Kinetic Measures Of Macrophage Chemotaxis
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Introduction
Macrophages play a central role in innate immunity as a first-line innate immune defense by migrating to inflamed tissues, where they clear microbes and repair injured tissues.

- Classically activated cells (M1) play an important role in fighting intracellular pathogens and tumors.
- Alternatively activated macrophages (M2) are involved in tissue repair, allergic reactions and tumor progression.

THP-1 is an immortalized cell line used as a model to study monocytes and macrophages.

- After exposure to PMA (phorbol-12-myristate-13-acetate), THP-1 cells start to differentiate into a macrophage phenotype with flat and amoeboid shape.

C5a is a protein fragment released from cleavage of complement component C5.

- C5a is an anaphylatoxin, causing increased expression of adhesion molecules on endothelium and increased vascular permeability.
- C5a is also a chemoattractant that plays a key role in increasing migration and adherence of neutrophils and monocytes to vessel walls.

Traditional in vitro methods for studying Macrophage cell migration include:

- Microfluidic Chemotaxis Assays: Researchers can see these, but they suffer from small gradients across the cell, low participation rates, and low throughput.
- Traditional Boyden Chamber Assays: This predominant industrial approach has good throughput (96-wells). However, the researcher can not easily visualize the process of cell migration, it requires many cells, and additional labeling or manual cell counting.

We have developed a new technology that eliminates most of the shortages of existing hemotaxis methods and provides a direct visualisation of the migration process as it occurs.

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<th>Obstacle</th>
<th>ClearView solution</th>
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<td>Macrophage cell numbers in tissues</td>
<td>Requires 20 times less cells than traditional Boyden chamber</td>
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<td>Isolated isolation and activation procedure</td>
<td>Multiple experiments can be run simultaneously due to automated image acquisition and analysis</td>
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<td>Participation rate</td>
<td>Negligible background noise with bottom-side micros</td>
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IncuCyte® ClearView™ Cell Migration Plate

Isolatable Chemotaxis – The ClearView Plate incorporates a pinhole smooth membrane surface enabling acquisition of gh-definition, phase-contrast images. Standard Boyden chamber surfaces are not easily amenable to imaging.

Isolatable chemotaxis was used in an assay that requires significantly fewer cells compared to traditional Boyden Chamber Assays.

Migrate Density – The combination of a long-term, isolatable gradient and the interest in visualising chemotaxis as resulted in an assay that requires significantly fewer cells compared to traditional Boyden Chamber Assays.

Integrin Signaling – In the ClearView Plate, cells are required to migrate to the pores. This requires integrin activations with the substrate that likely are not required in traditional Boyden Chamber experiments.

Shell-free – Migration without fouling, staining or cell scraping steps.

Automated Image Processing – The unique design of the bottom View Plate facilitates quantitation of cells on top and bottom of the membrane.

IncuCyte® ClearView™ Cell Migration Plate

Evaluation of the gradient. A 10kD dextran (labeled with Alexia Fluor® 594) was added to the ClearView reservoir plate at a concentration of 10 μM to establish gradients over zero, 24, 48, and 72 hours. Measurements of diffusion were made by sampling the insert wells and measuring fluorescent intensity on a microplate reader. N=3.

THP-1 Response to C5a Before and After Differentiation

5,000 THP-1 cells were seeded in each well on a fibronectin-coated surface. For A, C and E the chemotactractants were added to the reservoir plate immediately after cell settling. For B, D and E the cells were incubated in 5 ng/ml PMA for 48 hours prior to addition of chemotactractant.

A and B: Representative images of THP-1 cells seeded on the ClearView plate at T=0 of the experiment. Compare the round shape of the cells resembling that of monocytes in A to more flattened shape in B, indicating that the cell are actively interacting with the surface.

C and D: Kinetic curve of concentration-dependent responses to C5a. N=3.

E and F: C5a agonist curve at 30 h.

M1 and M2 Primary Macrophages

2,000 Macrophage cells were seeded in each well on a Matrigel-covered surface.

Figures A and C: The cells were activated into M1-like phenotype by incubating in 50 ng/ml GM-CSF for 6 days followed by 1 ng/ml LPS + 20 ng/ml IFNγ for one more day.

Figures B and D: The cells were activated into M2-like phenotype by incubating in 50 ng/ml M-CSF for 6 days followed by 10 ng/ml IL-4 for one more day.

A and B: Representative images of M1 and M2 – like cells before seeding on ClearView plate. Note that the M1 population have egg yolk morphology, while M2 have a mixed population of flat egg - shaped and spindle - shaped cells. This difference in morphology in M1 vs. M2 cells is consistent with previous observations (Young et al., The Journal of Immunology, 1990).

C and D: Kinetic curve of responses to C5a. N=3.

E and F: Table of other chemotactractants tested.

*α: Always responds to the chemotactractant

**α: The response is irregular depending on donor and isolation variables.

- No response to the chemotactractant.

Summary and Impact

- While differentiated macrophage-like THP-1 cells had a strong concentration-dependent response towards C5a, no response was observed in THP-1 cells before differentiation, suggesting biological significance of monocyte maturation in C5a-mediated chemotaxis.

- Both M1 and M2-like cells were found to have a response towards a number of chemotactractants, with M1-like cells generally having a slower response as compared to M2-like cells.

- Macrophages displayed a lag phase before the cells start to migrate across the filter, which was longer with higher concentrations of chemotactractant.

- This lag is a function of the cells and not an artifact of the system, since other cell types do not display this behavior.

- The present study proves traditional single end time point assay to be insufficient for investigating Macrophage chemotaxis and provides new information about kinetics of macrophage migration.

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