

Dual role of microglia in Alzheimer's disease and polarizing effect of cytokines

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Abstract. Background: Alzheimer's disease (AD) is a neurodegenerative disease characterized by abnormal accumulation of A β and neuroinflammatory responses. Microglia are key immune cells in the central nervous system and have important regulatory functions in the control of neuroinflammation and neurodegenerative diseases such as Alzheimer's disease. Methods: Microglia have both neuroprotective and neurotoxic effects, but the mechanism by which they protect or damage the nerve remains unclear. Here, I used an in vitro cell culture approach to investigate the role of microglia in neuroinflammation and its regulation of amyloid- β peptides. The same methods were used to investigate the effect of cytokines on microglial polarization and the clearance of amyloid- β . Expected conclusion: Cytokines such as IL-4 and TGF- β can promote the polarization of microglia to M2 direction and enhance its anti-inflammatory effect. Pro-inflammatory factors can induce its polarization to M1 direction and aggravate the degree of neuroinflammation. Therefore, microglia can not only clear amyloid beta, but also damage the nerve and accelerate the process of Alzheimer's disease. By studying this mechanism, I will find ways to promote the large-scale conversion of microglia to M2, which will provide new directions for Alzheimer's disease research and help develop new treatments.

Keywords: Alzheimer's disease, amyloid beta, microglia, cytokine, inflammatory response.

1. Introduction

1.1. Amyloid beta production and interaction with microglia

A β production is primarily governed by breaking down the amyloid precursor protein (APP). Subsequent to the activities of β -secretase and γ -secretase, A β monomers emerge and later converge to create neural plaques. A range of elements, encompassing genetic aspects, environmental influences, and inflammatory reactions, govern this procedure.[1]

The accumulation of A β not only directly causes neurotoxicity, but also activates microglia, triggering chronic inflammation and further aggravating nerve damage. In neuroinflammation, microglia are activated, showing morphological and functional changes. This activation is usually accompanied by alterations in cell surface markers, such as up-regulation of CD68 and CD11b. When microglial cells sense pathological signals, they rapidly activate and change their morphology. Activated microglia release various inflammatory mediators, such as cytokines IL-1 β , TNF- α , IL-6 and chemotactic factors to recruit other immune cells and enhance local inflammation. [2] Due to their phagocytic ability, microglia can clear amyloid β plaques in the brain and exert neuroprotective effects.

Excessive stimulation or ongoing inflammation in neurons could result in neurotoxicity and harm. [3] Considering the twin functions of microglial cells in neurodegenerative conditions investigating their roles and control mechanisms could shed light on creating novel therapeutic strategies.

1.2. Relationship between M1,M2 polarization and neuroinflammation

The activation of microglia can be divided into two main types: M1 and M2. In the process of neuroinflammation, the polarity states M1, M2 of microglia cells have significant impacts on neuroprotection and damage. M1 microglia are typically activated in acute inflammation, primarily by releasing pro-inflammatory cytokines IL-1 β , TNF- α to clear pathogens and damaged cells. Nonetheless, an overabundance of M1 activation may trigger a relentless cycle of neuronal harm and inflammation. [4] Conversely, M2 microglia are crucial in the repair and anti-inflammatory processes during the advanced stages of inflammation, aiding in the nervous system's restoration through the release of anti-inflammatory substances IL-10, TGF- β and fostering neuroprotective actions. [5]

1.3. Interaction of cytokines with microglia

Cytokines play an important regulatory role in the interaction between microglia and neurons. For example, Antiinflammatory cytokines such as IL-10 and TGF- β can promote the polarization of microglia to M2 type and enhance its ability to clear A β . IL-1 β , TNF- α and other pro-inflammatory factors may lead to the transformation of microglia into M1 type, and then trigger neuroinflammation. Therefore, regulating the levels of cytokines may become a strategy for the treatment of Alzheimer's disease. [6]

1.4. Role of signaling pathways

A variety of signaling pathways impact the activation of microglia and the governance of A β . As an example, activating NF- κ B, JAK/STAT, and MAPK. The pathway of NF- κ B signaling is crucial in the formation of M1-type microglia, and inhibiting this pathway could improve the polarization of these microglia, potentially reducing neuroinflammation and the buildup of A β . JAK-STAT: [7] NF- κ B: [8]

2. Experimental design

2.1. Objective

To explore whether microglia plays a protective or aggravating role in different degrees of neuroinflammation, because microglia has a dual role.

