VIROLOGY

Structural and Functional Characteristics of Lymphocytes in Chronic Carriers of Tick-Borne Encephalitis Virus

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Study of the surface phenotype, structure of plasma membrane, and activity of DNA repair system in peripheral blood lymphocytes from patients with different clinical forms of tick-borne encephalitis associated with long persistence of the virus revealed a pronounced imbalance between lymphocyte subpopulations, increased microviscosity of the plasma membrane lipid phase, and decreased activity of the DNA repair system in lymphocytes.

Key Words: *tick-borne encephalitis; lymphocyte; membrane; DNA repair*

Clinical practice demonstrates that manifestations of tick-borne encephalitis (TBE) are highly polymorphic. A great variety of clinical forms (from clinically asymptomatic virus carriership to lethal cases) can be explained by different intensity of immune reactions aimed at elimination of the virus and infected cells. It was previously shown that virus induces considerable changes in nonspecific and specific resistance. These changes are an important component of the pathological process, which can course as an acute, chronic, or asymptomatic infection [8].

The maintenance of immunological reactivity depends on the structure and function of immunocompetent cells. An important mechanism ensuring virus persistence is imbalance between cellular and humoral components of the immune response, which is caused by changes in the physicochemical and functional characteristics of T and B lymphocytes. We investigated the structure and functions of lymphocytes in patients with chronic carriership of TBE virus.

MATERIALS AND METHODS

Thirty-two patients with TBE (13 men and 19 women) aged 23-50 years were examined: 20 with chronic (more than 6 months) TBE virus antigenemia (CVA) without clinical manifestations and 12 chronic TBE virus carriers with blurred clinical symptoms. The disease was diagnosed on the basis of the results of clinical epidemiological, serological (indirect hemagglutination, enzyme immunoassay), molecular genetic (PCR), and instrumental methods. Control group comprised 17 male and female donors of the same age. Venous blood for analysis was collected after overnight fasting and stabilized with heparin (25 U/ml).

The total counts of lymphocytes in the peripheral blood were evaluated by the standard hematological methods. Lymphocyte subpopulations were evaluated by the lymphocytotoxic test [7] with monoclonal antibodies (Sorbent). The results were evaluated by estimating the relative and absolute counts of dead cells. Spontaneous lysis did not exceed 4-5%. Lym-

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phocytes were isolated on a Ficoll-Verografffin density gradient (1.077 g/cm^3) [6]. The lymphocyte suspension was diluted with Hanks' solution to a concentration of 2×10^5 cells/ml.

Microviscosity of lymphocyte plasma membrane was studied using by the fluorescent method [2] with pyrene, a nonpolar lipotropic probe located in deep layers of the plasma membrane. Fluorescence was measured on a Hitachi-MPF-4 fluorometer in a standard 1-cm cuvette (pyrene was added to lymphocytes 10 min before measurement) at 340 nm excitation wavelength (3×3 nm slit). Pyrene fluorescence spectrum has two peaks (370 and 390 nm) corresponding to pyrene monomer (F_M), and 470-nm corresponding to eximer form of the probe (F_E). The coefficient of pyrene eximerization was estimated by F_E/F_M ratio. This parameter reflects mobility of the lipid carbohydrate chains and the hydrophobic volume of plasma membrane (i.e. membrane microviscosity) [9].

Activity of the lymphocyte DNA repair system was evaluated by scintillation radiometry [3]. Two bactericidal lamps DB-15 (λ=254 nm) served as UV sources. The lamps were placed at a distance of 30 cm from the object (15 J/m^2 irradiation dose and 1.6 J/sec irradiation power). The samples were prepared as described previously [3], experimental samples were exposed to UV during 15 sec, control samples were not exposed. The samples were incubated for 2 h at 37°C in a culture medium with ^{3}H-thymidine (10 μCi/ml). The intensity of DNA synthesis was evaluated by the stimulation index (SI) determined by the ratio of radioactivity (cpm) of UV exposed to unexposed samples.

The data were statistically processed using Student's *t* test and nonparametrical Mann—Whitney's test [4].

RESULTS

The studied forms of infection suggest the involvement of immune mechanisms as a factor limiting virus reproduction, on the one hand, and promoting the realization of the infectious process, on the other [5,8,11]. The study of the surface phenotype of lymphocytes showed a significant decrease in the relative count of CD3$^+$ lymphocytes (p<0.05) and a significant increase of the absolute and relative counts of CD16$^+$ and CD72$^+$ lymphocytes in patients with subclinical TBE in comparison with donors (Table 1). Chronic TBE carriership with clinical manifestation of the infection was associated with a statistically significant decrease of CD8$^+$ percentage and significant increases in the relative and absolute counts of the peripheral blood CD72$^+$ and CD16$^+$ lymphocytes in comparison with the control (Table 1). The detected changes can be indicative of TBE virus capacity to stimulate proliferation of B cells carrying CD72$^+$ receptor. However natural killers (CD16$^+$) prevent unlimited proliferation of infected B cells, thus clearing the organism from intracellular viruses. Weak immune response insufficient for elimination of the virus and virus-infected cells creates conditions for long-term persistence of the virus in immunocompetent cells [5].

