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Modulating effect of HERV-W ENV on peripheral blood monocytes in patients with different types of multiple sclerosis

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The aim of the study was to determine the effect of the HERV-W ENV glycoprotein on the functional and metabolic characteristics of intact and stimulated peripheral blood monocytes, depending on the type of multiple sclerosis (MS), which can contribute to our understanding of their pathogenetic role in the development of neurological disorders and to design of novel therapeutic agents.

Materials and methods. To determine the reserve capacity index (Rci) of cytokine production, HERV-W ENV glycoprotein, *E. coli* lipopolysaccharide (LPS) or ssRNA40/LyoVec were added to the monocyte-enriched cell suspension as agonists of TLR4 and TLR7/8. The study also included the evaluation of arginase activity in cell lysates of the monocyte fraction. The study involved 37 patients with relapsing-remitting type and 19 patients with progressive type of MS. The control group included 32 healthy individuals.

Results. In patients with MS of both groups, the reserve capacity of monocytes to produce IL-1β was lower than in healthy individuals, while the reserve capacity to produce TNF-α and IL-10 was almost twice as high as in the control group. This index for IL-1RA in monocytes obtained from the group with progressive MS and stimulated with HERV-W ENV was 2 times lower than the control; thus increased IL-1β values provoke chronic inflammation. The highest arginase activity in patients with relapsing-remitting MS was observed when monocytes were cultured with HERV-W ENV and LPS. In patients with progressive MS, during the cultivation of monocytes with LPS, arginase activity was significantly reduced relative to the control.

Conclusions. The results obtained indicate functional and metabolic changes in peripheral blood monocytes upon stimulation with TLR4 and TLR7/8, which may be associated with the disease course. In progressive MS, stimulation with HERV-W ENV leads to an imbalance of pro-inflammatory and regulatory cytokines, contributing to a chronic inflammation and, as a consequence, to a more severe course of the disease.

Ключові слова:
HERV-W, розсіяний склероз, цитокіни, Toll-подібні рецептори.

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Модулювальна дія HERV-W ENV на моноцити периферичної крові в пацієнтів із різними типами перебігу розсіяного склерозу

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Мета роботи – визначити вплив глікопротеїну HERV-W ENV на функціональні та метаболічні особливості інтактних і стимульованих моноцитів периферичної крові залежно від типу перебігу розсіяного склерозу (РС), що є дуже важливим для розуміння їхньої патогенетичної ролі в розвитку неврологічних розладів і для розроблення нових терапевтичних засобів.

Матеріали та методи. Для визначення індексу резервної здатності продукції цитокінів у збагачену моноцитами суспензію клітин додавали глікопротеїн HERV-W ENV, ліпополісахарид *E. coli* або ssRNA40/LyoVec як агоністи TLR4 і TLR7/8. Оцінювали активність аргінази в лізатах клітин моноцитарної фракції.

У дослідженні взяли участь 37 пацієнтів із рецидивно-ремітуючим та 19 осіб із прогресуючим типом РС. Контрольна група – 32 здорові особи.

Результати. У пацієнтів із РС обох груп резервна здатність моноцитів до продукції IL-1β була меншою, ніж у здорових осіб, а резервна здатність до продукції TNF-α та IL-10 майже вдвічі вища за показник контрольної групи. Резервна здатність моноцитів до IL-1RA у групі пацієнтів із прогресуючим РС під дією HERV-W ENV вдвічі нижча за контроль, а отже підвищені показники IL-1β спричиняють хронічне запалення. Найбільшу активність аргінази в пацієнтів із рецидивно-ремітуючим РС спостерігали під час культивування моноцитів за наявності HERV-W ENV і LPS. У хворих на прогресуючий РС при культивуванні моноцитів з LPS аргіназна активність вірогідно знижена щодо контролю.

Висновки. Результати дослідження вказують на функціональні та метаболічні зміни моноцитів периферичної крові при стимуляції TLR4 і TLR7/8, що можуть бути асоційовані з типом клінічного перебігу захворювання. При прогресуючому перебігу РС індукція HERV-W ENV призводить до порушення балансу прозапальних і регуляторних цитокінів, спричиняючи хронічне запалення, а отже тяжчий перебіг захворювання.

