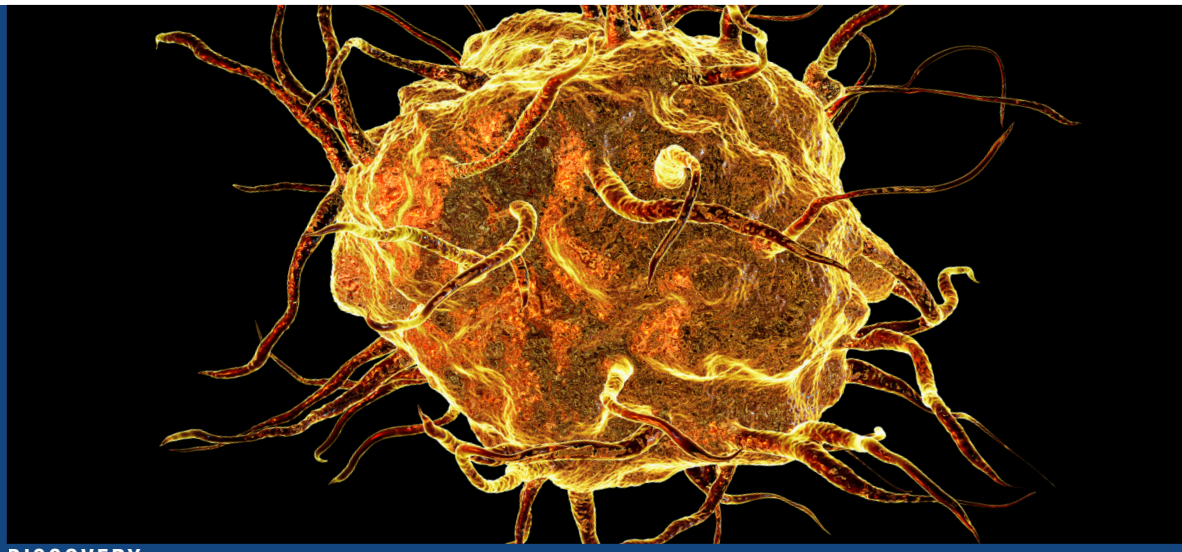
charles river

Summary

Powerful new *in vitro* assays are a translational tool to study the potency of biologics or small molecules as modulators of immune responses. We have developed a chemotaxis assay mimicking the migration of monocytes isolated from the peripheral blood of healthy human donors to assess the complex biology of monocytic migration.



DISCOVERY

Complex Biology *In Vitro* Assays: Immunology

Chemotaxis Assay Monocytes

Monocytes are innate immune cells involved in the resolution of tissue injuries, inflammation and infections. Monocyte migration is driven by chemokines including the stromal cell-derived factor 1α (SDF-1α), which is released by residential cells upon tissue damage [1-4].

SDF-1α, also known as CXCL12, allows monocytes to migrate through the vascular or lymphatic endothelium to the peripheral injured or inflamed tissue. Here, monocytes can further differentiate into macrophages or dendritic cells [5], and signal through the SDF-1α receptor, CXCR4. Although the recruitment of monocytes is essential for mounting effective immune responses and restoring tissue homeostasis, it also plays a pivotal role in the development of several inflammatory disorders and tumor development.

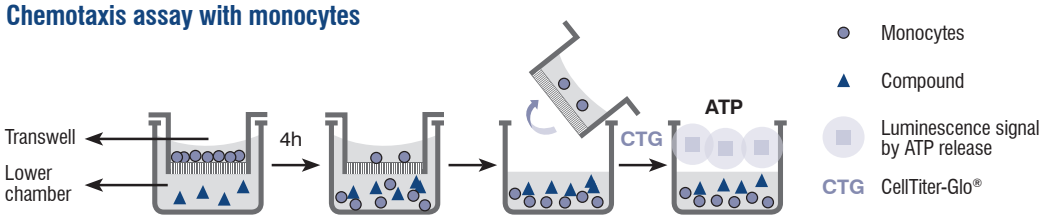
Hence, monitoring the response of human monocytes to chemokines is key for the identification of candidate drugs To study monocyte migratory mechanisms and test the effects of drug candidates on the modulation of monocytic migration, we have developed a chemotaxis assay using primary human monocytes freshly isolated from blood of healthy donors.

