THE SPATIAL AND TEMPORAL DYNAMICS OF MICROGLIA CELLS FOLLOWING TRANSIENT FOCAL CEREBRAL ISCHEMIA

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INTRODUCTION

In an ischemic stroke, cerebral ischemia and cell death induce a series of inflammatory responses in the Central Nervous System (CNS) collectively known as neuro-inflammation. Microglia cells play a critical role in neuro-inflammation. They are the major resident immune cells in the CNS and the major trigger of the innate immune response. Microglia cells communicate with astrocytes, neutrophils, dendritic cells, T and B lymphocytes, and pericytes, and participate in the inflammation in the brain parenchyma, the blood brain barrier, and inside the blood vessel. Interestingly, the activated microglia cells can be pro-inflammatory and anti-inflammatory. In the pro-inflammatory state, they release pro-inflammatory cytokines, reactive species and proteases to induce inflammation, while in the anti-inflammatory state, they release not only anti-inflammatory mediators, but also growth factors and neurotropic factors to reduce inflammation and facilitate neurogenesis and neuroplasticity for recovery. They also contribute to recovery by acting as phagocytes. Stroke therapies that target neuro-inflammation show great promise as neuro-inflammation takes days to develop and may persist for months, which translates to a long time-window for therapy. Microglia cells polarize into a spectrum of phenotypes that have different levels of pro-inflammatory and/or anti-inflammatory activity in response to the CNS microenvironment. Therefore, it is valuable to characterize the spatial-temporal dynamics of microglial cell polarization post cerebral ischemia.

METHODOLOGY

Male, wild-type Sprague Dawley rats (12 weeks old, 250-280 g) were obtained from Laboratory Animal Services Center, CUHK. Pre-surgery, we assessed their neurological functioning according to the Bederson Scale and the Modified Neurological Severity Scores (mNSS). We induced transient cerebral ischemia in the rats by inserting a 4-0 silicone-coated suture through the external carotid artery and the internal carotid artery to occlude the middle carotid artery. After 60 minutes, the filament was removed to facilitate reperfusion. Post-surgery, the animals were left to recover for 1/ 2/ 3/ 5/ 7 days. After assessing their neurological functioning again, we sacrificed them under anesthesia. We cut the brain tissue into 5μm sections and stained them with Ionized calcium binding adaptor molecule (Iba1) and Transmembrane protein 119 (Tmem119) immunofluorescent staining. Anti-Iba1 antibodies provides good immunofluorescent resolution of microglia cell morphology. However, Iba1 is also expressed on macrophages. Therefore, the microglia cell specific Tmem119 was also used. Immunofluorescent images were acquired with the Nikon Eclipse-Ti inverted microscope. Image J was used to quantify the number of microglia cells and cell processes on each cell and measure the length of microglia cell cell processes and the sur-face area occupied by each cell.

RESULTS AND DISCUSSION

40 rats were used in the study. 35 rats received the MCAO surgery and 5 rats received the sham surgery. The mortality rate of the MCAO surgery and the sham surgery were 5/35= ~14.2% and 0% respectively. All rats became active again within the first 1.5h post surgery. They were weak, lethargic and disoriented. They were tremulous as they tried to return to the prone position. The rats that received the MCAO surgery also had ipsilateral ptosis and hemiparesis. The rats that received the sham surgery did not display these deficits.

Figure 1. A rat showing ipsilateral hemiparesis after MCAO surgery

Figure 2. A rat showing ipsilateral ptosis after MCAO surgery

On the Bederson Scale, all rats scored 0 before the surgeries. The rats that received the MCAO surgery and were sacrificed on D1, D2, D3, D5 and D7 scored 3, 3, 3, 2, and 2 respectively. The rats that received the sham surgery scored 0 before they were sacrificed. The neurological deficits suggested a high likelihood that a stroke had been induced. They also showed that motor function improved with time. For the Modified Neurological Se-verity Scores, a higher score reflects more severe neurological deficits. All rats scored 0 before the surgeries. The rats that received the sham surgery scored 0 before they were sacrificed. The rats that received the MCAO surgery and were sacrificed on D1, D2, D3, D5 and D7 scored 17, 14, 12, 7, and 4 respectively. This again showed that neurological functioning improved with time. The sub-scores suggested that different domains of neurological functioning improved at different rates.

TTC staining showed that the brains of rats that received the MCAO surgery had observable infarcts. The rats that were sacrificed on D1 had an infarct covering over 50% of the ipsilateral brain that extended from the cortex into the hippocampus and the striatum. The infarcts in those that were sacrificed on later days receded laterally and shrunk in size, suggesting that a continuous repair process was present in the CNS.

