Cytogenetic Disorders in Peripheral Blood Lymphocytes of Patients with Febrile Form of Tick-Borne Encephalitis

N. P. Pirogova, V. V. Novitskii, O. V. Mikhailova, M. Yu. Khlusova, L. V. Babaeva, O. V. Voronkova, A. P. Mel'nikova, M. R. Karpova, and L. V. Lukasheva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 1, pp. 71-74, January, 2004
Original article submitted June 10, 2003

An appreciable increase in the number of aberrant lymphocytes was detected in the peripheral blood of patients with febrile form of tick-borne encephalitis (TBE). This increase peaked during week 2 of the infectious process and was paralleled by a decrease in the count of natural killer cells. By the end of the acute period of neuroinfection the number of cells with structural chromosome aberrations decreased, but still surpassed the control (donor blood). Genetically unstable cells were eliminated predominantly at the expense of lymphocytes with chromatid disorders, while the count of CD16-expressing lymphocytes increased. The intensity of reparative DNA synthesis was suppressed during the entire acute period of tick-borne encephalitis.

Key Words: *tick-borne encephalitis; lymphocytes; chromosome aberrations; reparative DNA synthesis*

The problem of genome instability induced by viral infections attracts attention because of obligate intracellular location of the pathogens and their cooperation with cell nucleus [12]. Normally, the count of cytogenetically modified cells varies within a wide range, while exposure to exogenous and some endogenous mutagens more than 10-fold increases their number [2-4]. Evolutional role of viruses as mutagens is determined by their pathogenicity. Manifestations and consequences of virus-induced injuries at different levels of organization of biological objects (from subcellular to organism level) largely depend on the tropism of infectious agent and type of infected cell [1]. Lymphotropic viruses, *e.g*. TBE virus, can modify immunological reactions and modulate tolerance to infectious and non-infection agents [5,8]. The balance between aggressive effects of pathogens and evolutionally developed resistance to these effects, *e.g*. repair of damaged DNA, determines the final effect of infection [11,13].

We studied the cytogenetic status of peripheral blood lymphocytes in patients with acute TBE during traditional therapy.

MATERIALS AND METHODS

Eighteen patients (male and female, aged 22-55 years) with TBE (febrile form, medium and severe degree) were examined on days 5-7 and 25-30 of disease. The diagnosis was based on anamnesis data (the fact of tick sucking), clinical picture, and serological findings (enzyme immunoassay). Therapy of TBE included daily intramuscular injections of donor anti-TBE immunoglobulin (titers 1:80-1:160) in a dose of 0.1 ml/kg for 3-4 days and iodantipyrine (0.1-0.3 g 3 times a day for 9 days). Control group consisted of 14 donors of the same age and sex.

Venous blood was analyzed. Metaphase plates of peripheral blood lymphocyte chromosomes were prepared by the air drying method using colchicine, and

Department of Pathophysiology, Siberian State Medical University, Ministry of Health of Russian Federation, Tomsk

0007-4888/04/1371-0061

the intensity of extra (reparative) DNA synthesis was studied as described previously [7]. The percentage of aberrant cells, number of aberrations of different type per cell, incidence of chromosome and chromatid aberrations per 100 cells were studied in cytogenetic analysis. Gaps were registered, but not as aberrations. All analyzed metaphases contained 46 chromosomes.

Peripheral blood leukocytes and lymphocytes were counted by the standard hematological methods. Natural killer cell (CD16) population in peripheral blood was evaluated in the lymphocytotoxic test using monoclonal antibodies (Sorbent) [6].

The results were statistically processed using Mann—Whitney's nonparametrical test.

RESULTS

The maximum number of aberrant lymphocytes in the peripheral blood of patients with febrile TBE was observed on days 5-7 of the disease (Table 1). The number of aberrations of different types per cell increased in comparison with donor cells. The aberrations detected were chromosome and chromatid. The incidence of chromosome aberrations was more than 6-fold higher than in the control. Chromosome aberrations presented as increased (in comparison with donors) incidence of paired fragments and appearance of dicentric and annular chromosomes (Table 1). Peripheral blood lymphocytes with chromatid aberrations were presented mainly by cells with single fragments. The incidence of single fragments more than 17-fold surpassed the normal.

One week after the start of therapy no changes in the counts of peripheral blood leukocytes and lymphocytes were detected in patients with febrile TBE (Table 2). However, the number of cells expressing CD16 antigens markedly decreased.

The intensity of reparative DNA synthesis in the peripheral blood lymphocytes of patients with febrile TBE significantly decreased on days 5-7 of the disease: the index of DNA repair stimulation was about 55% of the control level (Table 2).

A significant decrease in the count of leukocytes and a tendency toward a decrease in the count of peripheral blood lymphocytes were observed on days 25-30 of the disease (Table 2). This was paralleled by decreased percentage of aberrant lymphocytes, which however still surpassed the control values ($p<0.001$). The decrease in the number of aberrant cells was paralleled by a decrease in the number of aberrations of different types per cell and increase in the relative number of natural killer cells in comparison with the previous analysis (Tables 1, 2).

