СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ АКТИВАЦИИ ЭНДОГЕННОГО РЕТРОВИРУСА ЧЕЛОВЕКА *HERV-E λ 4-1* ПРИ АУТОИММУННОЙ ПАТОЛОГИИ

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Резюме. Учитывая наличие у эндогенных ретровирусов человека иммуномодулирующих свойств – (1) способности к активации врожденного иммунного ответа нуклеиновыми кислотами HERVs; (2) антигенности молекулы протеина оболочки транскрипционно-компетентных эндогенных ретровирусов, вызывающей поликлональную активацию лимфоцитов; (3) отсутствие экспрессии HERVs и продукции протеинов в тимусе во время формирования иммунной толерантности, что позволяет рассматривать эти вирусы как аутоантигены или неоантигены, представлялось актуальным исследовать ассоциацию репликационно-компетентного эндогенного ретровируса человека *HREV-E λ 4-1* с течением ряда аутоиммунных заболеваний – рассеянного склероза, ревматоидного артрита и системной красной волчанки. Целью настоящей работы было сравнительное исследование частоты активации эндогенного ретровируса человека *HERV-E λ 4-1* в мононуклеарных клетках крови при рассеянном склерозе, ревматоидном артрите, системной красной волчанке, а также хронических непрогрессирующих заболеваниях нервной системы и дегенеративно-дистрофическом заболевании костно-мышечной системы. Мононуклеарные клетки периферической крови выделяли при помощи центрифугирования венозной крови на градиенте плотности фиколла 1,078 г/см^3. Экспрессию гена *envelope HERV-E λ 4-1* выявляли методом обратно-транскриптазной полимеразной цепной реакции. Было обнаружено, что частота экспрессии гена *envelope HERV-E λ 4-1* при хронических непрогрессирующих заболеваниях нервной системы, также, как и при дегенеративно-дистрофическом заболевании суставов сопоставима с частотой экспрессии у условно-здоровых лиц. Однако частота экспрессии гена *envelope HERV-E λ 4-1* при аутоиммунных заболеваниях значительно превышала таковую и у условно-здоровых, и при невоспалительных заболеваниях. Максимальные значения частоты экспрессии отмечались при активном рассеянном склерозе, значительно превышающие показатели при системной красной волчанке и ревматоидном артрите в стадии обострения. Причем частота экспрессии в состоянии ремиссии рассеянного склероза была значительно ниже показателя при ремиттирующем течении в стадии обострения, а также при прогредиентном течении. Оценка частоты экспрессии гена *envelope HERV-E λ 4-1* при различных степенях тяжести рассеянного склероза выявила ее максимальные показатели при степени тяжести III и IV-V, как при ремиттирующем, так и при прогредиентном течении

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рассеянного склероза. Таким образом, активация эндогенного ретровируса человека *HERV-E λ 4-1* ассоциирована с течением аутоиммунных заболеваний – рассеянного склероза, ревматоидного артрита, системной красной волчанки и положительно коррелирует с активностью и степенью тяжести рассеянного склероза.

Ключевые слова: эндогенный ретровирус HERV-E λ 4-1, экспрессия, рассеянный склероз, ревматоидный артрит, системная красная волчанка, мононуклеарные клетки крови