Currently, there is a limited information on the effects of human endogenous retroviruses (HERVs) on the innate immune system, which represents the first line of defense against viruses and acts mainly through the detection of invariant microbial molecular patterns by pattern recognition receptors (PRRs) expressed on antigen-presenting cells (APCs), such as monocytes, macrophages and dendritic cells, as well as on other cell types. Examples of PRRs include transmembrane Toll-like receptors (TLRs), which can recognize various microbial products and act as a central element of the innate immune response to different classes of pathogens [1,2].

HERVs make up 8 % of the human genome and are derived from retroviral infections which integrated into mammalian germline cells millions of years ago [3]. HERVs are usually epigenetically silenced, but transactivation by exogenous viral infection, such as herpes virus type 6 (HHV-6), Epstein-Barr virus, or other viruses epidemiologically associated with multiple sclerosis (MS), can promote their (re)expression [4]. Studies have shown that human endogenous retrovirus type W (HERV-W) negatively affects the differentiation and remyelination of oligodendroglial progenitor cells through the pathogenic envelope protein HERV-W ENV. It has been shown that in multiple sclerosis, HERV-W ENV is present in myeloid cells associated with axons [5]. HERV-W ENV has been shown to induce a degenerative phenotype in microglial cells of patients with progressive multiple sclerosis, leading to the close spatial association of these cells with myelinated axons. Moreover, in HERV-W ENV-stimulated myelinated cocultures, microglia were found to structurally damage myelinated axons.

Elevated concentrations of HERV-W ENV protein, RNA, and/or DNA were detected in serum, cerebrospinal fluid, and brain in both clinically isolated syndrome (CIS) and clinically defined MS [6]. In addition, the HERV-W ENV presence was found to correlate with faster clinical progression of the disease and with an increased conversion into secondary progressive MS [7]. The HERV-W ENV glycoprotein inhibits myelin repair, hampering differentiation of oligodendroglial progenitor cells by inducing nitrosative stress through the activation of Toll-like receptor 4 (TLR4) [8]. In MS, monocytes are involved in both autoimmune inflammation and neurodegeneration, producing proinflammatory cytokines and molecules [9,10].

Therefore, HERV-W ENV may play a role in the pathogenesis of multiple sclerosis, so our study was aimed at analyzing the effect of this glycoprotein on the activity of monocytes at the functional level. We hypothesize that the expression of endogenous retrovirus HERV-W may contribute to inflammatory conditions, thereby fueling the autoimmune disorder.

Aim

To determine the effect of the HERV-W ENV glycoprotein on the functional and metabolic characteristics of intact and stimulated peripheral blood monocytes, depending on the type of multiple sclerosis, which can contribute to our understanding of their pathogenetic role in the development of neurological disorders and to design of novel therapeutic agents

Materials and methods

Immunological studies were conducted in the Laboratory of Clinical Immunology and Allergology of the State Institution “Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine”. The study included 56 patients with a verified diagnosis of “multiple sclerosis” established in the Department of Neuroinfections and Multiple Sclerosis of the State Institution “Institute of Neurology, Psychiatry and Addiction Medicine of the National Academy of Medical Sciences of Ukraine”. All patients were divided into two groups depending on clinical course of the disease:

1. A group of patients with relapsing-remitting MS (RRMS), n = 37 (16 men and 21 women), the mean age of patients was 35.9 (32.3; 38.54) years; included patients with a disease duration of at least 12 months who had at least one MS exacerbation or one paramagnetic contrast center on MRI 12 months prior to randomization;

2. A group of patients with progressive MS (PMS), n = 19 (8 men and 11 women), the mean age was 39.4 (34.6; 43.7) years. In PMS, there is no remission after the onset due to the formation of a progression stage, although patients may experience periods of stabilization of the disease without relapse or new MRI activity.

For 6 months before the collection of biological material, patients had not received any therapy with the drugs that could modify the course of the disease.

The control group consisted of 32 healthy individuals (11 men and 21 women) with a mean age of 36.2 (33.4; 38.6) years. Criteria for inclusion in the control group were the absence of acute infections for at least 1 month before taking biological material, the absence of chronic inflammatory, allergic and autoimmune diseases.

The biological material was blood samples obtained from patients with multiple sclerosis and almost healthy people. All individuals who participated in the study gave their voluntary written consent to participate in the study. All participants gave their voluntary written consent to take part in the study.

