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ИССЛЕДОВАНИЕ ЦИТОКИНСИНТЕЗИРУЮЩЕЙ ФУНКЦИИ МОНОНУКЛЕАРНЫХ КЛЕТОК КРОВИ БОЛЬНЫХ РАССЕЯННЫМ СКЛЕРОЗОМ ПОД ДЕЙСТВИЕМ ОЛИГОПЕПТИДА КОНСЕРВАТИВНОГО РЕГИОНА ЭНДОГЕННОГО РЕТРОВИРУСА HERV-E λ 4-1

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Резюме. Учитывая данные об ассоциации эндогенного ретровируса человека I класса субгруппы HERV-E λ 4-1 с рассеянным склерозом, аутоиммунным заболеванием, сопровождающимся нейровоспалением, изменением уровня нейротрансмиттеров, прогрессирующей неврологической дисфункцией, а также способность данного ретровируса к репликации и продукции протеинов в потенциальными иммуномодулирующими свойствами, целью данной работы было сравнительное исследование цитокинсинтезирующей функции иммунных клеток крови условно здоровых лиц и больных рассеянным склерозом под действием синтетического 17-аминокислотного олигопептида, гомологичного консервативному региону гидрофобного трансмембранного протеина p15E HERV-E λ 4-1. Объектом исследования были 40 больных, 17 мужчин в возрасте 38,0 (31,0-47,0) лет и 23 женщины в возрасте 39,0 (31,0-50,0) лет с установленным диагнозом рассеянного склероза (G 35, МКБ-10), удовлетворяющим критериям McDonald 2005, в модификации 2010 г., непрерывно-прогредиентным типом течения заболевания и длительностью 17,0 (14,0-18,0) лет и 30 условно-здоровых лиц, 12 мужчин в возрасте 32,0 (23,0-43,0) лет и 18 женщин в возрасте 36,0 (29,0-46,0) лет. Было проведено открытое обсервационное одноцентровое когортное контролируемое рандомизированное исследование. Обнаружено, что под действием ретровирусного олигопептида стимулировалась спонтанная продукция IL-1β, IL-6, TNFα, IFNγ и IL-2 МНК доноров в культуре, но не изменялась таковая IL-4 и IL-10. В то же время спонтанная и митоген-стимулированная продукция всех исследуемых цитокинов не изменялась под действием контрольного олигопептида. Культивирование РНА-стимулированных МНК доноров в присутствии ретровирусного олигопептида, по сравнению с контрольным, сопровождалось увеличением высвобождения IL-1β, IL-6 и TNFα в культуральный супернатант. Больные рассеянным склерозом характеризовались более высоким содержанием IL-1β, IL-6 и IFNγ в культуральном супернатанте нестимулированных митогеном МНК, по сравнению с условно-здоровыми лицами, а также более высокой продукцией IL-6 и IFNγ в ответ на стимуляцию РНА. У больных рассеянным склеро-

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зом ретровирусный олигопептид, в отличие от контрольного, стимулировал спонтанную продукцию IL-1β, IL-6, TNFα и IFNγ, не изменяя таковой IL-4 и IL-10. Полученные результаты свидетельствуют о провоспалительных свойствах синтетического олигопептида, гомологичного консервативному региону гидрофобного трансмембранного протеина p15E HERV-E λ 4-1, что, вероятно, является одним из механизмов реализации патологических свойств эндогенного ретровируса человека HERV-E λ 4-1 при рассеянном склерозе.

Ключевые слова: рассеянный склероз, непрерывно-прогредиентное течение, эндогенный ретровирус человека, олигопептид, мононуклеарные клетки крови, функциональная активность, цитокины

INVESTIGATION OF BLOOD MONONUCLEAR CELLS CYTOKINE-PRODUCTION FUNCTION FROM PATIENTS WITH MULTIPLE SCLEROSIS TREATED WITH THE ENDOGENOUS RETROVIRUS HERV-E λ 4-1 CONSERVATIVE REGION SYNTHETIC OLIGOPEPTIDE

