REACTION BIOLOGY

MACROPHAGE CELL-BASED ASSAYS

- Macrophage Polarization Assay
- Phagocytosis Assay
- Monocyte Killing Assay
- Multiplex Cytokine Assay

Macrophages, integral to the innate immune system, specialize in phagocytosis of pathogens and diseased cells, as well as secreting pro-inflammatory agents. In cancer and chronic inflammation macrophages often gain an anti-inflammatory phenotype and contribute to cancer progression.

Macrophages are characterized by high plasticity, and it has been shown that their polarization state and function can be modulated therapeutically. Therefore, macrophages targeting therapies are considered as next generation cancer immunotherapies.

At Reaction Biology, our scientists have devised a comprehensive array of *in vitro* assays tailored to studying macrophage function, specifically focusing on their involvement in the tumor microenvironment.

Macrophage Polarization Assay

Format: Flow cytometry

Phenotype via surface marker & cytokine release

Phagocytosis Assay

Format: Flow cytometry

Analysis of cellular response & live-cell imaging using labeled bioparticles

Monocyte Killing Assay

Format: Plate based detection

Quantifying tumor cell viability through luciferase expression

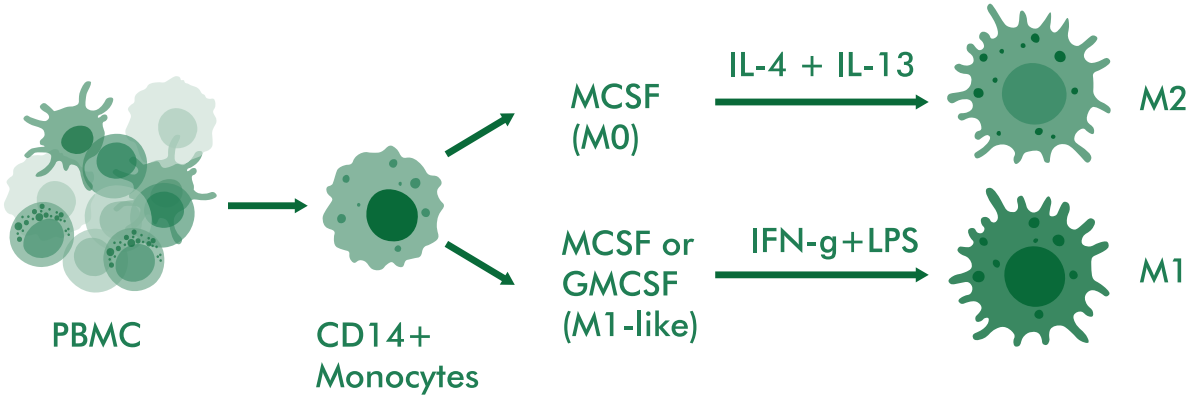
Multiplex Cytokine Assay

Format: Sandwich ELISA

Quantifying cytokine response

Macrophage Polarization Assay

Test the effect of your immunotherapeutic compound on macrophage differentiation, within the tumor microenvironment or in response to inflammation and autoimmunity.

