ImmunoTools *special* Award 2014

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Is the key to healing inside ourselves?

Abstract

Preliminary studies have shown how BDSCs (Blood Derived Stem Cells), from few milliliters of peripheral blood, currently represent an innovative and promising source of adult stem cells, quickly available and able to differentiate into several cell types since their plasticity. The BDSCs obtained applying a simple, natural and fast deprogramming method (Alaimo et al., 2013; Marfé et al., 2012) do not present problems of immune response or reject after inoculation. The aim of this project is to investigate, whether the lymphocyte fraction, isolated from the whole blood, undergoes to the same deprogramming process that occurred for the whole nucleated component (Marfé et al., 2012). Moreover, I will investigate when and how this happens, and if parameters such as age and sex may influence this process. The results obtained will provide useful information to develop, in a future, more effective and personalized therapies.

Aim

Zhao et al. in 2003 have shown that obtaining pluripotent stem cells from the monocyte fraction is possible. Most recent data (Alaimo et al., 2013; Marfé et al., 2012) have demonstrated how pluripotent adult stems (BDSCs) can also be obtained from the whole nucleated blood component.

The aim of my project will be to verify if the lymphocyte fraction is able to assume stemness features under specific conditions, in the case of success, which classes of lymphocytes are involved in this process.

Depending on the results, we will be able to verify whether the standard deprogramming process of nucleated cells, previously evaluated, is the same for the lymphocyte fraction.

Analysis will be conducted at different time points (0, 24, 48, 72 hours) in order to test if parameters such as sex and age influence, not only the rate of deprogrammation, but also the number of deprogrammed cells during the 72 hours.

Experimental design

Samples will be obtained by taking blood from healthy patients randomly selected in the Italian population. The parameters considered during this study will be sex and age. Each group (male / female) will be composed by 40 individuals including: 10 people from the 0-11 age class, 10 from the 11-21 age class, 10 from the 21-65 age class and 10 over 65.

Initially, Lymphoprep™ will be used both to purify the nucleated cells from the blood and for an initial isolation of the lymphocyte fraction. In order to isolate the lymphocytes with a greater accuracy, the cellular component previously purified will be sorted for specific lymphocyte markers ($CD3^+$, $CD4^+$, $CD8^+$,$CD20^+$,$CD25^+$) and the cells will be counted per mL of blood. The sorted lymphocyte population will be then deprogrammed according to our protocol (Marfé et al., 2012) using rhMCSF and rhLIF. During this process, the cells will be monitored at different time points (0, 24, 48 and 72 hours) using CD3, CD4, CD8, CD20, CD25, CD19, CD34, CD105 to check changes in the number and type of surface markers expressed, and to understand whether the whole lymphocyte fraction undergoes the deprogrammation or if it happens only partially, and how long the process takes to complete.

Finally, in order to confirm the pluripotency of cells obtained after deprogrammation protocol it will be carry out a reprogrammation protocol toward an hepatic lineage using rhHGF, rhBMP-2, rhEGF, rhIGF-I, rhIGF-II since we have already performed it in a recent study (Alaimo et al., 2013). Moreover, hepatic differentiation allows a crosscheck because can be evaluated both by molecular analyses and by functional assays as PAS staining and Urea assay.