Microglia cells were present throughout the cortex and the striatum. There were no statistically significant differences in the number of microglia cells in the ipsilateral side and the contralateral side, and the cortex and the hippocampus. On the contralateral side, the number increased from D1 to D3, and decreased afterwards. On the ipsilateral side, the number fluctuated with time and did not form a trend. More data is needed to ascertain its statistical significance.

The total and average number of microglia processes were lower on the ipsilateral side than the contralateral side. These observations probably reflect more microglia cell activation on the ipsilateral side, as the 'amoeboid' form typical of activated microglia cells have less and shorter processes. The average number of microglia processes across the cortex and hippocampus had similar standard deviations of between 1.5 and 2.0. This suggest that microglia cell populations consist of cells with different number of processes across different regions in different time points, potentially due to the need for these cells to perform diverse functions. The trend of smaller variations in the average number of microglia processes seen on the ipsilateral side than the contralateral side is also compatible with this postulation, as more microglia cells on this side may be activated as they are closer to where the ischemia occurred. The total and average lengths of microglia processes were lower on the ipsilateral side than the contralateral side. The average lengths of microglia processes had similar standard deviations of around 30 um. These observations align with the hypothesis we suggested above. The total and average surface area occupied by microglia cells were smaller on the ipsilateral side than on the contralateral side. When microglia cells are activated, they become globular in shape to perform phagocytosis, thus the cell body should increase in size. Accordingly, the surface area occupied by microglia cells, is expected to increase. However, this was not seen. One possible reason is that the reduction of surface area caused by the shortening and reduction of microglia processes was nullified by the increase caused by the change of microglia cell shape. The standard deviation of average surface area was smaller on the ipsilateral side than the contralateral side. The observation that microglia cells have more uniform surface area on the ipsilateral side align with the hypothesis we suggested above.

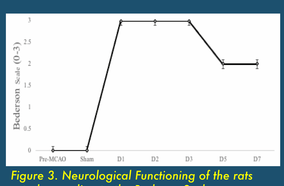

Figure 3. Neurological Functioning of the rats rated according to the Bederon Scale

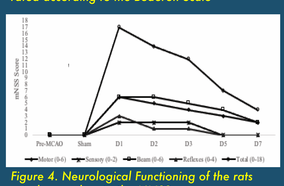

Figure 4. Neurological Functioning of the rats rated ac-cording to the MNSS

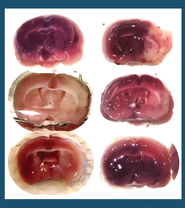

Figure 5. Brain sections after treat-ment with TTC. Top Left: Sham; Top Right: D1; Middle Left: D2; Middle Right: D3; Lower Left: D5; Lower Right: D7

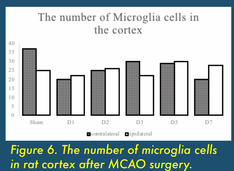

Figure 6. The number of microglia cells in rat cartex after MCAO surgery.

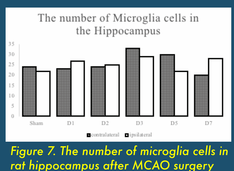

Figure 7. The number of microglia cells in rat hippocampus after MCAO surgery

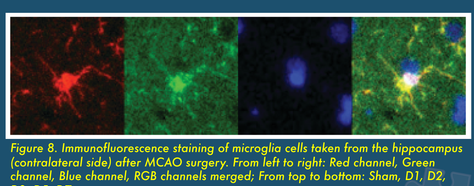

Figure 8. Immunofluorescence staining of microglia cells taken from the hippocampus (contralateral side) after MCAO surgery. From left to right: Red channel, Green channel, Blue channel, RGB channels merged; From top to bottom: Sham, D1, D2, D3, D5, D7.

CONCLUSION

Ischemic stroke presents a heavy disease burden globally and novel therapeutics that improve post-stroke outcome are needed. In our study, we developed and functionally verified an animal model that could produce infarct and model focal cerebral ischemia. We confirmed the observation that microglia cells exhibit di-verse cell morphology after focal cerebral ischemia. We also found out that the physical characteristics fluctuated post-MCAO surgery but did not show any trends. The changes in the physical characteristics of the microglia cells suggest that there is a change of microglia cell phenotypes with time following transient focal cerebral ischemia. Future research can better characterize these changes. RNA profiling and Western Blotting can also be used to identify and define the microglia cell phenotypes present following transient focal cerebral ischemia.

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