Immunophenotypic profile and viability of bone marrow mononuclear fraction of horses

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Abstract

Horses are commonly affected by musculoskeletal, neurological and reproductive injuries. In order to treat these injuries, studies involving therapy with mesenchymal stem cells (MSCs) from bone marrow (BM) as well as the derived mononuclear fraction (MF) are increasing. The use of this uncultivated fraction from BM for therapeutic use has resulted in satisfactory improves, similar to the ones observed in cultured cells. The advantages of using the MF include practicality, low cost and no need for cultivation. The aim of this study was to characterize immunophenotypically bone marrow MF of horses by flow cytometry using a panel of clusters of differentiations (CD44, CD90, CD105, and MHC-II CD18/CD11a) containing markers commonly used to characterize cultured equine MSCs. Furthermore, we also aimed to determine the viability of fresh equine MF for therapeutic use. Bone marrow samples from 4 horses were collected by aspiration of the fifth sternebra after sedation and local anesthesia. After collection, all samples were filtered and centrifuged at 250g. To the remaining material was added low glucose DMEN in ratio 1:1, and subsequently it was slowly added the Histopaque 1077 (Sigma® USA) the mixture. After centrifugation at 350g for 30 minutes, the MF was aspirated and washed twice with low glucose DMEN. After the last wash, the pellet of mononuclear cells was reserved to perform the viability analysis (n = 4) and immunophenotypic characterization (n = 3). For immunophenotypic characterization of MF were used monoclonal antibodies mouse anti-rat CD90 with FITC, mouse anti-horse CD44 with FITC, mouse anti-human CD105 with FITC, mouse anti-dog CD18 with alexa fluor 647 and mouse anti-horse MHC classe II (abD serotec®, UK). The analysis was performed by flow cytometer LSR FORTESSA (Becton Dickinson® and Company, USA), being counted 10.000 events and control of autofluorescence. It was adopted as positive markers expression above 2%. The viability test was performed by staining with 0.4% trypan blue to estimate the integrity of the cell membrane by the ratio between the number of unstained cells to the total cell number. Data regarding immunophenotypic analysis and viability are expressed as mean and SEM. Immunophenotypic analysis revealed positive expression of markers CD18 (67 ±12,3%), CD44 (61,6 ±8,1%), CD90 (29,3 ±8,4%), MHC-II (22,5 ±7,2%) and negative for CD105 (1,7 ±0.4%). The analysis of cell viability showed excellent outcome for fresh MF (97,0±2,1%). In experimental conditions, the cells of BM mononuclear fractions have showed excellent viability and this is an important feature for cryopreservation and therapeutic use. It is worth mentioning that it has not been possible to predict the proportion of progenitor

cells in the MF, since many of these CD are also expressed in leukocytes. Thus, it is important to identify markers that are expressed exclusively on stromal progenitor cells.

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