

APPLICATION NOTE

Evaluate anti-inflammatory compounds using a multiparametric THP-1 cell-based assay

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Introduction

Macrophages originate from blood monocytes that leave the circulation and penetrate various tissues where they differentiate into macrophages. They are involved in the removal of pathogens and phagocytosis of dead cells. In addition, they initiate inflammation by releasing cytokines that activate vascular cells and facilitate macrophage adhesion to blood vessels and migration into the tissues. Differentiated THP-1 cells have been widely used as an *in vitro* model of macrophages in studies of macrophage involvement in inflammatory responses.¹ The human monocytic cell line THP-1 can be differentiated into macrophages by phorbol 12-myristate 13-acetate (PMA) and activated by bacterial lipopolysaccharides (LPS). Activated THP-1 cells change morphology and become more adherent. They also secrete inflammatory cytokines

as a result of the cascade of cell signaling events initiated by LPS. The expression levels of cytokines are important physiological readouts for cell-based models of inflammation.

We present results from a multiparametric THP-1 cell-based assay. We used phenotypic imaging on the ImageXpress® Pico Automated Cell Imaging System to assess cell morphology and adhesion. We also used low-volume enzyme-linked immunosorbent assays (ELISAs) run on the Pu-MA System, and detected on the SpectraMax® iD5 Multi-Mode Microplate Reader, to measure secreted cytokines and evaluate effects of compounds on inflammatory responses. The multiparametric assay workflow is shown in Figure 1.

Benefits

- Measure multiple analytes where sample volume is limited
- Reduce reagent use by 5-10X and deliver results in less than three hours
- Observe macrophage differentiation using automated imaging

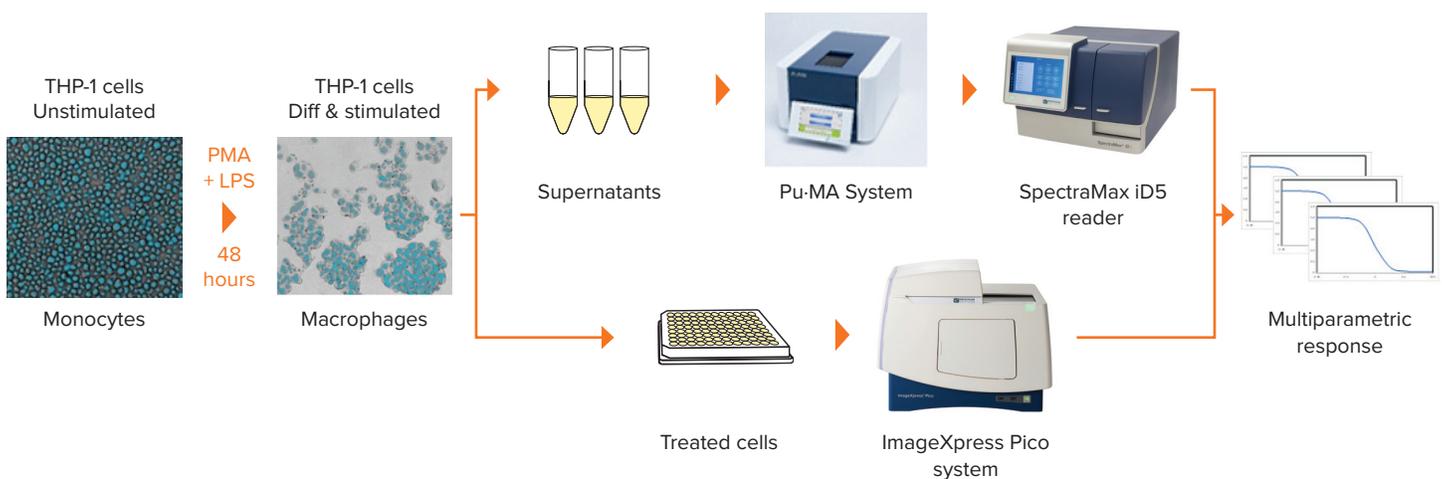


Figure 1. Multiparametric assay workflow. The ImageXpress Pico system, Pu-MA System, and SpectraMax iD5 reader are used to phenotypically image macrophage formation and quantify levels of secreted cytokines to evaluate effects of pharmacological compounds on the inflammatory response.

