Functional roles of extracellular vesicles derived from microglia with diverse activation states

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Microglia respond to all types of CNS injury and acquire different activated phenotypes, participating not only in mechanisms of injury but also in tissue repair (1). However the mode(s) of action of these cells in fostering or inhibiting CNS repair is still largely unclear.
Here, we investigated the action of extracellular vesicles (EVs) released by microglia with diverse activation states (2) on Oligodendrocyte Precursor Cells (OPCs) and hippocampal neurons. Fluorescence analysis of OPCs exposed to EVs together with the proliferative marker EdU showed that EVs produced by pro-inflammatory cells limit OPC proliferation, while EVs released by pro-regenerative microglia tend to increase it. Stronger proliferative action was observed *in vivo* upon delivery of EVs derived from pro-regenerative microglia to mice with focal myelin lesions, with a significant increase in the density of proliferating NG2+ cells at the lesion site. Immunocytochemistry and western-blot analysis of markers of mature oligodendrocytes revealed that EVs derived from both inflammatory and pro-regenerative microglia, but not from unstimulated cells, promote OPC maturation *in vitro*, with EVs released by pro-regenerative microglia displaying higher differentiation activity and significantly fostering myelin deposition in an *in vitro* system of OPCs co-cultured with DRG neurons. Globally these results show that through EVs, pro-regenerative microglia and, at lesser extent, pro-inflammatory and resting cells may exert a beneficial action on OPCs, promoting their differentiation and myelin formation.
Conversely, a clear detrimental action of EVs derived from inflammatory microglia was observed in cultured hippocampal neurons, highlighting a previously unrecognized role of microglia-derived EVs in inflammation-induced synaptic alteration. Indeed, immunofluorescence and western blotting analysis for synaptic markers showed that EVs secreted from inflammatory but not pro-regenerative microglia decrease the density of dendritic spines and cause destabilization of excitatory synapses. Molecular mechanisms underlying such synaptic alterations wil be discussed.

References and acknowledgements
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