A Cytometric Assay for Macropinocytosis in RAW 264.7 Cells

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Background
Macropinocytosis

Methods

• Cell culture sapphire flash capes to form vesicles 0.2-5 μm in diameter which contain significant volumes of extracellular fluid/solutes
• Stimulated by growth factors, chemokines, certain pathogens
• Dependent on PLC, PKC, PI3K, actin, clathrin/CRS
• Macropinocytosis Tracer = FITC dextran, 10-42 kDa

Measurement of Macropinocytosis

Cytometric Quantitation of FITC-Dextran Uptake

• 10 minute stimulation

Inhibitor Effects on Uptake

Macropinocytosis is a rapid functional response to cell

An Alternate Microplate-based Assay was too insensitive

Microplate Assay of Macropinocytosis RAW264.7 Cells

Ligand Response Summary

- RAW264.7 cells exhibit spontaneous macropinocytosis, and this is increased within 10 minutes in response to multiple ligands.
- Macropinocytosis in RAW264.7 cells can be quantified by flow-cytometry. Fluorescence assays using a microplate assay lack sufficient sensitivity.
- Macropinocytosis can be assayed on 26-48 samples/day/technician.
- The assay for macropinocytosis can be adapted for single-cell assays.
- Macropinocytosis is a rapid functional response to cell signaling that may be of use to the Alliance for Cellular Signaling.

Microscopic Assessment of Macropinocytosis

Tracer is observed in Macrophages at 10 minutes

Rapid Kinetics of Ligand Responses

Timecourse of Macropinocytosis by RAW264.7

Timecourse of Macropinocytosis by BMDC

Ligand Responses

MCSF Dose Response

Antagonists
10 minute Macropinocytosis in RAW264.7

Ligands

- MCSF, UTP, PAF, R-848, CpG, pIpC
- LPS/LBP, LPA, S1P, ISO, TER, PGE, EST

Response Summary

- Ligand Concentrations:
  - UTP, PAF, R-848, CpG
  - LPS/LBP, LPA, S1P
  - ISO, TER, PGE, EST

- Fold Increase
  - MCSF + UTP = <additive
  - MCSF + PAF = at 1.25-1.5 fold:

- Ligand Responses:
  - Macrophages
    - Macropinocytosis
    - Efflux of High MW molecules is slow in M
    - Macrophages

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