In order to avoid the effects of immunity factors, the virus can actively suppress the expression of its own antigenic markers on the surface of infected cell, which destabilizes the membrane structure [5,11]. One of effective methods for detecting structural changes in membranes is the fluorescent probe method [2]. A significant decrease of pyrene eximerization degree was detected in TBE patients in comparison with donors (Table 2). Comparison of the mean pyrene eximerization coefficients in patients with different clinical forms of

TABLE 1. Peripheral Blood Lymphocyte Subpopulations in Patients with TBE CVA ($X\pm m$)

Parameter		Donors	Patients with TBE CVA	
			with clinical symptoms	asymptomatic
Total lymphocyte count, 10^9/liter		1.81±0.14	2.49±0.14***	3.15±0.21**
	%	36.38±2.02	45.50±2.84	40.60±2.50***
Lymphocyte subpopulations	CD3$^+$, 10^9/liter	0.96±0.11	1.18±0.05	1.10±0.91
	%	4.50±3.20	46.10±2.57	42.90±1.95***
	CD8$^+$, 10^9/liter	0.39±0.05	0.43±0.05	0.55±0.05
	%	23.00±2.51	16.80±0.98***	16.70±1.04
	CD16$^+$, 10^9/liter	0.20±0.01	0.50±0.07***	0.79±0.11*
	%	16.13±2.36	27.20±1.60***	30.80±2.55*
	CD72$^+$, 10^9/liter	0.34±0.10	0.69±0.06**	0.56±0.07***
	%	14.38±1.54	31.10±2.21***	30.20±3.09*

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

TABLE 2. Biophysical Characteristics of Plasma Membrane and DNA Repair in Lymphocytes from Patients with TBE CVA ($X \pm m$)

Group		Pyrene eximerization, arb. units	SI of DNA repair system, arb. units
Donors		1.061±0.025	1.91±0.15
Patients with TBE CVA	with clinical symptoms	0.797±0.069*	1.32±0.17***
	asymptomatic	0.942±0.055***	1.25±0.09**

TBE showed no statistically significant difference (Table 2). The detected changes indicate increased microviscosity of the lipid phase of lymphocyte membrane, which is fraught with inhibition of such functionally significant membrane processes as receptor binding to second messengers and ligands, decrease of many enzymes activities, and, as a result, inhibition of the lymphocyte immune functions [12-14].

Structural changes in the membrane matrix can be responsible for cell dysfunctions. However the pathological effect of the virus depends not only on the severity of structural impairment of the plasma membrane as the most sensitive component of the cell, but largely on the efficiency of the intracellular reparative systems, ensuring the genetic homeostasis [3]. Study of activity of DNA repair system in peripheral blood lymphocytes in asymptomatic virus carriers and patients with chronic TBE carriership with blurred clinical symptoms showed a significant decrease of the SI values by 39 and 31%, respectively, in comparison with donors (Table 2). Attenuation of the repair processes results in accumulation of DNA defects in immunocompetent cells. It leads, among other things, to an increase in the concentration of protein product gene *p53*, which can arrest cell division and trigger the receptor and cytochrome *c*-dependent cascades of caspase activation [1]. Another mechanism of lymphocyte apoptosis is possible, when poly(ADP-ribose)polymerase (PARP) becomes the target for caspases. This enzyme is involved in DNA repair and catalyses poly (ADP-ribosylation) of DNA-associated proteins. Excessive activation of PARP in case of multiple DNA breaks leads to suppression of glycolysis and mitochondrial respiration, notably reducing the content of intracellular NAD^+ and causes lymphocyte death [10]. Hence, decreased activity of lymphocyte DNA repair system indirectly points to increase of lymphocyte apoptosis and, as a result, to impairment of the mechanisms regulating the immune response in TBE patients.

These data indicate pronounced structural and functional rearrangement of the lymphocyte population during long-term persistence of TBE virus. Obviously, changes in lymphocyte subpopulations, increased microviscosity of lipid phase of their plasma membrane, decreased activity of DNA repair system indicate imbalance of the structure and function of immunocompetent cells under conditions of infectious process. On the other hand, deficiency of lymphocytes can be an important factor in long-term persistence of the virus.

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