Microglial Extracellular Vesicles: Indicators and propagators of post-stroke neuroinflammation

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Microglia are the brain's resident immune cells and play a critical role in the response to neurological injury including stroke. Microglia are highly dynamic, adopting a variety of phenotypes that mediate post-stroke tissue repair and chronic inflammatory signaling. Importantly, a prolonged pro-inflammatory phenotype post-stroke is associated with secondary neurodegeneration and cognitive impairment. Currently, our ability to assess and potentially modify microglia activity post-injury is limited by our inability to clinically measure microglia phenotype. To address this limitation, we used *in vitro* models to characterize extracellular vesicles released from microglia (MEVs) and validated blood-based measurement of circulating MEVs in a preclinical model of ischemic stroke. MEVs were collected from microglia supernatant *in vitro* after exposure to lipopolysaccharide (LPS) after which surface protein and cargo were evaluated using qPCR, western blot and transmission electron microscopy. MEVs from LPS-exposed cells were applied to naïve microglia and pro-inflammatory gene expression was measured. Stroke was modeled in 3-month Fischer 344 rats using a focal injection of Endothelin-1 with plasma collected at baseline and 28-days post-injection after which circulating MEV proteins and cargo were measured using nanoflow cytometry. MEVs released from microglia following LPS exposure *in vitro* have altered surface proteins and carry pro-inflammatory molecules that reflect their phenotype. MEVs released from LPS-exposed cells have signaling capacity and propagate pro-inflammatory signaling to naïve cells in a dose-dependent manner. Circulating MEVs can be rapidly measured in plasma using nanoflow cytometry with antibodies against surface proteins and cargo and remain elevated 28-days post-stroke. The use of circulating MEVs as indicators of microglia activity allows for non-invasive, rapid measurement of microglia phenotype with potential application to many neurological diseases. Future work is required to better understand the relationship between MEVs, pro-inflammatory signaling and neurological outcomes.