Isolation of peripheral blood mononuclear cells (PBMCs). Isolation was performed by the method of G. Rousselet, adapted to small blood volumes [11]. Separation was performed using double Percoll gradient (Sigma, USA). To prepare 100 mL of Percoll isoosmotic solution, 41.5 mL of Percoll with ρ = 1.131 g/cm^3 were dissolved in 48.5 mL of distilled water, 10 mL of 1.5 M NaCl solution were added and mixed thoroughly. To prepare 100 mL of Percoll hyperosmotic solution, 48.5 mL of Percoll with ρ = 1.131 g/cm^3 were dissolved in 41.5 mL of distilled water, 10 mL of 1.6 M NaCl solution was added and also mixed thoroughly.

Isolation cells of the monocyte fraction. To isolate cells of the monocyte fraction, 1 mL of the prepared mononuclear suspension was carefully layered on 3 mL of Percoll hyperosmotic solution and centrifuged at 580 g for 15 minutes at +20 °C. The cell layer was carefully removed from the phase interface using a plastic Pasteur pipette, resuspended in RPMI-1640 medium to 12 mL and centrifuged at 350 g for 7 minutes at +20 °C. After removal of the supernatant the procedure was repeated. After centrifugation the supernatant was removed, and the pellet was resuspended in 3 mL of complete RPMI

medium. The resulting suspension was adjusted to 5×10^6 cells/mL and divided by 0.1 mL. The number of monocytes in the suspension, determined with anti-CD14 PE-labeled monoclonal antibodies (EXBIO Praha, Czech Republic), was 75 % or more. Cell viability after trypan blue staining was at least 98 %.

TNF-α, IL-1β, IL-1RA, IL-10, IL-12p70, IL-12p40 concentrations in culture supernatants were determined using test systems "Alpha-TNF ELISA Best" A-8756, "Interleukin-1 beta ELISA Best" A-8766, "IL-1 receptor antagonist ELISA Best" A-8764, "Interleukin-10 ELISA Best" A-8774 manufactured by CJSC «Vector-Best-Ukraine» and test systems Human IL-12p70 Human ELISA Kit, IL-12 p40 Human ELISA Kit (Invivogen, USA).

For the series exposed to HERV-W ENV, LPS, and ssRNA40/LyoVec, the Reserve capacity index (Rci) of each cytokine production was calculated as the ratio of the cytokine content in the series with the exposure to the corresponding stimulator compared with that value in the series with intact cells (Rci_{HERV-W}, Rci_{RNA}, and Rci_{LPS}). For each experimental series, the ratio of IL-1RA and IL-1β production $(IL1RA/IL1β)_{INT}$, $(IL1RA/IL1β)_{HERV-W}$, $(IL1RA/IL1β)_{RNA}$, and $(IL1RA/IL1β)_{LPS}$ was also calculated.

Cultivation of cells of the monocyte fraction. Cells of the monocyte fraction were cultured in four parallel series: a) with the addition of ERVW-1 recombinant protein (MyBiosource, США) at concentration 1 μg/mL as a stimulator of TLR4, b) with the addition of *E. coli* lipopolysaccharide ("Sigma-Aldrich", USA) at concentration 1 μg/mL as a stimulator of TLR4, c) with the addition of a complex of resistant to nucleases guanine and uracil-enriched phosphothioate-modified ribooligonucleotide and cationic lipid single-stranded RNA ssRNA40/LyoVec (Invivogen, USA) at concentration 1 μg/mL as a stimulator of TLR7/8, d) control samples, cultured without an inducer. Monocytes suspension was cultured in 96-well plates at concentration 10^5 cells/mL in a final volume of 0.2 mL for 24 hours at +37 °C in an atmosphere of 5 % CO_2.

After incubation, the suspension was centrifuged at 350 g for 7 minutes at +20 °C, the precipitate was used to determine the cytokine levels and arginase activity and the supernatant – to determine the concentration of nitrites.

