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IFN-β influences the phenotype of B lymphocytes in Multiple Sclerosis patients

Along my PhD study, I am investigating the mechanisms underlying the effects induced by IFN-β therapy on B cell phenotype in patients affected by Relapsing-Remitting Multiple Sclerosis (RRMS).

MS is commonly considered a T cell-driven disorder. However, many evidences have recently suggested how B lymphocytes are central players in the development and pathogenesis of this disease. B cells are also the natural target of Epstein-Barr virus (EBV) infection and this well correlates with recent clinical and epidemiological data supporting an association of EBV infection with MS development. EBV establishes latent infection in memory B cells, phenotypically identified as $CD19^+/CD27^+$ through the expression of two different latent genes, latent membrane protein 2a (LMP2a) and EBV nuclear antigen 1 (EBNA1).

Given the antiviral and immunomodulatory properties of Interferon (IFN)-β (in the formulation of Betaferon), one of the most used first-line disease- modifying drugs for the treatment of MS patients, the main objective of my PhD project is the characterization of the effect of this therapy on B lymphocytes, being them both the major EBV reservoir and a key pathogenetic cell type in MS.

To this aim, blood samples from RRMS patients will be collected at baseline and 1 month after the beginning of IFN-β.

Along this study we first evaluated whether IFN-β treatment might affect the percentage of circulating total B cells by performing FACS analysis on isolated PBMC. Preliminary data show a selective reduction after IFN-β therapy in circulating total $CD19^+$ B cells, with a particular decrease in the memory B cell compartment ($CD19^+/CD27^+$). We would like to further investigate this interesting result by increasing the number of patients analyzed and also by testing whether IFN-β effect is directed specifically on B lymphocytes or if this therapy may also affect the percentage of other cell types, such as $CD3^+$ T cells or $CD14^+$ monocytes.

Next, to define the responsiveness of the enrolled patients to IFN-β treatment, the expression of MxA (Myxovirus protein A) and OAS2 (2'-5' oligoadenylate synthetase), two genes with antiviral activity classically used as biomarkers of type I IFN

response, was investigated by real time RT-PCR in isolated B cells. In line with these observations**,** we also found that IFN-β therapy reduces the levels of the EBV latent transcript LMP2A, reinforcing the previous hypothesis that IFN-β exerts part of its therapeutic effects through an anti-EBV mechanism by targeting B cell compartment. Indeed, by reducing the number of circulating EBV-infected memory B cells, classically considered the reservoir of the virus in humans, IFN-β might subsequently control EBV expression as well. Based on these findings, in our proposal we would investigate in detail the mechanisms responsible for this selective reduction of memory B cells upon IFN-β treatment.Several working hypotheses have been proposed to investigate this phenomenon, namely: the shedding of CD27 from B cell membrane, the increased expression of CXCR4 and CXCR3, two chemokine receptors involved in the migration of B cells from the peripheral blood to lymphoid organs or inflamed tissues, and finally the specific induction of apoptosis in these cells. Two of these hypotheses have been already tested.

First of all we investigated whether the reduction of CD27 expressing B cells was due to an increase in the shedding of CD27. Indeed, it has been demonstrated that the levels of soluble CD27 (sCD27) are increased in a variety of immunopathological diseases, including primary Sjogren's syndrome and B-cell malignancies. However, the levels of sCD27, analyzed by ELISA in the sera of MS patients before and after IFN-β therapy, were unchanged. Furthermore, the expression of CXCR4 and CXCR3 was analyzed by flow cytometry on circulating CD19+ B cells. These chemokine receptors are interesting since CXCR4-expressing B cells may return from the bloodstream to the bone marrow after the differentiation into Ab-producing cells, while the migration of B cells into inflammatory tissues is mainly dependent on CXCR3. Also in this case we did not find a specific effect of IFN-β treatment on the expression of both CXCR3 and CXCR4 in B cells. Based on these evidences, our interest is now focused on the possibility that IFN-β might induce specifically apoptosis in memory B cells of MS patients.

To this aim, we would use two different approaches. At first we will test whether $CD19^+/CD27^+$ memory B cells display an enhanced expression of the apoptotic marker CD95 (or FAS) following IFN-β therapy, since it has been shown that a treatment with this cytokine can induce this receptor on other cell types. Furthermore, we will also evaluate the induction of apoptosis in memory B cells directly analyzing the phosphatydyl-serine exposure by using Annexin-V. To better discriminate the necrotic cells from the early or late apoptotic ones the 7-actinomycin D (7AAD) will be also included in the analysis. In the case where CD95 expression would result increased in memory B cells upon IFN-β therapy, a stimulation of PBMC obtained from IFN-β-treated MS patients with an anti-FAS antibody will be also performed to evaluate the biological relevance of this induction and the frequency and apoptosis of $CD19^+/CD27^+$ memory B cells analyzed by flow cytometry. Furthermore, we might also study the specific response to IFN-β therapy through the increase in the expression of the surface activation marker CD38 (an interferon-inducible gene), after therapy, on naïve or memory B cells to assess whether this may correlate with apoptosis mediated by continuous activation after IFN-β administration.

The use of various monoclonal antibodies directed against the surface markers listed above together with Annexin V might allow us to evaluate in deep the variation in the percentage of the immune cells, and in particular B cells, in peripheral blood of MS patients undergoing Betaferon therapy. This approach will help us to confirm our

preliminary data showing a specific action of IFN-β on B lymphocytes and to extend our study on the mechanisms underling this phenomenon.

In particular, to carry out this program we are planning to use the following combinations of antibodies listed in IT-Box-139:
1) CD19-FITC/CD3-PE/CD14-APC: to discriminate following IFN-β therapy the specific reduction in percentage of B cells as compared to T cells or monocytes.
2) CD19-FITC/CD20-PE/CD27-APC: to evaluate the percentage of naïve versus memory B cells before and after therapy.
3) CD19-FITC/CD38-PE/CD27-APC: to assess the response to IFN-β therapy and activation status of B cells by analyzing the expression of the IFN-inducible activation marker CD38.
4) CD19-FITC/CD95-PE/CD27-APC: to test whether memory B cells of IFN-β-treated MS patients would induce the apoptotic marker CD95.
5) Annexin V-FITC/CD19-PE/CD27-APC/7AAD: to discriminate live, apoptotic or dead circulating memory B cells before and after IFN-β
therapy in MS patients.
6) IgG1, IgG2a or IgG2b FITC-, PE- or APC-conjugated will be used as non-specific human control as needed.