ImmunoTools *special* Award 2019

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Role of EphB2 and EphB3 on the Immunomodulatory Capacity of Human Mesenchymal Stem Cells.

Background

Mesenchymal Stem Cells (MSCs) are a heterogenous cell population described in culture by their adhesion to plastic surfaces, differentiation in mesodermal cell linages (mainly chondrogenic, adipogenic and osteogenic), and the expression or absence of specific cell surface markers (CD44, CD45, CD105, CD73, CD29, CD90, to name a few)[1]. In recent years, MSCs are being used as therapeutic agents taking advantage of their ability to differentiate and their immunomodulatory properties[2].

It has been determined that MSCs can intervene in tissue regeneration in two ways: directly, differentiating the necessary mesodermal cells, or taking advantage of their immunomodulatory properties[3]. However, we do not understand the mechanisms that regulate these properties, although it points to the importance of cell-cell interactions[4] and the secretion of various inhibitors of immune activity such as transforming growth factor beta 1 (TFG- β1), prostaglandin E2 (PGE2), indolamine 2,3-dioxygenase (IDO), nitric oxide (NO) and interleukin 10 (IL-10), among others[5]. Some studies propose that these factors that produce MSCs are induced *in vitro* by an increase in the concentration of IFN-γ produced by activated lymphocytes[4]. In this way, they regulate, among other effects, the blockage of the proliferation and activation of T and B lymphocytes, as well as NK cells, dendritic cells and macrophages[6].

Then, considering that Eph receptors and their ligands has been demonstrated to intervene on cell-cell contact, the immunomodulatory capacity of MSCs could be affected directly or indirectly by these molecules. On this regard, some preliminary results in our laboratory indicate that murine MScs, lacking EphB2 or EphB3 receptors, have a reduced immunomodulatory capacity and they modify the expression level of CD19 cell marker in splenocytes, when compared with WT MSCs.

Objectives

On this new project we want to confirm these observations on human MSCs derived from bone marrow using blockage of EphB2 and EphB3 receptors to simulate the KO condition of murine MSCs. Additionally, we want to characterize the B cells population to determine if the interaction of peripheric lymphocytes with either $EphB2^{-/-}$ and $EphB3^{-/-}$ MSCs alters the maturation of these lymphocytes.

Methods

The first step in this project is to determine the phenotype of MSCs from human donors by flow cytometry using two panels as follow:

1°: CD-44-FITC, CD45-PerCP, CD29-PE and CD105-APC (**ImmunoTools**).

2°: CD34-FITC, CD19-APC, and CD73-PE (**ImmunoTools**).

Thus, we will assure that the cell populations are positive for CD44, CD29, CD105 and CD73, and negative for CD45, CD34 and CD19.

We also need to assess the viability of the cultures with Annexin V-APC (**ImmunoTools**), to ensure the highest viability before starting the blockage and co-culture.

Once we confirmed the phenotype and viability of the MSCs, we will then isolate lymphocytes from human blood and culture them with Phytohemagglutinin (PHA) to activate them, then we will co culture this cells with MSCs treated with EphB2 and EphB3 specific blockage for 24, 48 and 72 hours. Then we will remove the lymphocytes from each well washing with culture medium and transferring to a FACS tube for the analysis using flow cytometry.

We will analyze then T and B cells, determining the percentage of $CD4^+$ and $CD8^+$ T cell subpopulations, together with mature, immature and memory B cells. Thus, we will need to do two different labeling panels more:

1°: CD3-PerCP, CD4-FITC, CD8-PE and CD19-APC, to analyze the T cells populations (**ImmunoTools**).
2°: CD19-APC, CD24-FITC and CD38-PE, to determine the different population of B cells mentioned before (**ImmunoTools**), because memory B cells are $CD24^{High}CD38^{low}CD19^{+}$, mature B cells are $CD24^{int}CD38^{int}CD19^{+}$ and immature B cells are $CD24^{High}CD38^{High}CD19^{+}$.

Then we want to measure the expression of TGF-B1, IDO and iNOS in the MSCs after being in contact with the activated lymphocytes to determine if the difference in the immunomodulatory capacities are due to a cell-cell interactions or a soluble factors that are not release in response of the presence of activated lymphocytes.

Once, we understand the role of EphB2 and EphB3 on the immunomodulatory capacities of MSCs we would like to use this information on the development of immunological therapies in the future.

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includes 25 reagents

FITC conjugated anti human	CD4, CD24, CD34, CD44,Mouse IgG1, Mouse IgG2b
PE conjugated anti human	CD8, CD29, CD38, CD73, Mouse IgG1
PerCP conjugated anti human	CD3, CD45, Mouse IgG1
APC conjugated anti human	CD19, CD105, Annexin V, Mouse IgG1, Mouse IgG2b

DETAILS more AWARDS

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