MARKER OF INTRATHECAL IMMUNE ACTIVATION FOR THE EVALUATION OF THE IFN-β EFFICACY IN RELAPSING-REMITTING MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an immune-mediated chronic inflammatory demyelinating disorder of the central nervous system characterized by a disruption of blood-brain barrier and expansion of activated self-reactive lymphocyte clones in CNS. Formation of perivascular inflammatory lymphoid infiltrates promotes focal microglia activation, demyelination and axonal loss. Specific MS lesions could be visualized on MRI of the brain or spinal cord as T2 hyperintensive or gadolinium-positive T1 sites. Location, number and size of the lesions determine the individual neurological symptoms. The rate of disease progression is also individual, but the accumulation of neurodegenerative changes leads to severe disability. The most common clinical form (~ 85% of cases at disease onset) is relapsing-remitting multiple sclerosis (RRMS), characterized by relapse events followed by partial or complete remission.

There is no effective therapy for MS currently, but several disease modifying drugs (DMDs) have been shown to ameliorate the disease course, although the individual treatment response remains unpredictable.

The evaluation of DMD's therapeutic efficacy is based on the Expanded Disability Status Scale (EDSS), which reflects the rate of disability progression according to clinical and radiological features, namely the number of clinical exacerbations and new sites of specific lesions on the MRI after the first year(s) of therapy. Considering MS progression is not a linear process influenced by complex of divers (mostly poorly defined) genetic and environmental factors, use of EDSS scoring strictly speaking can't be regarded as completely objective method.

Interferon beta (IFN-β) is the first DMD introduced in clinical practice in the 1990s. Due to its relatively good safety profile, it remains the first-line therapy for patients with RRMS. Its efficacy was confirmed by numerous studies showing significant reduce in the relapse rate, areas of lesions, and disease activity. However, clinical response varied among individuals, and about 20% of IFN-β recipients haven't improvement (so called none-responders)[1]. Moreover, there are evidences suggestive of possible deleterious effect of IFN-β in some patients [2, 3]. So, it is highly desirable to have ability for more objective assessment of the therapeutic intervention efficacy, and preferably it would have been done earlier than in a year after treatment initiation.

The first useful quantitative marker that negatively correlates with the effectiveness of IFN-β therapy is the titers of IFN-β neutralizing antibodies (NAb) [4]. Any biopharmaceutical are potentially capable of inducing an immunogenic response characterized by the accumulation of antibodies specific to an active biomolecule. Different types of approved recombinant IFN-β preparations have different antigenic properties. The process is also affected by individual immunological reactivity, dose and route of administration, but in general, NAb are formed in 2–40 % of cases during the first 6–12 months of treatment, reducing drug bioavailability, and, accordingly, its therapeutic efficacy.

Nevertheless, the results of retrospective studies indicate that NAb-positive patients represent minority among none-responders, suggesting the antibody mediated drug bioavailability reduction is obviously not the main cause of a suboptimal response to IFN-β [5].

A common disadvantage of the method is the use of marker that is not directly related to the pathogenesis of multiple sclerosis as well as to the biological effects of IFN-β. Actually, despite the long history of recombinant IFN-β using, the mechanisms that provide its therapeutic effect in MS remains unclear. IFN-β is a pleiotropic cytokine. Its binding to the IFNAR receptor on the surface of various types of immune cells leads to the activation of several transcriptional factors regulating the expression of more than hundred known genes (interferon regulated genes, IRGs) [6] whose products affect the numerous immune processes. The complex system of negative feedback mechanisms, which provides control of the immune system activation at different levels of the signaling cascade, greatly complicates the understanding of the nature of the pharmacotherapeutic effects of long-term IFN-β therapy.

The differences in the mRNA expression profiles of peripheral blood mononuclear cells (PBMC) in patients who responded or did not respond to IFN-β therapy [7] was shown, but an algorithm that allows distinguishing these two categories has not been determined.

The expression levels of some IRGs (e.g. MxA, GPR3, IL17RC, ISG15, TRAIL, OASL, IFIT1, IFIT2, RSAD2, OAS3, IFI44L, TRIM22, IL10, CXCL10, STAT1, OAS1, OAS2, IFNAR1, IFNAR2, IFN-β, ISG20, IFI6, PKR, IRF7, USP 18) was proposed for monitoring of IFN-β therapy in MS patients. The most promising results were shown for MxA (Myxovirus Resistance Protein A) and its mRNA expression levels [8], which correlate well with NAb's positivity and, under certain conditions, may be more appropriate than determining the titers of IFN-β-neutralizing antibodies. The LFIA test system was developed, which allows detection 96% of the IFN-β responders with 89% specificity using a cut-off level of 100 μg/L for an elevated MxA-concentration [9].

