Chronic Tick-Borne Encephalitis Virus Antigenemia: Possible Pathogenesis Pathways

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Study of the proliferative potential and immunophenotype of lymphocytes and cytokine-producing capacity of mononuclear cells in patients with chronic tick-borne encephalitis virus antigenemia showed changes in T cell lymphoproliferative response, relative content of T cells and T helper inductors, immunoregulatory index, and imbalance in the production of immunoregulatory Th1 and Th2 cytokines.

Key Words: *tick-borne encephalitis virus; lymphocytes; lymphocyte blast transformation test; immunophenotype; cytokines*

Tick-borne neuroinfection remains highly incident in Siberian and Far-Eastern regions, and the area of prevalence of *Ixodes persulcatus*, carriers of tick-borne encephalitis virus (TBEV), increases every year. Recent studies revealed a pathomorphosis of TBEV with a trend to predominance of more mild forms (latent and febrile) and clinically asymptomatic virus carriership [1,3,4].

TBEV belongs to neurotropic viruses, but similarly to other viruses causing persistent infections, it is tropic to lymphoid tissue. Nucleotide sequences of viral RNA are detected in monocyte/macrophages, granulocytes, T and B cells of the peripheral blood [1,3].

Internalization of the virus into the host cell is primarily an "alarm signal" mobilizing congenital and adaptive immune mechanisms aimed at limitation and elimination of the infection. Infection of lymphocytes is very important, because it can appreciably modify functional activity of immunocompetent cells and immune response [8,12]. The mechanisms leading to different clinical manifestations and outcomes of infection caused by the same agent (from complete cure to transformation into clinically manifest chronic neuroinfection) remain not quite clear [3].

We evaluated the proliferative potential, immunophenotypical characteristics of peripheral blood lymphocytes, and production of immunoregulatory cytokines by mononuclear cells in patients with chronic TBEV antigenemia with and without clinical manifestations.

MATERIALS AND METHODS

The study was carried out on 32 patients (18 men and 14 women aged 18-45 years) with chronic TBEV antigenemia without clinical manifestations (15 patients) and with minimum clinical symptoms (17 patients). In each case the diagnosis was verified by clinical epidemiological, instrumental, serological (enzyme immunoassay), and molecular genetic (PCR) tests. Control group consisted of 19 age- and sex-matched donors. Heparin-stabilized (25 U/ml) venous blood was collected after overnight fasting and analyzed.

Total peripheral blood lymphocyte count was evaluated routinely [6]. Immunocytochemical evaluation of lymphocyte subpopulations by cell differentiation markers ($CD3^+$, $CD4^+$, $CD8^+$, $CD56^+$, $CD22^+$) was carried out using Dako kits [9].

Suspension for lymphocyte blast transformation test was prepared as described previously [2]. Difco

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phytohemagglutinin (PHA; 0.01 mg/ml) was added into the suspension. The test results were expressed as the percentage of unchanged, transitional, and blast lymphocyte forms [5].

The concentrations of IL-2, IL-4, IL-6, IL-10, IL-12, IFN-γ, and TNFα in the culture media were measured. Mononuclear cells were isolated in Ficoll-Paque density gradient (Pharmacia) and cultured (2×10^6 cell/ml) in RPMI-1640 with 10% inactivated FCS, 0.3 mg/ml L-glutamine, 10 mM HEPES (Flow), and 100 μg/ml gentamicin at 5% CO_2 for 24 h. Cytokine production was stimulated by adding PHA (Difco) into the medium. Cytokine content in supernatants was evaluated by enzyme-linked immunosorbent assay (Cytimmune, Procon). Optical density was measured on a Multiscan EX microplate reader (ThermoLabSystems). Cytokine concentrations were calculated by a calibration curve. Stimulation index, *i.e.* the ratio of stimulated to basal production, was calculated for evaluation of cell potential to immunocytokine production.

Normal distribution of variables was verified using the Kolmogorov—Smirnov test. The means were compared using Student's *t* test (for normal distribution) and Mann—Whitney's *U* test (for distribution other than normal). Analysis of correlations was carried out by calculating Spearman's *r* coefficient.

RESULTS

The interaction of the agent with the host depends on many components, in particular, pathogenic characteristics of the infectious agent and the capacity of the immune system to respond adequately to a foreign agent. Quantitative and functional changes in T and B cells are the most important immunity disorders in chronic viral infections [12].

