Cytokines and Intrathecal Synthesis of Oligoclonal IgG in Viliuisk Encephalomyelitis and Multiple Sclerosis Patients

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Interleukin 6(IL6), interferon gamma (INFγ) and interleukin 18 (IL18) were investigated in cerebrospinal fluid (CSF) and serum of Viliuisk encephalomyelitis (VE), multiple sclerosis (MS) and other neurological diseases patients. IL 18 and INFγ production in VE and MS were significant different. There had been a significant increase of IL 18 in CSF of VE patients and INFγ in CSF and serum of MS patients. High level of INFγ in CSF of MS patients was associated with presence of oligoclonal IgG.

Key words: Viliuisk encephalomyelitis, multiple sclerosis, cytokines, intrathecal synthesis of oligoclonal IgG.

The aethiology and pathogenesis of the disease of Viliuisk encephalomyelitis (VE) remain unclear up to the day. Histopathologic findings in the cases of acute and chronic inflammation in brain and spinal cord of died VE patients specify long-lasting inflammatory process [11]. Investigation of cerebrospinal fluid (CSF) of VE patients have revealed intrathecal synthesis of oligoclonal IgG, but cause of local immune response remains unknown [15].

Some researchers suggest significant role of immunogenetic factors in VE pathogenesis [3, 5, 8]. Changes in lymphocytes population and depression of interferon-α system were shown in blood of VE patients [2, 4]. Significant association was found between VE and two single nucleotide polymorphisms (SNPs) in third intron of interferon gamma (INFγ) gene [3].

Last years in the Yakut population other disease of nervous system in pathogenesis of which immunopathologic mechanisms play an essential role began to extend - multiple sclerosis (MS). Changes in cytokines production may have important contribution in the development of immunopathologic process in central nervous system (CNS) and influence to local immune response [6, 7].

The purpose of research was comparative analysis of the levels of proinflammatory cytokines in blood and liquor of VE and MS patients and their association with intrathecal synthesis of oligoclonal IgG.

Materials and methods

Three groups of neurologic patients have been investigated:

Viliuisk encephalomyelitis. There were analysed blood serum and liquor of 16 VE patients surveyed in the hospital and during expeditions of Institute of Health. All VE patients had the chronic form of disease with duration from 3 till 54 years (on the average 22.3±13.4 years). This group consisted of 14 individuals of the Yakut and 2 of the Evenk nationality, 6 women, 10 men, in age from 38 till 74 (on the average 51.9±11.2 years).

Multiple sclerosis. There were analysed blood serum and liquor of 11 MS patients undergone medical examination in the Republican hospital №2 - the Center of emergency medical aid. Seven patients had remitting, 1- primary-progressing and 3 – secondary forms of disease with duration from 1 till 18 years (on the average 7.1±5.3 years). This group consisted of 9 individuals of the European Russian and 2 of the Yakut nationality, 6 women, 5 men, in age from 17 till 55 (on the average 38.5±12.3 years).

Other neurologic diseases (Oth.n.d.). This group consisted of 9 patients with other not inflammatory diseases of nervous system with the following diagnoses: sensitive dystonia - 1 person, hydrocephaly - 1, insult - 2, dyscirculatory encephalopathy - 2, epilepsy - 3 individuals, all of them of the Yakut nationality, 7 women, 2 men, in age from 18 till 72 (on the average 40.1±19.0 years).

Intrathecal synthesis of IgG was studied using the method of isoelectric focusing as previously described [10]. Results were estimated as positive when oligoclonal (two or more) bands were identified in the CSF but not in the accompanying serum of the same patient.

In blood serum and liquor of the patients contents of interferon gamma (INFγ), interleukin-6 (IL-6), interleukin-18 (IL-18) were investigated by means of immune-enzyme analysis with use of commercial kit of Joint-Stock Company "Vector-best", Russia and the equipment of Joint-Stock Company "ПИК-Н", Russia.

Statistical processing was made by means of the program Statistika 8.0. Results were described by calculation of medians and 25 and 75 procentiles. The significance of the results was determined using of nonparametric criteria of Kruscal-Wallis, Mann-Whitney and Wald-Wolfowitz.

Results

Intrathecal synthesis of oligoclonal IgG

Oligoclonal bands of IgG were identified in 9 from 16 VE patients. Duration of illness of these patients was 15,3±4,9 years. Patients with negative results have more long duration of illness (31,1±15,9 years) (p=0,02). These results suggest early received data [1].

In the group of MS intrathecal synthesis of IgG was determined in 6 from 11 patients. Five of them have remitting and one primary progressing forms of disease, all European. Five

patients have negative results, two of them have optic form, one atypical (pseudotumor), one remitting and one secondary progressing forms of disease. Two individuals without oligoclonal bands were Yakut, other European nationalities.