A COMPARATIVE STUDY OF HUMAN ENDOGENOUS RETROVIRUS *HERV-E λ 4-1* ACTIVATION IN AUTOIMMUNE PATHOLOGY

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Abstract. Considering the presence of immunomodulatory properties of human endogenous retroviruses, namely (i) the ability to activate the innate immune response by HERVs nucleic acids; (ii) the antigenicity of transcriptionally competent endogenous retroviruses envelope protein molecule, which causes polyclonal activation of lymphocytes; (iii) the absence of HERVs expression and protein production in the thymus during the immune tolerance formation, which allows us to consider these proteins as autoantigens or neoantigens, it seemed relevant to investigate the association of replication-competent human endogenous retrovirus *HERV-E λ 4-1* with course of some of autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus. The aim of this work was a comparative study of the human endogenous retrovirus *HERV-E λ 4-1* activation frequency in blood mononuclear cells in multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, as well as in chronic nervous system non-progressive diseases and the degenerative-dystrophic disease of the musculoskeletal system. The peripheral blood mononuclear cells were isolated by the venous blood centrifugation on Ficoll density gradient of 1.078 g/cm^3. Expression of the *HERV-E λ 4-1 envelope* gene was detected by reverse transcriptase polymerase chain reaction. It was found that the *HERV-E λ 4-1 envelope* gene expression frequency in the chronic non-progressive diseases of nervous system, as well as in degenerative-dystrophic joint disease, is comparable to the expression frequency in conditionally healthy individuals. However, the *HERV-E λ 4-1 envelope* gene expression frequency in autoimmune diseases significantly exceeded that in conditionally healthy individuals and in non-inflammatory diseases. The maximum values of expression frequency were observed in active multiple sclerosis, significantly higher than in systemic lupus erythematosus and rheumatoid arthritis in the acute stage. Moreover, the expression frequency in the remission stage of multiple sclerosis was significantly lower than in the acute stage of the relapsing-remitted course, as well as in the progredient course. Estimation of *HERV-E λ 4-1 envelope* gene expression frequency at different severity levels of multiple sclerosis revealed its maximum rates at III and IV-V severity levels, both in relapsing-remitting and progressive course of multiple sclerosis. Thus, activation of the human endogenous retrovirus *HERV-E λ 4-1* is associated with the course of autoimmune diseases, namely multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus; it positively correlates with the activity and severity of multiple sclerosis.

Keywords: endogenous retrovirus HERV-E λ 4-1, expression, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, blood mononuclear cells

Introduction

The ability of human endogenous retroviruses (HERVs) to regulate immune response has made it possible to consider these genome elements as potentially involved in autoimmune disease development. Altered expression of HERVs is also considered to be as one of autoimmune disorders triggers. It was confirmed by the presence of increased levels of proviral RNA and antibodies to some of HERVs proteins in the sera of many autoimmune diseases patients, namely multiple sclerosis (MS), systemic lupus erythematosus (SLE), type 1 diabetes mellitus, rheumatoid arthritis (RA) [3, 5, 8, 10]. Thus,

the spectrum of HERVs associated with autoimmune diseases and the mechanisms of its direct involvement in diseases pathogenesis are still far from being fully understood.

Among the classical mechanisms of virus-driven autoimmunity, such as molecular mimicry and epitope spreading, special attention is given to the role of HERVs nucleic acids in direct activation of innate immunity and in the epigenetic modulation of interferon status. HERVs mechanism of action cannot be fully explained either by de novo insertional mutagenesis, or by the viral particle's formation. It has been suggested that potential HERVs pathogenicity may be realized through the presence of their proviral DNA in the genome, acting as a regulatory sequence that changes the neighboring and distant genes expression. Moreover, HERVs proviral DNA may be considered as a binding site for transcription factors. Therefore, HERVs potential effects will be limited to some genomic window around the primary insertion site of the provirus [1, 7]. However, there is evidence which supports a more global HERVs mechanism of action.

Some HERVs, in particular *HREV-E λ 4-1*, are able to encode an envelope protein, and its presence in a number of autoimmune diseases has been identified. In addition, it was suggested that HERVs envelope proteins mechanisms of action are based on antigenicity of their molecule, that possibly causing lymphocyte's polyclonal activation, ie. they function as "superantigens". Moreover, proteins encoded by HERVs, which are a part of human genome, should be considered as autoantigens or neoantigens, since they are not expressing in the thymus during the immune tolerance formation [6, 7, 12]. It was shown that the sequence similarity between *HERV-W* envelope proteins and myelin can induce an immune response in MS [11].

Retroviral nucleic acids and viral proteins can be perceived by various pattern recognition receptors, such as Toll-like (TLR) or NOD-like receptors [14], which leads to the induction of autoimmunity [15]. A direct interaction between some HERVs and TLR proteins has been shown. For example, the envelope protein of *HERV-W* binds to TLR4 and CD14 and stimulates the pro-inflammatory cytokines production, including IL-1β, IL-6, and TNFα [9]. Although the majority of HERVs sequences are nonfunctional, some of HERVs loci are coding and can be activated when exposed to some external and internal environmental factors [4].

The aim of this work was a comparative study of the human endogenous retrovirus *HERV-E λ 4-1* activation frequency in blood mononuclear cells of patients with multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, as well as with chronic non-progressive diseases of the nervous system and with degenerative-dystrophic disease of the musculoskeletal system.