Hematological analysis revealed a significant increase in the mean values of the absolute and relative counts of peripheral blood lymphocytes in TBEV carriers with and without clinical symptoms of viral neuroinfection in comparison with the corresponding parameters in donors. The signs of changed functional activity of T lymphocytes were detected in TBEV carriers. Specific mitogenic stimulation (PHA) activated T cell proliferation. The mean content of blast-transformed lymphocytes increased significantly in patients with chronic TBEV antigenemia compared to donors (Tables 1, 2).

The reactions of the immune system to the virus can be divided into congenital (determined by reactivity of the complement system, mononuclear phagocytes and natural killers) and adaptive (forming during response to specific antigens) [7,11]. The most important property of the adaptive immunity system is selective involvement of lymphocytes expressing specific antigen-carrying receptors into the immune response. The study of immunophenotypical characteristics of lymphocytes in patients with chronic TBEV antigenemia revealed decreased relative contents of mature T cells ($CD3^+$) and T helper inductors ($CD4^+$), and decreased immunoregulatory index ($CD4^+/CD8^+$) compared to the corresponding parameters in donors (Table 1). Our findings are in line with the results of other studies, indicating that chronic infection caused by

TABLE 1. Subpopulation Composition of Peripheral Blood Lymphocytes in Patients with Chronic TBEV Antigenemia ($X\pm m$)

Parameter		Donors	Patients with chronic TBEV antigenemia	
			without clinical symptoms	with minimum clinical symptoms
Total lymphocyte count	10^9/liter	1.95±0.17	2.92±0.37***	2.80±0.45***
	%	33.74±1.32	45.47±3.74*	44.81±1.24**
$CD3^+$	10^9/liter	1.59±0.32	1.60±0.2	1.07±0.13
	%	66.17±1.87	49.50±5.21*	58.33±1.07**
$CD4^+$	10^9/liter	0.56±0.14	0.67±0.28	0.43±0.07
	%	42.45±3.11	20.50±1.70*	24.11±3.48*
$CD8^+$	10^9/liter	0.71±0.15	0.86±0.20	0.55±0.10
	%	28.93±1.26	24.40±3.06	31.22±5.12
$CD56^+$	10^9/liter	0.42±0.11	0.50±0.13	0.31±0.06
	%	17.23±1.59	14.51±2.28	16.44±2.01
$CD22^+$	10^9/liter	0.35±0.05	0.54±0.15	0.28±0.04
	%	15.72±0.77	15.12±2.51	15.67±1.91
$CD4^+/CD8^+$		1.48±0.09	0.83±0.10*	1.04±0.31**

Note. Here and in Tables 2, 3: *$p<0.001$, **$p<0.01$, ***$p<0.05$ compared to donors.

TABLE 2. Proliferative Activity of Peripheral Blood Lymphocytes (%) in Patients with Chronic TBEV Antigenemia ($X \pm m$)

Lymphocyte form	Donors	Patients with chronic TBEV antigenemia	
		without clinical symptoms	with minimum clinical symptoms
Blast-transformed	71.46±1.05	79.83±1.42*	76.33±1.84***
Transitional	20.85±1.24	13.67±1.08*	14.75±1.56**
Unchanged	7.69±0.61	6.50±0.68	8.92±1.01

TBEV is associated with weak ineffective antiviral response of T cells with an appreciable decrease of the helper/suppressor coefficient [7]. The selective damage of T cell-mediated immunity can be associated with TBEV propagation in the thymus [1].

An important parameter characterizing the function of the immune system in chronic viral infection is production of cytokines by T cells. The cytokines ensure cell-cell cooperation and positive and negative immunoregulation. The type of the immune response to viral infection depends on the predominant participation of Th1 and Th2 lymphocyte subclasses, differing by the spectra of cytokines produced by them. The cytokine imbalance oriented at the Th1 and/or Th2 type immune response is pathogenetically significant for chronic transformation of the viral infection and for elimination of the virus and recovery [11,12].

