfere with AM functions; it augments AM adhesion, phagocytosis, and oxidant production *in vitro* [10]. Our group has previously demonstrated the presence of PAF in the bronchoalveolar lavage fluid (BALF) of young Thoroughbred (TB) racehorses in advanced race training, which was associated with evidence of oxidative stress and interfered with AM phagocytosis and oxidant production [4].

Our research group focuses on establishing cell therapies for asthmatic humans and horses, as many individuals do not respond to current treatments using corticosteroids. We have recently demonstrated the usability of autologous bone marrow-derived mononuclear cells (BMMNCs) in horses with severe asthma [11], a model used to investigate severe neutrophilic asthma [12]. Intratracheal instillation of BMMNCs resulted in decreased respiratory effort and neutrophils in the BALF, increased expression of the anti-inflammatory IL-10, and maintenance of AM function [11]. However, for clinical perspectives of allogeneic and ready-to-use treatments in humans and horses, it is important to understand the effects of mesenchymal stromal cells (MSCs) on the inflammatory process and the immune response in asthma.

Therefore, considering that AM orchestrate the pulmonary immune response, we designed a preliminary study to comparatively investigate the effects of BMMNCs and bone marrow derived MSCs (BMMSCs) on AM function. In addition, we aimed to investigate whether their mechanism(s) of immunomodulation would involve PAF inhibition.

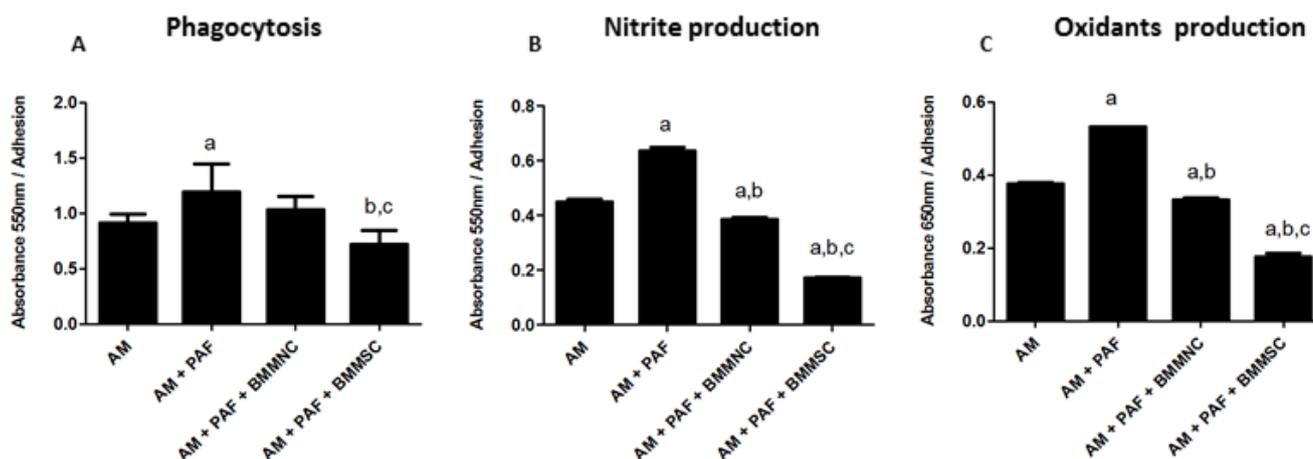
## MATERIAL AND METHODS

The BMMNCs used in the present study had been previously obtained [11] and were maintained in liquid nitrogen for approximately 45 months. The cells had a viability of 74% as indicated by trypan blue staining. This finding substantially exceeds our previously reported observation that BMMNCs maintain acceptable viability after five months of frozen storage [13]. Moreover, the BMMSCs used in the present study were successfully cultured from these BMMNCs, using an established protocol [14]. The study was approved by the Ethical Committee on the Animal Use of the Pontifical Catholic University of Paraná (PUCPR; approval number 01217).

