ImmunoTools *special* Award 2014

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Progranulin-linked immunomodulation: towards an understanding of cellular and molecular pathomechanisms involved in frontotemporal lobar degeneration

Progranulin (GRN) is a pluripotent growth factor with key roles in many cellular processes including wound healing and angiogenesis. GRN has also been suggested to exert a neuroprotective role based on the fact that mutations, causing GRN haploinsufficiency, are a major cause of frontotemporal lobar degeneration (FTLD). In this disorder, patients show a progressive deterioration of memory, behaviour and/or language skills. Currently there are no means to alleviate, slow down or stop the FTLD-pathology. An increasing body of knowledge indicates that GRN exerts its neuroprotective effects through the modulation of neuroinflammation. In support of this idea, we showed an accelerated gliosis beginning at 6 months of age in GRN-deficient mice (*Wils et al, J Pathol 2012*). These mice are also more susceptible to neurodegeneration following central nervous system injury accompanied with neuroinflammation (*Martens et al, J Clin Invest 2012*), whereas GRN overexpression models are protected from lipopolysaccharide-induced cytotoxicity due to an up-regulation of anti-inflammatory IL-10 expression (*Tao et al, Brain Res 2012).* However, the exact pathomechanisms underlying the deregulated inflammatory state due to GRN depletion remain to be characterized.

Given their prime role in both cytokine secretion and clearing of apoptotic neurons and debris, microglia have attracted great interest for the generation of an anti-inflammatory, neuroprotective milieu. After two decades of clinical trials, it is clear that further optimization efforts are still needed to fully unlock the therapeutic targeting of these cells. Recently, evidence has emerged that microglia and macrophages display multiple phenotypes that are classified into two principal states of activation: the pro-inflammatory (M1) and the anti-inflammatory (M2) subtypes.

Such an understanding has not yet been established for FTLD, but could explain that while GRN knockout macrophages display an increased phagocytosis rate of yeast particles (*Kao et al, PNAS 2011*), an increased vulnerability to infections in GRN-knockout mice was reported by others and us (*Wils et al, J Pathol 2012; Yin et al, J Exp Med 2010*). Studying M1/M2 ratios and activities in GRN knockout settings will, therefore, be of great mechanistic and clinical significance.

In this project we hypothesize that GRN-depletion encompasses alterations in the intrinsic functionality of these subtypes. For this, *in vitro* and *in vivo* sets of experiments are being carried out on both GRN-knockout and wild-type settings. The microglia/macrophage phenotypes will be studied by flow cytometry, for which the ImmunoTools antibodies (CD11b, CD18, CD29, CD32, CD80) will be of great convenience. Labelled isotype matched immunoglobulins will be used as controls. Murine cytokines by ImmunoTools would be a valuable asset in the culture (rm M-CSF) and polarization (rm IFNgamma for M1, IL-10 for M2) of microglia/macrophages for further *in vitro* functionality studies.

In addition, we intend to deploy in this context the polarization and activity of T cells, including the bidirectional cross-talk with microglia/macrophages. A better insight in the prevalence of subtypes of both cell types, as well as the molecular signals involved, would contribute to pursuing an immune modulatory approach with the highest clinical potential. ImmunoTools antibodies will be employed to identify T-cell subsets (CD3e, CD8a, CD4, CD25) and their activation status (CD62L, CD134, CD154).

We believe that these results will be instructive in our understanding of the complex GRN biology, especially in its role in (neuro-)inflammation, and contribute to the identification of key pathways to target FTLD diseases. Reagents of ImmunoTools will be of great value to effectuate the proposed experiments, which I am conducting as part of my doctoral studies.

ImmunoTools *special* AWARD for **Zoë Van Acker** includes 24 reagents

FITC - conjugated anti-mouse CD8a, CD11b, CD18, CD25, CD32, CD62L, CD80, CD134, isotype control IgG2b,

PE - conjugated anti-mouse CD3e, CD8a, CD18, CD25, CD29, CD32, isotype control IgG2b,

PerCP - conjugated anti-mouse CD4,

APC - conjugated anti-mouse CD3e, CD4, CD11b, isotype control IgG2b,

recombinant mouse cytokines: rm IFNgamma, rm IL-10, rm M-CSF

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