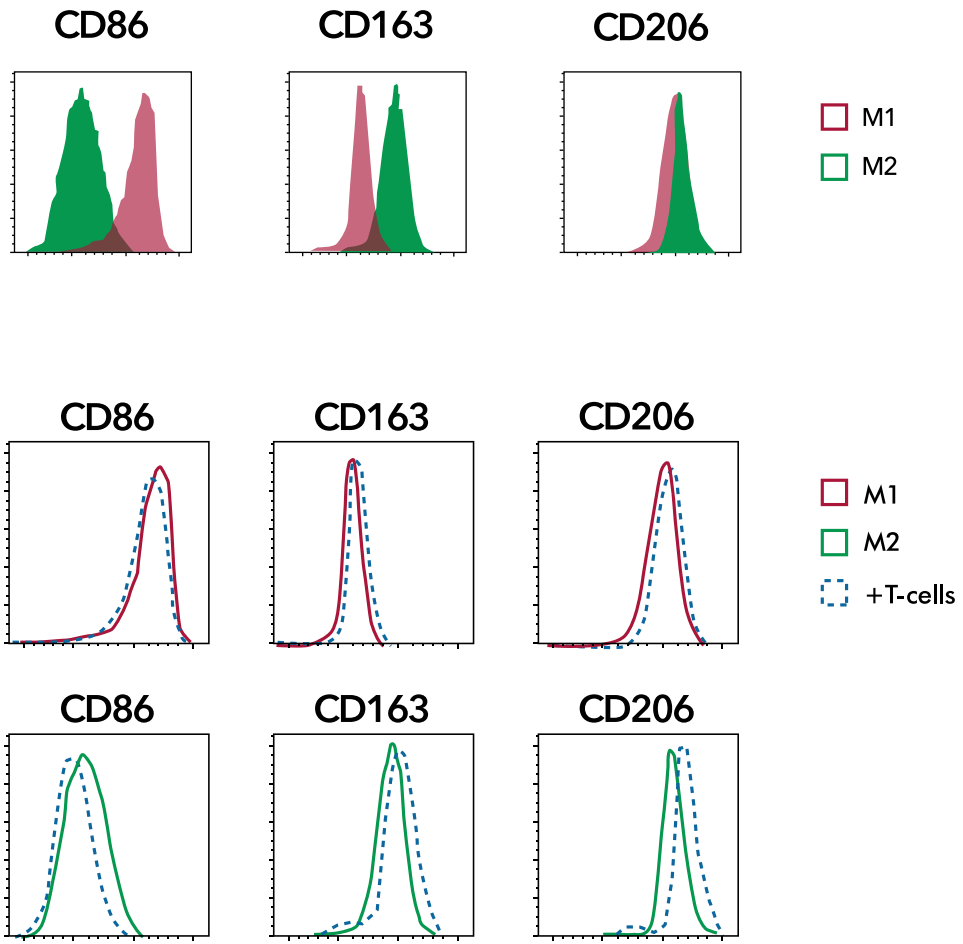


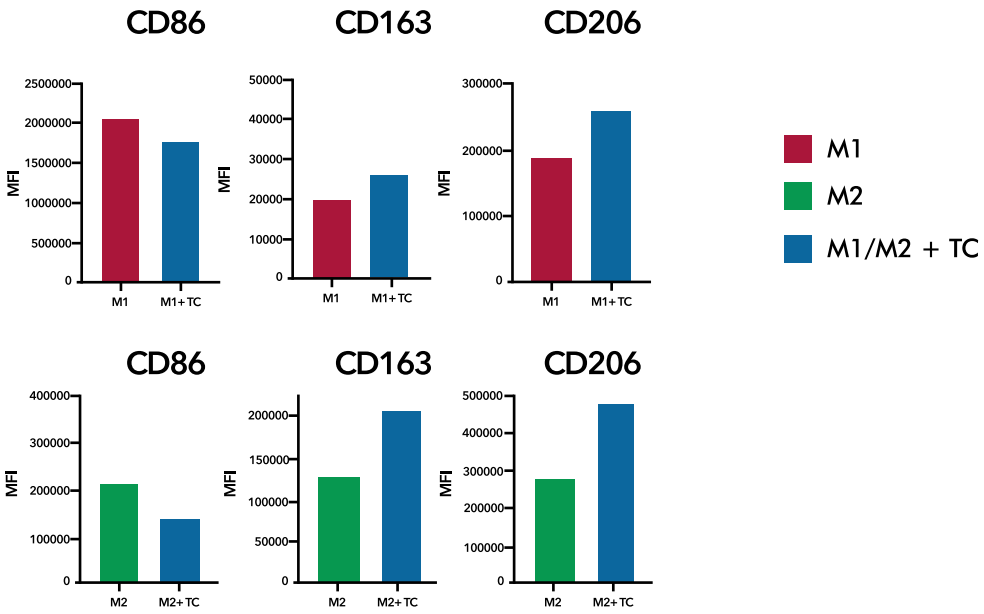
Workflow depicting the isolation of CD14+ monocytes from donor PBMCs and their polarization into M1 and M2 macrophages

Experimental workflow

- Isolation of CD14+ monocytes from healthy donor PBMCs.
- Culturing cells under specific conditions to promote polarization into:
  - Pro-inflammatory M1 macrophages
  - Anti-inflammatory M2 macrophages