Materials and methods

Examination of patients and collection of the material was carried out in the Clinic of Research Institute of Fundamental and Clinical Immunology, the Research Institute of Clinical and Experimental Lymphology, Branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences and the Novosibirsk's NIITO named after. Ya. L. Tsivyan.

The study included analysis of:

– 96 healthy volunteers, 45 men and 51 women with an average age 38.0 (24.0-46.0) years;

– 205 unrelated patients with a diagnosis of MS (G35) established in accordance with the McDonald criteria (2010, 2017) and confirmed by magnetic resonance imaging. Forty-five people were in the stage of disease remission (21 men and 24 women, with an average age 36.0 (29.0-43.0) years), and 160 people – in the stage of MS exacerbation (76 men and 84 women with an average age 38.0 (30.0-43.0) years), with a disease duration of 2-18 years, an average age of the disease onset in both groups of 25.0 (23.5-31.5) years, corresponding to the inclusion/exclusion criteria and signed a voluntary informed consent;

– 26 patients with an established diagnosis of systemic lupus erythematosus (SLE) (M32) in the acute stage (12 men and 14 women with an average age 33.0 (27.0-42.0)) years);

– 53 patients with rheumatoid arthritis (RA) (M05), in the acute stage (25 men and 28 women with an average age (34 (28-46) years)

– patients with chronic non-progressive organic diseases of the nervous system (CND) with static, motor, mental and speech disorders, including 16 patients (8 men and 8 women with an average age 36 (18-33) years) with children's cerebral palsy (CCP) (G80), and 20 patients (12 men and 8 women with an average age 34 (27-43)) years) with long-term consequences of spinal injury, in some cases in combination with craniocerebral injury (T91.3)

– 24 patients with deforming osteoarthritis (DOA) (M16-M17) (14 men and 10 women with an average age 46 (38-53) years).

The control groups were formed from healthy volunteers, the patients with relapsing-remitting type of MS in remission stage, patients with CCP, long-term consequences of spinal injury and DOA. The study groups were formed from the patients with autoimmune diseases in acute stage, namely MS, SLE, and RA.

The study protocol was developed in accordance with the Helsinki Declaration of the World Medical Association "Ethical principles for conducting scientific medical research involving humans" as

TABLE 1. COMPARATIVE FREQUENCY OF *HERV-E λ 4-1 ENVELOPE* GENE EXPRESSION IN BLOOD MONONUCLEAR CELLS OF AUTOIMMUNE DISEASE PATIENTS

Patient group	Expression of *HREV-E λ 4-1 envelope* gene, persons	Expression of *HREV-E λ 4-1 envelope* gene, persons, %
Conditionally healthy, n = 96	3	3.13
Children's cerebral palsy, n = 16	1	6.25
Spinal injury, n = 20	2	10.0
Deforming osteoarthritis, n = 24	1	4.17
Multiple sclerosis, remission, n = 45	11	24.4**
Systemic lupus erythematosus, n = 26	8	30.77*
Rheumatoid arthritis, n = 53	22	41.5*
Multiple sclerosis, relapsing course, exacerbation, n = 82	51	62.2* ***
Severity I-II, n = 29	17	58.6*
Severity III, n = 31	20	64.5* ***
Severity IV-V, n = 22	18	81.8* ***
Multiple sclerosis, progressive course, n = 78	58	74.4* ***
Severity I-II, n = 27	17	62.9*
Severity III, n = 28	22	81.5* ***
Severity IV-V, n = 23	20	86.9* ***

Note. Statistical significance of differences (F – Fisher's test): *, $p < 0.05$ between control groups and study groups; **, $p < 0.05$ between control groups; ***, $p < 0.05$ between study groups.

amended in 2013 and the "Rules of Good Clinical Practice", approved by the Russian Federation Ministry of Health Order No. 200n dated April 1, 2016. This work was approved by local ethical committees.

Peripheral blood mononuclear cells (PBMCs) were isolated by venous blood centrifugation on a Ficoll density gradient of 1.078 g/cm^3 (Lymphocyte separation medium, MP Biomedicals, LLC, Eschwege, Germany) at a ratio of 3:1, at 1500 rpm within 45 min. Cells collected from the interphase were washed thrice with 199 medium and precipitated by centrifugation. Isolation of total RNA was carried out by the phenol extraction method, using the test system VectoRNA – extraction (Vector-Best, Novosibirsk). The obtained DNA amplification was carried out in a programmable thermocycler "Tertsik", (DNA-technology, Moscow), using pairs of oligonucleotide primers to the human endogenous retrovirus *HERV-E λ 4-1 envelope* gene, homologous to the conservative regions of antiparallel DNA chains. The resulting cDNA fragments were analyzed by electrophoresis in 2% agarose gel with the addition of 0.00001% ethidium bromide (VectoDNA-EF, Vector-Best, Russia). The resulting cDNA segment was detected as a discrete band after electrophoretic separation of cDNA molecules. Samples with a visible cDNA band in the gel corresponding to the expected amplicon size were considered as positive.

