IMMUNE RESPONSE HETEROGENEITY AND CLINICAL EVOLUTION OF MULTIPLE SCLEROSIS PATIENTS TREATED WITH INTERFERON BETA OVER A DECADE

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Multiple Sclerosis (MS) is one of the most frequent neurological pathologies and represents one of the major causes of non-traumatic invalidity in the young adult. A chronic, inflammatory, immune-mediated and neurodegenerative disease of the central nervous system (CNS), MS combines complex mechanisms in the aspect of initiating and maintaining the axonal demyelination phenomenons together with neurodegeneration in the brain parenchyma and spinal cord.

It's multifactorial etiology is a subject of numerous studies in our days. Until now, only three factors were proved certain to play a direct role in developing MS: seropositivity for Ebstein Barr Virus, a history of infectious mononucleosis and smoking. These three factors, together with a low level of vitamin D in a patient with a genetic susceptibility for MS can be the trigger for the immune responses responsible for inflammatory and neurodegenerative mechanisms.

The entwining of the inflammatory and neurodegenerative processes represents the basis of MS' physiopathology. Thus, early inflammation which acts as a key factor of demyelination processes evolves in an inversely proportional manner with the neurodegeneration, both independent. Thanks to the studies of Compston (2006) and Leray (2010), the physio-pathological evolution now recognizes two stages: the first one, early in the progression of the disease is dependent on focal inflammation and the second, which augments later in the progression of the disease is dependent on residual inflammation and neurodegeneration, and independent of the first. Numerous controversies exist nowadays regarding the relationship between these two processes. The outside – in hypothesis stipulates the fact that the inflammation is the process that activates all the mechanisms of neuronal destruction by peripheral activation of the T lymphocytes, enabling their penetration in the CNS. The inside – out hypothesis states that the neurodegenerative processes appear at the onset and are maintained by the action of the inflammatory mechanisms. Supporting data for the inside out hypothesis are found in studies upon the effects of disease modifying therapies (DMTs), which play their role in the inflammatory, active MS phase but have modest or null effects in the progression of the disease.

The immune pathogenesis of MS is the result of the complex interaction between innate and adaptive immunity. The agents of innate immunity are dendritic cells (DC), NK cells (Natural Killer) and macrophage population of the CNS. The adaptive immunity is mediated trough TCD4+ and CD8+ cells, respectively B cells. The specific activation and differentiation of naïve T cells is coordinated in the periphery by DC and antigen presenting cells (APC). These types of cells are normally found in the teguments and other areas that have a direct contact with the exterior environment, with an important role to interact and take the antigen and transport them to the lymphatic ganglia. Here, the antigen will make contact with naïve T cells and will promote antigen-specific immunity or contrary, immune tolerance. Naïve T lymphocytes, after they are peripherally activated, under the influence of various transcription factors will differentiate in effector T cells: Th 1 and Th17 with proinflammatory properties and Th2 with anti-inflammatory properties, with an important role in identifying and neutralizing the pathogenic agent recognized as a non-self. Subsequently, some lymphocytes will differentiate in regulatory T cells, that play a role in discriminating the self from the non-self. Chronologically, the first event is represented by the peripheral activation of the T lymphocytes, in the lymphatic tissues under the influence of APC. The susceptibility of the lymphoid cells to react to presented antigens is mediated by the thymus, by a negative selection of strong auto-reactive T cells, in order to prevent autoimmunization. But, a separate category of T cells was described in patients predisposed to MS, as being more sensitive to the crossed reaction with the myelin antigens, which gives them a highly proinflammatory potential.

After activation, the T lymphocytes CD4+ will leave the lymphatic ganglions, enter the blood stream and in the CNS they will pass through the blood brain barrier (BBB). The cerebral tissue is privileged by the action of the immune cells, but in SM, they will pass in the brain parenchyma and start to secrete proinflammatory cytokines such as IFNγ and TNFα that stimulate the expression of adhesion molecules ICAM-1 and VCAM-1, passing through the tight junctions between the endothelial cells. The last obstacle is represented by glia limitans, a basal membrane composed by astrocyte endings. In the CNS, the T lymphocytes will be reactivated by local APCs and by secreting proinflammatory cytokines (IFNγ, IL-17, TNFα) will activate the microglia, maintaining the BBB breakdown and recruitment of other inflammatory cells, such as NK and B lymphocytes. The result is a complex inflammatory process, a result

of the interaction between cytokines, antibodies, proteases (ex MMP-9), toxic mediators such as ROS and glutamate, all of them leading to the initiation of the demyelinating processes.

The pharmacological approach of MS has three congruent plans: treatment of relapses, symptomatic treatment and DMTs. The Interferon β 1b (IFNβ1b) was the first DMT approved for the treatment of MS for RRMS in 1993, following a double blind, placebo-controlled study on 372 RRMS patients that received 50mcg sc IFNβ1b every other day, 250mcg sc IFNβ1b every other day or placebo. The efficiency of IFNβ1b upon disability progression, recurrence rates and evolution were uncontestable, in 1999 it was also approved for the treatment of secondary progressive MS (SPMS).