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Abstract. Considering to the data of class I human endogenous retrovirus HERV-E λ 4-1 subgroup association with multiple sclerosis, an autoimmune disease accompanied by neuroinflammation, changes in the neurotransmitters level, progressive neurological dysfunction, as well as the ability of this retrovirus to replicate and to produce proteins with potential immunomodulatory properties, the aim of this work was a comparative study of the blood immune cells cytokine synthesizing function in conventionally healthy individuals and multiple sclerosis patients under the synthetic 17 – amino acid oligopeptide homologous to the hydrophobic transmembrane protein p15E HERV-E λ 4-1 conserved region influence. The 40 patients, 17 male persons aged 38.0 (31.0-47.0) years old and 23 female persons aged 39.0 (31.0-50.0) years old with an established diagnosis of multiple sclerosis (G 35, ICD-10), corresponding to the McDonald 2005, modified in 2010 criteria with a continuously progressive disease course and the disease duration of 17.0 (14.0-18.0) years, and 30 conditionally healthy individuals, 12 male persons aged 32.0 (23.0-43.0) years old and 18 female persons aged 36.0 (29.0-46.0) years old were the objects of the study. An open-label, observational, single-center, cohort, controlled, randomized trial was conducted. It was found that the donor's blood mononuclear cells IL-1β, IL-6, TNFα, IFNγ and IL-2 spontaneous production in culture was stimulated, but that of IL-4 and IL-10 did not change under the retroviral oligopeptide influence. At the same time, the spontaneous and mitogen-stimulated production of all studied cytokines did not change under the control oligopeptide influence. The PHA-stimulated donor's blood mononuclear cells cultivation in presence of the retroviral oligopeptide, as compared to the control one, was accompanied by an increase in the IL-1β, IL-6 and TNFα release into the culture supernatant. The multiple sclerosis patients were characterized by IL-1β, IL-6 and IFNγ higher content in the mitogen-unstimulated blood mononuclear cells culture supernatant, compared with conditionally healthy individuals, as well as by a higher production of IL-6 and IFNγ in response to PHA stimulation. The retroviral oligopeptide, in contrast to the control one, stimulated the IL-1β, IL-6, TNFα and IFNγ spontaneous production without altering that of IL-4 and IL-10 in multiple sclerosis patients. The obtained results indicate that the synthetic oligopeptide homologous to the conserved region of the hydrophobic transmembrane protein p15E HERV-E λ 4-1 has the pro-inflammatory properties, which is probably the one of human endogenous retrovirus HERV-E λ 4-1 pathological abilities realization mechanism in multiple sclerosis.

Keywords: multiple sclerosis, continuously progressive disease course, human endogenous retrovirus, oligopeptide, blood mononuclear cells, functional activity, cytokines

Introduction

An increased incidence has been observed in most economically developed countries in recent decades for the multiple sclerosis (MS), an autoimmune demyelinating disease of the nervous system, accompanied by neuroinflammation, changes in the neurotransmitters level, progressive neurological dysfunction, cognitive impairment, affective disorders, as well as the irreversibility of neurological symptoms which are characteristic of this disease, insufficient relevance of modern methods for the pathological process activity monitoring, incomplete certainty and multifactorial nature of the etiology and pathogenesis mechanisms [1, 2, 3, 4, 7, 10, 13, 14], determine the relevance of new potential etiological factors and their involvement in the MS pathogenesis identification and research.

Among the MS autoimmune inflammation triggers in the nervous system, endogenous retroviruses (ER) are considered as some of the most relevant being capable of polyclonal T-lymphocytes activation [8, 9, 11, 12, 15]. These retroviruses are an integrated in the provirus form of exogenous retroviruses and are a type of the genome mobile elements – RNA retrotransposons, DNA sequences that make up to 8% of the human genome distributed in more than 700,000 discrete loci. Usually, the retroelement activity in the human genome is repressed by both genetic and epigenetic mechanisms. However, in the evolution process some of them acquired the pathogenic properties and the ability to replicate as a result of mutations and recombinations, to form the virion structure and produce viral proteins with immunomodulatory properties and fulfill the role of superantigens – to form antigenic epitopes by the molecular mimicry mechanism recognized by the immune system cells. Inflammation and the immune system activation, in turn, are factors that modulate ER transcription, since their promoter regions contain binding sites for transcription factors, involved in the formation of oxidative stress response, which inhibits the deacetylase activity stimulating simultaneously histone acetylation and activation of ER expression [5, 19]. ER RNA can be recognized by Toll-like receptors as pathogen-associated cues, which induces the type I interferon production. Taking into account our earlier data about the class I retrovirus of the HERV-E λ 4-1 subgroup (ER λ 4-1) association with the MS course, as well as its ability to replicate and produce proteins [6, 8, 15], **the aim of this work** was to compare blood immune cells cytokine-synthesizing function from apparently healthy individuals and MS patients treated with the synthetic 17-amino acid oligopeptide, the hydrophobic transmembrane protein p15E ER λ 4-1 conserved region homologous influence.

Materials and methods

The 40 patients, 17 males aged 38.0 (31.0-47.0) years old and 23 females aged 39.0 (31.0-50.0) years old with an established MS diagnosis (G 35, ICD – 10), corresponding to the McDonald 2005 criteria, modified in 2010, with a continuously progressive type of disease course and a disease duration of 17.0 (14.0-18.0) years, and 30 apparently healthy individuals, 12 males aged 32, 0 (23.0-43.0) years old and 18 females aged 36.0 (29.0-46.0) years old were enrolled to the study. An open-label, observational, single-center, cohort, controlled, randomized study to evaluate the synthesis of certain cytokines in vitro was conducted.