It should be emphasized that the decrease in the number of aberrations at this term was due to decrea-

TABLE 1. Cytogenetic Disorders in Peripheral Blood Lymphocytes of Patients with Febrile Form of Tick-Borne Encephalitis ($\bar{X}\pm m$)

Group	Number of metaphase plates	Aberrant cells, %	Number of aberrations of different types per cell	Incidence of chromosome aberrations per 100 cells				Incidence of chromatid aberrations per 100 cells	
				total	paired fragments	dicentric chromosomes	annular chromosomes	total	single fragments
Donors (n=14)	1400	1.31±0.35	0.013±0.003	0.652±0.200	0.652±0.200	0	0	0.658±0.190	0.658±0.190
Patients on days 5-7 of disease (n=18)	1800	10.00±0.64*	0.160±0.001*	4.38±0.65*	2.63±0.18*	1.38±0.42	0.125±0.010	11.75±0.80*	11.75±0.80*
Patients on days 25-30 of disease (n=18)	1800	7.0±0.7*+	0.092±0.008*+	3.3±0.4*	2.20±0.25*	0.40±0.16	0.70±0.15+	6.00±0.42*+	6.00±0.42*+

Note. Here and in Table 2: *$p<0.05$ compared to donors, +compared to the patients on days 5-7.

TABLE 2. Peripheral Blood Parameters and Extra DNA Synthesis in Patients with Febrile Tick-Borne Encephalitis ($\bar{X}\pm m$)

Group	Leukocytes, g/liter	Lymphocytes		$CD16^+$ lymphocytes		Stimulation index, cpm/ million cells
		g/liter	%	g/liter	%	
Donors (n=14)	6.15±0.16	1.99±0.11	32.40±2.67	0.22±0.02	12.10±1.32	1.96±0.21
Patients on days 5-7 of disease (n=18)	6.05±0.31	2.41±0.14	39.60±2.80	0.23±0.04	7.33±0.98*	1.08±0.05*
Patients on days 25-30 of disease (n=18)	5.41±0.21*	1.80±0.07$^+$	33.3±2.3	0.32±0.04	15.00±1.56$^+$	1.07±0.04*

sed incidence of chromatid aberrations presented mainly as single fragments. The incidence of chromosome aberrations on days 25-30 of the disease did not differ significantly from that on days 5-7 and surpassed the control values.

The index of stimulation of reparative DNA synthesis by the end of the acute period of the disease remained low and did not differ from the corresponding parameter on days 5-7 of the disease, which attests to negative effect of TBE virus on induction of extra DNA synthesis.

Hence, virus infection induced the appearance of aberrant lymphocytes in the peripheral blood. This parameter peaked one week after TBE onset and was paralleled by inhibition of reparative DNA synthesis. Presumably, the detected deficit of the relative count of natural killer cells at the initial stages of the disease (cells constitutionally determining elimination of genetically deficient cells) was responsible for accumulation of lymphocytes with abnormal chromosome structure. On days 25-30 of the disease the decrease in the peripheral blood lymphocyte count and of index of reparative DNA stimulation index were paralleled by a statistically significant decrease in the number of aberzrant cells, mainly with chromatid aberrations in the presence of somewhat increased count of CD16-positive lymphocytes. It can be hypothesized that cells with chromatid aberrations are actively eliminated from the body due to realization of the mechanism of recovery of genetic homeostasis associated with elimination of deficient cells by the priority effector systems of natural resistance of the body, the chief of which are natural killer cells [9,10].

The study was supported by the Council for Grants of the President of the Russian Federation for Supporting the Leading Scientific Schools of the Russian Federation, No. NS-1051.2003.4.

REFERENCES

1. E. A. Gileva, T. I. Buzhievskaya, and I. S. Stanova, *Genetika*, **37**, No. 4, 504-510 (2001).
2. A. D. Durnev, *Vestn. Rossiisk. Akad. Med. Nauk.*, No. 4, 70-76 (2001).
3. N. N. Il'inskikh, V. V. Novitskii, N. N. Vanchugova, *et al.*, *Micronuclear Analysis and Cytogenetic Instability* [in Russian], Tomsk (1991).
4. E. Yu. Moskaleva, I. A. Fedorova, and O. A. Nizenko, *Vestn. Rossiisk. Akad. Med. Nauk.*, No. 4, 12-17 (1993).
5. R. V. Petrov and R. M. Khaitov, *Ibid.*, No. 11, 18-21 (2000).
6. R. V. Petrov, R. M. Khaitov, and B. V. Pinegin, *Immunologiya*, No. 6, 6-9 (1994).
7. N. V. Ryazantseva, O B. Zhukova, V. V. Novitskii, *et al.*, *Byull. Eksp. Biol. Med.*, **134**, No. 11, 547-550 (2002).
8. I. S. Freidlin and P. G. Nazarov, *Vestn. Rossiisk. Akad. Med. Nauk.*, No. 5, 28-32 (1999).
9. S. B. Cheknev, *Immunologiya*, No. 6, 25-26 (1999).
10. A. A. Yarilin, *Vestn. Rossiisk. Akad. Med. Nauk.*, No. 4, 25-35 (1999).
11. R. W. Pero, B. Widegren, B. Hogstedt, and F. Mitelman, *Mutat. Res.*, **83**, No. 2, 271-289 (1981).
12. T. L. Reeder, R. J. Bailey, C. W. Dewald, and A. Tefferi, *Blood*, **101**, No. 5, 1981-1985 (2003).
13. C. Schmitz, B. Axmacher, and U. Zunker, *Acta Neuropathol.* (Berl.), **97**, No. 5, 71-81 (1999).