Characterization of human iPSC-Derived Macrophages reveals the hallmarks of macrophage morphology and function

Background

The human immune system is a complex network of organs and cells that collectively recognize potentially dangerous foreign substances, to protect us against infection and disease. Circulating peripheral blood monocytes and their differentiation into tissue-specific macrophages at the site of infection plays a crucial role in this immune response.

Specifically, granulocyte-macrophage colony-stimulating factor (GM-CSF), released by a variety of cells, stimulates monocyte differentiation into inflammatory-macrophages. These macrophages are highly aggressive against foreign bodies, engulfing and destroying any harmful substances via phagocytosis. Additionally, macrophage colony-stimulating factor (M-CSF, or CSF-1), which is continuously secreted by cells to regulate the production of monocytes in the body, leads to the differentiation of monocytes into anti-inflammatory macrophages. These are involved in wound healing and tissue repair, such as the removal of apoptotic cells via efferocytosis.

Consequently, macrophages are important cells to study in biomedical research, helping to characterize immunological function and immune systems disorders, such as rheumatoid arthritis and lupus, as well as uncover new medicines for these diseases.

Advances in stem cell biology are now enabling the generation of macrophages from human induced pluripotent stem cells (hiPSCs) for disease research and drug discovery, resulting in several advantages over traditional immortalised cell lines (e.g. THP-1) and peripheral blood sources. These hiPSC-Derived Macrophages can therefore offer an enhanced physiologically-relevant cellular model to advance disease research, facilitating the discovery of anti-inflammatory treatments.

Key points

- Macrophages play a crucial role in the human immune response and are a significant cell of interest in biomedical research into immune disorders and drug discovery.
- Exciting advances in stem cell biology are now enabling macrophages to be differentiated from monocytes derived from human induced pluripotent stem cells(hiPSCs), which can enhance the reproducibility and reliability of your research compared to traditional sources.
- This study characterized hiPSC-Derived Macrophages (Axol Bioscience) to validate their morphological, molecular and functional features, offering insights into their suitability for immune disease research and for the discovery of anti-inflammatory treatments.
- Flow cytometry and immunocytochemistry revealed that hiPSC-Derived Macrophages express the relevant cell surface markers, CD45, CD14 and CD11b, and two assays showed they display normal phagocytic and efferocytic responses.
- Therefore, hiPSC-Derived Macrophages are a physiologically-relevant, functionally-validated cellular model for studying apoptosis and immunological function, and can potentially facilitate the advancement of drug discovery to help patients with inflammatory diseases.

Aims and objectives

- To describe the morphological, molecular and functional characteristics of human iPSC-Derived Macrophages (Axol Bioscience) differentiated from hiPSC-derived monocytes.
- Morphological and molecular analyses were performed using flow cytometry (fluorescence-activated cell sorting) and immunocytochemistry, including characterizing whether the relevant molecular features and cell surface markers, CD45, CD14 and CD11b, were expressed by the hiPSC-Derived Macrophages. HLA-DR (Human Leukocyte Antigen - antigen D Related), a major histocompatibility complex, was undetected in the hiPSC-Derived Macrophages as expected.
- In collaboration with Essen Bioscience (a Sartorius Company), functional characterization of the hiPSC-Derived Macrophages was conducted using two different assays, each measuring typical macrophage characteristics: 1) phagocytosis and 2) efferocytosis.

Materials and Methods

Human iPSC-Derived Macrophage culture

Human iPSCs were intermediately differentiated to Human iPSC-derived monocytes using a novel serum-free and defined growth factor-based protocol. The hiPSC-derived monocytes were then plated into a 96-well plate with a seeding density of 15,000 cells per well and differentiated to produce terminally differentiated human iPSC-Derived Macrophages (Axol Bioscience) containing large vesicles. The directed terminal differentiation to macrophages takes six days, so to avoid overactivation of the macrophages at day 14, they were used within seven days upon arrival at the bench.

Flow cytometry - Fluorescence-activated cell sorting (FACS) analysis

At day seven of differentiation, cells were detached using cold 1x phosphate-buffered saline (PBS) containing 5mM ethylenediaminetetraacetic acid (EDTA). Markers investigated were CD45 (APC fluorophore, ImmunoTools), CD14 (PE fluorophore, ImmunoTools), CD11b (APC fluorophore, ImmunoTools) and HLA-DR.

