**AUTHORS**

Michela Deleidi3,4, Bianca Marchetti1,2, Federico Bertoli3,4, Carmela Giachino1

1Neuropharmacology Laboratory, Oasi Research Institute-IRCCS, Troina, Italy

2 Biomedical and Biotechnological Sciences, Pharmacology Section, University of Catania-Italy

3 Mitochondria and Inflammation in Neurodegenerative Diseases, DZNE, Tübingen-Germany

4 Hertie Institute for Clinical Brain Research, University of Tübingen

**ABSTRACT**

Ex vivo culture of spleen derived macrophages

**PROTOCOL**

Spleens were dissected from abdominal cavity and filtered through a 40-μm nylon strainer. Red cell lysis buffer was used to remove red cells. A single splenic cell suspension then was obtained Cells were cultured in Roswell Park Memorial Institute (RPMI) medium 1640 RPMI 1640 (BioConcept 1-41F01-I) supplemented with 10% FBS, 2mM L-Glutamine and antimicrobials (Penicillin-Streptomycin Pen 10'000 IU/ml Strep 10 mg/ml and amphotericin B (250 μg/ml BioConcept). Mouse microglia BV2 cells from Elabscience (No.: EP-CL-0493) were cultured in parallel for each spleen culture preparation and served as controls. Briefly, BV2 cells were maintained in Roswell Park Memorial Institute (RPMI) medium 1640 supplemented (BioConcept 1-41F01-I) with 10% FBS (FBS-02-0500), 2mM L-Glutamine 5-10K50-H) and antimicrobials (Penicillin-Streptomycin Pen 10'000 IU/ml Strep 10 mg/ml and amphotericin B (250 μg/ml) (BioConcept 4-01F00-H). Spleen macrophages (SPMs) differentiate into the M1 phenotype after stimulation with LPS (100 ng/ml) ± IFN-γ (10 ng/ml). For BV2 stimulation, RPMI was replaced by Dulbecco’s Modified Eagle Medium (DMEM) High Glucose. (BioConcept, 1-26F03-I).