Constant activation of lymphocytes during long-term persistence of the virus leads to an increase in IL-4 content and, in turn, to gradual predominance of the Th2 type immune response [13]. This is confirmed by a significant increase in the mean values of spon-

TABLE 3. Basal and Stimulated Production of Cytokines by Mononuclear Cells (pg/ml) and Stimulation Index (SI) in Patients with Chronic TBEV Antigenemia ($X \pm m$)

Parameter		Donors	Patients with chronic TBEV antigenemia	
			without clinical symptoms	with minimum clinical symptoms
IL-2	basal	42.70±7.38	55.25±7.51	45.78±5.16
	stimulated	198.30±20.81	104.75±9.10**	95.33±2.61*
	SI	5.35±0.52	2.03±0.20***	2.30±0.25*
IL-4	basal	58.90±6.43	88.50±3.65**	116.33±3.93*+
	stimulated	145.90±15.19	200.25±10.73***	237.33±14.40*
	SI	2.56±0.19	2.28±0.13	2.06±0.14
IL-6	basal	78.70±7.99	145.00±6.24*	175.00±13.54*
	stimulated	413.90±29.78	410.00±12.63	442.11±18.27
	SI	5.95±0.83	2.27±0.125**	2.71±0.34**
IL-10	basal	63.23±7.04	53.01±5.40	55.58±4.68
	stimulated	156.93±14.07	133.38±5.62	142.33±7.51
	SI	2.60±0.25	2.79±0.40	2.65±0.17
IL-12	basal	65.80±4.44	54.20±3.94	89.70±2.74*+
	stimulated	599.59±29.12	468.50±20.37**	276.89±9.76*+
	SI	9.60±1.01	9.01±0.83	3.11±0.14*+
IFN-γ	basal	106.00±7.52	146.25±9.25**	124.11±4.52
	stimulated	272.50±19.61	351.88±23.60***	327.22±18.56
	SI	2.58±0.10	2.48±0.24	2.66±0.18
TNFα	basal	103.65±16.22	50.38±5.05***	77.86±7.39+++
	stimulated	342.85±60.86	162.38±9.79***	158.89±11.75***
	SI	3.71±0.95	3.36±0.25	2.12±0.15***++

Note. *p<0.001, **p<0.01, ***p<0.05 compared to the corresponding parameters in patients without clinical manifestations.

taneous and PHA-stimulated production of IL-4 in patients with chronic TBEV carriership without clinical symptoms and with clinical manifestation of neuroinfection in comparison with the corresponding parameters in donors (Table 3).

Activated Th2 lymphocytes can stimulate the secretion of IL-6 and IL-10 activating the humoral immunity and simultaneously suppressing IL-2 production by T cells. Spontaneous production of IL-6 significantly increased in chronic TBEV carriers without clinical symptoms and with clinical manifestation of the disease. The mean index of stimulation of IL-6 production in these patients decreased more than 2-fold in comparison with the corresponding parameter in donors (Table 3). IL-2 and IL-12 promote differentiation of Th0 lymphocytes towards the Th1 type immune response and increase the yield of cytotoxic T lymphocytes involved in the immune response to intracellular viruses [10,12,14], but PHA-stimulated production of these cytokines was notably decreased in all examined patients.

Sharply pronounced *in vivo* and *in vitro* antiviral activity of IFN-γ and TNFα, Th1 cytokines potentiating the cellular immune response, is proven [13]. TNFα production (both spontaneous and PHA-stimulated) decreased by more than 30% in all patients, but this decrease was more pronounced in chronic TBEV carriers with clinical manifestations of the infection. TNFα alone and in complex with other cytokines participates in activation of cytotoxic processes in cells exhibiting a potent proinflammatory effect [12,15]. Secretion of IFN-γ, which acts as a synergist of IL-2 and IL-12 increased in chronic carriers of TBEV without clinical manifestations of the disease. Increased production of IFN-γ activation of humoral immune response, and stimulation of antibody production can protect only from virus penetration into the internal medium and virus-tropic host cells. But in case of intracellular penetration of the viruses, the key role is played by factors of cellular immunity. Suppression of cellular and predominance of humoral immune response, incapable of completely eliminating the agent, is presumably an important factor promoting long-term persistence of TBEV in the body and development of chronic infection.

The ratio of Th1 and Th2 cytokines in patients with long-term persistence of TBEV was evaluated by the analysis of correlations. A direct relationship between production of IFN-γ and IL-4 by mononuclear cells was revealed (r=0.718, p<0.01). IFN-γ and IL-4 are cytokines of the differentiation and proliferation stage of Th1 and/or Th2 lymphocytes and the direction of the immune response (cellular/humoral) is determined by their relative ratio. Analysis of individual concentrations of Th1 and Th2 cytokines showed that their content in TBEV carriers was higher than in donors, which attests to imbalance in Th1 and Th2 cytokine production.

Hence, chronic TBEV antigenemia with clinical symptoms and without clinical manifestations of neuroinfection is associated with increased lymphocyte proliferation potential, decreased relative content of mature T cells and T helper inductors, reduced immunoregulatory index, and with imbalance in Th1 and Th2 cytokine production.

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