An increase in IL-8, IL-1 β and TNF- α secretion was observed in THP-1 cells stimulated with PMA and LPS. To evaluate effects of anti-inflammatory compounds, cells were additionally treated with the p38 MAP kinase (MAPK) inhibitors SB202190 and pyrrolidine dithiocarbamate (PDTC), and the antibiotic moxifloxacin. SB202190 acts on the JAK/STAT and NF κ B pathways². The antioxidant PDTC suppresses activation of NF κ B³. Moxifloxacin inhibits IL-8 and TNF α by inhibiting NF- κ B, ERK, and JNK activation⁴. Inhibition of inflammatory responses by the compounds was measured by quantifying changes in cell adhesion and in cytokine and chemokine secretion. Concentration-dependent decreases in expression were seen for the selected compounds. Effects on cell morphology and adhesion were also assessed by phenotypic analysis of cell images.

Materials

- THP-1 cells (ATCC)
- PMA (Sigma)
- LPS (Sigma)
- SB202190 (Sigma)
- PDTC (Sigma)
- Moxifloxacin (Sigma)
- ELISA antibody pairs for IL-8, IL-1 β , and TNF α (BioLegend)
- ImageXpress Pico Automated Cell Imaging System (Molecular Devices)
- Pu-MA System (Protein Fluidics)
- SpectraMax iD5 Multi-Mode Microplate Reader (Molecular Devices)

Methods

- THP-1 cells were plated at 20,000 cells per well of a 96-well microplate and incubated for 48 hours. They were then stimulated with a mix of PMA and LPS (0-5 pg/mL of PMA, and 0-100 pg/mL LPS) for 24 hours.
- Anti-inflammatory compounds were added two hours prior to stimulation.
- After incubation, 60 μ L of supernatant was taken from each well for ELISA. The samples were analyzed fresh or stored at -70°C for subsequent analysis.

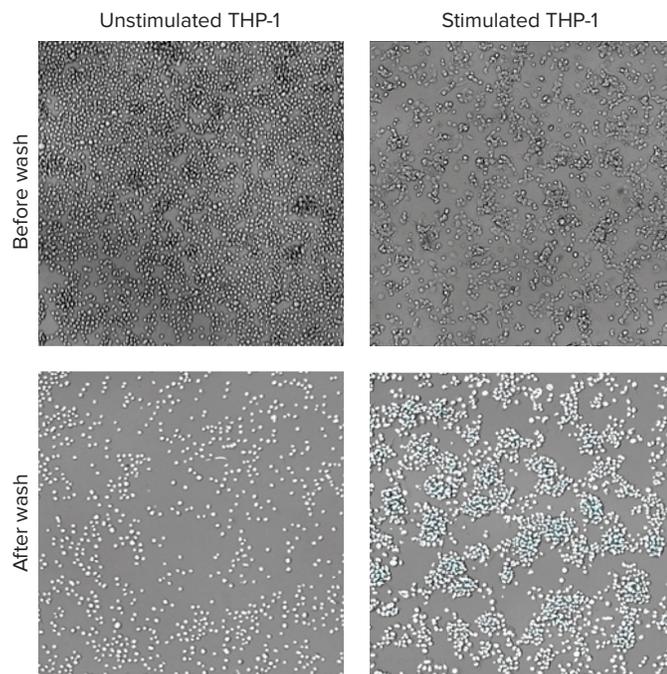


Figure 2. Transmitted light images of unstimulated (left) or stimulated (right) THP-1 cells before (top) and after (bottom) removal of non-adherent cells. Analysis masks for cell counting in CellReporterXpress software are shown in white (bottom).