Statistical data processing was carried out using the software package "Statistica 10.0" (StatSoft, USA). Comparison of gene expression frequencies between the studied samples was carried out using the Fisher's exact F-test. The statistical significance level was taken at $p < 0.05$. The sample was checked for normal distribution using the Kolmogorov-Smirnov test. To study the correlation relationships, the method of the Spearman rank correlation coefficient calculating was used.

Results and discussion

The results of the frequency of *HERV-E λ 4-1 envelope* gene expression study in PBMC of patients with MS, SLE and RA, compared with patients with CND and DOA, are presented in Table 1.

It was found that the *HERV-E λ 4-1 envelope* gene expression frequency in PBMC of patients

with chronic non-progressive diseases of the nervous system, as well as with DOA, is comparable to the expression frequency in PBMC of healthy individuals, but less than in MS in remission stage. The frequency of the *HERV-E λ 4-1 envelope* gene expression in autoimmune diseases (MS, SLE, RA) significantly exceeded parameters in the control groups. The maximum of expression frequency observed in active MS, exceeding those in SLE and RA in the acute stage. Moreover, the expression frequency in the stage of MS remission was significantly lower than its rate in the relapsing course in the acute stage and in the progredient course of MS. Estimation of the *HERV-E λ 4-1 envelope* gene expression frequency in various MS severity levels revealed its highest values in severity III and IV-V, both in relapsing-remitting and progressive MS. The frequency of the *HERV-E λ 4-1 envelope* gene expression positively correlated with MS activity, as well as with its severity ($r = 0.75$ and $r = 0.78$, respectively).

Thus, the human endogenous retrovirus *HERV-E λ 4-1* activation is associated with the course of autoimmune diseases, namely MS, RA and SLE. Expression of the *HERV-E λ 4-1 envelope* gene positively correlates with the MS activity and severity.

Our data on human endogenous retrovirus *HERV-E λ 4-1* activation in autoimmune diseases are consistent with the results of the study, indicating an increase of mRNA *HERV-E* clone *4-1* expression in $CD4^+$T cells in patients with SLE, which positively correlated with the diseases activity. Among the possible mechanisms of *HREV-E λ 4-1* involvement in the pathogenesis of these diseases, activation of the Ca^{2+}/calcineurin (CaN)/NFAT1 and E2/ER-α signaling pathways and hypomethylation of the 5'LTR DNA of the *HERV-E 4-1* clone have been described. Clone *HERV-E 4-1* also has been implicated in the disease pathogenesis through the microRNA MiR P-302d/methyl-CpG-binding domain protein 2 (MBD2) expression, DNA hypomethylation, and IL-17 signaling pathway via its 3'LTR [13].

In addition, HERVs are known to interfere with various processes related to the nervous system development and functioning: human sodium-dependent type 1 neutral amino acid transporters (hASCT1) and hASCT2 have been recognized as cellular receptors for *HERV-W* Env. Both receptors play a role in the glutamatergic transmission regulation in the brain. Also, the *HERV-W envelope* gene overexpression activated the Ca^{2+}-activated K^+ conduction channel in human neuroblastoma cells through the cAMP response element (CREB), which suggests a significant role of the HERV in the regulation of neuronal activity in the nervous system diseases. Activation of HERV transcription (the *HERV-W envelope* gene expression) regulated the brain-derived neurotrophic factor (BDNF) expression, neurotrophic tyrosine kinase receptor type 2 (NTRK2), and dopamine D3 receptor (DRD3) genes [2]. Such mechanisms may be involved in formation of pathological process in MS and other autoimmune diseases involving *HERV-E λ 4-1*.

Conclusion

Thus, the involvement of human endogenous retrovirus *HERV-E λ 4-1* in the course of autoimmune diseases, especially multiple sclerosis, opens new perspectives for the development of pathogenetically determined therapy.

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