Determination of arginase activity and nitrite content in peripheral blood monocytes. Arginase activity in cell lysates was determined by the method of Classen et al. [12]. After removing the supernatant from the wells of the plate, the pellets with cultured cells were carefully washed with 0.2 μL of phosphate buffered saline, after careful removing of the washing solution 0.1 mL of a 0.1 % aqueous solution of Triton X-100 (Sigma-Aldrich, USA) was added to the wells and left for 15 minutes on a shaker for cell lysis. Then 0.1 mL of 50 mM Tris-HCl buffer (pH = 7.5; Sigma-Aldrich, USA) and 0.01 mL of 100 mM $MnCl_2$ solution were added. 0.1 mL of lysate from the wells was transferred to "safelock" tubes and incubated for 7 minutes at 56°C for activation of arginase. Then, 0.1 mL of a 0.5 M arginine solution (pH = 9.7; Sigma-Aldrich, USA) was added to each tube and incubated at t = +37 °C for 120 minutes (series without adding an inductor, with the addition of LPS and ssRNA40/LyoVec). The reaction was stopped by adding 0.8 mL of stop reagent to test tubes, and 0.9 mL to test tubes with 0.1 mL of standard solutions containing 0.0 μg, 7.5 μg, 15.0 μg, 30.0 μg, 45.0 μg and 60.0 μg of urea. To each tube 40 μl of 6 % alcoholic solution of α-isonitrosopropiophenone was added and vortexed for 5 seconds, then sequentially incubated for 30 minutes at t = +95 °C and for 30 minutes at t = +4 °C. After incubation, the urea content in the samples was determined on Stat Fax 303 plus (USA) with a filter installed at 545 nm.

The concentration of nitrites in the supernatants was determined by the method of Griess [12]. 0.1 mL of culture supernatants were transferred to the wells of a 96-well plate, mixed with 0.1 mL of Griess reagent and incubated for 30 min at t = +20 °C in darkness. Absorption was read on Stat Fax 303 plus (USA) with 545 nm filter installed and the nitrite concentrations were calculated according to a standard calibration curve. The results obtained were presented in μmol NO/10^5 cells.

Statistical processing of the obtained data was performed using STATISTICA 11.0 (StatSoft Inc.) and XLSTAT 19.6 (Addinsoft). The normality of the data distribution in the groups was checked using the W-Shapiro–Wilk test. The Mann–Whitney U-test was used to determine the significance of differences in the studied samples. The critical value to assess the significance across these findings was 0.05. Data in the text and tables are presented as medians, 1 and 3 quartiles: Me (Q25 %; Q75 %).

Results

The study of the functional activity of peripheral blood monocytes in patients with different types of MS was performed by modeling their activation with agonists, namely, by HERV-W ENV glycoprotein, *E. coli* lipopolysaccharides for TLR4 activation, and by ssRNA for TLR7/8 activation.

Evaluation of the immunomodulatory effect of the HERV-W ENV glycoprotein revealed significant differences in the reserve ability of monocytes to produce *in vitro* major proinflammatory and regulatory cytokines in patients with different types of the disease (*Table 1*).

It has been found that the reserve capacity of monocytes for TNF-α production in patients with MS was significantly higher than in healthy individuals. The value of Rci_{HERV-W} was 1.6 times higher in patients with a recurrent type of MS than in the control group, and 2.2 times higher than in patients with a progressive type of the disease. When ssRNA40/LyoVec and LPS were added as inducers, the value of Rci in RRMS and PMS groups was 3 times lower than in the control group.

Stimulation with HERV-W ENV glycoprotein led to an increase in production of IL-1β in MS patients, significantly more pronounced than in healthy people in the control group. In particular, HERV-W ENV-stimulated IL-1β production in patients with PMS was 1.6 times higher than that observed in the control group (412.3 (337.9; 564.6) pg/mL vs. 263.5 (171.2; 315.6) pg/mL in the control, $P < 0.05$). The level of stimulated IL-1β production in the RRMS group was 1.5 times higher compared with the control group (318.3 (247.4; 469.5)

Table 1. Reserve capacity index (Rci) of proinflammatory and regulatory cytokine production by peripheral blood monocytes upon stimulation with glycoprotein HERV-W ENV, ssRNA40/LyoVec and lipopolysaccharide *E. coli* in patients with relapsing-remitting multiple sclerosis (RRMS) (n = 37), progressive multiple sclerosis (PMS) (n = 19), and healthy individuals (control) (n = 32), Me (Q25 %; Q75 %)

