PROINFLAMMATORY CYTOKINES mRNA EXPRESSION IN DEPENDENCE OF SUPPRESSIVE EPITOPE OF RETROVIRAL TRANSMEMBRANE p15E PEPTIDE ACTIVATION AT MULTIPLE SCLEROSIS PATIENTS

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INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system with the autoimmune component. It is characterised by discrete, multifocus lesions of myelin degradation. All phenotypes of lymphocytes and activated macrophages are represented in the inflammatory infiltrates.
Myelin basic protein reactive T cells, macrophages and microglia produce a wide range of factors, wich may influence oligodendrocyte function during the demyelinating process. The complex of proinflammatory cytokines is, probably, the major factor of demyelination (1,3,7).
Different bacterial and viral agents may play a role of triggering factors, predecessing the autoimmune reactions with myelin antigens (9, 15). Not virus, per se, is a key factor in the disease development, but the immune reaction on its persistence (13). In this context, we pay attention on endogenous retroviruses,due to retroviral genes products play the certain role in development of some immunopathilogical conditions. Genomes of vertebrates carry a variety of endogenous retrovirus sequences (ERV), as normal constituents in their genome (6). Most often endogenous retrovirus sequences are defective, some of them contains stop codons in the reading frams, or truncated, preventing their expression as viral particles (14). At the same time, in some conditions, endogenous retroviruses may exert their activity. The observations, that endogenous retroviruses are involved in the regulation of immune response have focussed attention on these genetic elements as factors, potentially involved in the development of autoimmune diseases (4,5). In particular, some interrelations have

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been found between rheumatoid arthritis and ERV – 3 (12). A characteristic feature of infections with retroviruses is the incorporation of retroviral DNA into the genome of the infected cells. On the other hand, it is possible that retroviruses have been involved from normal genetic elements.
Several endogenous retroviruses are normally transcribed in peripheral blood mononuclear cells and in brain tissue – HRES – 1, HERV – K, ERV3, ERV3 – Kruppel (10). Others mediate immunosupression through the production of immunosuppressive retrovirus – related proteins, like p15E (2, 16).
The aim of our study was to investigate the level of expression of mRNA for the suppressive epitope of retroviral transmembrane p15E peptide in peripheral blood mononuclear cells of patients with relapsing – remitting form of multiple sclerosis; to estimate the level of expression of proinflammatory cytokines in infected and non – infected patients; to evaluate the results of the treatment by the profile of proinflammatory cytokine expression in peripheral blood mononuclear cells from infected and non – infected patients.

MATERIALS AND METHODS

Samples of peripheral blood were taken from 44 multiple sclerosis patients 23 – 67 years old, 28 of them were women, 16 – men. In all cases it was clinically definite multiple sclerosis, the relapsing – remitting form. The disease duration of these patients ranged from 6 months to 18 years. All of this patients suffer from different degree of neurological disorders.
Mononuclear cells were obtained from the samples of peripheral blood by Ficoll – Paque gradient density centrifugation (pharmacia, Uppsala, Sweeden). Cytoplasmic RNAs were isolated by the method, described by (8). The predeterminated amounts of RNA were treated with reverse transcriptase (Medigen Lab, Russia), in amounts, corresponding to 20U per 1 μg of RNA, in mixture of 10 μl, containing 1 mM each dNTP (Medigen Lab., Russia), 5mM magnesium chloride (Sibensim, Russia), 50 mM potassium chloride (Sibensim, Russia), 5U RNAse inhibitor (Boehringer Mannheim, Biochemica), oligonucleotides mixture (Vector – Best, Russia) and 10mM Tris – HCl, pH 8,3 (Medigen Lab, Russia) (11). After the incubation at 42°C for an 1 h, the samples were boiled for 5 min and then placed on ice. Then the samples were exposed to amplification in reaction mixture, containing 0,4 μM of upstream as well as downstream primers (Vector – Best, Russia), 0,625 U of Taq DNA polymerase (Medigen Lab., Russia), 0,2 mM of each dNTP (Medigen Lab., Russia), 2 mM magnesium chloride (Sibensim, Russia), 50 mM potassium chloride (Sibensim, Russia), 10mM Tris – HCl, ph 8,3 (Medigen, Russia). For the amplification a temperature cycling device from DNA – Technology Lab., Russia, was used. Amplifications were carried out for 35 cycles each consisting of 1 min at 94° C, 1 min at 58° C, and 1 min at 72° C. After the amplification the amounts of 10 μl of the amplified samples were subjected to agarose gel electrophoresis. Visualisation of the amplification products have been done with ethidium bromide.

