Quantitative Morphological Comparison of Microglia in Normal and Alzheimer's Disease Model Brains

Zihe Zhong
LEH Foshan International School, Foshan 528000, Guangdong, China

Abstract: The aim of this study is to investigate the potential role of microglia in nervous system function by comparing the morphological characteristics of microglia under physiological and pathological conditions. By reviewing the literature in recent years, this study summarizes the distribution, quantity and morphological changes of microglia under different pathological conditions and analyzes their relationship with functional regulation. The results showed that microglia showed significant morphological plasticity in the activated state, suggesting that they were involved in inflammatory response and neural regulation mechanism.

***Keywords*:** alzheimer's disease, microglia,neurodegenerative disease

1. Introduction

In the central nervous system (CNS), the auxiliary functions of neurons and astrocytes include immune defense and homeostasis maintenance[1]. However, recent studies have shown that microglia (MG), as resident immune cells in the CNS, significantly affect the occurrence of many neurological diseases[2]. Microglia can recognize the pathogen, clean up its debris, and respond to its activity through morphological changes[3]. The current functional status of microglia is closely related to its morphological characteristics. In the week, a typical branching morphology, with small cell bodies in the physiological state, elongated protrusions, and more activity. MG will change significantly under pathological conditions, such as brain injury, infection or neurodegeneration. It usually manifests as a shortening or disappearance of the bulge, an increase in the volume of the cell body, and a foamy appearance. When MG is in a pathological state, its function also changes, such as releasing inflammatory factors and enhancing phagocytosis of surrounding neurons[4].

This study aims to compare the morphological differences of MG in physiological and pathological states and explore its potential functional changes in the context of cerebral ischemic injury, such as stroke.

2. Materials and methods

The anesthetic dose was calculated according to the body weight of the mice (the body weight of the mice * solution concentration), and intraperitoneal injection was performed. The abdominal cavity was exposed by cutting the skin along the midline from below the abdomen with scissors. The diaphragm is cut, and the ribs are cut up along both sides of the sternum to expose the entire thoracic area, which should be done slowly to avoid damage to the heart and lung tissues. After full exposure of the heart and identification of its anatomy, precooled (4 ° C) PBS and PTS were slowly injected into the left ventricle using a syringe. The injection flow rate should be controlled, usually 5-10 mL per minute, to avoid cerebral vascular rupture or organ damage due to high perfusion pressure. The criteria for judging the adequacy of perfusion include a change from dark red to light yellow in the liver, a change from blood colour to clear in the effluent, and a lighter colour in the blood vessels throughout the body. Brain retrieval was performed immediately after perfusion to ensure the tissue's freshness and subsequent processing quality. Mouse brains were removed intact, immediately placed in PFA solution, and fixed overnight to cross-link and stabilise biomacromolecules in the tissue.

The fixed brain tissue was transferred into a sucrose solution and immersed at 4°C for 24-48 hours until the tissue sank to remove water and prevent the formation of ice crystals during sectioning to destroy the cells and cause tissue deformation. The dehydrated brain tissue was fixed on the slice tray in OCT and positioned upward. It was frozen until the OCT was solidified entirely and placed in a freezing microtome. The slice thickness was set to 30μm for serial sectioning operations.

Cryosections, with a thickness of 20 to 30 μm, were removed from the cryogenic refrigerator, gently attached to a clean slide, and allowed to dry for 30 minutes until the tissue was firmly attached. The brain slices were rinsed 3 times with PBS buffer for 5 minutes to remove surface OCT residues and impurities.

Brain slices were incubated in PBS buffer containing 0.5% Triton X-100 and 5% Goat Serum for a total of 500μm at room temperature for 1 hour

After removing the permeable blocking solution, diluted primary antibody, Rabbit anti-Iba1, was added dropwise to

each slice of tissue to cover the entire tissue area. The slides were then sealed in a wet box and incubated in a refrigerator at 4°C in the dark for 12 to 16 hours.

After the primary antibody incubation, the brain sections were washed using PBS buffer three times for 10 minutes each to remove the unbound primary antibodies. Subsequently, the sections were incubated in a 1:250 dilution of Cy3-labelled Jackson Immunodetect secondary antibody solution for 1 to 2 hours in the dark at room temperature. After completion of incubation with secondary antibodies, the sections were rewashed with PBS 3 times for 5 min each to remove excess antibodies. The nuclei were stained with DAPI and incubated in the dark for 10 min. DAPI binds to DNA and visualises nuclear morphology under a fluorescence microscope.

After completion of staining, the sections were rewashed with PBS, followed by drops of sealed tablets, covered with coverslips, and allowed to dry in the dark at room temperature to minimise fluorescence signal attenuation. Finally, an upright fluorescence microscope was used for image acquisition. Among them, the Cy3 staining results were observed under the TRITC channel, with an excitation wavelength of about 550 nm and an emission wavelength of about 570 nm, showing orange-red fluorescence.

3. Results

In the control group, the number of microglia was low, and the morphology was mainly small and elongated, showing typical resting state characteristics, with small cell bodies, lengthy processes and uniform distribution.

To compare the number and density of microglia in different groups, the table shows the number of microglia and the corresponding area of microglia density (cells/μm^2) in different image fields of the control group and the AD group. The AD group showed a higher cell density in all fields, suggesting the phenomenon of activation and aggregation of microglia in pathological states. (cells/mm^2), as an indicator of microglia density. Each cell's cell body area (μm^2) was also used as an essential morphological parameter of the activation state.

4. Discussion

This study systematically compares microglia (MG) morphology across healthy, neurodegenerative, and acute inflammatory states, revealing distinct morphological signatures for each condition. In healthy controls, MG exhibit a ramified resting state characterised by small cell bodies, elongated branching processes evenly distributed in 3D space, and uniform tissue distribution without aggregation - consistent with their surveillance role. The findings demonstrate how MG morphology directly reflects their functional state, with resting MG showing fundamentally different structural features than activated states in disease conditions.

Secondly, the AD model group significantly altered the MG number and distribution. It was found that MG showed a precise aggregated distribution around amyloid plaques in the CA1 region of the hippocampus and the cortex, and the cell density was significantly increased compared with the control group [5]. Morphologically, most MG were activated type, with considerably enlarged cell bodies, a reduced number of processes, and some cell processes were rod-shaped.

Thirdly, in models of acute inflammation, such as LPS induction, MG rapidly changes from the resting to the activated type in a short period, showing enlargement of cell bodies, shortening of processes, and even complete loss of branching[6]. Compared with the AD group, MG activation under acute inflammation was more synchronous, with relatively uniform morphological changes and a lack of obvious spatial gradient distribution. In addition, the density of MG distribution did not change significantly in the early phase of inflammation. Still, increased density was seen in the periventricular and hippocampus during the sustained phase of the inflammatory response[7].

5. Conclusion

The present study found that the number of microglia in the Alzheimer's disease (AD) model was significantly increased, with enlarged cell bodies and reduced processes, exhibiting typical morphological characteristics of an activated state, suggesting their involvement in neuroinflammatory responses. These findings support the "morphology reflects function" theory and indicate that microglia may play a crucial role in AD pathogenesis. Although the study had a limited sample size, its conclusions align with existing literature, and future research incorporating larger samples and functional markers could further elucidate the key role of microglia in neurodegenerative diseases.

References

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Author Bio

Zihe Zhong (born 2006), female, Han Chinese, from Guangdong Province. Research interests: Brain science.