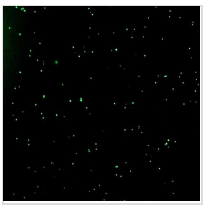

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Chlamydia pneumoniae-Induced Neuroinflammation Cell Model Using Lyophilized Cell-Free Supernatant

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Chlamydia pneumoniae (Cpn) is a gram-negative intracellular pathogen that causes a variety of pulmonary diseases, and there is growing evidence that it may play a role in Alzheimer's disease (AD) pathogenesis. Cpn can interact functionally with host histones, altering the host's epigenetic regulatory system by introducing bacterial products into the host tissue and inducing a persistent inflammatory response. Because Cpn is difficult to propagate, isolate, and detect, a modified LPS-like neuroinflammation model was established using lyophilized cell free supernatant (CFS) obtained from infected cell cultures, and the effects of CFS were compared to LPS.

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protocol

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HEp-2 human epithelial carcinoma cell line (ATCC CCL-23)
Chlamydia pneumoniae (ATCC 53592)
Pathfinder Chlamydia Culture Confirmation System (Cat. No. 30701, Bio-Rad, Germany)

1 HEp-2 cells were used as a host to inoculate Chlamydia pneumoniae. A 6-well plate of 1X106 HEp-2 cells was seeded 48 hours prior to inoculation with Chlamydia pneumoniae (ATCC 53592).

2 The suspension of elementary bodies diluted in infection medium was added directly to wells.

3 The mixture was centrifuged at 1500 × g for 1 h.

4 The plate was incubated for 1 h at 37 °C in the presence of 5% CO2.

5 Current medium was discarded.

6 Cells were washed with 300 µL Hanks Balanced Salt Solution.

7 500 µL fresh medium was added to the wells.

8 The plate was incubated for 72 hours.

9 The inclusion bodies were confirmed using the Pathfinder Chlamydia Culture Confirmation

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System (Cat. No. 30701, Bio-Rad, Germany) according to the kit manual.

10 The cells were imaged using the Cytation 3 Cell Imaging Multi-Mode Reader (BioTek, USA).

11 The number of inclusion-forming units per milliliter (IFU/ml) in HEp-2 cells was used to determine the infectivity titers of chlamydial stocks and 1x106 HEp-2 monolayers in 6-well plate were contaminated with Chlamydia pneumoniae suspended in inoculating media at 1 multiplicity of infection (MOI) ratio.

12 Cell-free supernatant was collected from the wells, lyophilized and stored in aliquots at -80 °C.

13 The lyophilized cell-free supernatant was weighed to prepare a main stock in the desired ratio and used to trigger the inflammatory response.

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