IN VITRO CHARACTERIZATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED MACROPHAGES

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Introduction

Human induced pluripotent stem cell-derived macrophages (hiPSC-macrophages) (Axol Bioscience) offer a physiologically relevant model for modelling infection and immuno-oncology screening *in vitro*.

hiPSC-macrophages are a type off immune cell (leukocyte or white blood cell) differentiated from hiPSC-derived monocytes. Macrophages play an essential role in phagocytosis, immunity and have an anti-inflammatory role in a variety of tissue types throughout the body. Macrophages are, thus, able to clear up cell debris, microbes and foreign substances through engulfing or internalising and destroying them by a process referred to as acidification. Moreover, when recruited to the site of injury or infection, macrophages have the ability to differentiate into tissue specific macrophages such as microglia, which are part of the nervous system.

Here we present data on the differentiation and characterization of hiPSC-macrophages. We determined seeding parameters and optimal differentiation conditions of hiPSC-derived monocytes to hiPSC-derived macrophages. The hiPSC-derived macrophages were, subsequently, assessed for expression of specific macrophage cell surface markers , using a cytokine array. Phagocytosis was then assessed using zymosan particles.

Here we have demonstrated that hiPSC-macrophage offer a suitable, reliable and reproducible alternative to human blood derived macrophages for, not only, infection and immuno-oncology studies, but also for co-culture studies for tissue regeneration and repair studies.

Materials and methods

hiPSC-macrophage culture: Human iPSCs were differentiated to monocytes, using a novel serum-free and defined growth factor based protocol. Monocytes were, then, plated (seeding density 15,000 cells per well of a 96 well plate) and differentiated to produce terminally differentiated human derived iPSC macrophages (Axol Bioscience) containing large vesicles.

Phagocytosis assay: Macrophages were co-incubated with either a 1:100 or 1:250 dilution of blue FluoSpheres and LPS (0.1 to 30 ng/ml), 5µM Cytochalasin D or control conditions. The cells were imaged live at 30, 60 and 90 min. At 2 hours, cells were fixed and stained with CellMask and imaged using InCell 2200, with quantification performed using the InCell Developer Toolbox and data presented as the total intensity of beads within the cells.

FACs analysis: At day 7 of differentiation, cells were detached using cold 1xPBS containing 5mM ethylenediaminetetraacetic acid. Markers used were CD45 (APC fluorophore, ImmunoTools), CD14 (PE fluorophore, ImmunoTools) and CD11b (APC fluorophore, ImmunoTools).

Immunocytochemistry: Macrophages were fixed in 4 % PFA for 15 min at room temperature, permeabilised with 0.3 % Triton-X-100 and blocked for 1 hour. Primary antibody, IBA1, was incubated over night at 4°C, washed and incubated with secondary antibody for 1 hour, at room temperature, washed and counterstained with DAPI.

Morphology of hiPSC-macrophages

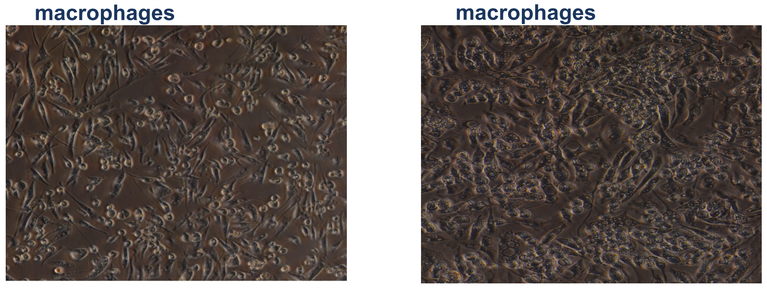

Figure 1: Morphology of differentiating monocytes to macrophages on day 3 and day 7
During maturation (differentiation, the macrophages become less elongated and have large vesicles. Vesicles start to appear by day 3. At day 7, macrophages are terminally differentiated and numerous large vesicles can be observed.

