ImmunoTools *special* Award 2015

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Maternal Immune Activation (MIA) effects on behaviour phenotypes in the offspring

My project is focused on the study of pathological behaviour phenotypes in adult offspring due to inflammatory processes occurring during pregnancy. In the last 20 years a wide numbers of evidences has highlighted the correlation between infection contracted by women during pregnancy and neurological disorders in children; this has been called from the media the “winter baby phenomenon”.

Further investigation about pregnant and embryo inflammatory status are very needed since pregnancy infection are known to increased risks for autism spectrum disorders *(Meyer et al., 2006; Harvey and Boksa, 2012)*, schizophrenia *(Miller et al., 2013)* and depression *(Enayati et al., 2012)* in adult offspring.

Based on these rationale I am going to study the possibility that a single inflammatory event during pregnancy may alter neurodevelopment, leading to neurologic disorders in the offspring by using the Poly I:C (polyinosinic-polycytidylic acid) and LPS (lipopolysaccharide) models of inflammation. Poly I:C is a TLR3 (Toll-like receptor 3) agonist while LPS in a TLR4 (Toll-like receptor 4) agonist. These are generally recognized as virus and bacterial infection mimicry agent respectively *(Boksa etc al 2010; Zhao and Schwartz et. al., 1998).*

MIA (maternal immune activation) is driven by cytokines such as IL6, IL1β and TNFα. These cytokines are produced by both the mother itself and directly by fetal membranes *(Ashdown et al., 2006; Mandal et al., 2013)*. A rapid rising of IL6 and IL1β cytokine levels can be observe in the described models by ELISA (blood samples) few hours after the treatments. I could use ImmunoTools ELISA kit to evaluate the levels of TNFα, another major regulated cytokine, in both dam blood samples and embryos (mouse ELISA-set for TNFα). This cytokine are already known to have great importance during the postnatal period and particularly during breastfeeding *(Liu et al, 2014)*.

Behaviour preliminary data on adult offspring mice showed deficit in memory (i.e. novel object recognition) and sociability test for both the inflammatory model used even if with specific differences.

To better investigate the contribution of IL6 and IL1β on initial inflammation, given obtained data the most release cytokines, we will administrate alternatively rm IL6 or rm IL1β from ImmunoTools to pregnant mice using as readout offspring mice behaviour phenotypes.

Observed pathological phenotypes could be the result of the acute inflammatory hit during embryogenesis or even of a chronic inflammatory status. To assess this hypothesis we will use CX3CR1-GFP mice as animal model to analyse activation status of microglia. To perform this analysis we will sort brain GFP-positive cells and use flow cytometry to characterize offspring adult isolated microglia for cell activation markers such as CD11b and CD80 by ImmunoTools antibodies (APC - conjugated anti-mouse CD11b, PE - conjugated anti-mouse CD80 and relative isotype control IgG2b).

Moreover we will use LysM-GFP mice to assess inflammatory status of immune periphery cells and to distinguish between microglia and possible inflammatory contribution in the brain of infiltrate macrophage cells performing the same analysis as before collecting cells from both brain and peripheral tissue.

In order to do that I will need a substantial amount of cytokines and antibodies making this opportunity by ImmunoTools more valuable. Results from these experiments will contribute to elucidate how inflammation affects neurodevelopment and pathological behaviour phenotypes in adults hoping to make a direct and clear link with inflammatory events during pregnancy.

ImmunoTools *special* AWARD for **Marco Rasile** includes 25 reagents

APC - conjugated anti-human CD9, CD63, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b

FITC - conjugated anti-mouse CD9, CD81, CD90, isotype control IgG2b

PE - conjugated anti-mouse CD80, CD90, CD117, isotype control IgG2b

APC - conjugated anti-mouse conjugated anti-mouse CD11b, isotype control IgG2b

mouse TNF-a ELISA-set, for 96 wells, (each 3 reagents)

recombinant mouse cytokines: rm EGF, rm IL-1beta, rm IL-6, rm PDGF-AA, rm PDGF-BB, rm VEGF DETAILS more AWARDS