ImmunoTools *special* Award 2019

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Murine iPSC-derived microglia and macrophages as *in vitro* tools to investigate and modulate neuroinflammation

Background

Neuroinflammation is defined as the development of an inflammatory response within the central nervous system (CNS). Neuroinflammation is a hallmark of many neurological pathologies, including both traumatic diseases, such as stroke and spinal cord injury, and neurodegenerative disorders, such as Alzheimer's disease, amyotrophic lateral sclerosis (ALS) and Parkinson's disease (*Di Sabato et al., 2016*).

As CNS resident innate immune cells, microglia are centrally involved in the development and evolution of neuroinflammation as they are immediately activated upon detection of injury. Moreover, following neurotrauma the integrity of the blood brain barrier is often compromised, causing circulating inflammatory monocytes to infiltrate and colonize the inflamed brain tissue (*Kanazawa et al., 2017*; *Milich et al., 2019*; *Yamasaki et al., 2014*). Within the inflammatory lesion, microglia and infiltrating monocytes display multiple activation states which are generally situated between two spectral ends of functional polarisation: the 'classical activation', which is characterized by secretion of pro-inflammatory molecules and the 'alternative activation', which is associated with pro-regenerative and anti-inflammatory functions (*Mosser and Edwards, 2008; Murray, 2017*). Hence, activated myeloid cells can either contribute to disease progression or promote protection and regeneration. In this context, the use of immunomodulating compounds able to promote microglia and infiltrating monocytes alternative activation is expected to become a new therapeutic approach for multiple CNS disorders (*Cherry et al, 2014; Dooley et al., 2016; Hamzei Taj et al., 2018; Le Blon et al., 2016*).

Project description

Reliable *in vitro* modelling of neuroinflammatory processes is currently one of the major challenges in biomedical research. The main aim of this project is to develop murine induced pluripotent stem cells (iPSC)-derived cell culture models able to recapitulate the distinct activation behaviour of microglia and infiltrating monocytes during neurotrauma.

Murine iPSC-derived microglia are generated using a newly developed procedure which recapitulates microglia ontogenesis *in vitro*. This protocol relies on sequential administration of rmSCF, rmVEGF, L929/bEnd5-conditioned medium, rmIL3 and rmGM-CSF from ImmunoTools. iPSC-derived microglia are co-cultured with astrocyte-committed embryonic-brain derived neural stem cells (NSC) (cultured with rmEGF and rmFGF-2 from ImmunoTools), which provide the environmental cues necessary to sustain microglia identity *in vitro* (*Quarta et al, 2019*). Murine iPSC-derived macrophages are obtained by differentiation of iPSC-derived embryoid bodies in medium supplemented with L929-conditioned medium, rmIL3 and rmGM-CSF from ImmunoTools (*Quarta et al, 2019*).

These iPSC-derived myeloid populations will be used as *in vitro* tools for the validation of novel immunomodulatory therapies to treat CNS injuries or diseases. Specifically, we will evaluate the therapeutic potential of cytokines interleukin 4 (IL4) and interleukin 13 (IL13). To achieve this goal, iPSC-derived microglia and iPSC-derived macrophages cultures will be stimulated *in vitro* (e.g. using IFNγ, rmIL-1β or rmTNFα from ImmunoTools) to mimic myeloid cells activation during neurotrauma. Additionally, rmIL4 and rmIL13 from ImmunoTools will be administered at different time-points pre- and post-insult. Analysis of microglia/macrophage polarisation markers will be performed both on mRNA and protein level and secretion of pro- and anti-inflammatory cytokines (such as TNFα and IL6) will be evaluated using ELISA kits from ImmunoTools.

This study will contribute to elucidate the mechanism of IL4/IL13-induced microglia and macrophage polarization and to optimize therapeutic strategies based on IL4/IL13-mediated immunomodulation. Moreover, the established iPSC-derived microglia and macrophages cellular models will be highly instrumental as *in vitro* platform to implement innovative immunomodulatory therapies for CNS disorders.

ImmunoTools *special* AWARD for **Alessandra Quarta** includes 25 reagents

PE - conjugated anti- mouse CD11b, CD54, Gr-1, isotype control IgG2b, Annexin-V

mouse ELISA-sets: IL-6, TNFalpha

recombinant mouse cytokines: rm EGF, rm FGF-b / FGF-2, rm GM-CSF, rm IFNgamma
rm IL-1beta, rm IL-4, rm IL-13, rm MCP1 / CCL2, rm SCF
rm TNFalpha, rm VEGF-A.

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