ImmunoTools *special* Award 2018

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The study of classical and alternative activation in human induced pluripotent stem cell-derived microglia and macrophages

Neurodegenerative diseases and traumata are all comprised of one similar component, i.e. neuroinflammation. An insult in the CNS will trigger the brain's immune system, with its key players being brain-resident microglia and infiltrating macrophages. Only recently, it has become clear that these phagocytes play distinct roles and can exhibit different functions when it comes to neuroinflammation. With regard to blood-borne macrophages, several studies have revealed their ability to adopt various phenotypes, ranging from a "pro-inflammatory" M1 to an "anti-inflammatory" or "alternatively activated" M2 phenotype, in both *in vitro* and *in vivo* studies. Microglia, however, have mainly been shown to adopt these similar phenotypes *in vitro*, while *in vivo* evidence is still sparse. Nonetheless, increasing evidence specifies the importance of a correct phenotype of CNS phagocytes at the right time and place during inflammation. Therefore, many studies nowadays are investigating immune modulation as a potential therapeutic strategy to treat CNS pathologies. Indeed, one may reasonably argue that forced M2 polarisation could offer a beneficial therapeutic effect on the neurological disease outcome.

Here, the existence of reliable pre-clinical models for human neuro-immune research would be desirable. The classical approach to pre-clinically study neurodegenerative processes and to validate new neuro-protective compounds for human application is done by a combination of *in vitro* experiments using cell lines or primary cells, and (large) animal experimentation. However, this approach has several limitations in terms of validity and ethical consideration. First, the use of cell lines is debatable, since these are in general oncogenically transformed cells which only partly mimic the original cell type. Second, primary neural cells, like microglia, are extremely difficult to obtain from healthy human brain tissue.

Third, due to high costs associated with animal experimentation and differences between human and rodent physiology and pathology, obtained pre-clinical data may not be as reliable as one would desire.

Therefore, we aim to develop an in vitro assay to study and modulate activation states in human neuro-inflammation by using human induced pluripotent stem cell-derived microglia and macrophages.

In vitro differentiation of hiPSC-derived microglia will be performed according to a recently described protocol by *Muffat et al*. Briefly, hiPSC lines, following a short pre-differentiation step along the neuro-ectodermal lineage, will be cultured in a specific microglia differentiation medium supplemented with interleukin (IL)-34 and macrophage-colony stimulating factor (M-CSF). Under these culture conditions, hiPSCs develop yolk sac-embryoid bodies, from which microglia precursors can be harvested and maintained in culture. According to current literature, hiPSC-derived microglia will be characterized by immunocytochemistry and flow cytometry to demonstrate the following expression pattern: $CD45^{low}$, CX_3CR1^{high}, $TMEM119^{high}$, $CCR2^{low/neg}$, $HLA\text{-}ABC^{pos}$, $CD11b^{pos}$, $CD14^{neg}$ and $CD16^{pos}$.

In vitro differentiation of hiPSC-derived macrophages will be performed according to a protocol described by Karlsson *et al*. Briefly, hiPSC cell-derived embryoid bodies will be cultured in medium supplemented with M-CSF and IL-3. Following isolation of monocyte precursors from these embryoid bodies, further differentiation towards macrophages will be established under stimulation of M-CSF. hiPSC-derived macrophages will be further characterized by immunocytochemistry and flow cytometry to demonstrate the following expression pattern: $CD45^{high}$, $CX_3CR1^{low/neg}$, $CCR2^{high}$, $CD11b^{pos}$, $HLA\text{-}ABC^{pos}$, $CD14^{neg}$ and $CD16^{pos}$.

hiPSC-derived microglia and macrophages will be evaluated by means of transwell migration assays, investigating their response to CX_3CL1 and CCL2. As microglia express CX_3CR1, they will migrate towards CX_3CL1, and macrophages which express CCR2, will migrate towards CCL2. Second, phagocytosis assays will be performed. Lastly, M1/M2-priming experiments will be performed, creating a pro-inflammatory environment by lipopolysaccharide/interferonγ stimulation and an anti-inflammatory environment by IL-4 stimulation. Next, cell phenotype will be analysed by flow cytometry, were we expect M1-activated macrophages/microglia to have the following expression profile: $CD86^{+}$ $CD80^{+}$ $TNFa^{+}$ $HLA\text{-}DR^{hi}$ $CD206^{int}$ $IL\text{-}1RI^{+}$, and M2-activated macrophages/microglia to have the following expression profile: $CD163^{+}$ $CD206^{hi}$ $IL\text{-}1RII^{+}$ $HLA\text{-}DR^{int}$.

ImmunoTools *special* AWARD for **Debbie Le Blon** includes 21 reagents

APC – conjugated anti-human CD14, CD16, CD80

FITC - conjugated anti-human CD11b, CD14, CD16, CD45, CD86, HLA-ABC, HLA-DR

PE - conjugated anti-human CD11b, CD45, CD163, HLA-DR, TNF-alpha

PerCP - conjugated anti-human CD45

recombinant human cytokines: rh IFN-gamma, rh IL-3, rh IL-4, rh MCP-1/CCL2, rh M-CSF

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