2.2. The Design of Experiments

Microglia and neurons were cultured in 6-well plates with same concentration. The experiment was divided into experimental group and control group. Control group : no stimuli are added. Experimental group: The first one add LPS (1 μ g/mL) Mimics low levels of neuroinflammation and the second add TNF- α (10 μ g/mL) Mimics high levels of neuroinflammation. Then, the treated microglia were cultured with neuronal cells. The specific process is as follows microglia were removed and washed with PBS, 2 mL of neurons were added to each cultured for 24 hours. In the last 12 hours after co-incubation, add A β 1-42 with 0.5 μ M to each well. After co-culture, MTT assay was used to evaluate cell activity. 20 μ L MTT solution (5 mg/mL) was added to each well and continued to be cultured for 4 hours. Then, add 150 μ L DMSO, mix it and Put it in the enzyme marker. [9] Next we have to detect of inflammatory factors. Centrifuge with 300 g, 10 min to remove cell residue. Use ELISA kits IL-1b to detect the concentration of inflammatory factors in the medium. [10] Then extract protein and Western Blot: The cells were washed with PBS, followed by lysis buffer which is RIPA buffer + protease inhibitor at 200 μ L per well for 10 min. And Centrifuge in 12000 \times g, 10 minutes, take the supernatant, and determine the protein concentration. Use SDS-PAGE gel to electrophoresis. And, transfer the membrane and Antibody incubation. [11] Finally, analyse data: The cell activity, inflammatory factor concentration and target protein expression were compared among all groups.

2.3. Expected result1

A group of microglia with high inflammation may have a more neuroprotective effect, effectively clearing amyloid beta.

2.4. Objective2

Prove that cytokines can promote microglia to M2 and play a role in neuroprotection.

1. Select C57BL/6 mice aged 2-4 weeks. First, the mice were anesthetized, and then the brains of the mice were removed under sterile conditions. The removed brain tissue was cut into small pieces and placed into a centrifuge tube. Filtration was performed, and the suspension was taken for centrifugation at 1500 rpm for 5 min at 4 ° C. Cultures were made with 5 mL DMEM/F-12 medium and cell concentrations were recorded.[12] Microglia were seeded into 12-well culture plates with 1 mL of medium per well.

2. experimental group: add IL-4 with 10 ng/mL concentration and the culture continued for 48 hours. Control group: At the same time, give the same volume of DMEM/F-12 + 10% FBS

3. extract RNA: use PBS wash the cells and then Add 1 mL TRIzol, mix well, lysate the cells.[13] Add 200µL chloroform, shake gently, let stand for 5 min, centrifuge with 12000 rpm, 15 min, 4°C. Remove the supernatant and place it in a new centrifuge tube. The RNA was precipitated and determine the RNA concentration.

4. PCR: reaction mixture include DNA 1 µL, RNA primer 1 µL, SYBR Green qPCR Master Mix 10 µL, water 8µL. 95°C, 15s in Denaturing, and 60°C, 30s in annealing and extending. Last, analysis Running data.[14]

5. protein extraction and concentration determination: RIPA cracking buffer 200 µL was added for cracking for 20 min. Then Centrifuge at 12000 rpm for 10 minutes, take the supernatant, pour it into a new centrifuge tube, and store it at -80°C. BCA protein concentration assay kit was used. The sample and the standard solution were added to the 96-well plate, and then the BCA reagent was added, mixed, and stored at 37°C for 30 minutes. Finally, the 96-well plate was put into the enzymometer to determine the absorbance.

6. SDS-PAGE gel electrophoresis: first, Preparation of 10% SDS-PAGE gel and 4% polyacrylamide. Then, A 30 µg protein sample is mixed with a 4× buffer and boiled for 5 minutes to denature. The sample is put into the electrophoresis apparatus for electrophoresis.[15]

7. Transfer the membrane and Antibody incubation: The electrophoretically separated proteins were transferred from the gel to the PVDF mode. Appropriately diluted primary antibody Arg1, CD206 was incubated at 4 °C for 12 hours. Then wash with PBS to remove unbound primary antibodies. Next, add HRP tagged secondary antibodies with 1 hour, and wash with PBS. [16]

8. analyse data : The gene expression data of the experimental group and the control group were analyzed, and the expression levels of Arg1 and CD206 were compared. The protein expression levels of M2 polarization markers Arg1 and iNOS were compared to find the difference between the experimental group and the control group.

2.5. Expected result 2

IL-4 can promote the M2 polarization of microglia.

3. Conclusion

3.1. Conclusion 1

Through the first experiment, we can learn whether microglia has more neuroprotective than destructive effects or more destructive effects in different degrees of neuroinflammation. Therefore, we can further explore the influence of neuroinflammation degree on microglia polarization in the future. Thus, microglia was able to transform more into M2, clearing amyloid beta, and further developing drugs that could treat neurodegenerative diseases such as Alzheimer's disease.

3.2. Conclusion 2

Approaches targeting cytokines and their communication pathways might surface as innovative therapeutic techniques, such as the use of anti-IL-1 β and IL-6 inhibitors, or the direct delivery of cytokines like IL-4 and IL-13. Microglia play an important role in the pathogenesis of neuroinflammation and Alzheimer's disease. They regulate the accumulation and clearance of A β through different polarization states, reflecting their dual roles between neuroprotection and neurotoxicity. At the same time, regulating the levels of cytokines to promote the transformation of microglia M2 also has an important potential role in the treatment of Alzheimer's disease, which can not only inhibit the inflammatory response and enhance the clearance function, but also may change the pathological process of the disease.

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