ImmunoTools *special* Award 2014

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Immunomodulatory properties of a novel MSCs population obtained from human umbilical cord UC (UCMSCs)

In the last decade, the use of stem cells for therapy in clinics defined an important tool to treat functional and structural defects of organs and tissues. In particular, the design and the obtaining of tissue substitutes engineered (ETS) with stem cells (SCs) represents an innovative therapeutical strategy that Tissue Engineering proposes as alternative to traditional synthetic prosthesises. ETS are designed specifically for each patient; they are formed by an inert scaffold and autologue stem cells supporting their integration in recipients. Besides bone marrow that is at this moment the main source of stem cells for therapy, various niches of stem cells have been identified in humans. Moreover, at different phases of human development, SCs resulted of different typologies both as distribution as potentials. For the possible immediate clinical use, recently a big interest is focusing on adult stem cells (ASCs), that are multipotent cells identified in skin, umbilical cord and adipose tissue. In contrast to totipotent stem cells which isolation involves the embryo's distruction, ASCs are obtained from adult tissues by medical procedures as biopsy, bone marrow aspiration and liposuction. Recently, MSCs have been shown to possess immunomodulatory properties. in vitro studies have demonstrated that MSCs inhibit the production of interferon (IFN)-γ and tumor necrosis factor (TNF)-α,increase the level of IL-10 limiting T cell expansion, inhibit natural killer cells monocytes and mature dendritic cells.

As reported in Burra et al (2012) our group isolated and characterized a novel MSCs population obtained from human umbilical cord UC (UCMSCs). In order to better define the usage of UCMSCs in human, we purpose to characterize the immunoreactivity of this UC population using a multidimensional methodological approach aimed to morphological, immunophenotipycal and molecular analysis by cytometry of co-cultures prepared with UCMSCs and human lymphocytes.

In details, the project will be developed as following described:

- Peripheral blood samples will be obtained from healthy donors (Padova University Hospital) while umbilical cords will be donated from Cittadella Hospital (Padova);
- Transport and delivery of samples to Cell Biology Research Unit-Dept of Pharmaceutical and Pharmacological sciences, Padua University

- Cell extraction. Lymphocytes will be isolated by Ficoll gradient separation with standard procedures. UCMSCs will be obtained with explant method. UCMSCs will be analyze by flow cytometry (FCM) for the expression of CD14, CD29, CD31, CD34, CD40, CD45, CD54, CD73, CD90, CD105, CD117, CD133, CD166 and HLA class I and II. As negative controls, corresponding isotype control antibodies will be used.
- Co-cultures. Before preparing co-coltures lymhocytes will be characterized morphologically and immunophenotypically for the expression of CD3, CD4, CD8, CD25, FoxP3. The interaction among UCMSCs and lymphocytes will be explored using two different methods: cell-cell interaction by seeding in the same plate both types of cells and indirect interaction by secreted factors culturing cells in insert system.
FCM will be performed to characterize lymphocytes and UCMSCs for the expression of antigen above reported. The percentage variations within the subsets of immune cells co-cultured with UCMSCs will be measured at different time points (3-5-7 days) by FCM and compared with lymphocytes cultured alone.
- Cell immunomodulatory activity under inflammation. As previously reported (Krampera et al. 2006), co-coltures will be treated with INFγ using low dose (25ng/ml) and high dose (500ng/ml) for 48 hours. Negative controls will be prepared using INFγ-untreated cells. IDO is one of the molecular switches controlling the balance between regulatory T (T_{reg}) and effector T cell responses and COX2 potentiates the differentiation of T_{reg} cells. Based on these evidences, INFγ-treated and -untreated samples will be analysed by qPCR for the expression of CXCL9, COX2 and IDO.
- Analysis of expressed cytokines. In order to evaluate the effect of UCMSCs on lymphocytes, the expression of specific cytokines will be evaluated by FCM. In particular, after blocking of cells with Golgi Plug, the intracellular accumulation of presence assay for the following cytokines: IFNγ, IFNα, IL-1a, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-18. In parallel, lymphocytes and UCMSCs not cocultured will be used as negative controls. ImmunoTools Award would be an important contribution to realize this project.

ImmunoTools *special* AWARD for **Debora Bizzaro** includes 24 reagents

FITC - conjugated anti-human CD13, CD14, CD16, CD29, CD31, CD40, CD45, CD71, HLA-DR, HLA DP, Control-IgG1,

PE - conjugated anti-human CD105, HLA-ABC, Control-IgG2a,

APC - conjugated anti-human CD34, CD54, IL6, Control-IgG1, Control-IgG2b,

Anti-human 3 colour reagent: CD3/CD4/CD45, CD3/CD8/CD45,

recombinant human cytokines: rh IFNgamma, rh GM-CSF, rh G-CSF

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