The mechanism of action of IFNβ1b in MS is not fully understood. It reduces the number of myeloid DC in the peripheral blood stream, it decreases the production of IFNγ, reduces the activity of APC that inhibits the activation of T lymphocytes. The inhibition of MCH class II in the periphery, by modulating the co-stimulating molecules such as CD80 and CD28 in the APC, decreases the peripheral activation of T lymphocytes. One of the most important indirect effects is mirrored in the umoral layer, by inhibiting the proinflammatory cytokines and by stimulating the anti-inflammatory cytokines, it shifts the immune response from a Th1 derived to a Th2. Numerous studies centered their objectives upon in vivo and in vitro effects of IFNβ. The in vitro studies demonstrated that by modulating DC, B and T lymphocytes, it prevents the differentiation of T lymphocytes towards Th17, a strong proinflammatory subclass of effector T cells, by directly inhibiting IL-1β, IL-23 and TGF-β and concomitantly promotes the anti-inflammatory cytokine secretion such as IL-27 and IL-10. High serum levels of IL-17 correlated with an suboptimal response to IFNβ therapy.

Thus, knowing the heterogeneity of the immune response in the MS patients, together with the intricate mechanisms of inflammation and demyelination, the present study wished to identify the clinical and immunological characteristics of MS patients treated more than 10 years with IFNβ1b.

The first study encompassed the correlations between the serum levels of various pro and anti-inflammatory cytokines with the clinical aspects of the patients: disability duration, EDSS, annual recurrence rates (ARR), and subsequently to identify the differences based on gender, with the purpose that these biomarkers be used as biomarkers for treatment's response. We included 70 patients diagnosed with MS, treated with IFNβ1b for more than 10 years in the Regional MS center in Targu Mures, Neurology 1 clinic. The patients were evaluated both clinically and serologically based on a pre-mixed 15 cytokine panel, Bioplex Pro Human Th17 Cytokine Panel, using multiple xMAP method.

The conclusions of the first study strengthen the indication to administer IFNβ1b in patients that present with a favorable evolution, by drastically lowering the ARR. The major cytokines implicated in the pathogenesis of MS, IL-10 and IL17F showed no major statistical results, due to the fact that they are mainly expressed in the initial stages of the disease. Serum levels of TNFα correlated with EDSS, indicating a higher risk of disability and IL-6 proves its active proinflammatory role, being closely correlated with ARR.

An interesting aspect was found: the co-modulation between sCD40L and IL-31 that opened the gates to new hypothesis for research. The interrelation between these two cytokines, sCD40L with proinflammatory properties and IL-31, presumably anti-inflammatory, was described once before, by Guerrero-Garcia (2018), when they proposed using low IL-31 serum levels as a favorable biomarker to responders to DMTs. This co-modulation was described in the abovementioned study in patients with RRMS, but in our study it also correlated for SPMS.

The immune response differences based on gender are of high and actual importance. After analyzing the immune profile, we noticed that TNFα is an inflammation biomarker expressed both for female and male population. Among the biomarkers proposed for female patients, there were IL-17F and TNFα and for the male patients, IL-6 and TNFα. IL-25, due to its polymorphism dependent on the exon sequences, might indicate neurodegeneration in male patients.

The second part of the present thesis studied the clinical characteristics of the MS patients and assessment of disability, such as defining the favorable/unfavorable prognostic factors, respectively the research upon a paradoxal entity, benign MS (B-MS) by analyzing a lot of patients with a minimal

disability score after more than 10 years of treatment with IFNβ1b. The latter were evaluated based on their ambulatory parameters, using T25FW test, cognition: symbol digit modalities test (SDMT) and depression and anxiety by Hamilton questionnaire.

The results proved a reduction in disability accumulation after keeping the IFNβ1b therapy for a long period of time. Younger age at onset (30 years) was a favorable clinical biomarker together with initiating treatment at a younger age and as soon as the diagnosis is made, by taking advantage of the concept of therapeutic window of opportunity. IFNβ1b does not directly influence the progression in the SP phase but actively reduces the inflammatory activity with benefic effects regarding disability accumulation. The onset with motor signs is associated with a poorer prognosis while the onset with brainstem symptoms correlates with a better prognosis.

The patients from the supposed B-MS group presented the following favorable indictors: female gender, young age at onset, onset with sensory symptoms, optic neuritis or brainstem symptoms such as diplopia. In these patients, a significant positive correlation was found between the levels of IL-10 and IL017, that suggest the persistence of inflammatory activity with high IL-10 levels induced either by IFNβ1b treatment (perfect treatment responders) or by the fact that patients had high basal levels of IL-10 that maintained a benign evolution. IL-6 maintains its specificity, being positively correlated with ARR. Cognition assessment using SDMT might unmask early cognitive decline, but further studies are needed in order to standardize the test and the patients have to be longitudinally followed.

The unicity of this studied lot reigns in the possibility of active following these patients. A significant number of studies that include imaging, biologic and serologic criteria are ongoing both international and locally. The disponibility of clinical biomarkers is being completed with inflammation biomarkers, the subject of the first part of the present thesis, but the real challenge is represented in identifying the neurodegenerative and progression biomarkers.

The present work, at this time is one of the few studies in literature that combines the immune profile with the clinical characteristics of the patients with a long disease evolution, treated with the same DMT for more than a decade. The results aid in collecting practical data that can be used by the neurologist in everyday practice and open the gates to numerous research opportunities not only upon the immune profile, but also on a clinical perspective.

The study of mechanisms and their application at a individual level represents the stone for the foundation of modern, personalized therapies in MS.