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Immune stimulation of human induced pluripotent stem cells (hiPSC)-derived glia with lipopolysaccharide (LPS)

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We use this protocol and it's working

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Abstract

Human induced pluripotent stem cells (hiPSC) are widely used as a human development and disease model system. However, for the development of optimized disease models as platforms to uncover new drugs, human patient-derived iPSCs must effectively differentiate into cell states that faithfully recapitulate hallmarks of diseased cells and tissues. Lipopolysaccharide (LPS) is a potent activator of immune cells, including B cells, monocytes, macrophages, and other LPS-reactive cells like microglia and astrocytes. LPS can trigger the secretion of cytokines like interleukin-6 (IL-6), interleukin-1β (IL-1β), or TNF-α, the expression of inflammatory markers (HLA-DR, CD68), changes in cell morphology, and activation pathways to respond to the immune challenge. The mechanism behind LPS involves the activation of toll-like receptor 4 (TLR4) and nuclear factor kB (NFkB). Considering that LPS is a pro-inflammatory bacterial antigen widely used in the literature as an activation stimulus, this protocol describes the application of LPS to activate iPSC-derived microglia in vitro. We propose the use of LPS as a positive control for microglia activation assays (e.g., phagocytosis assay, cytokine production and secretion), and as a phenotyping tool to investigate disease-mutated microglia by comparing to disease-relevant stimuli. As an analysis endpoint, this protocol suggests two main readouts: morphology index and expression of inflammatory biomarkers in fixed cells, and measurement of IL-6 in cell culture media.

Materials

1. Cell culture vessels (PerkinElmer, CellCarrier Ultra 96, Cat. No. 6055300)
2. RPMI 1640 medium (Thermo Fisher, Cat. No. 11875093) or Advanced RPMI 1640 medium (Thermo Fisher Scientific, Cat. No. 12633020)
3. PBS Solution (to aliquot LPS; Life Technologies, Cat. No. 14200-166)
4. Fetal Bovine Serum (Fisher Scientific, Cat. No. SH30084.03HI)
5. GlutaMAX supplement (Thermo Fisher, Cat. No. 35050061) to supplement cell culture media
6. Lipopolysaccharide (LPS) (Sigma-Aldrich, Cat. No. L2654-1MG)
7. Vortex
8. Sonicator (water bath)

REAGENT INFORMATION AND PREPARATION

1 **Lipopolysaccharide (LPS) aliquot preparation**

1.1 **LPS solubility:** LPS are molecules that form micelles in every solvent. This explains the hazy solutions observed in water and PBS. Organic solvents will not give clearer solutions.
At very low concentrations (<0.1 mg/mL in water), LPS tends to stick to containers of certain types of glass or plastic. If the LPS concentration is > 1 mg/mL, adsorption to the sides of the vial is negligible. Therefore, we recommend preparing 1 mg/mL LPS in PBS of 100 µL and store in polypropylene microcentrifuge tubes.

1.2 **LPS stability in solution:** Solutions (1 mg/mL) in PBS/saline or tissue culture medium can be stored frozen in aliquots (recommended for long-term storage). Repeated freeze/thaw cycles are not recommended. Prepare single-use aliquots. Store at -20 °C or -80 °C.
LPS is stable in Phosphate-Buffered Saline (PBS) at 1 mg/mL at 0-4 °C for a month.
Before making any dilutions, aliquoting or adding it to cell medium, it is crucial to vortex and sonicate your stock tube immediately.

2 **LPS resuspension and aliquot preparation**

2.1 Add 1 mL of PBS to the 1 mg vial of lyophilized LPS;

2.2 Vortex and sonicate for 5-10 min in continuous pulse (frequency 20-40kHz).

2.3 Check if the suspension looks uniform. Aliquot immediately.

2.4 Storage at -20 °C or -80 °C.

LPS STIMULATION OF IMMUNE CELLS

3 Most incubations with LPS can be done for less than 24 hours.
The working concentration range is **0.1 to 5.0 ng/mL** (dilute in fresh media), depending on the

cell type and cell culture media (with or without serum). We recommend titrating your system to see what concentration and time points work best.
Serum-free media may require higher LPS concentrations (5.0 to 50 ng/mL).

PROCEDURE

4 Day -1

4.1 Plate your cells in the desired culture vessel (seeding range 30,000 to 65,000 cells/cm2) with the desired coating and desired number of replicates (we recommend triplicates for each condition, cell line, etc). We suggest a 96-well plate. Let them attach overnight.
OBS: We recommend this step one day before stimulation as cell attachment won't be influenced by activation, therefore the seeding among experimental groups will be more uniform.

5 Day 0

5.1 Label two flasks: one labeled 'stimulated' (activated) and the other labeled 'non-stimulated' (control).

5.2 Prepare complete cell medium by adding serum to a final concentration 10% for RPMI 1640, or 0.5% for Advanced RPMI. Supplement with 1% GlutaMAX if needed. Bring medium to 37°C.
Optional: Supplement media with 1% penicillin-streptomycin (5,000 units/mL).

5.3 Prepare the volume based on the flask/well size you will be using. For a 96-well plate, we recommend 200 µL of cell suspension per well. Divide the complete medium between the two flasks, making sure you have enough medium volume for both conditions.

5.4 Thaw the LPS aliquot and sonicate for 5-8 min, continuous pulse. Afterward, pipette directly to final cell culture media or a pre-dilution with PBS and use within 30 min. It is advised that the PBS-LPS volume pipetted into cell culture media does not surpass 15% of the total volume.

5.5 Add lipopolysaccharide (LPS) at the desired concentration into the stimulated (activated) flask. Add PBS (vehicle) in the control medium flask. Aspirate cell medium from the wells and add the new cell medium (control and stimulated) on top of attached cells.

5.6 Incubate both flasks for 24 h or the desired time in a 5% CO2 incubator at 37°C.

6 Day 1 (and forward)

6.1 Collect media for IL-6 dosage. We recommend fixing cells afterward to evaluate morphology and activation markers.