EXPANSION AND PURIFICATION OF PERIPHERAL BLOOD STEM CELLS: PHENOTYPIC CHARACTERIZATION

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Introduction

Stem cells are widely used in the clinical veterinary practice to treat tendonitis and orthopaedic injuries. In the majority of the cases, autologous stem cells are derived from bone marrow or even from adipose tissue of animals, following expensive and time consuming protocols. These require in vitro propagation prior to transplantation, and are affected by stem cells yield decreases with animal ageing. Up to now, there are no reports of equine/dog adipose tissue derived stem cells phenotype, although commercial availability of so-called adipose tissue derived stem cell therapy is rapidly taking place.

In this paper we present a new method to obtain stem cells directly from mammal´s peripheral blood cells (Patent N. IT RM 2006A000498).

Our protocol is highly innovative as it allows to generate autologous stem cells in a quick and easy way. In our hands cells undergo few or not replication cycles, thus limiting all risks related to in vitro propagation.

Matherial and method

Sampling

Sixty animals between horses and dogs, males and females of all ages were analyzed and blood samples (5-7 ml) were collected in tubes containing heparin or EDTA as anticoagulant. Under laminar flow blood was diluted in NH4Cl Lysing Buffer and washed with PBS to remove any

red cells contamination. The cells were then incubated for at least 72 hours (37°C) with MCSF and analyzed by flow cytometry to evaluate phenotype and absolute number.

Cytofluorimetric analysis

200 µL of each sample was incubated for 1hr at RT with saturating amounts of antibodies, washed once and analyzed by flow cytometry or sorted by using BDFACSAria II.

Results

We present the phenotypic analyses after in vitro expansion by FACSAriaII cytofluorimetric analysis on sorted stem cells. We identify at least three subpopulations

-A typical hematopoietic stem cell population, positive for CD90, CD117 and CD34

-A "mesenchymal stem cell like" population, showing CD105, CD50 expression

-A "pluripotent-like stem cells" population, expressing the intracellular transcription factors Sox2, Oct3/4 and Nanog.

These factors have been shown to be strongly involved in the maintenance of the undifferentiated state of pluripotent cells.

In addition to the above mentioned markers we also found some positivity for CXCR4, CD29, CD49e and CD49b molecules.

Conclusion

Isolation of pluripotent stem cells from peripheral blood provides a non-invasive source of stem cells with potentially superior cellular characteristic to other stem cells used in veterinary with regard to immune tolerance, proliferative potential and differentiation potency.

The ability to obtain undifferentiated cells in less of 72 hours and the possibility to deliver them both locally and systemically should make them valuable candidates for a lot of therapy protocols.

In vivo these cells display regenerative tissues capability and have the property to resolve some metabolic pathologies. The signals required to re-differentiate these cells into specific cell types are still unclear and under study. Nevertheless, the beneficial use of these stem cells is quite clear. In fact, in vivo they show the ability to regenerate different tissues including tendons, ligaments, bones, cartilages and muscles (skeletal and cardiac). Treatment with our cells leads to improvement or even complete remission of many different pathologies. None of the

animal that we successfully treated in the past five years has shown any side effect such as phenomena of rejection, infection or the onset of teratomas.

Once administered in animals our cells differentiate "in vivo" (and not "in-vitro", as in other methods using growth factors and/or chemical stimuli). M-CSF derived stem cells acquire phenotype and function of macrophages, lymphocytes, epithelial , endothelial, neuronal and hepatocyte cells, according to the needs and pathologies of the treated animals

References

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