Cross-talk between microglia and oligodendroglial progenitors in cerebral ischemia: implications for new purinergic strategies to brain repair

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Oligodendrocytes, the myelin-forming cells in the brain, are severely affected by ischemia [1], contributing to stroke-associated deficits. The possibility to implement spontaneous post-injury repair mechanisms by targeting myelin still represents an unexplored field. In this respect, GPR17, a P2Y-like receptor transiently expressed on Oligodendrocyte Progenitor Cells (OPCs) has emerged as a target to implement stroke repair through stimulation of OPC maturation [2]. Results obtained by fate-mapping analysis using the conditional GPR17-iCreERT2xCAG-eGFP transgenic mice showed that the subpopulation of adult OPCs expressing GPR17 (GFP^+-cells) represents "a reserve pool" that is maintained for repair purposes after brain damage [3]. In particular, we recently demonstrated that, after brain ischemia, GFP^+-cells actively respond to injury increasing their proliferation rate and migratory capacity. However, at later stages, only a low percentage of these cells undergoes maturation [4]. This limited post-stroke repair is likely due to local unfavourable inflammatory milieu mediated by macrophages and resident microglia, which participate to post-ischemic inflammation assuming both detrimental and beneficial phenotypes.

Here, we aimed at: (i) characterizing the spatio-temporal distribution of GFP^+-cells in relation to microglia/macrophage polarization in transgenic mice after middle cerebral artery occlusion (MCAo); (ii) exploring the cross-talk between microglia and OPCs, by assessing how vesicles released extracellularly (EVs) by microglia, polarized toward a pro- or anti- inflammatory state, influence OPC behaviour.

In vivo studies showed that GFP^+-cells accumulate at the border of the ischemic lesion starting from 72h after ischemia, when microglia and macrophages show both pro- and anti-inflammatory features. One week after stroke, the absolute number of pro-inflammatory cells increases, while myeloid cells with pro-regenerative phenotype do not significantly change. *In vitro* studies pointed out that EVs produced by pro-inflammatory microglia only slightly limit OPC proliferation, whereas EVs produced by pro-reparative microglia tend to increase it. Preliminary data showed that all types of EVs (from unstimulated, pro-inflammatory or pro-regenerative microglia) are able to induce OPC migration, indicating that EVs provide attractive guidance cues independently of the activation state of donor microglia. Interestingly, EVs from pro-regenerative microglia have a higher chemotactic effect on the subpopulation of cells expressing GPR17, suggesting that EVs may also contain purinergic signals able to influence OPC migration via GPR17. Finally, exposure to EVs from either pro- or anti-inflammatory microglia (but not resting cells) promote OPC maturation. However, only EVs released by pro-regenerative cells significantly foster myelin deposition in an *in vitro* system of OPCs co-cultured with DRG neurons.

Shedding light on these signals is important for developing combined therapeutic interventions where a purinergic approach, aimed at implementing recovery after stroke, is potentiated by agents promoting a better microglia phenotype with pro-regenerative effects on OPCs.

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