Assay of phagocytic function in primary murine microglia

Noel Derecki (ncd3z@virginia.edu)
Kipnis Lab, University of Virginia
James Cronk (jcc2cm@virginia.edu)
Kipnis Lab, University of Virginia
Jonathan Kipnis (kipnis@virginia.edu)
Kipnis Lab, University of Virginia

Method Article

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Abstract

Here, we describe a method for determining phagocytic function of microglia isolated from mouse brains.

Procedure

To determine the phagocytic ability of microglial cells, we prefer to use apoptotic neural cells as targets, since they should most closely approximate natural microglial targets in vivo. 1. Dissociate neural progenitor cells in 0.1M PBS + 2mg/ml papain \(Worthington) at 37º C for 30 m. 2. Treat dissociated neural progenitor cells \(NPC) with UV light for 15-20 minutes; NOTE: this should be done in a thin plastic petri dish; thick plastic \(eg. 15 or 50 ml conical tube) will block a significant portion of UV light, leading to suboptimal apoptosis. 3. Wash NPC in 50 ml conical tube with 40 ml PBS \(RT), spin at 1200 RPM, 7 m, RT, low brake. Decant supernatant. 4. Resuspend NPC in 5 ml RT 0.1M PBS 5. Add 5 ul sta 5\(6)-TAMRA, SE \(Invitrogen) to NPC, vortex immediately and let incubate at 37º C in dark for 15 m. 6. Wash 3X \(as in Step 3) with 40 ml ice-cold 0.1M PBS. 7. Resuspend in 1 ml DMEM/F12 \(Invitrogen) + Anti-anti \(Invitrogen); count cells using a hemacytometer. Dilute or concentrate to 5 X 10^6 per ml. 8. Feed onto microglial cells \(previously allowed to adhere to glass cover slips in 24-well plates) in 900 ul DMEM/F12 + 10% fetal bovine serum \(Atlanta Biologicals) + Anti-anti at a ratio of 10 NPC:1 microglial cell. NOTE: We typically perform experiments using 5 X 10^4 microglia per coverslip such that cells are spaced sufficiently to allow later quantification, thus 5 X 10^5 NPC would be fed on in 100 ul DMEM/F12 + Anti-anti. 9. Place cells in incubator, at 37º and 5% CO2 10. At desired timepoints \(eg. one, two and five h) remove plates from incubator 11. In a sterile hood, remove cover slips with forceps, dip 20 X briskly in warm 0.1M PBS \(to remove non-phagocytosed NPC), then place cover slips in 1 ml 4% PFA in a 24-well plate. Replace remaining cover slips in incubator until next desired time-point. 12. Fix cells on cover slips in dark for 20 m. Wash 3X with 0.1M PBS, at RT. 13. Block cover slips in 10% serum in PBS containing 0.3% Triton X-100 and 0.5% BSA, 1 h, RT in dark. 14. Incubate with anti-CD11b \(eBioscience), 1:100) for 1 h at RT, in dark. 15. Wash cover slips 3X with 0.1M PBS, RT. 16. Incubate with desired secondary antibody \(1:1000, Invitrogen) for 1 hour at RT. 17. Wash cover slips 1X with 0.1M PBS, RT. 18. Incubate in DAPI \(1:20,000) 5 m. 19. Wash cover slips 3X with 0.1M PBS, RT. 20. Mount cover slips with Aqua-Mount \(Lerner Laboratories) on slides. Let dry O/N before imaging. 21. Typically, a “phagocytic index” can be calculated by dividing the total area of microglial cells \(CD11b+) by the total area of labeled NPC. Alternatively, number of phagocytosed targets/cell may be used to quantify phagocytosis. 22. To check validity of phagocytosed \(internal) vs. adhered targets, Z-stacks may be imaged on confocal microscope.

Timing

NPC dissociation, 30 m NPC labeling, 45 m Incubations, variable \(1+ h) Immunohistochemistry, 3 h

Troubleshooting

Problem: Little or no phagocytosis observed Solution 1: NPC were not adequately irradiated; use stronger UV source, extend irradiation time, confirm cell death by dye or antibody-based assay \(eg. annexinV, PI). Solution 2: Insufficient incubation time; extend incubation.

Anticipated Results

Microglia are highly phagocytic cells. Even after a short period of time, high levels of phagocytosis may be seen, as in Figure 1.

Figures

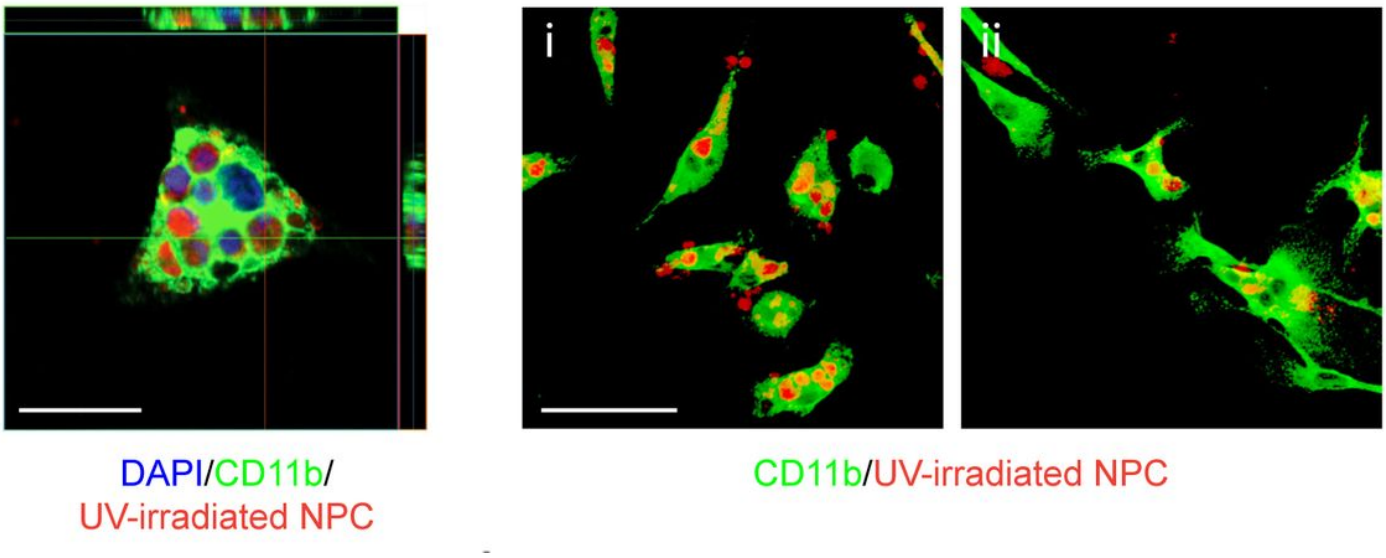

Figure 1

Microglial Phagocytosis At left is shown a representative Z-stack indicating that UV-irradiated TAMRA-labeled NPC (red) have been phagocytosed and are situated inside of microglia. Immunolabeling for CD11b is shown in green and nuclei are indicated by DAPI (blue). At right are shown representative images of microglia immunolabeled with CD11b (green) after having phagocytosed UV-irradiated TAMRA-labeled NPC (red) for 2 h.