Standardization of the use of mononuclear cells in association with alveolar macrophages for in vitro study

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Abstract

The alveolar machophage is the resident cell lineage in lungs and it is responsible for phagocytosis in respiratory diseases, such as asthma and a similar disease in horses, the Recurrent Airway Obstruction (RAO). Since the treatment with corticosteroids presents various side effects, the cell therapy is an option, but there are no studies in horses with RAO. Therefore, this study aimed to standardize the optimal concentration and the moment of addition of bone marrow-derived mononuclear cells (BMMCs) of horses for the in vitro study of alveolar macrophage phagocytosis. In order to obtain the alveolar macrophages, three samples of bronchoalveolar lavage fluid of horses were collected, centrifuged at 340 g for 10 minutes at 4 °C and the cell concentration was adjusted to 5x106 cells/mL. The BMMCs were obtained from the fifth sternebra in previous collections and adjusted to concentrations of $1x10^3$, $1x10^4$, $1x10^5$ and $1x10^6$ cells/mL. The association of alveolar macrophages with BMMCs was analyzed by phagocytosis assay in a 96-well plate, stimulated by zymosan at the four concentrations of BMMCs and in two different moments, each sample in triplicate. On moment 1 (M1), 100uL of BMMCs were allowed to adhere for one hour along with 100uL of alveolar macrophages and on moment 2 (M2) the BMMCs were added after stimulation of macrophages by zymosan. A triplicate with only alveolar macrophages and a triplicate for each BMMCS concentration, with no association with macrophages, were used as the control group. The result was obtained by absorbance at 550nm. Statistical analysis was performed by Kruskal-Wallis test followed by Dunn's test. The results for M1 and M2 were not statistically significant (P>0,05), probably due to the small number of samples, but it was possible to observe a variation between the moments. M1 caused an increase in phagocytic activity and M2 caused its decline, which shows promise for a reduction in the inflammatory response. It was observed that the absorbance in controls for only alveolar macrophages and only BMMCs was similar, being the result of M1 the sum of alveolar macrophages and BMMCs in controls. For M2 the absorbance values decreased whereas the BMMCs alone already generated response. Also related to the M2, two concentrations of BMMCs are more efficient; they are $1x10^3$, quoted in the literature for similar studies in human asthmatics and $1x10^6$, which was the

most significant outcome for this study. Promising results for cell therapy in respiratory diseases by its influence on the activity of alveolar macrophages after stimulation can be observed.

Ethics Committee: CEUA-PUCPR n° 788.