The group of other neurologic diseases consisted of patients with not inflammatory diseases, who had no intrathecal synthesis of IgG.

Cytokines IL-6, INFγ, IL-18

Results of cytokines IL-6, INFγ, IL-18 analysis are shown in table 1.

The content of IL-6 in serum did not differ in three investigated groups of patients (p=0.16 by criterion of Kruscal-Wallis). Increased level of IL-6 (more then 10 pg/ml) in serum was determined in serum of 3 individuals in the group of VE patients. This fact may reflect predisposition of these patients to systemic inflammation, not related with investigated pathology. Content of IL-6 in liquor was examined only in VE patients and it did not reveal essential increase.

INFγ level in serum of VE and Oth.n.d. groups was not higher than in healthy donors (not more than 10 pg/ml). Content of INFγ in serum of MS patients was statistically significantly higher in comparison with VE and Oth.n.d. groups (p=0.01) (fig. 1).

In liquor content of INFγ protein was increased in three groups of patients in comparison with serum (table 1) that testified to course of inflammatory process in brain of these patients. Despite of increased content of INFγ this parameter was statistically significantly lower (p=0.006) in VE patients in comparison with MS patients and Oth.n.d group (fig.1). This fact specifies limitation of production of protein of this cytokine in VE patients in comparison with other neurologic diseases, that possibly is the specific feature of VE pathology.

Content of IL-18 in serum did not differ in three groups whereas in liquor statistically significant distinctions were revealed (table 1). Thus the highest values were revealed in VE patients (fig. 2). This is the second specific feature of VE pathology that gives the evidence of participation of cells of brain tissue producing cytokine IL-18 in pathogenesis of this disease.

Cytokines and intrathecal synthesis of IgG

The analysis of cytokine status of patients depending on revealing intrathecal synthesis of IgG shows that in three groups of patients with intrathecal synthesis of IgG or at its absence the level of cytokines IL-6, INFγ and IL-18 in serum does not change. The positive association with intrathecal synthesis of IgG was revealed in liquor only in MS patients and only with cytokine INFγ (p=0.02) (fig.3). Other cytokines (IL-6, IL-18) do not show such dependence.

Our results showed some peculiarities of immune response in brain of VE and MS patients. As known local immune response develop with participation of both cells of peripheral

immune system and cells of brain tissue. In inflammation condition T-and B-cells migrate across blood-brain barrier from peripheral circulation and after activation T-lymphocytes into Th2 cells begin to produce IgG intrathecally. Activated microglia and astrocytes carry out antigens presentation function in brain [14]. These glial cell populations stimulate T-lymphocytes proliferation and produce INFγ and IL-18 respectively [13]. IL-18 is one of primary factor of induction INFγ by microglia [9]. Glial populations have different efficiency to restimulate distinct T cells subsets, like that microglia may activate Th1 and Th2 cells, whereas astrocytes mainly Th2 [12]. This peculiarity will manifest in forming of cell-mediated or humoral immune response in brain.

Lower level of IL-18 and high level of INFγ in CSF of MS and oth.n.d. groups in comparison with VE patients shows, that immune response in CNS of these patients is mainly cell-mediated.

High level of IL-18 and significantly low level of INFγ in CSF suggest that VE patients form preferentially humoral type of immune response in CNS. It seems that VE patients have problem with production of INFγ that fill in activation and proliferation of astrocytes (gliosis). The present data confirm early received association between VE and INFγ gene.

Conclusion

VE patients are characterized by raised levels of IL-18 and INFγ content in liquor whereas in serum these parameters remain at the level of healthy people. Despite of raised level INFγ in VE patients this parameter is statistically significantly lower ($p=0.006$) in comparison with MS patients and patients with other neurologic diseases. It gives evidence of restriction of production of protein of this cytokine in VE patients in comparison with other neurologic diseases that is specific feature of VE pathology. VE patients do not have association of INFγ level with intrathecal synthesis of IgG.

In VE patients changes in intrathecal content of IL-18 are more expressed. In CSF its level is considerably higher than in MS patients and in group of other neurologic diseases. Contents of IL-18 in blood serum do not differ in three groups. Also there is no association of IL-18 level with intrathecal synthesis of IgG.

In MS patients content of INFγ is increased both in serum and in CSF. The highest level of INFγ is marked in CSF at presence of intrathecal synthesis of oligoclonal IgG that specifies positive association of these parameters.

The received results reflect distinctions in mechanisms of immonopathology of brain at VE and MS. At MS increased production of INFγ both in blood and in liquor, but at VE intrathecal synthesis of IL-18 have most importance in development of immonopathology.

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Table 1.