Cell surface markers analysis

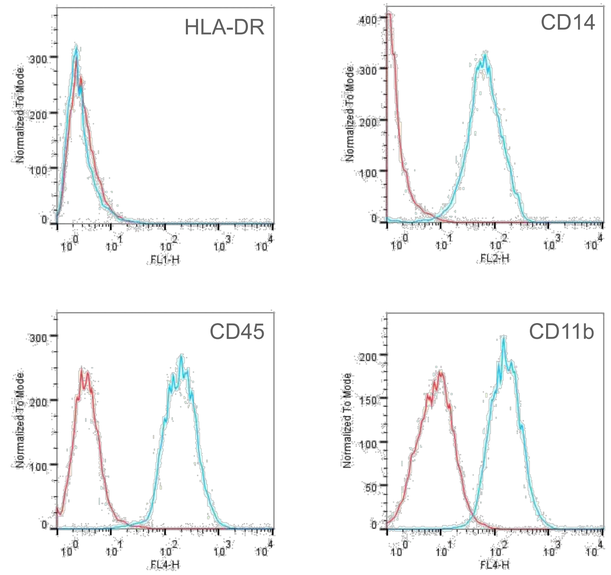

Figure 2. The analysis of cell surface markers exhibited by macrophages
Axol hiPSC-macrophages exhibit strong positivity for CD45, CD14 and CD11b cell surface markers, which are all present in mature macrophages.

Macrophage marker expression

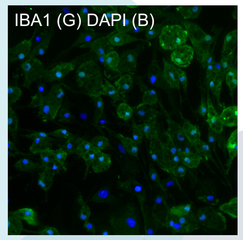

Figure 3. Expression of the IBA1 protein was confirmed in Axol iPSC-Derived Macrophage using immunocytochemistry.
hiPSC-Macrophages were cultured in Macrophage Maintenance Medium for 11 days before staining for IBA1 and counterstained with DAPI.

Inhibition and stimulation of phagocytosis in Axol hiPSC-Derived Macrophages

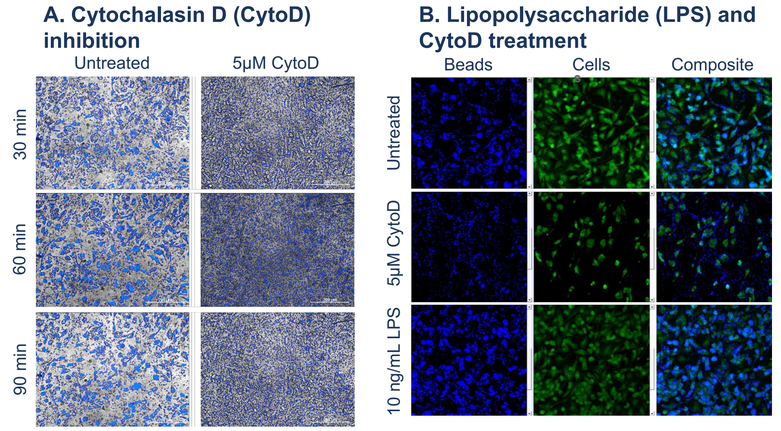

Figure 4. Phagocytosis by hiPSC-derived macrophages
Analysis reveals inhibition of phagocytosis following CytoD treatment and activation of phagocytosis following LPS treatment. *A;* The blue FluoSpheres can be seen in large vesicles of the macrophages in the untreated culture. In comparison, the CytoD-treated culture shows smaller macrophages with the DAPI beads in the medium revealing an inhibition of phagocytosis. *B;* Treatment with 10ng/ml LPS shows a significant increase in phagocytosis; whereas treatment with CytoD shows a significant inhibition of phagocytosis. *C;* A good inhibition curve of phagocytosis was observed following treatment with CytoD. *D;* Graph showing the total bead fluorescence per cell in untreated, CytoD treated and LPS treated cultures. Data from Dr Will Stebbeds and Dr Graham Smith, Charles River Laboratories.

Conclusions

The generation of hiPSC-macrophages from hiPSC-derived monocytes provides a much purer population of macrophages, offering a reproducible and reliable cell type.

Axol hiPSC- Derived Macrophages have been shown to:

- exhibit expected morphology with large vesicles and elongated shape.
- express key characteristic markers such as IBA1, CD45, CD14 and CD11b.
- have highly phagocytic behaviour that can be stimulated by LPS and inhibited by CytoD.

Through the characterisation and analysis of the developed hiPSC-derived macrophages, we have shown that these cells can be used as a viable alternative to blood derived macrophages for a wide range of applications including infection and immuno-oncology studies.

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