Briefly, BALF of two clinically healthy TB horses was aseptically collected and processed as previously described [4] to obtain AM, maintained in phosphate-buffered saline (PBS). BMMNCs and BMMSCs were in Iscove's Modified Dulbecco's Medium (IMDM, Sigma). Cells were plated in quadruplicate in 96-well plates ( $1 \times 10^5$  cells/well) and incubated at 37°C for 1 h. Then, the cells were washed and divided into four treatment groups: non-treated AM, AM+PAF (100 nM/well), AM+PAF+ $1 \times 10^5$  BMMNCs/well, and AM+PAF+ $1 \times 10^5$  BMMSCs/well. The cells were subjected to assays for cell adhesion, phagocytosis [4,5,10], nitrite (Griess Reagent; ThermoFisher Scientific, Waltham, MA, USA), and oxidant production (CellROX<sup>®</sup> Deep Red; ThermoFisher Scientific) using an ELISA plate reader (VersaMax<sup>®</sup>; Molecular Devices, Sunnyvale, CA, USA) at the relevant wavelengths. Cell adhesion was determined to normalize the other AM functions assayed in each group [10]. The data were analyzed using one-way ANOVA followed by the Tukey *post-hoc* test, with  $p < 0.05$  considered significant.

## RESULTS AND DISCUSSION

The results are shown in Figure 1. Consistently, PAF significantly increased phagocytosis as well as nitrite and oxidant production in the AM ( $p < 0.01$ ). In the presence of BMMNCs, nitrite and oxidant production were significantly reduced to levels even lower than those in the AM group ( $p < 0.01$ ); however, there was no difference in phagocytosis. Finally, in the presence of BMMSCs, all AM functions were significantly reduced, and the effects were significantly greater than those of BMMNCs ( $p < 0.0001$ ).



**Figure 1.** Modulatory effect of BMMSCs on PAF-stimulated equine alveolar macrophages (AM). Non-treated AM ( $1 \times 10^5$  cells/well) were used as a control, PAF was used at 100 nM/well, BMMNCs or BMMSCs were additionally added at  $1 \times 10^5$  cells/well. Prior to the treatments, AM were incubated with PAF at 37°C for 1 h. A. Phagocytosis of AM. <sup>a</sup> $P = 0.002$  vs. AM; <sup>b</sup> $P = 0.002$  vs. AM+PAF; <sup>c</sup> $P = 0.001$  vs. AM+PAF+BMMNCs. B. Nitrite production by AM. <sup>a</sup> $P = 0.030$  vs. AM; <sup>b</sup> $P = 0.028$  vs. AM+PAF; <sup>c</sup> $P = 0.030$  vs. AM+PAF+BMMNCs. C. Oxidant production by AM. <sup>a</sup> $P < 0.0001$  vs. AM; <sup>b</sup> $P < 0.0001$  vs. AM+PAF; <sup>c</sup> $P < 0.0001$  vs. AM+PAF+BMMNCs.

PAF can stimulate AM to eliminate inhaled particles from the airways [10]. However, in the case of pulmonary inflammation, PAF is produced uncontrolledly and in excess by the inflammatory cells because of tissue phospholipid oxidation due to oxidative stress [5]. The investigated TB horses, although clinically healthy, were in active race training and probably, the AM were in an active state, as previously demonstrated in a similar population [4]. The BMMNCs modulated the airway immune response, reducing the production of oxygen and nitrogen reactive species by AM and maintaining AM phagocytosis in a non-activated status, in line with our previous observations, where AM phagocytosis decreased in asthmatic horses treated with BMMNCs [11]. The modulatory effects of BMMSCs on the Th1/Th2 airway immune response have been previously demonstrated in humans and involve positive interference with oxidative stress [15].

## CONCLUSION

In conclusion, cell therapy immunomodulates equine AM activity, with BMMSCs showing better results than BMMNCs, and thus, has therapeutic potential for equine asthma.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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