However, other independent pharmacogenetic study has shown that IRGs expression level in Nab-negative patients did not allow identifying the group of IFN-β none-responders [10]. The probable explanation is that the majority of IRGs (including MxA, which is an element of nonspecific antiviral resistance) haven't direct relation to the MS pathogenesis, and their expression levels reflect just the presence of a biological response to IFN-β, but do not characterize its influence on the disease course. In addition, Rudick R.A. et al (2011) [2] using the

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panel with 166 IRGs demonstrated that the biological response to IFN-β in the group of patients with low therapeutic efficacy was higher than in patients whose state was improved during therapy. Therefore, it can be assumed that some patients with poor response to therapy may have relatively high levels of IRGs induction, including MxA.

Principal feasibility of cerebrospinal fluid (CSF) markers of inflammation, axonal damage, and demyelination for evaluation of DMD efficacy in patients with progressive forms of multiple sclerosis (PPMS, SPMS) was demonstrated [11,12]. Sufficient decrease of CSF osteopontin (OSP) from baseline to week 60 (as well as decreasing of some other CSF biomarkers, e.g. cell count, index IgG, CXCL13 and MMP9) was detected in an open-labeled, phase 2A, proof-of-concept trial of Natalizumab in progressive MS [11]. However, in a similar study of the methylprednisolone efficacy, there was no significant decline of CSF OSP level (as well as levels of others CSF markers, with the exception of MMP9) in 12 and 60 weeks of treatment despite the presence of clinical signs of improvement [12].

Recently, the responsiveness of several CSF inflammatory markers (in particular sCD27, sCD21, IL-7, IL-12p40, IL-10 and TNF-α) to treatment with both natalizumab and methylprednisolone in progressive MS was demonstrated [13], and sCD27 (soluble form of CD27 receptor) was validated as sensitive and reliable biomarker of intrathecal inflammation.

Traditionally, multiple sclerosis considered to be T-cell mediated disease in view of the prevalence of CD4+ Th1 / Th17 and CD8+ T lymphocytes in CNS perivascular infiltrates.

However, the experience of different DMD application indicates that suppression of T-cell immunity (e.g., the neutralization of IL-12 / IL-23p40 inhibiting Th1 and Th17 polarization) does not significantly affect the disease activity [14]. T lymphocyte depletion also does not have a positive therapeutic effect unless it is accompanied by a concomitant depletion of B-lymphocytes. One of the most effective "anti-T" DMD is Alemtuzumab — monoclonal antibodies specific for CD52, predominantly expressed on the surface of both T and B lymphocytes.

Finally, remarkable therapeutic effect of B cell depletion therapy (BCDT) with monoclonal antibodies to CD20 (rituximab, ocrelizumab) and CD19 (inebilizumab) led to the correction of the existing paradigm of MS immunopathogenesis [15].

The BCDT effect is obviously not due to inhibition of antibody production, as the plasma cells (the major antibody producers) do not express CD20. The most likely targets are other functions of B cells, in particular their ability to process and present antigens (particularly, autoantigens) to T cells in the context that determines the direction of their differentiation [16]. The context is provided by surface expression of costimulatory or co-suppressing molecules (CD70, CD80, CD86, CD38, OX40L, PD-L1) and production of pro- or anti-inflammatory cytokines (lymphotoxin-α, TNF-α, IL-6, GM-CSF, IL-10, IL-35, TGF-β). Certain subpopulations of B-lymphocytes are capable of inducing effector T cells apoptosis and anergy [17]. The ability to regulate proliferation and activation of conventional antigen presenting cells (APCs) of the monocyte-macrophage lineage was also shown, which may further advance B-cell ability to modulate local T cell response [18].

B-lymphocytes were found in meninges, parenchyma and CSF of MS patients, and their number increased during exacerbations. Ectopic lymphoid follicles were found in the meninges and brain parenchyma in patients with progressive forms of MS, which is likely to be the main source of resident pathogenic lymphocyte populations. Clinical exacerbations are accompanied by an active intrathecal migration of antigen-experienced memory B cells with a concomitant decrease of their number in peripheral circulation [19], and the migratory populations of memory B-cells considered being the main driver for immunopathological changes in RRMS [20].

It should be noted that memory (CD27+) B-cells itself (in particular, the recently characterized population of GM-CSF+CD27+CD24highCD25highCD86highCD49dhigh CD38low [21]) are the major source of inflammatory stimuli, while less mature (CD27-), particularly transient (CD19+CD20+IgD+CD27-CD24hiCD38hi transitional B-cells), as well as the terminally differentiated plasma cells (CD138hiCD22-), generate mostly tolerogenic signals [22, 23].

Generally, accepted explanation of BCDT therapeutic effect in MS is that relatively rapid recovery of the B-cell population after the depletion occurs due to less mature forms, ensuring at least temporary prevalence of tolerogenic stimuli over the activational ones.

