OP-064 Mutual interactions between multipotent adult progenitor cells (Multistem® cells) and macrophages

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Question

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the CNS in which infiltrated macrophages and microglia are thought to be the primary effector cells. Effector mechanisms of activated macrophages and microglia include the phagocytosis of myelin, and secretion of inflammatory and toxic mediators, such as reactive oxygen and nitrogen species, which affect axonal and myelin integrity. Therapeutic strategies to modulate myeloid cell-mediated neuroinflammation are currently explored. Among other approaches stem cell-based strategies are under investigation. Multistem® (rMS) is a clinical product of cells isolated under multipotent adult progenitor cell (MAPC) culture conditions. So far, MAPCs and rMS cells have been used in several experimental approaches, such as experimental stroke, traumatic brain injury and several other neuroinflammatory diseases, with promising results. In a model of spinal cord injury, it was shown that MAPCs can shift macrophages from a proinflammatory M1 to the anti-inflammatory M2 (alternatively activated) phenotype. Mesenchymal stem cells including rMS seem to be more potent in secreting immunomodulatory molecules such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) following licensing with proinflammatory cytokines. In this project, we aim to explore the mutual interactions between macrophages and rMS cells *in vitro* in terms of downregulation of macrophage functions relevant for MS disease promotion.

Methods

rMS cells derived from Lewis rats and provided by ReGenesys BVBA (Heverlee, Belgium) were seeded in macrophage medium for 24 h to obtain conditioned media. rMS cells were seeded in two different conditions: either 50 (CM50) or 100 µl (CM100). Serial dilutions of rMS-conditioned medium were applied to the rat alveolar macrophage cell line NR8383. In each condition, 100 ng/ml LPS was added to induce NO production. Cell contact-mediated effects of rMS on NO production by NR8383 cells or primary macrophages (isolated from the peritoneum of naive Lewis rats) were tested in coculture experiments. Therefore, rMS cells were added in increasing ratios to NR8383 cells, ranging from 1:0.02 to 1:2. Finally, effects of supernatant derived from activated NR8383 cells on rMS NO production and gene expression was explored.

Results

While rMS-conditioned medium was able to downregulate LPS-induced NO production by macrophages in a dose-dependent manner, NO production was upregulated in the coculture set up. Supernatant of LPS-activated NR8383 induced the production of NO by rMS as well as the upregulation of iNOS gene expression, explaining the upregulation in NO production observed in the coculture set up. Finally, rMS following incubation with supernatant of LPS-activated NR8383 supernatant upregulates molecules such as COX-2, an enzyme responsible for the production of prostaglandin E2 (PGE-2).

Conclusion

rMS-conditioned medium is able to downregulate the production of NO,which is a hallmark of classically activated macrophages. Additionally, macrophages can prime rMS *in vitro* to express molecules (e.g., COX-2) that are involved in PGE-2-mediated mechanisms by which stem cells can modulate the activation of macrophages . Further experiments also need to be performed to confirm possible modulation of macrophage phenotype and functionality *in vivo*.