Immunocytochemistry

Macrophages were fixed in 4% paraformaldehyde (PFA) solution for 15 minutes at room temperature, permeabilized with 0.3% Triton-X-100 and blocked for 1 hour. The primary antibody, IBA1, was incubated overnight at 4ºC, washed, and the secondary antibody was incubated for 1 hour at room temperature, washed, and counterstained with 4',6-diamidino-2-phenylindole (DAPI) or phalloidin fluorescent stains.

Phagocytosis Assay

This study was conducted in collaboration with Essen BioScience (a Sartorius company). Axol hiPSC-Derived Macrophages were seeded at increasing densities from 1,875 up to 30,000 cells per well in an Essen® ImageLock plate. At day 10, the hiPSC-Derived Macrophages were co-incubated with a range of concentrations of IncuCyte® pHrodo® green E. coli bioparticles® (Essen BioScience, a Sartorius company) - specifically, 10, 5 or 2.5 µg per well (these bioparticles only fluoresce when engulfed by the hiPSC-Derived Macrophages). Phase and green fluorescence images were captured in the IncuCyte S3 every 30 minutes for 18 hours at 10x magnification. Phagocytosis was quantified using IncuCyte® software, which applied a processing definition to remove background fluorescence, allowing accurate segmentation of the fluorescence image.

Efferocytosis Assay

This study was conducted in collaboration with Essen BioScience (a Sartorius company). HiPSC-Derived Macrophages were seeded and cultured at 15,000 cells per well in an Essen® ImageLock plate. At day 10, the hiPSC-Derived Macrophages were treated with three different densities of apoptotic Jurkat cells (immortalized human T-lymphocytes -). These were added at densities of 200,000, 100,000 and 50,000 cells per well and only fluoresced when engulfed by the hiPSC-Derived Macrophages.

To induce apoptosis, Jurkats were treated with 10 µM camptothecin, washed to remove the compound and labelled using an IncuCyte® pHrodo® Red Cell Labelling Kit (Essen BioScience, a Sartorius company). Phase and red fluorescence images were captured in the IncuCyte S3 every 20 minutes for 24 hours at 10x magnification.

Results

Morphological and molecular characterization

Rounded monocytes were found to transition into hiPSC-Derived Macrophages with two distinct morphologies: 1) small and round, and 2) elongated (**Figure 1**). The FACs analysis and immunocytochemistry revealed that the hiPSC-Derived Macrophages displayed the molecular characteristics required for macrophage activation and phagocytic/efferocytotic function, and showed similar morphological features to macrophages *in vivo*.

Specifically, the IBA1 antibody, and DAPI and phalloidin fluorescent staining, revealed the presence of receptors in the hiPSC-Derived Macrophages associated with cell motility, enabling macrophages to migrate to find and engulf apoptotic cells and foreign substances (**Figure 2**).

In addition, unstimulated hiPSC-Derived Macrophages expressed the relevant cell surface markers, CD45, CD14 and CD11b and demonstrated strong positivity for these, as well as showing minimal expression of HLA-DR markers, thus reflecting the unprimed culture conditions (**Figure 3**).

Functional characterization

Axol hiPSC-derived macrophages demonstrated efficient phagocytosis of both *E. coli* bioparticles and apoptotic Jurkats. Bioparticles and apoptotic cells were labelled with a pH-sensitive fluorophore which experiences fluorescence enhancement upon encountering the decreased pH of the phagolysosome. Phagocytosis is therefore reported as an increase in fluorescence intensity within the cytoplasm of the macrophage.

A density-dependent uptake of *E. coli* bioparticles was observed and at high bioparticle density the hiPSC-Derived Macrophages were found to be more active, with the onset of phagocytic behavior occurring earlier in these cells compared to cultures with lower densities of *E. coli* bioparticles (**Figure 4**).

Axol hiPSC-Derived Macrophages were also observed to phagocytose apoptotic cells (efferocytosis). It was also noted that hiPSC-Derived Macrophage activity was dependent on the number of apoptotic cells added to the culture; the macrophages engulfed fewer apoptotic cells over 24h when seeded in cultures with 100,000 and 50,000 cells, respectively (**Figure 5**).

Under these conditions hiPSC-Derived Macrophages responded more rapidly when cultured with apoptotic Jurkat cells (i.e., within 10 minutes) compared to when they were cultured with *E. coli* (i.e., within 4 hours), demonstrating similar activity levels to those expected *in vivo*.