RESULTS

After the amplification with the β-actin primers and subsequent electrophoretic examination, all samples from peripheral blood mononuclear cells produced the fragment with the expected size. These fragments were registrated as bands with the similar intensity of staining in the agarose gel. 20 samples of peripheral mononuclear

cells from 44 were amplifiable with the primers. All of them produced a fragment of the expected size (692 bp). This group of patients were immunosuppressed. The quantity of CD3 lymphocytes were 729 ± 121 cells in 1 μl (N 1000 – 2000 cells/μl), CD4 lymphocytes – 375 ± 98 cells/μl (N 600 – 1000 cells/μl), CD8 lymphocytes – 274 ±87 cells/μl (N 300 – 700 cells/μl), B – lymphocytes – 74 ±19 cells/μl (N 100 – 500 cells/μl), the phagocytic activity was reduced. The group of patients without the activation of the endogenous retrovirus genome characterised with higher quantity of T and B lymphocytes, but it is not exceed the normal level. In infected group of patients the level of expression of proinflammatory cytokines was the same: IL – 1 at 16 patients (80%), IL – 6 at 14 patients (70%), TNF - α at 12 patients (60%). Non – infected group of patients demonstrate the lower level of proinflammatory cytokines expression: IL – 1 at 5 patients (20,9%), IL – 6 at 7 patients (29,0%), TNF - α at 5 patients (20,9%). After the treatment the expression of mRNA for the suppressive epitope of retroviral transmembrane p15E peptide observed at16 of patients (36,4%). At patients, expressing the mRNA for the suppressive epitope of retroviral transmembrane p15E peptide, the level of proinflammatory cytokines mRNA expression was: IL – 1 at12 patients (75%), IL – 6 at 11 patients (69%), TNF - α at 15 patients (94%). At non – infected patients the level of mRNA for proinflammatory cytokines expression was significantly lower: IL – 1 at 4 patients (14,2%), IL – 6 at 5 patients (17,9%), TNF - α at 3 patients (10,8%).
At the group of patients, expressed the mRNA for the immunosuppressive epitope of retroviral transmembrane p15E peptide notwithstanding to the treatment, the quantity of lymphocytes remains low: CD3 903±117 cells/μl, CD4 650±114 cells/μl, CD8 290±101 cells/μl, B lymphocytes 138±66 cells/μl. At the same time, the non – infected group of patients demonstrates the increasing of the quantity of lymphocytes to normal level. The increasing of the quantity of lymphocytes and decreasing of the level of mRNA for proinflammatory cytokines expression correlates with the reduction of neurological disorders: the defeats of pyramidal tract, coordination disorders, sensitivity violations, pelvium organs defeats, optical nerve disorders, decreasing of the size of demyelinating lesions.

DISCUSSION

At the present study we have investigated the level of expression of mRNA for the suppressive epitope of retroviral transmembrane p15E peptide and compared the expression of mRNA for proinflammatory cytokines at relapsing – remitting multiple sclerosis patients infected and non-infected with endogenous retrovirus, using RNA – PCR followed by agarose gel electrophoresis and ethidium bromide staining of the products of amplification. Our results indicate that the mRNA for the suppressive epitope of retroviral transmembrane p15E peptide expresses in perpheral blood mononuclear cells at 45% of relapsing – remitting multiple sclerosis patients with high level of proinflammatory cytokines mRNA expression. Due to the important role of the proinflammatory cytokines in myelin damage this data indicates that this group of patients possesses more hard course of the disease. Moreover, we achieved evidence that the expression of mRNA for the suppressive epitope of retroviral transmembrane p15E peptide prevented the decreasing of the mRNA expression for the proinflammatory cytokines in peripheral blood mononuclear cells after the treatment. This observations permit to elaborate new approaches to the therapy of relapsing – remitting forms of multiple sclerosis, direct to the inactivation of

endogenous retroviruses and subsequent decreasing of the level of proinflammatory cytokines.

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