Phagocytic Functions of the Blood in Patients with Acute Tick-Borne Encephalitis

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We studied metabolic and functional activity of peripheral blood phagocytes from patients with febrile tick-borne encephalitis. Phagocytic activity of neutrophils determining nonspecific resistance of the organism progressively increased, while metabolic activity decreased. Secretion of nitric oxide by peripheral blood mononuclear phagocytes was suppressed, while phagocytic functions of these cells remained unchanged.

Key Words: *tick-borne encephalitis; neutrophils; monocytes; phagocytosis*

Tick-borne encephalitis (TBE) is a neuroinfection characterized by high mortality rate, severe residual symptoms, and tendency to chronization [2,12,14]. Reticuloendothelial cells play an important role in the initial stage of infection. Variability of clinical symptoms and immunosuppression that accompany viral diseases are associated with the direct adverse effect and replication of pathogenic agents in immune and phagocytic cells of the organism [4,6,8].

Reproduction of TBE viruses in the site of inoculation is followed by their dissemination with the blood and lymph to various organs and tissues. Most circulating viruses are found in the plasma; some viruses are integrated with leukocytes. In some instances the type of virion dissemination determines clinical symptoms of TBE, which is related to the presence of excess amounts of toxic products formed after degradation of cells and tissues and biologically active substances in the blood. The symptoms of TBE include microcirculatory disturbances, renal failure, endogenous intoxication, and polyorgan insufficiency [3,11]. The development of structural and functional disturbances in organs and systems during infectious processes is related to incomplete phagocytosis of the pathogenic agent and its biochemical cleavage via enzymatic degradation, lipid peroxidation, and nitration of macromolecules [4,8,11].

Here we studied functional and metabolic characteristics of peripheral blood phagocytes from patients with TBE.

MATERIALS AND METHODS

We examined 35 febrile patients with moderate and severe TBE (men and women, 18-55 years) on days 1-3 (initial stage), 5-7 (peak), and 25-30 (clinical convalescence) of the disease. The diagnosis was made by anamnesis (tick bite), clinical symptoms of the disease, and serologic tests for the presence of TBE viruses in the peripheral blood (enzyme immunoassay). The control group included 20 healthy donors (men and women, 18-55 years).

The contents of glycogen, lipids, and nonenzymatic cationic proteins were measured in the venous blood [10]. Alkaline and acid phosphatases and nonspecific esterase activities were measured as described elsewhere [9]. The mean cytochemical coefficient (ACC) was calculated.

To evaluate phagocytic activity of neutrophilic granulocytes, they were incubated with a 1-day-old culture of *Staphylococcus aureus* H-209 at 37°C.

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We examined smears and estimated the ratio of active neutrophils, engulfing capability of neutrophils (ECN), and index of completeness of phagocytosis [3,5].

Expression of Fcγ and C3β receptors on monocytes was studied by EA- and EAC-rosette formation with sheep erythrocytes [7,13]. Phagocytic activity of monocytes was determined by changes in optical density of the lysing solution on a SEF-4 device at λ=540 nm. To obtain supernatants the mononuclear cells were isolated in a density gradient of Ficoll-Urografin (ρ=1077 g/cm^3) and cultured in RPMI-1640 medium (Sigma) containing 10% inactivated fetal bovine serum, 0.3 mg/ml L-glutamine, 5 mM HEPES, and 100 μg/ml gentamicin at 37°C and 5% CO_2 for 24 h. $NO^•$ production was stimulated by the addition of *E. coli* lipopolysaccharide (026:B6, Sigma) [1, 11]. The supernatant (100 μl) and equivalent volumes of sulfanilamide and N-(1-naphthyl)-ethylene amide in 2.5% orthophosphoric acid (ICN) were placed in a 96-well plate. Optical density was measured on a Multiscan device at 550 nm.

The results were analyzed by Mann—Whitney *U* test.

RESULTS

In the initial period of TBE the contents of lipids and nonenzymatic cationic proteins, alkaline phosphatase activity, and ratio of active peripheral blood neutrophils were lower than in healthy donors. Acid phosphatase activity markedly increased, which was probably associated with stimulation of lysosomal enzyme synthesis during phagocytosis [4,11]. Phagocytic activity and ACC for monocyte nonspecific esterase decreased in this period (Table 1).