Cytokines	Series	Patients group		Control
		RRMS	PMS	
TNF-α	LPS	31.8 (28.5; 37.2)*	35.3 (30.2; 43.3)*	120.1 (108.4;131.6)
	ssRNA	43.8 (39.6; 47.2)*	35.6 (31.7; 40.8)*	131.1 (110.4; 148.7)
	HERV-W	35.2 (31.4; 39.6)*	46.8 (42.7; 51.1)*	21.4 (18.5; 25.6)
IL-1β	LPS	7.8 (5.3; 9.6)*	12.5 (10.2; 16.4)*	58.1 (49.5; 66.8)
	ssRNA	9.3 (6.7; 11.6)*	20.2 (16.3; 24.7)*	61.6 (57.2; 65.9)
	HERV-W	5.4 (3.9; 7.1)*	7.2 (5.5; 9.4)*	33.6 (29.4; 37.5)
IL-1RA	LPS	24.6 (21.3; 28.1)	10.4 (8.5; 12.7)*	22.3 (18.4; 25.1)
	ssRNA	23.8 (20.9; 26.7)*	21.4 (19.5; 24.6)*	12.7 (10.3; 15.2)
	HERV-W	17.2 (15.6; 20.5)	7.6 (5.3; 9.0)*	15.4 (12.6; 17.9)
IL-10	LPS	9.3 (7.2; 12.6)	5.4 (3.6; 6.8)*	16.5 (13.2; 18.6)
	ssRNA	4.2 (3.1; 5.8)*	1.6 (0.8; 1.9)*	12.3 (10.3; 15.1)
	HERV-W	7.2 (5.2; 9.3)	6.7 (4.5; 8.8)	4.5 (3.2; 6.0)
IL-12p70	ЛПС	9.1 (7.7; 10.8)*	0.9 (0.5; 1.1)*	2.5 (2.2; 3.6)
	ssRNA	8.3 (6.5; 10.8)*	1.2 (0.8; 1.5)	3.1 (2.6; 3.9)
	HERV-W	9.2 (8.1; 11.3)*	1.3 (0.8; 1.5)*	2.7 (2.0; 3.3)
IL-12p40	LPS	1.6 (1.1; 2.0)	2.3 (1.7; 2.9)*	1.3 (1.0; 1.9)
	ssRNA	1.9 (1.2; 2.3)*	2.4 (1.9; 2.9)	3.6 (3.0; 4.1)
	HERV-W	1.5 (1.1; 1.8)	1.3 (0.8; 1.9)*	2.7 (2.2; 3.4)

*: P < 0.05 when compared with the control group.

Table 2. Arginase activity of peripheral blood monocytes of patients with relapsing-remitting multiple sclerosis (RRMS) (n = 37), progressive multiple sclerosis (PMS) (n = 19) and healthy individuals (control) (n = 32), mU/10^5 cells, Me (Q25 %; Q75 %)

Series	Patients group		Control
	RRMS	PMS	
Non-stimulated cells	2.51 (2.24; 2.86)	2.42 (2.11; 2.73)	2.24 (2.01; 2.54)
HERV-W ENV-stimulated cells	9.15 (6.73; 12.15)	9.34 (8.77; 10.46)*	5.73 (4.13; 7.64)
LPS-stimulated cells	10.41 (8.23; 11.54)	6.22 (5.15; 8.56)*	11.54 (10.03; 12.18)
ssRNA40/LyoVec-stimulated cells	6.59 (5.82; 8.21)	8.74 (6.37; 10.27)	5.46 (3.55; 7.14)

*: P < 0.05 when compared with the control group.

pg/mL vs. 213.9 (186.5; 256.3) pg/mL in the control). The ratio of stimulated IL-1β production to its spontaneous production was significantly reduced relative to control, namely, by 3.7 and 5.5 times in the RRMS and PMS groups, respectively.

Stimulation of IL-1β production during incubation of monocytes with the addition of ssRNA40/LyoVec was more pronounced in the group of patients with a progressive type of MS (P < 0.05 compared with control). At the same time, the values of Rci_{LPS} and Rci_{RNA} had significant differences both between the groups of RRMS and PMS, and between the groups of patients and control group. Rci_{RNA} in the PMS group was 2.2 times higher than in the RRMS group (P < 0.05 when compared between groups of patients), and 3 times lower than in the control group (P < 0.05 when compared between PMS group and control).

In the group of patients with recurrent type of MS, Rci_{RNA} was reduced even more relative to the control indicator, namely, by 6.6 times. Decreased reserve capacity of monocytes to produce TNF-α and IL-1β compared to this indicator in healthy individuals may, on the one hand, indicate changes in the subpopulation of cells producing these cytokines, and on the other hand, indicate the "depletion" of these cells, the presence of impairment in MyD88-mediated signaling and NF-κB activation [13].