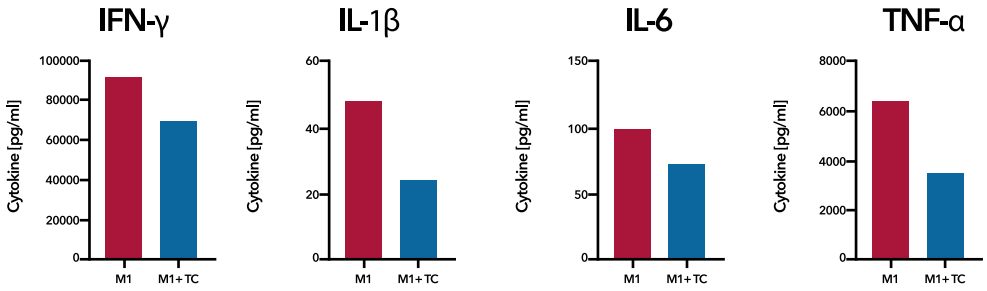
- Verification of the differentiation process through flow cytometry, identifying key surface markers such as CD86, CD163, and CD206 to distinguish macrophage phenotypes





Assessment of macrophage differentiation into specific phenotypes through the analysis of M1/M2 phenotype markers using flow cytometry. TC=Test compound

- Combine cell phenotype with functional assays and cytokine measurements indicative of M1 and M2 types



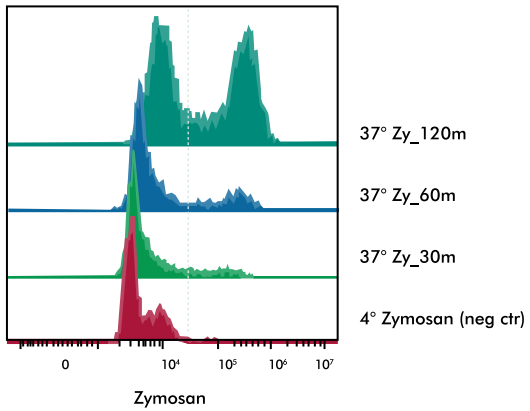
Multiplex cytokine analysis to assess signature phenotype of macrophage differentiation and polarization

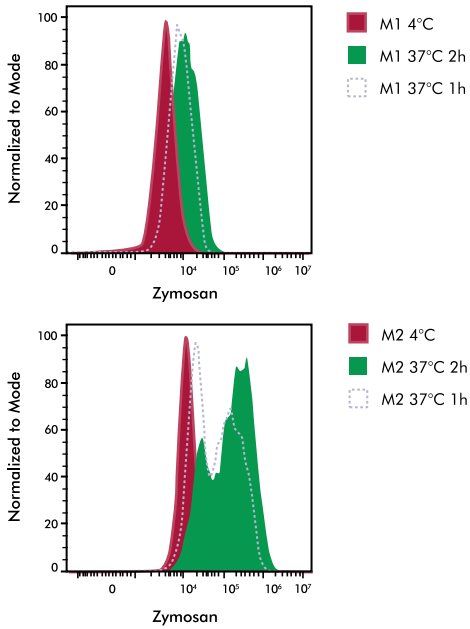
Phagocytosis Assay

Assess the phagocytic capabilities of various monocyte/macrophage subtypes utilizing labeled bioparticles

Experimental workflow

- Use of fluorescently tagged bioparticles, such as Zymosan-pHrodo and Ecoli-pHRodo
- Assessment of phagocytic activity by measuring internalization of flow cytometry analysis or real-time measurements using microscopy
- Comparing phagocytic capacity of various macrophage types or macrophage treatments





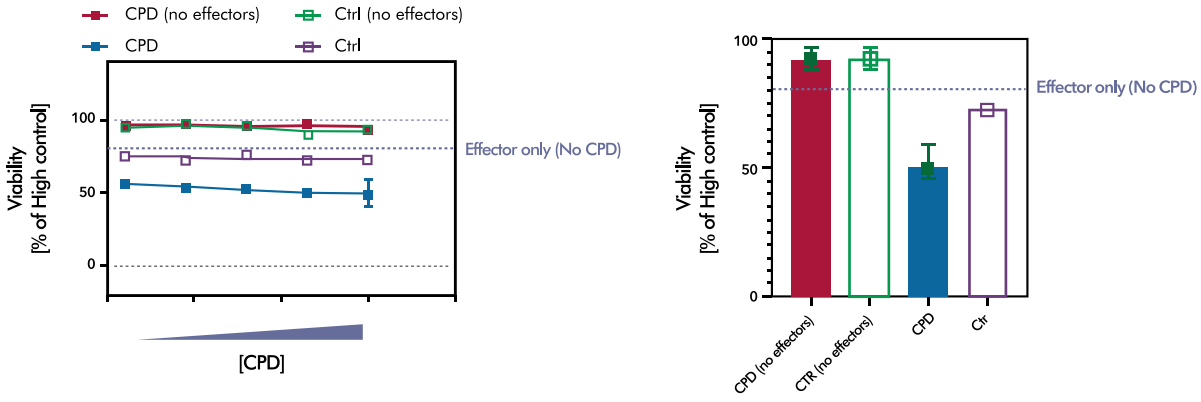
Representative histograms depicting monocyte (left) and polarized macrophage (right) phagocytosis using zymosan reporter particles as measured by flow cytometry

