ImmunoTools *special* Award 2014

Jan Boddaert, Dhr.**,** PhD-student

Supervisors:
Prof. dr. Samir Kumar-Singh, MD, PhD;
Prof. dr. Patrick Cras, MD, PhD

Cytology and histology - Medical microbiology, Molecular and cellular pathology unit, Campus Groenenborger - Room G.U.108b, Groenenborgerlaan 171 - 2020 Antwerpen - Belgium

Cellular and molecular characterization of post-stroke immunodepression

Acute cerebrovascular accident or stroke is the third most common cause of death worldwide and the main cause of disability in developed countries. The risk for stroke patients to develop severe infectious complications is as high as 30%, of which pneumonia is the most important and fatal infection contributing to ≈20% of all in-hospital deaths. Though the precise mechanisms are unknown, one of the key events leading to this high morbidity and mortality due to infectious complications is the development of systemic, stroke-induced immunosuppression (SIIS). SIIS is characterized by severe leukopenia, lymphocyte apoptosis, and blood cytokine alterations. While other cell-types such as Tregs and iNKT-cells have also been implied, stroke-induced cytokine alterations were initially suggested to be driven by a switch from the pro-inflammatory Th1 to anti-inflammatory Th2 cells. However, whether it is indeed a straightforward Th1 to Th2-type cytokine shift remains unknown as, for instance, the classical Th2 cytokine IL-4 was not found to be altered in a recent study on stroke models. We believe that the development of “post-stroke infections” heavily skews the observed post-stroke cytokine profile.

Also, while T cell mediated immunity is generally accepted to be impaired by ischemic brain injury, the precise T-cell response, temporal alterations of the T cell subpopulations and the underlying mechanisms remain to be understood. In a very recent study, Gu and colleagues showed that post-stroke decrease of B-cells and PBMCs did not occur in athymic rat, underscoring the importance of understanding the evolution of the post-stroke T cell population. Based on our hypothesis that the behaviour of Th17 cells with the capability of

transforming into iTreg cells could be important in the generation of post-stroke immunodepression, we are studying the behaviour of $CD4^+$ T helper subpopulations in the acute and sub-acute phase after cerebral ischemia in our already established mouse model of middle cerebral artery occlusion (MCAO) and results will be validated in human stroke patient samples.

With the use of specifically selected antibodies form ImmunoTools, we will be able to apply flow cytometric analysis to first validate the occurrence of immunosuppression in our mouse stroke model and in human stroke patient samples. To investigate the influence of post-stroke infection on the post-stroke cytokine profile, animals – that also develop spontaneously develop post-stroke infections within 3 days after reperfusion – will either be treated with prophylactic antibiotics immediately after MCAO or inoculated with *E. coli* to induce a controlled infection. Peripheral blood samples will be collected and key cytokines for different T helper responses will be studied using ELISA-assays developed by ImmunoTools.

Concerning the cellular post-stroke response, we will identify the stable/activated T cell population of T cells with flow cytometry using a negative/positive selection with annexin V/CD69, provided by ImmunoTools. The ImmunoTools multicolour combination antibodies for flow cytometry analysis will allow us to further study the behaviour of the different T-cell subtypes (Th1, Th2, Th17 and Treg) in blood and lymphoid organs at specific time-points within 24h and up to 5 days post-stroke.

We believe, that our studies on the temporal and phenotypic profile of the post-stroke T cell population, focusing on Th17/iTreg cells, could provide important insights in post-stroke systemic immunosuppression.

ImmunoTools *special* AWARD for **Jan Boddaert** includes 19 reagents

FITC - conjugated anti-human CD11b, CD19, CD25, CD69, Annexin V,

multicolour combinations anti-human:
CD3 FITC / CD4 PE / CD45 PerCP
CD3 FITC / CD8 PE / CD45 PerCP

human IL-4 ELISA-set for 96 wells, human IL-6 ELISA-set for 96 wells, human IL-12p40 ELISA-set for 96 wells (each 3 reagents),

FITC - conjugated anti-mouse CD11b, CD19, CD25 DETAILS more AWARDS