Cytokine production in culture supernatants of blood mononuclear cells (MNCs) from apparently healthy individuals and MS patients was carried out by the solid-phase enzyme-linked immunosorbent assay method using ELISA-BEST (Koltsovo, Novosibirsk, Russia) and Pro Con (St. Petersburg, Russia) test systems, according to the manufacturer's instructions. For that purpose, the patient MNCs were collected from venous blood added with heparin and isolated by centrifugation on a Ficoll density gradient (Lymphocyte separation medium, ICN Biomedicals Inc.) at 1500 rpm. within 40 minutes.

Cells harvested from the interphase were washed in 199 culture medium, pelleted by centrifugation and resuspended at a concentration of 20×10^6 / ml in RPMI-1640 culture medium containing 10% human blood serum AB (IV), 10 mM Hepes buffer, 4×10^{-5}M 2-mercaptoethanol, 2 mm L-glutamine, 40 μg/ml gentamicin, and cultured at 37 °C and 5% CO^2 in an atmosphere for 24-72 hours, depending on the experimental conditions. The research protocol was developed in accordance with the Helsinki Declaration by the World Medical Association "Ethical principles for conducting of scientific medical research with human participation", as amended in 2013 and with the "Rules of Good Clinical Practice" approved by the Russian Federation Ministry of Health Order No. 200n, dated of 01.04.2016.

The 17-amino acid retroviral or control (with the reverse amino acid sequence) oligopeptides at suboptimal concentration were introduced into the cell culture for 24 hours after the onset of cell culture with or without the suboptimal mitogen concentration (50 μg/ml) (Phytogemagglutinin-L Phaseolus vulgaris (PHA), Sigma Aldrich), determined in a series of preliminary experiments.

Statistical data processing was carried out using descriptive statistics, comparative analysis, on the base of nonparametric Mann–Whitney U test, with the commercial software package "Statistica 10.0" (StatSoft, USA) use. Results were presented as a median and an interval between 1 and 4 quartiles (Me ($Q_{0.25}$-$Q_{0.75}$) %). Differences were considered as statistically significant at $p < 0.05$. Calculating

sample size principles: sample size was not calculated previously.

Results and discussion

The data obtained on ER λ 4-1 effect assessing blood donors MNC baseline cytokine production are presented in Table 1.

We found that under the retroviral oligopeptide exposure, the spontaneous production of IL-1β, IL-6, TNFα, IFNγ and IL-2 by donor MNCs was stimulated, but that of IL-4 and IL-10 did not. At the same time, spontaneous production for all studied cytokines did not change after treating with control oligopeptide. The suboptimal PHA concentration

TABLE 1. RETROVIRAL OLIGOPEPTIDE EFFECT ON CONDITIONALLY HEALTHY PERSONS BLOOD MONONUCLEAR CELLS CYTOKINE PRODUCTION, Me ($Q_{0.25}$-$Q_{0.75}$)

Cytokines	Spontaneous production, pg/ml			PHA-induced production, pg/ml		
	0.9% NaCl	Control oligopeptide	Retroviral oligopeptide	0.9% NaCl	Control oligopeptide	Retroviral oligopeptide
IL-1β	24.3 (19.9-27.8)	26.7 (23.1-31.2)	171.0 (182.2-214.8)*	151.9 (57.4-245.1)^	134.4 (78.3-165.6)	190.9 (156.2-219.4)*
IL-6	15.1 (12.4-17.6)	17.8 (15.3-19.5)	20.5 (19.4-23.4)*	22.8 (18.5-23.4)^	19.1 (17.2-23.1)	257.3 (232.4-276.9)*
TNFα	5.4 (2.0-7.8)	2.4 (2.1-2.6)	37.6 (34.1-39.5)*	148.5 (143.1-154.7)^	144.2 (138.9-147.6)	215.5 (182.4-248.4)*
IFNγ	8.10 (7.6-8.9)	8.1 (7.6-8.4)	78.5 (41.3-105.8)*	16.0 (11.5-19.3)^	16.0 (13.3-19.6)	27.5 (16.9-30.4)
IL-4	1.0 (0.8-1.4)	1.2 (0.9-1.4)	2.3 (0.9-2.5)	12.4 (9.8-14.6)^	12.8 (11.8-14.9)	10.8 (8.4-11.8)
IL-10	1.5 (1.2-1.6)	1.6 (1.5-1.8)	1.2 (0.7-1.7)	37.9 (30.3-40.6)^	34.5 (31.0-36.8)	33.8 (29.0-35.1)
IL-2	0.6 (0.5-0.6)	0.6 (0.5-0.7)	9.4 (8.9-10.3)*	10.6 (9.5-11.7)^	9.8 (8.2-11.4)	10.8 (8.8-12.1)

Note. n = 30; * – p < 0.05 between the control and retroviral oligopeptide; ^ – p < 0.05 between the spontaneous and PHA-induced production (Mann–Whitney U test).