HERV-W ENV glycoprotein-induced production of IL-1RA increased both in the control group and in patients with RRMS and PMS. The level of stimulated IL-1RA production in patients with RRMS was 1.8 times higher than in the control group (P < 0.05); in patients with PMS, this indicator did not differ from the control values. It is noteworthy that the value of Rci_{HERV-W} in the group of PMS was 2 times lower than in the control, which may indicate a decrease in the reserve capacity of monocytes to synthesize this protein in patients with PMS; thus, against the background of elevated IL-1β levels in the PMS group, this imbalance provokes chronic inflammation.

The rate of Rci_{LPS} of IL-1RA production in group of patients with PMS was reduced by 2.1 times compared with the control. In ssRNA40/LyoVec-induced production of IL-1RA, the reserve capacity indices in the groups did not differ from the control. Patients with MS also had a significant reduction in $(IL1RA/IL1β)_{INT}$ relative to the control. In the RRMS group, the value of $(IL1RA/IL1β)_{INT}$ was 1.2 (0.9; 1.4) units, in the PMS group 2.3 (1.8; 2.5) units, while in the control group it constituted 9.1 (7.8; 9.6) units. $(IL1RA/IL1β)_{HERV-W}$ was 5.3 (4.9; 5.6) units in the RRMS group, 2.5 (2.1; 2.9) units in the PMS group. The ratio of LPS-induced production of IL-1RA and IL-1β in patients with PMS was significantly reduced compared with the control.

In patients of the RRMS group, the $(IL1RA/IL1\beta)_{LPS}$ was 3.4 (3.1; 3.6) units. The value of $(IL1RA/IL1\beta)_{RNA}$ in patients of the RRMS group constituted 2.8 (2.5; 3.1) units, in the PMS group 2.5 (2.2; 2.7) units, in the control group 2.2 (2.0; 2.5) units ($P > 0.05$ when comparing groups of patients and controls, as well as groups of patients with each other).

Stimulation with HERV-W ENV led to an increase in IL-10 production in the PMS group, and it is important to note that in patients of both groups the value of Rci_{HERV-W} was almost twice higher than in the control group, in contrast to the series treated with other stimulants.

The level of LPS-induced IL-10 production in observed groups of patients with MS did not differ from the control on the background of a moderate decrease in reserve capacity index. With ssRNA40-induction, the content of this cytokine in the RRMS and PMS groups was reduced relative to the control by 3 and 6.3 times, respectively ($P < 0.05$). Rci_{RNA} was reduced by 2.9 times in the RRMS group and 7.7 times in the PMS group, $P < 0.05$.

Therefore, the greatest effect on IL-10 production was observed when monocytes obtained from patients with progressive type of MS were stimulated with HERV-W ENV glycoprotein.

The level of IL-12p70 production in the cultivation of intact monocytes in both groups of patients with MS was significantly lower than in the control. In the RRMS group, the low level of IL-12p70 production by intact cells was accompanied by increased Rci_{HERV-W} values relative to the control (9.2 (8.1; 11.3) units vs. 2.7 (2.0; 3.3) units in control, $P < 0.05$), Rci_{LPS} (9.1 (7.7; 10.8) units vs. 2.5 (2.2; 3.4) units in the control, $P < 0.05$), and Rci_{RNA} (8.3 (6.5; 10.8) units vs. 3.1 (2.6; 3.9) units), $P < 0.05$). At the same time, in patients with a progressive type of disease, stimulation of monocytes did not lead to a significant increase in IL-12p70 production, which may indicate the depletion of IL-12-producing cells against the background of high spontaneous production of this cytokine.

During incubation of monocytes, we evaluated both the production of the IL-12p70 dimer and the production of the IL-12p40 subunit, which is synthesized mainly by the cells of the monocyte fraction of PBMCs. In intact cells of patients with PMS, against the background of increased production of IL-12p70 compared with the group of RRMS, there were an elevated levels of production of the IL-12p40 subunit, namely 3 times higher than in the RRMS group and 1.7 times higher than in the control. Stimulation of monocytes with LPS led to a significant increase in IL-12p40 production, namely by 2.7 times. In the RRMS group, a reduced reserve ability to synthesize the IL-12p40 subunit was observed.

The modulating effect of TLR4 and TLR7/8 agonists on the production of TNF-α, IL-1β and IL-10 by PBMCs obtained from patients with different types of MS reflects the persistence of the detected imbalance of immunoregulatory cytokines; in patients with a progressive type of the disease, there was a tendency to more pronounced production of proinflammatory cytokines against the background of reduced reserve ability to synthesize anti-inflammatory cytokines.

