ImmunoTools *special* Award 2019

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Phenotypic characterization of B cells in Multiple Sclerosis patients treated with rituximab or ocrelizumab before the treatment and after the repopulation

Background

Multiple sclerosis (MS) has been traditionally considered a T cell-mediated pathology, but the important role of B cells in its pathogenesis is now widely accepted. In fact, the majority of lesions contain infiltrating B lymphocytes with antibodies deposition and, furthermore, clonally expanded, class-switched and somatically hypermutated B cells are present in the cerebral spinal fluid, central nervous system parenchyma and meninges [1].

Several defects in B cells from MS patients have been demonstrated. For example, in MS there is an abnormally high proportion of circulating B cells with increased expression of CD80 [7], a molecule that, together with CD86 binds the T-cell activation marker CD28 sustaining T cell activation. On the other hand, anti-inflammatory B cells, commonly defined as “Regulatory B cells” and mainly identified for their capacity to produce IL-10 and by the expression of different surface markers (e.g CD24, CD38, CD5, and CD1d) [5], are reduced in patients with remitting-relapsing MS (RRMS) [6].

Indeed, B-cell depleting therapies are currently approved for the treatment of both primary progressive MS (PRMS) and RRMS with an efficient suppression of acute inflammatory activity for RRMS and the slowing-down of lesions in PRMS [1]. Monoclonal antibodies directed against CD20 (rituximab, ocrelizumab and ofatumumab) deplete immature and mature B cells, sparing plasma cells and hematopoietic stem cells [2]. Since antibody-secreting cells do not express CD20 on the surface, the efficacy of B-cell depleting therapy has been attributed to antigen-

presentation and cytokine production, rather than on the ablation of immunoglobulin production. As a proof of concept for the central role of B cell-specific antigen presentation to T cells, animal models are resistant to MOG-induced experimental autoimmune encephalitis when B cells lack MHC-II [3].

To date, the functional status of repopulating B cells after anti-CD20 treatment is under investigation and largely unknown, especially for ocrelizumab. Of note, this point has a great significance in clinical practice since it can determine the need to repeat over time B-cell depletion or to stop it if an “immunological resetting” occurred. For example, B cells from MS patients are polarized to an inflammatory phenotype (e.g. IL-6 and GM-CSF production rather than IL-10), but after rituximab therapy, there is skewing towards an anti-inflammatory condition [4].

Objective

Based on these premises, the aim of my project is to phenotypically characterize the B cell subsets in patients with MS before and during the therapy with ocrelizumab or rituximab.

Methods

Patients with PRMS that did not receive other treatments before the therapy with ocrelizumab or rituximab and age- and a sex-matched healthy volunteer will be recruited. B cells counts will be monitored over time by enumerating CD20 and CD19 B cells. Repopulating B cells in the peripheral blood will be evaluated by addressing the percentages of primarily naïve and immature B cells (CD27-Naïve B cells, CD24hi CD38hi transitional B cells) as well as memory B cells and plasmablasts. After B-cell reconstitution, I will characterize these cells for the expression of markers related to T cell activation. In the specific, I will determine the levels of T-cell costimulatory signals such as CD80, CD86 and CD40, MHC-II on different B cell subsets (**ImmunoTools**). Since my expertise is related to the Regulatory B cells field [8],[9] I will also focus on markers related to these cells: CD24, CD38, CD27, CD25, CD5, CD9. Lastly, since I will analyse samples from patients receiving anti-CD20 therapies, I will include anti-CD20 Ab in the staining, but also markers specific for T cells (CD4, CD8, CD25) to possibly correlate them to B cell repopulation.

I kindly ask **Immunotools** to provide the reagents listed below, I chose the same antibodies with different fluorophores in order to set up the best combination.

ImmunoTools *special* AWARD for **Silvia Tonon** includes 25 reagents

FITC - conjugated anti-human	CD19, CD80, CD86, CD40, CD20, CD9, CD27
PE - conjugated anti- human	CD19, CD80, CD40, HLA-DR, CD20, CD25, HLA-II, HLA-DR, CD38
PerCP - conjugated anti-human	CD20, CD8, HLA-DR, CD5
APC - conjugated anti-human	CD27, CD24, CD19, CD4

DETAILS more AWARDS

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