TABLE 2. MULTIPLE SCLEROSIS PATIENTS BLOOD MONONUCLEAR CELLS CYTOKINE PRODUCTION UNDER THE RETROVIRAL OLIGOPEPTIDE INFLUENCE, Me ($Q_{0.25}$-$Q_{0.75}$)

Cytokines	Spontaneous production, pg/ml			PHA-induced production, pg/ml		
	0,9% NaCl	Control oligopeptide	Retroviral oligopeptide	0,9% NaCl	Control oligopeptide	Retroviral oligopeptide
IL-1β	47.8 (29.3-77.1)	36.4 (15.8-42.2)	192.0 (164.2-246.1)*	122.8 (66.1-241.2)^	125.9 (82.1-166.0)	235.3 (143.7-250.0)*
IL-6	35.1 (25.2-48.2)	39.1 (22.3-49.4)	110.7 (67.1-133.5)*	123.4 (65.2-154.2)^	119.6 (95.8-124.4)	264.9 (219.8-293.2)*
TNFα	7.9 (4.0-11.5)	9.9 (7.3-13.9)	44.6 (25.2-58.8)*	139.5 (106.0-199.6)^	146.0 (117.2-158.9)	223.6 (180.0-250.0)*
IFNγ	18.1 (12.0-29.2)	22.3 (17.0-37.8)	78.7 (48.4-106.3)*	29.2 (16.3-37.4)^	28.1 (13.2-36.1)	34.4 (17.0-37.2)
IL-4	1.9 (0.7-3.4)	2.3 (0.9-3.2)	2.9 (1.2-4.6)	11.7 (8.2-16.7)^	12.4 (10.1-16.9)	10.2 (4.6-10.1)
IL-10	2.6 (1.3-3.6)	1.6 (0.4-2.7)	2.9 (0.7-3.9)	33.9 (27.5-41.4)^	32.0 (26.1-40.2)	37.4 (24.3-38.6)
IL-2	1.8 (0.6-3.4)	1.7 (0.5-2.9)	1.7 (0.8-1.5)	12.0 (8.4-14.6)^	9.2 (7.8-13.7)	12.1 (7.5-15.5)

Note. As for Table 1.

resulted in increased level for all cytokines studied. Exposure to control oligopeptide had no changes in PHA-stimulated cytokine production. However, the PHA-stimulated donor MNCs cultured with the retroviral oligopeptide were accompanied by increased release of the IL-1β, IL-6 and TNFα into the culture supernatant.

The results of the MS patient MNCs cytokine-synthesizing function after exposure to retroviral oligopeptide are presented in Table 2.

MS patients were characterized by a higher level of IL-1β, IL-6, and IFNγ in the of mitogen-unstimulated MNCs culture supernatant compared with those from apparently healthy individuals ($p < 0.05$, Mann–Whitney U test), as well as by higher production of IL-6 and IFNγ ($p < 0.05$, Mann–Whitney U test) in response to PHA stimulation.

The suboptimal PHA concentration was accompanied by the increased production of all cytokines studied in the culture supernatant. The retroviral *vs* control oligopeptide stimulated the IL-1β, IL-6, TNFα and IFNγ spontaneous production, without altering that of IL-4 and IL-10 in MS patients. The control oligopeptide did not affect the cytokines level in the supernatant of PHA-stimulated MNCs. However, the culture of MS patients PHA-stimulated MNCs with the retroviral oligopeptide was accompanied by further increase in production of IL-1β, IL-6, and TNFα into the culture supernatant.

Thus, the results shown above indicate about sequence-specific pro-inflammatory properties of a synthetic oligopeptide homologous to the conserved region of the hydrophobic transmembrane protein p15E ER λ 4-1, which might serve as one of the pathological properties enabled by the human endogenous retrovirus HERV-E λ 4-1 in multiple sclerosis and accompanying its low-grade production and neuroinflammation. The detection of the increased cytokines production mainly by Th1, Th17 and macrophages involved in the MS pathogenesis after exposure to the retroviral oligopeptide confirms its potential role in the pathogenesis of this disease and can serve as a perspective target for pathogenetically justified therapy.

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