At the next stage of our work, we studied the differences in the functioning of the arginase and NO-synthase pathways of L-arginine metabolism in intact and TLR4- and TLR7/8-activated peripheral blood monocytes in patients with different types of MS.

Moderate basal arginase activity ($P > 0.05$ relative to the control) was found in groups of patients with relapsing-remitting and progressive types of MS. The arginase pathway of L-arginine metabolism was predominant in the intact cells of the monocyte fraction of PBMCs, as evidenced by the low level of NO production in all groups (<0.1 μmol NO/10^5 cells).

Under the action of the stimulating HERV-W ENV glycoprotein, arginase activity increased by 3.6 times relative to the intact cells in the RRMS group, 3.8 times in the PMS group, and 2.6 times in the control group ($P < 0.05$).

Cultivation with LPS led to an increase in arginase activity of monocytes compared with intact cells, constituting 4.1 times in the RRMS group, 2.6 times in the PMS group and 5.2 times in the control group, but when comparing the stimulation in the PMS group, arginase activity turned out to be below the levels in control ($P < 0.05$). Stimulation with ssRNA40/LyoVec also caused an increase in arginase activity, namely 2.6 times in the RRMS group, 3.6 times in the PMS group and 2.4 times in the control ($P < 0.05$).

Arginase activity during the cultivation of monocytes treated with various agonists depended on the type of the clinical course of MS. In patients with RRMS, the highest arginase activity was observed in monocyte culture in the presence of HERV-W ENV and LPS. In monocytes obtained from patients with PMS, cultivation with LPS led to a significant reduction of arginase activity relative to the control. Basal and stimulated production of nitric oxide by monocytes after 24 hours cultivation in all groups was <0.1 μmol NO/10^5 cells, indicating a predominance of the arginase pathway of L-arginine metabolism.

Discussion

HERV-W ENV protein has numerous effects on microglia, such as morphological changes in microglia in amoeboid cells, increased cell proliferation, stimulation of the secretion of proinflammatory factors, suppression of the expression of neuroprotective factors, and decreased myelin clearance. It has been shown that HERV-W ENV is present on TLR4-positive microglia in MS lesions closely associated with myelinated axons. In myelinated cocultures stimulated with ENV proteins, microglia are forced to contact with axons, which leads to the leakage of intraaxonal and myelin proteins. Taken together, these observations suggest that HERV-W ENV in the MS brain may induce myeloid cells to cause damage to myelinated axons. HERV-W ENV-mediated modulation of microglial cell polarization to a proinflammatory (M1) phenotype contributes to axonal damage and neurodegeneration in MS [5].

In the works of Rolland et al. and Saresella et al., when studying the mechanisms of the pro-inflammatory properties of the surface unit (ENV-SU) of the HERV-W/MS-associated retrovirus (MSRV) envelope protein, it was shown that ENV-SU is able to specifically activate monocytes through the pattern recognition receptors CD14 and TLR4. This activation is associated with the release of major pro-inflammatory cytokines such

as IL-1β, IL-6 and TNF-α [14,15]. In our study, we observed a similar secretion profile of IL-1β, IL-12 and IL-1RA in monocyte culture in the presence of HERV-W ENV and LPS.

HERV-W ENV protein may exhibit its TLR4-dependent proinflammatory effect in the central nervous system (CNS) and thus initiates and/or significantly exacerbates the disease. This idea is consistent with the data of Perron et al. [16] that the HERV- ENV glycoprotein is expressed in the white matter of MS brains and that the viral load detected in the cerebrospinal fluid increases with the progression of MS and thus can have an important prognostic value for the disease [17]. The effect of viral load was confirmed by our studies *in vitro* in a group with a progressive type of MS, for which it was shown that the HERV-W ENV glycoprotein causes an imbalance in the production of IL-1β/IL-1RA. Thus, the reduced value of the ratio of IL-1RA and IL-1β production during the cultivation of intact monocytes, as well as with the addition of TLR4 and TLR7/8 agonists in patients with MS may indicate a violation of auto- and paracrine regulation in IL-1RA/IL-1β system, and can be considered as one of the pathogenetic mechanisms of the formation of the pro-inflammatory context [18,19].

Mameli et al. have demonstrated that cytokines such as TNF-α and IFN-γ can activate the ERVWE1 promoter. The highest activation of HERV-W expression in glioblastoma cells was observed under the influence of TNFα. They also emphasized that the mechanism of activation of transcription by these cytokines is associated with increased binding of the p65 subunit of NF-κB to a sensitive element located in the enhancer region of the ERVWE1 promoter [20].

