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Development of a flow cytometry-based assay for the immunomodulatory properties of human Mesenchymal Stromal Cells

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Introduction

Human bone marrow derived mesenchymal stromal cells (hBM MSC) have been described as possessing immuno-modulatory properties, affecting immune cells in many ways. However, there is a commercial need for a reliable, rapid, quantifiable assay to assess their potency. For this propose, we developed a rapid flow cytometry-based screening whole blood assay.

Aim of the study

To determine the effects of MSC on the production of TNF-α and IL-12 by LPS-stimulated monocytes.

Methods

A number of factors were considered prior to optimizing the assay, including:

- Anticoagulant (Heparin; EDTA and Citrate)
- Blood dilution (1/2; 1/5; 1/10; 1/15; 1/20 and 1/30)
- LPS concentration (0.5ng/ml, 1ng/ml, 2ng/ml, 5ng/ml, 10ng/ml and 20ng/ml)
- Incubation time (4h, 6h, 8h and 24h)

Cell activation

Ultrapure LPS + Brefeldin A (0.6µg/mL) + RPMI + Peripheral Blood

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Incubation at 37°C in 5% CO_2

Intracellular staining

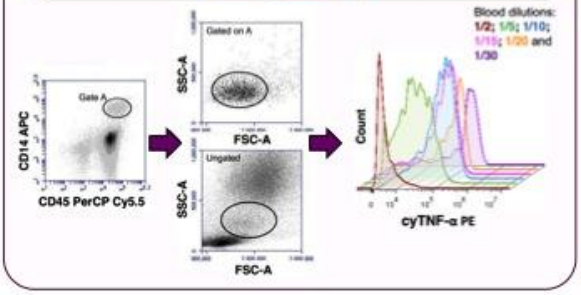
We used the IntraPrep Kit, from Beckman Coulter, according to the manufacturer's instructions.

We used **CD45**$^{PerCP.Cy5.5}$ and **CD14**^{APC} markers to identify monocytes and following fixation, analyzed the intracellular expression of **TNF-α**^{PE} or **IL-12**/IL-23p40^{PE}. After staining, samples were acquired using the BD Accuri C6 four color flow cytometer.

Data Analysis

Samples were analyzed using both Cflow, and FlowJo 7.6.1. software. Statistical analyses were carried out using Microsoft Office Excel.

Gating Strategy



Acknowledgements

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Results

Anticoagulant

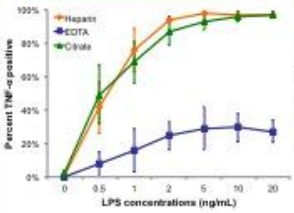

Figure 1 – Expression of TNF-α in Classical Monocytes. The blood was collected into 3 different tubes with anticoagulant . Monocytes were stimulated during 6h in different LPS concentrations, and were diluted x5 (n=4).

Blood Dilution

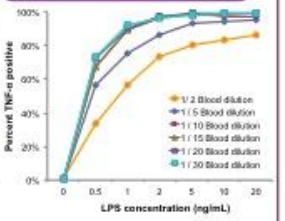

Figure 2 – Expression of TNF-α in Classical Monocytes. The blood was collected and diluted in Heparin. Monocytes were stimulated during 4h in different LPS concentrations (n=5).

Incubation Time

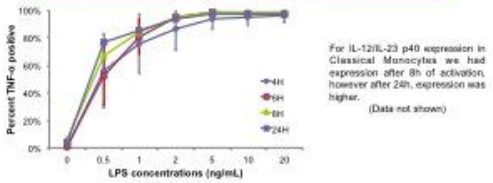

Figure 3 – Expression of TNF-α in Classical Monocytes. Monocytes were stimulated during 4h, 6h, 8h or 24h. The blood was collected in Heparin tubes and was diluted 5 times, and activated in different LPS concentrations (n=4).

MSC - Monocytes

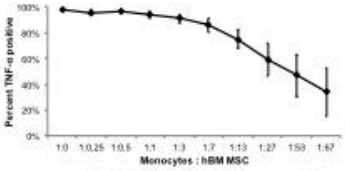

Figure 4 – Immunosuppression of TNF-α expressed by monocytes in the presence of MSC. Intracellular TNF-α expression by classical monocytes decreased in an MSC dose-dependent manner, in the presence of 1ng/mL LPS, at 1/10 blood dilution and after 6 hours of incubation. These results are an average of 3 different bone marrow donors and 5 different blood donors. Blood was collected in Heparin tubes (n=3).

Conclusions

- Heparin was the best anticoagulant in terms of cell activation. This was because anticoagulants containing divalent cation chelating agents removed Ca^{2+} which was found necessary for optimal monocytes activation.
- Detection of intracellular TNF-α expression was optimal at 6h whereas IL-12 became optimally detectable by 24h (data not shown).
- We detected optimal cytokine expression using a 1/10 blood dilution and 1ng/mL of LPS.
- We found promising preliminary results showing that MSC had a dose-dependent suppressive effect on monocyte activation.
- In conclusion, hBM MSC have the capacity to immunoregulate monocytes, and we have established a rapid, quantifiable assay to determinate the effects of hBM MSC on monocyte activation.