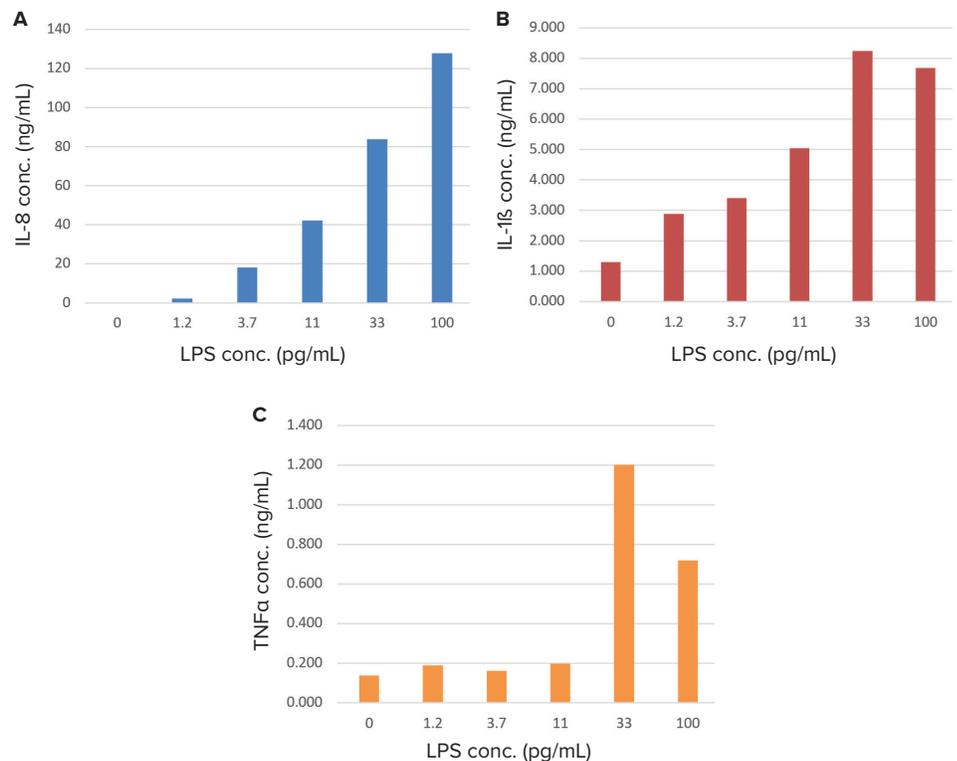


Figure 3. Chemokine and cytokine secretion in response to LPS. The concentrations of IL-8 (A), IL-1 β (B), and TNF α (C) increased in response to LPS.

- Supernatants were diluted 3:1 in assay buffer and analyzed for IL-8, TNF α , and IL-1 β using the Pu-MA System flowchips and reagents. Results were detected using the absorbance mode of the SpectraMax iD5 reader.
- Cells were imaged with transmitted light (TL) using the ImageXpress Pico system. Non-adherent cells were removed by washing twice with media. Cells in TL images were counted using CellReporterXpress™ Image Acquisition and Analysis Software.

Results

Upon stimulation with PMA and LPS, activated THP-1 cells adhered to the plate surface and upregulated cytokines. Cells were imaged using transmitted light with the ImageXpress Pico system to compare cell morphology before and after stimulation.

Phenotypic changes resulting from THP-1 stimulation are shown in Figure 2. Cell morphology changed from the small, rounded, non-adherent phenotype typically observed with monocytes to the larger, flatter, and more adherent macrophage phenotype. The number of adherent cells present after washing away the non-adherent cells was quantified using the ImageXpress Pico system and CellReporterXpress software.

The concentrations of the chemokine IL-8 and the cytokines IL-1 β and TNF- α secreted in response to cell stimulation were measured in supernatants using the automated, low-volume Pu-MA System. It uses small sample volumes (10-20 μ L) and existing antibody pairs. This enables one to measure multiple analytes where sample volume is limited. Increases in chemokine and cytokine secretion with cell stimulation are shown in Figure 3. Levels of IL-8 increased 30-fold, IL-1 β increased 6-fold, and TNF α increased 15-fold in stimulated cells compared to unstimulated cells.

We investigated the effect of several anti-inflammatory compounds on cell morphology, adhesion, and secretion of IL-8 by stimulated THP-1 cells. Changes in cell morphology caused by anti-inflammatory compounds were imaged using the ImageXpress Pico system (Figure 4). To measure changes in cell adhesion in response to anti-inflammatory