Content of IL-6, INFγ, IL-18 in serum and CSF of VE, MS and oth.n.d. patients.

Parameter	Group of patients	Serum, pg/ml		CSF, pg/ml	
		Me (25-75%)	n-	Me (25-75%)	n
IL-6	VE	4 (3.4-15.4)	11	1.25 (1.15-1.45)	12
	MS	5.7 (5.2-8.5)	10		
	Oth.n.d.	5.7 (5.5-8.2)	8		
INFγ	VE	4 (3-4)*	11	7 (3.5-9.5)*	12
	MS	7 (6-19)*	11	20 (10-30)*	11
	Oth.n.d.	5 (3.75-6.5)*	8	18 (19-18.5)*	9
IL-18	VE	139.5 (90-210)	14	15.5 (13-20)*	10
	MS	125 (110-166)	11	4.6 (4.15-5.1)*	8
	Oth.n.d.	141 (110-219)	8	4.6 (3.8-7.8)*	9

Me (25-75%) – median and quartiles, - n-number of samples, * - p<0,05

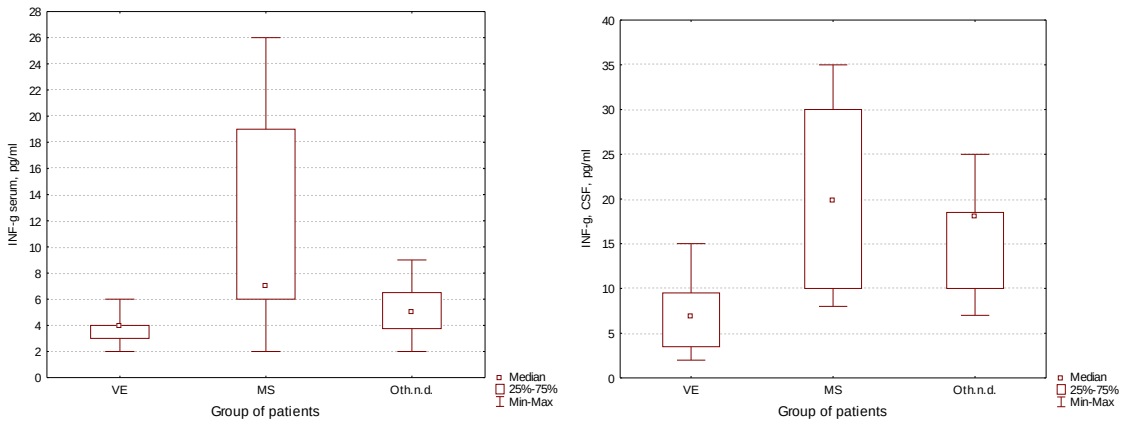

Fig. 1. Level of INFγ in serum and CSF of three groups of patients.

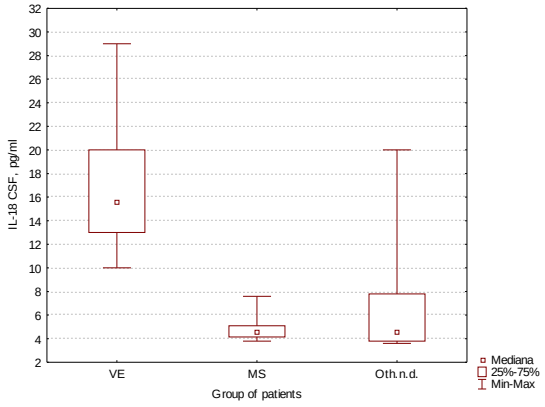

Fig. 2. Level of IL-18 in CSF of three groups of patients.

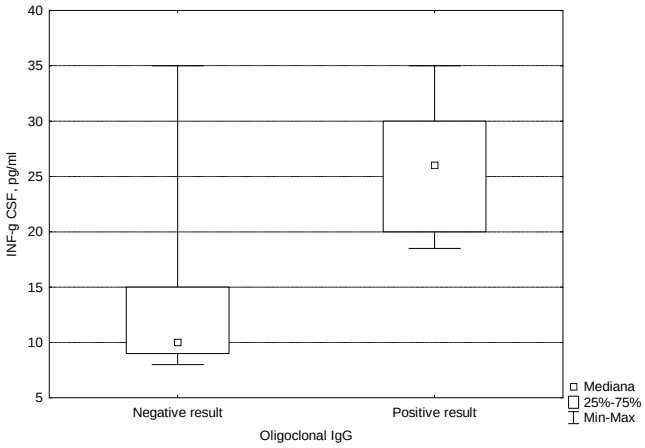

Fig. 3. Level of INFγ in CSF of MS patients with negative and positive results of oligoclonal IgG testing.

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