Assay Principle

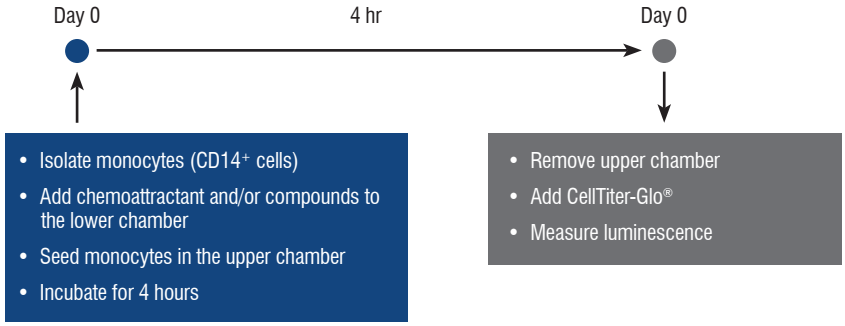
The chemotaxis assay evaluates the migration of monocytes through permeable supports (Transwell® or Boyden chamber) that contain 5.0 μm pore polyester membrane. Blood-derived human monocytes are isolated using magnetic beads coupled to an anti-CD14 antibody (positive selection) and seeded in the upper chamber of a 96-well Boyden chamber in serum-free medium, while chemoattractants and/or compounds are placed in the lower chamber. After 4 hours, monocytes that have migrated through the pores into the lower chamber are detected by measuring their cellular ATP levels using a luminescence-based reaction (CellTiter-Glo®, Promega) and read with a plate reader (EnVision™, PerkinElmer). The luminescent signal generated is directly proportional to the number of cells present in the lower chamber (Fig. 1).

Figure 1. Chemotaxis assay principle with human monocytes

Chemotaxis assay with monocytes



Assay Workflow



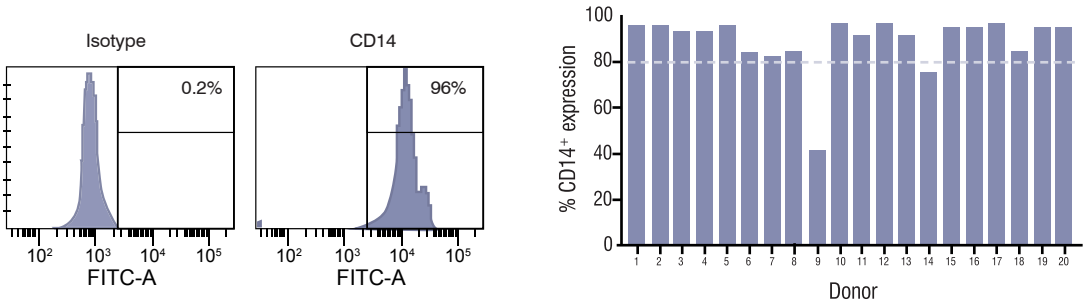
Assay Setup

Cell type	CD14⁺ cells (monocytes) from healthy human blood donors
Seeding density	50,000 cells/insert
Trigger	SDF-1α [10 nM]
Controls	Control: Serum-free medium (0.5% BSA) Vehicle: 0.1 % DMSO
Inhibitors	AMD3100 (CXCR4 inhibitor) in 8-point concentration-response curve
Time	4 hours
Readout	ATP levels (luminescent signal)

Quality Check (QC) of Monocytes

Flow cytometry is used to perform a quality check (QC) of freshly isolated monocytes. After isolation, monocytes are stained with an anti-CD14 antibody and its relative isotype control, and analyzed by flow cytometry (Fig. 2A). Donors that exhibit > 80% of CD14 expression will be used for the chemotaxis assay (Fig. 2B).