Figure 1: Phase images show morphology of differentiating human iPSC-derived monocytes (Day 0-7) which produce terminally differentiated human iPSC-Derived Macrophages (Axol Bioscience) containing large vesicles. Data courtesy of Dr Gillian Lovell and Tim Dale (Essen BioScience, a Sartorius company).

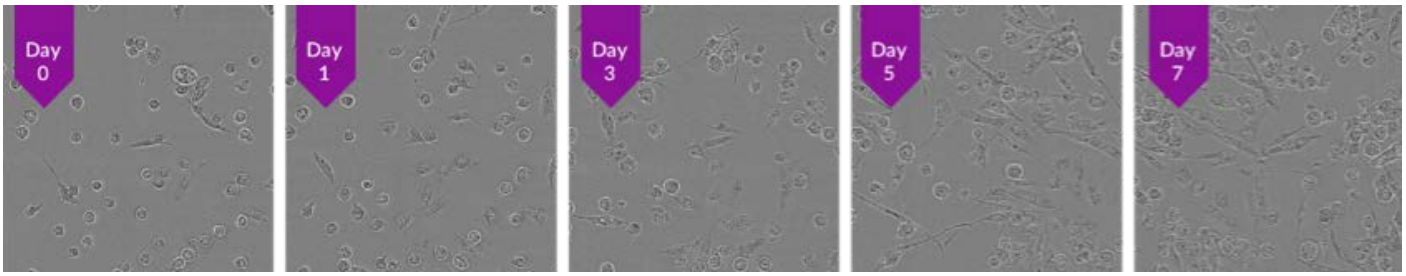


Figure 2: A) and B): Human iPSC-Derived Macrophages (Axol Bioscience) stained for IBA1 (green) and DAPI (blue). C): Human iPSC-Derived Macrophages (Axol Bioscience) stained for IBA1 (green), phalloidin (red) and DAPI (blue).

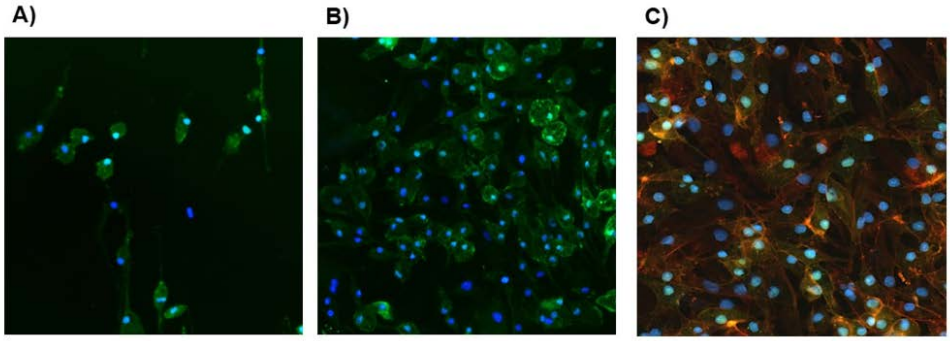
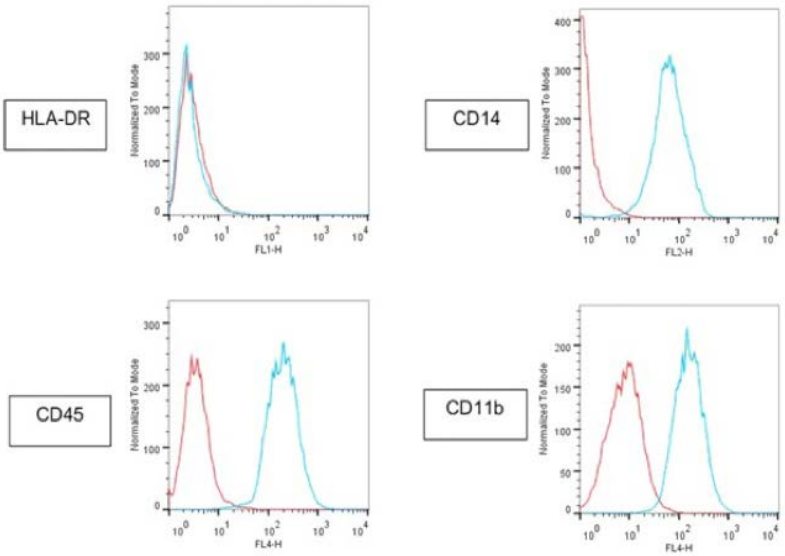


Figure 3: Expression of cell surface markers, HLA-DR, CD14, CD45 and CD11b, on hiPSC-Derived Macrophages (Axol Bioscience) detected by flow cytometry and immunocytochemistry.



