ImmunoTools *special* Award 2018

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Migration of regulatory T cells to the brain in multiple sclerosis

Background: Multiple sclerosis (MS) is a demyelinating, progressive disorder of the central nervous system (CNS) caused by an autoimmune response towards myelin antigens. Worldwide, 2.5 million people suffer from MS, with symptoms typically appearing between 20 and 40 years of age. It is the most common cause of neurological disability in young adults associated with low quality of life, life-long therapy and high medical costs (total mean annual cost of €45,300 per patient with moderate disease severity in Belgium in 2015). In MS, autoreactive $CD4^+$ T cells (TH cells) are identified as the main effector cells. The autoimmune response in MS patients is believed to start in secondary lymphoid organs, where dendritic cells present self-antigens to naive TH cells, leading to activation, differentiation into effector T cells (Teff) and migration to the brain. In healthy individuals, peripheral activation of autoreactive TH cells, which have escaped negative selection in the thymus, is suppressed by regulatory T cells (Tregs). One of the earliest steps in the pathogenesis of MS is the disruption of the blood brain barrier (BBB) mainly caused by TH_{17} cells. Under physiological conditions, the BBB protects the neuronal microenvironment by functioning as a physical barrier between the periphery and the CNS. The BBB is composed of a fine network of specialized endothelial cells (ECs), which are linked adjacently via adherens and tight junctions, limiting paracellular migration of molecules and cells. However, during active disease, the BBB is impaired and leaky in MS patients, favouring migration of immune cells into the CNS parenchyma. It is not clear whether BBB dysfunction precedes immune cell infiltration or leukocyte migration modifies BBB permeability. However, it is generally accepted that leukocytes produce cytokines, reactive oxygen species and enzymes which influence BBB function.

Methods implementing ImmunoTools reagents: My ultimate research goal is to elicudate the difference between migration to CNS between Teff and Tregs. First, I will start with human blood samples. I will receive blood samples from both healthy donors and MS patients. The peripheral blood mononuclear cells will be phenotyped using flow cytometry. Following human markers are used: FITC CD3, PE CD4, PerCP CD25, APC CD127, FITC CD11a, PE CD49d and PerCP CD62L. Results will elucidate differences in expression levels of adhesion molecules between both Tregs and Teff and healthy donors and MS patients. In addition, I will perform ELISA (IP-10 (CXCL10), human MCP-2 (CCL-8)) on supernatant of human brain endothelial cells. This will elucidate whether the endothelial cells produce chemokines and using migration assays with rh IP-10 /CXCL10 and rh MCP-2 / CCL8, how the immune cells react to these chemokines. To stimulate the cells, following cytokines will be used: rh

IFNgamma, rh TNF-alpha. From these results, I will perform *in vivo* studies to elucidate the mechanisms in more detail. After certain therapies, I will also look with flow cytometry at immune cells of these mice. Following markers are used: FITC CD3e, PE CD4, APC CD25, FITC CD11a, PE CD49d and APC CD62L.

ImmunoTools *special* AWARD for **Paulien Baeten** includes 23 reagents

APC - conjugated anti-human CD25, CD62L

FITC - conjugated anti-human CD3, CD11a, CD127

PE - conjugated anti-human CD4, CD49d

APC - conjugated anti-mouse CD25, CD62L

FITC - conjugated anti-mouse CD3e, CD11a

PE - conjugated anti-mouse CD4, CD49d

human ELISA-set (for one 96 plate): human IP-10 / CXCL10, human MCP-2 / CCL-8

recombinant human cytokines: rh IFN-gamma, rh TNF-alpha

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