Figure 2. Monocytes (CD14⁺ cells) quality check

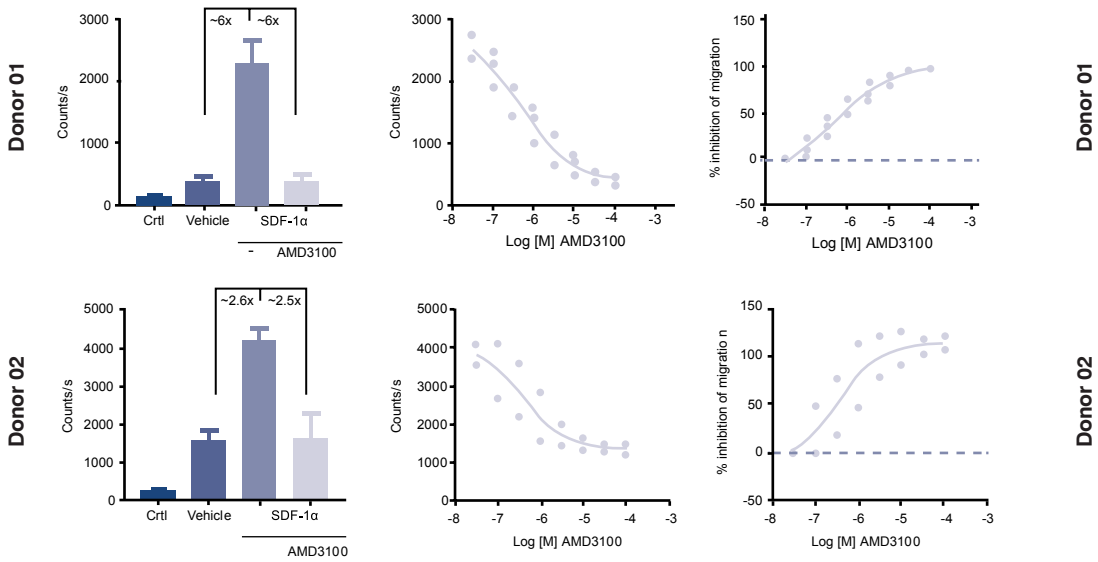


A. Representative flow cytometry histograms show CD14 expression on 96% of cells isolated from one donor (left histogram). Isotype control staining shows no non-specific binding (right histogram).

B. Percentage of CD14 expression on cells isolated from different donors (20 donors tested). Dotted line shows the minimal threshold level of acceptance (80%) for CD14 expression in a panel of 20 human healthy donors.

Performance of the developed assay was validated using the SDF-1α receptor antagonist, AMD3100 (CXCR4 inhibitor), as a positive control for inhibition of migration together with the two assay controls (Control: Serum-free medium [0.5% BSA]; Vehicle: 0.1% DMSO). Representative data obtained from monocytes (CD14⁺ cells) isolated from two healthy donors (Donor 01 and 02) are reported below (Fig. 3).

Figure 3. Monocyte chemotaxis responses



A. Assay windows obtained following the addition of the chemoattractant SDF-1α in the absence (-) and presence of the CXCR4 antagonist (AMD3100, 100 μM) for two donors (Donor 01 and 02). Assay controls (control:serum-free medium [0.5% BSA]; vehicle:0.01% DMSO) are reported.

B. Representative concentration-response curves of AMD3100 (left panel) and the relative curve of inhibition (right panel) for monocyte migration on two donors (Donor 01 and 02). Results are provided as percentage of inhibition normalized (PIN) values.

Summary

Migration of immune cells is essential for resolving tissue injuries, inflammation, and infections. This process is orchestrated by chemokines, such as SDF-1α, which can recruit monocytes and other immune cells. SDF-1α, a member of the C-X-C chemokine family, has been reported to be implicated in different pathologies including pancreatic cancer[6], Alzheimer's disease[7], atherosclerosis[8] and Sjögren's syndrome[9]. Therefore, a better understanding of the processes involved in migratory responses of monocytes would offer insight into potential treatments for autoimmune disorders and inflammation.

Here we demonstrate an optimized chemotaxis assay allowing the modulation of SDF-1α-induced migration of human monocytes isolated from healthy blood donors through a 5 μm pore size inserts of a Boyden chamber. SDF-1α at 10 nM strongly induced the migration of human monocytes that is inhibited by the CXCR4 antagonist (AMD3100) in a dose-dependent fashion. IC50 values were consistent across different donors (not shown). Using this assay, the migration of monocytes isolated from blood donors can be monitored to evaluate therapeutic candidates for the treatment of inflammatory conditions and other pathologies.

For our clients' scheduling convenience our Chemotaxis Assay with Monocytes will be performed on bi-monthly-basis upon receipt of compounds. Results are issued within 4 weeks following compound receipt.

Assay Reference code

OTS-212 Chemotaxis Monoytes

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