According to our data, the reserve capacity of monocytes to produce TNF-α in patients with MS was also higher than in healthy individuals, which may indicate changes in the subpopulation composition of cells producing these cytokines (reflecting the increased contribution of CD14+CD16++ monocytes).

The studies of Wang et al. have shown that TLR4 knockdown disrupts HERV-W ENV-induced TNF-α and IL-10 expression. Overexpression of HERV-W ENV results in upregulation of MyD88, transmitting a signal from TLR4. HERV-W ENV upregulates IL-10 and TNF-α expression by suppressing MyD88 production in glial cells [21].

The results of our work indicate a tendency towards activation of peripheral blood monocytes in patients with MS with their polarization towards proinflammatory M1 phenotype, at the same time there was an increased activation of arginase in monocytes upon stimulation with HERV-W ENV, especially pronounced in patients with a progressive course of the disease.

Plasticity of cells of monocyte origin is due to the dynamic integration of numerous signals coming from neighbouring pro- and anti-inflammatory cells, characterized by different metabolic states and differential regulation of phenotypic markers. Among them, the expression of such enzymes as inducible nitric oxide synthase (iNOS) and arginase-1 is commonly used as a signature marker for evaluation of pro- and anti-inflammatory polarization of monocytes, respectively [22–24].

The differential distribution of proinflammatory and anti-inflammatory monocytes at the borders of the CNS can significantly affect the functional activation of these cells invading the central nervous system during autoimmune inflammation. CNS barrier cells (blood-brain barrier (BBB) endothelial cells and vascular plexus epithelial cells) can directly regulate the transcription of NOS2 (encoding iNOS) and Arg1 (encoding arginase-1) in interacting monocytes. Namely, while stimulation of BBB endothelial cells with TNF-α and IFN-γ induces NOS2 expression in monocytes, IL-1β-induced activation of BBB endothelial cells leads to significant upregulation of Arg1 in monocytes [23,25].

Our data are consistent with the numerous data of the authors [1,2,5,15,16], that the MSRV envelope protein fraction contains a binding site for the cellular receptor and allows the virus to naturally interact with host cells. CD14 and TLR4 are evolutionarily conserved receptors that play a critical role in APCs activation and proinflammatory cytokine production in response to LPS [26]. Thus, CD14 and TLR4 are PRRs that interact to activate innate immunity in response to both bacteria and viruses. These receptors are involved in mediating the proinflammatory effect of the MSRV envelope protein, identifying the HERV-W family proteins as a putative new class of viral ligands for these PRRs.

Thus, our studies confirm that HERV-W ENV can activate the innate immune system through TLR4 and promote the development of a Th1-type immune response. This statement is consistent with the idea that due to its proinflammatory properties, the HERV-W envelope protein can participate in immunopathological cascades associated with chronic inflammatory and neurodegenerative diseases.

Conclusions

1. The study of TLR-mediated activation of cells of the monocyte fraction of PBMCs in patients with relapsing-remitting and progressive type of MS revealed a modulating effect of HERV-W ENV on the production of TNF-α, IL-1β, IL-12 and IL-10. In patients with MS of both groups, the reserve capacity of monocytes for IL-1β production was lower than in healthy individuals, while the reserve capacity for TNF-α and IL-10 production was almost twice as high as in the control group. It is important to note that in the PMS group the value of Rci_{HERV-W} for IL-1RA was 2 times lower than the control, so against the background of elevated IL-1β in the PMS group, this imbalance provokes chronic inflammation.

2. The study of the arginase pathway revealed a significant increase in the arginase activity in monocytes upon stimulation with TLR4 and TLR7/8 compared to the baseline, and it was more pronounced in patients with PMS upon monocytes incubation with HERV-W ENV.

3. In monocytes obtained from patients with the progressive type of MS, incubation with HERV-W ENV leads to the stimulation of the production of both proinflammatory and regulatory cytokines with anti-inflammatory action; it is considered as an important mechanism of disruption of intercellular cooperation, which can result in a biased

immune response, contributing to increased disease susceptibility and more severe course of MS.

Prospects for future research. Further studies on the effect of human endogenous retroviruses on the activation of mononuclear phagocytes in MS are very important for understanding the pathogenic and neuroprotective pathways in neurological disorders and for the development of new therapeutic agents.

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