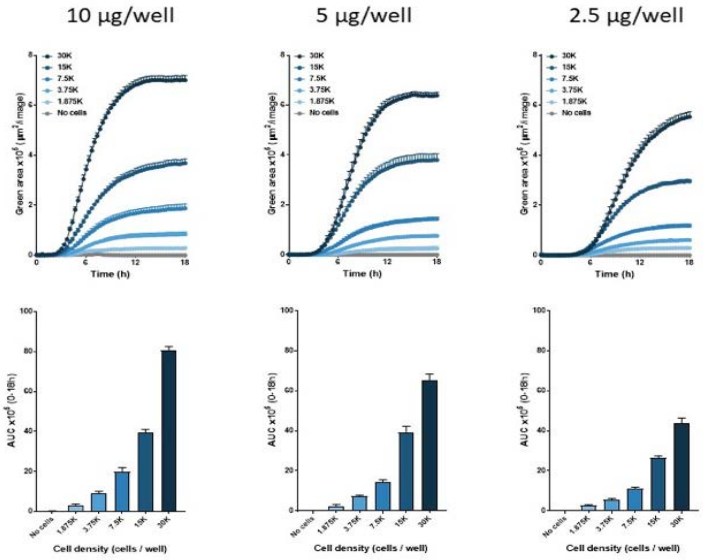


Figure 4: Phagocytosis of IncuCyte® pHrodo® green *E. coli* bioparticles® is bioparticle number dependent. HiPSC-Derived Macrophages (Axol Bioscience) were co-incubated with a range of densities of IncuCyte® pHrodo® green E. coli bioparticles® (Essen BioScience, a Sartorius company) (10, 5 or 2.5 µg per well) and analyzed for phagocytic activity. Data courtesy of Dr Gillian Lovell and Tim Dale (Essen BioScience, a Sartorius company).

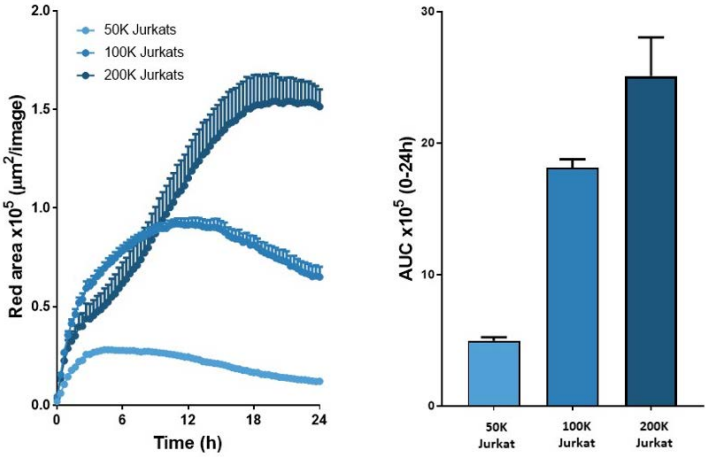


Figure 5: Efferocytosis of apoptotic cells is cell-number dependent. HiPSC-Derived Macrophages (Axol Bioscience) were treated with three different densities of apoptotic Jurkat cells (200,000, 100,000 and 50,000 cells per well) and scanned for efferocytic activity. Data courtesy of Dr Gillian Lovell and Tim Dale (Essen BioScience, a Sartorius company).

Conclusions

The data presented here strongly demonstrate that our human iPSC-Derived Macrophages display the morphological, molecular and functional characteristics expected from human macrophages. This includes having the molecular features relevant for performing phagocytosis and efferocytosis and predicted cell surface markers (CD45, CD14 and CD11b), as well as displaying normal phagocytic and efferocytic responses.

Our human iPSC-Derived Macrophages therefore provide an ideal physiologically-relevant, functionally-validated cellular model for studying apoptosis and inflammatory responses *in vitro*, as well as being a practical and attractive model for advancing research into immune system function and drug discovery for inflammatory diseases.

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