Finally, it has been shown that B-lymphocytes are targets for other DMDs, including IFN-β [24, 25, 30]. Like other Type I interferons, IFN-β increases the expression of BAFF (B-cell activating factor), stimulating the autoantibodies production in patients with SLE (Systemic Lupus Erythematosus) and NMO (Neuromyelitis Optica), the so-called "type I IFN diseases". In MS patients IFN-β also increases the expression of BAFF, but paradoxically reduces the activity of the inflammatory process [26].

Interestingly, in MS intrathecal production of BAFF observed (predominantly by astrocytes) and its concentration in CSF rise with the disease progression [27, 28], which could have an independent predictive value, as exogenous IFN-β may not have a positive effect in presence of elevated intrathecal concentration of BAFF.

The most significant IFN-β-induced immunophenotypic changes in PBMC are rising absolute and relative numbers of transient B-cells [29, 30] for which BAFF is a survival factor. This population is tolerogenic and produces significant amounts of IL-10 after activation of the CD40/STAT3 signaling cascade [31]. Simultaneous decrease in the memory B cells also observed [30, 32], and may be explained by FAS/TACI dependent apoptosis. In particular, IFNβ-inducible expression of apoptosis marker FAS-R (CD95) is more pronounced in the CD27+ memory B cells [32].

Such kind of phenotypic and functional changes of B-cell populations resembling those of BCDT could have similar effect, decreasing effector T-cell activity. In this context, CSF sCD27 validated for monitoring of

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DMD efficacy in progressive forms of MS [13], could be no less useful in assessment of IFN-β efficacy in patients with RRMS, as it actually reflects B-cell mediated T-cell activation [33].

CD27 is a type II transmembrane glycoprotein of the TNF receptor family (TNFRSF7) expressed on the surface of different lymphoid cells (most T-, antigen-experienced B- and NK-cells). Interaction of CD27 with its ligand CD70 contributes to lymphocyte activation, affecting their survival and differentiation. Extensive secretion of sCD27 is believed to be a mechanism that provides T-cell competition for CD70, which determines the duration of contact with APC during the antigen presentation process [34].

In vitro, elevation of sCD27 occurs after antigen-specific activation of T-lymphocytes, and, importantly, the level of sCD27 significantly increases when this activation occurs in the presence of B-lymphocytes [33]. Thus, CSF level of this marker can be used as a cumulative indicator of local B-mediated antigen-specific T cell activation.

In most IFN-β treated MS patients, the "peak of renewal" of the B-cell population is observed in 6th month of therapy [35], so, it could be supposed, monitoring of sCD27 CSF concentration changes may allows relatively early estimation of therapeutic intervention appropriateness [13, 36]. For conclusions that are more credible the broadening of research in this direction is highly desirable.

Conclusions. Managing of such complex disease as MS requires obtaining information about the main physiological processes in different compartments. Development and validation of biomarkers for optimization of therapeutic strategies in patients with MS is complicated by high individual variability of disease course and treatment response, as well as multiple issues concerning study design and results interpretation. Monitoring of CSF levels of sCD27, as an early treatment responsive marker of intrathecal immune activation, which correlates with clinical indicators of the course of the disease, shows very promising results and deserves closer attention of researchers and clinicians dealing with MS.

Marker of intrathecal immune activation for the evaluation of the IFN-β efficacy in relapsing-remitting multiple sclerosis

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Multiple sclerosis is an autoimmune neurodegenerative disease of the central nervous system characterized by a violation of the integrity of the blood-brain barrier, an expansion of autoreactive lymphocyte clones specific to the membrane proteins of the nerve cells, and the formation of focal perivascular lymphocytic infiltrates. Interferon beta (IFN-β) is the first-line disease modifying drug in the treatment of relapsing-remitting multiple sclerosis (RRMS). However, clinical response varied among individuals, and about 20 % of IFN-β recipients haven't improvement. Moreover, there are evidences suggestive of possible deleterious effect of IFN-β in some patients. The identification of biomarkers that can help in the early evaluation of the response to a patient's treatment is necessary primarily for the individualized treatment of multiple sclerosis. In particular, cell-specific biomarkers of intra-inflammatory inflammation, which correlate with clinical manifestations of multiple sclerosis, can serve as informative indicators of the course of the disease. As a candidate marker of this type, the content of the soluble form of CD27 (sCD27) - a second type transmembrane glycoprotein, belonging to the TNF receptor (TNFRSF7) and expressing on the surface of cells of the lymphoid series, in cerebrospinal fluid is considered. It can be used as a cumulative indicator that correlates with the process of B-mediated antigen-specific T cell activation and is suitable for an objective assessment of the therapeutic effect of IFN-β in PPMS, starting from 6 months after initiation of therapy. Monitoring of CSF levels of sCD27, as treatment responsive marker of intrathecal immune activation, shows very promising results.

Keywords: multiple sclerosis, biomarkers, IFN-β, CD27.

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