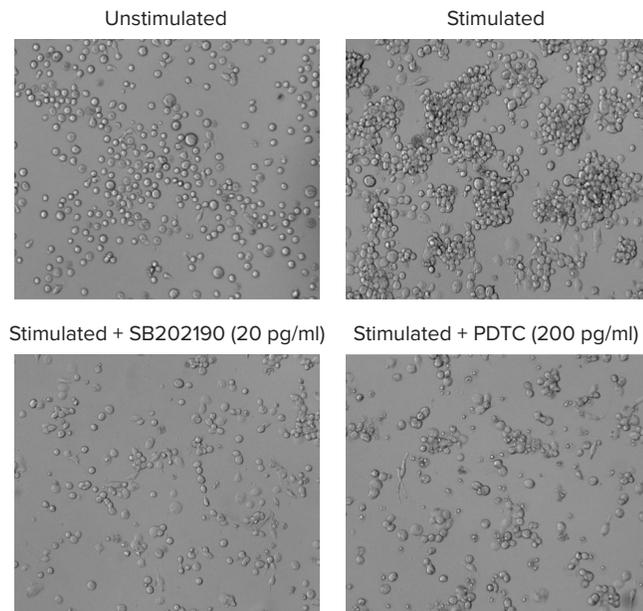


Figure 4. The effect of anti-inflammatory compounds on phenotypic response of THP-1 cells. Top: transmitted light images of THP-1 cells with or without PMA + LPS stimulation. Bottom: Stimulated cells incubated with anti-inflammatory compounds prior to PMA + LPS stimulation.

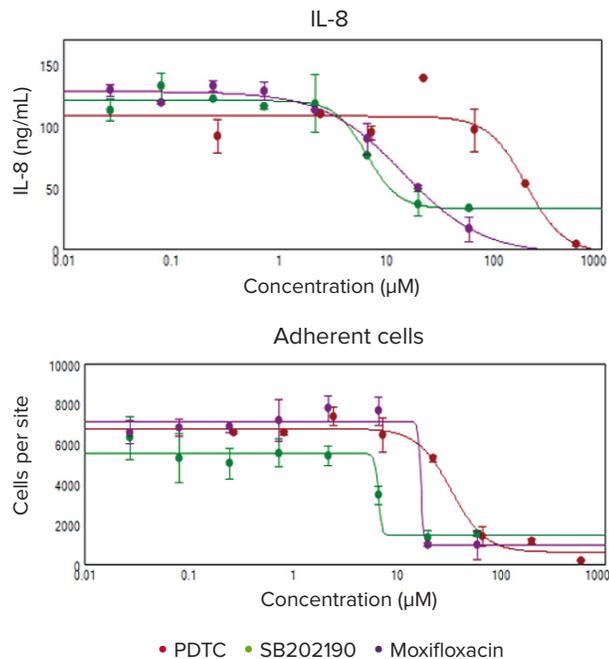


Figure 5. The effect of anti-inflammatory compounds on IL-8 levels and number of adherent cells.

EC ₅₀ , μ M	IL-8	IL-1 β	TNF α	Adhesion
PDTC	201	42	63	33
2B202190	6.6	9.8	6.4	6.6
Moxifloxacin	17.4	9.9	17.9	17.1

Table 1. EC₅₀ values derived from a 4-parameter fit using SoftMax Pro Software.

compounds, the number of adherent cells present before and after washing was determined by image analysis. Cells in the images were counted using CellReporterXpress software, and data were imported into SoftMax Pro Software so that EC₅₀ values could be calculated using a 4-parameter curve fit. Supernatants from these cells were then assayed using the Pu-MA System and SpectraMax iD5 reader to determine the effect of anti-inflammatory compounds on secretion of the chemokine IL-8. Treatment with SB202190, PDTC, and moxifloxacin decreased IL-8 secretion in a concentration-dependent manner and resulted in a decrease in the number of adherent cells (Figure 5). EC₅₀ values determined using a 4-parameter curve fit are shown in Table 1.

Conclusion

We present a multiparametric inflammation assay that uses a THP-1 cell model with the ImageXpress Pico—an automated cellular imaging system—the Pu-MA System—a novel, low-volume automated ELISA system—and the SpectraMax iD5 microplate reader. Treated cells were imaged with the ImageXpress Pico system, and CellReporterXpress software was used to count cells that adhered in response to stimulation. The Pu-MA System was used to perform immunoassays with existing ELISA antibody pairs using a microfluidic flowchip that reduces reagent use by 5-10X and delivers results in less than three hours. ELISA results were detected on the SpectraMax iD5 reader and results were analyzed with SoftMax Pro Software. Using this workflow, we monitored changes in the secretion of chemokines and cytokines (IL-8, IL-1 β , and TNF α) in response to three anti-inflammatory compounds. Observed differences in chemokine and cytokine levels were consistent with published mechanisms of action.

References

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