Monocyte Killing Assay

Investigate the efficacy of biologics by measuring their ability to kill target cells and induce monocytes to release inflammatory cytokines

Experimental workflow

- Isolation of CD14+ monocytes from PBMCs
- Co-culturing with target cells that express luciferase, at 10:1 (E:T ratio) effector to target ratio, meaning ten monocytes for every target cell
- Assessment of luciferase expression as a readout of viability of target cells
- Optional: detection of inflammatory cytokine secretion in cell culture supernatants



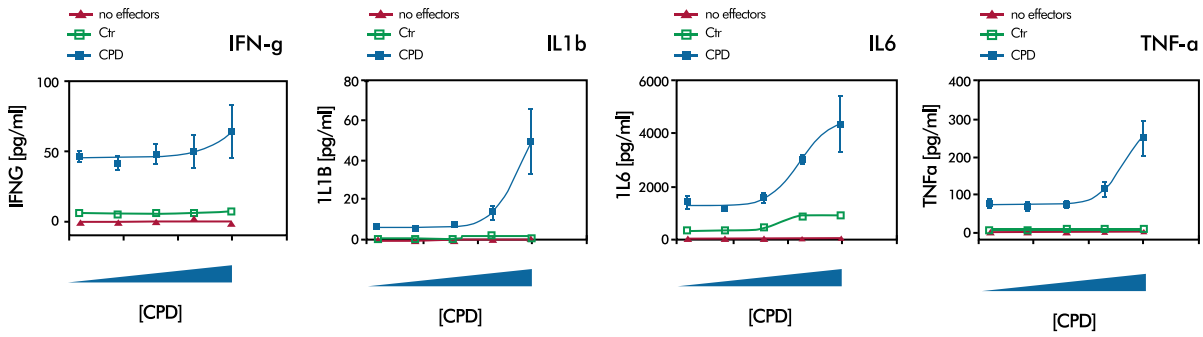
Representative data showing killing of luciferase-expressing target cells by CD14+ monocytes stimulated with increasing compound concentrations

Multiplex Cytokine Assay

Measure cytokines released upon differentiation or activation of immune response

Experimental workflow

- Isolation of CD14+ monocytes from PBMCs
- Co-culturing with target cells that express luciferase, at 10:1 (E:T ratio) effector to target ratio, meaning ten monocytes for every target cell
- Supernatant collection upon activation of immune response
- Cytokine level detection using electrochemiluminescent multiplex immunoassay (MSD)



Representative data demonstrating cytokine release detected in cell culture supernatants from monocytes co-cultured with target cells with increasing compound concentrations response. The cytokine release is detected using MSD multiplex assay

Let's Discover Together

Recombinant Proteins

- Kinase proteins
- Epigenetic proteins
- Substrates
- Custom-tailored protein production

Target-Specific Assays

- Biochemical and cell-based assays
- Enzymatic activity testing
- Protein: Protein Interaction assays
- Receptor Biology

Cellular Oncology

- 2D and 3D proliferation assays
- Drug combination screening
- Invasion and migration assays
- Angiogenesis assay

Biophysical Assays

- Surface Plasmon Resonance
- Thermal Shift Assay
- Isothermal Titration Calorimetry
- Microscale Thermophoresis

In Vivo Pharmacology

- In Vivo Hollow Fiber Model
- Xenograft models
- Orthotopic models
- Metastasis models

Safety & Adme-Tox

- Cardiac Safety Panel
- CYP inhibition
- PK/PD studies
- In Vitro Safety Panel

Integrated Drug Discovery

- Target research
- Hit identification
- Hit-to-Lead
- Lead optimization

Biomarker Discovery

- Genomic biomarkers
- Protein biomarkers
- Immunophenotyping

Immuno-Oncology

- In Vitro Killing Assays
- Syngeneic Mouse Models
- Propriatary Tumor Models
- Immunophenotyping

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Unveil the impact of your immunotherapy on macrophages in the tumor environment

– Let's discover together