TABLE 1. Phagocytic Activity of Neutrophils and Functional State of Monocytes in the Peripheral Blood from Patients with TBE ($X \pm m$)

Parameters		Healthy donors	Patients with TBE, stage		
			initial	peak	clinical convalescence
Ratio of active neutrophils, %		65.30±1.28	52.63±2.31$^+$	68.41±2.45$^+$	68.31±2.73$^+$
Engulfing capability of neutrophils, U		6.54±0.09	5.11±0.26	6.34±0.51	8.10±0.96*$^{+o}$
Index for completeness of phagocytosis, %		61.67±2.15	72.03±3.32	63.03±2.68	81.67±2.97*^{o}
Fcγ$^+$ monocytes, %		26.06±1.12	39.23±2.15*	31.75±1.39$^+$	42.50±2.26*
C3β$^+$ monocytes, %		28.84±0.94	34.54±3.85*	29.01±2.36	28.33±3.67
Phagocytic activity of monocytes, optical density units		119.3±3.3	92.6±2.9*	134.5±5.1$^+$	125.2±4.2$^+$
$NO^•$ production, μmol/ml	basal	7.44±0.77	—	3.50±0.44*	—
	stimulated	13.68±1.35	—	2.50±0.16*	—

Note. Here and in Table 2: $p<0.05$: *compared to healthy donors, $^+$compared to initial stage, ^{o}compared to peak of the disease.

TABLE 2. Enzyme Activity (ACC) and Contents of Glycogen and Lipids in Peripheral Blood Leukocytes from Patients with TBE ($\bar{X} \pm m$)

Parameters		Healthy donors	Patients with TBE, stage		
			initial	peak	clinical convalescence
Glycogen	neutrophils	2.170±0.114	2.450±0.053	2.147±0.121	2.549±0.042*^{o}
	monocytes	0.785±0.073	0.556±0.158	0.593±0.097*	0.794±0.077^o
Lipids	neutrophils	2.89±0.04	1.76±0.09*	2.58±0.15$^+$	2.42±0.07$^+$
	monocytes	0.49±0.16	0.43±0.11	0.55±0.07	0.575±0.180
Alkaline phosphatase		1.388±0.104	0.985±0.148*	0.455±0.131*$^+$	0.475±0.219*
Acid phosphatase (neutrophils)		0.645±0.024	0.991±0.013*	1.012±0.007*	1.191±0.087*
Nonspecific esterase (monocytes)		1.238±0.062	1.090±0.096*	1.123±0.073	1.138±0.123
Nonenzymatic cationic proteins		2.120±0.113	1.074±0.051*	1.552±0.143*	1.595±0.201*

At the peak of TBE we observed a slight increase in ACC for lipids and content of nonenzymatic cationic proteins (compared to the initial stage). However, these parameters did not reach the control value. In this period ACC for alkaline phosphatase progressively decreased, which was probably related to recruiting of young neutrophilic leukocytes into the circulation [4]. The ratio of active granulocytes returned to normal. Glycogen content in monocytes from patients markedly decreased compared to the control (Table 2). At the peak of TBE expression of Fcγ receptors and phagocytic activity of monocytes increased compared to the initial stage of the disease. $NO^{\bullet}$ production during stimulation of cultured mononuclear cells from healthy donors increased by 2 times compared to the baseline level. However, at the peak of the disease $NO^{\bullet}$ secretion by intact and stimulated monocytes was suppressed (Table 1). The inhibition of $NO^{\bullet}$ synthesis aggravates the symptoms of TBE. $NO^{\bullet}$ deficiency promotes persistence of the pathogenic agents in host cells and provokes recurrences or progredient course of the disease [2,4].

During convalescence glycogen content surpassed that in healthy donors and patients at the peak of TBE. The amount of lipids in cells returned to normal (Table 2). Acid phosphatase activity increased, while alkaline phosphatase activity and content of nonenzymatic cationic proteins decreased. In this period the ratio of active neutrophils was higher than at the peak of TBE. ECN and index for completeness of phagocytosis markedly surpassed these parameters in healthy donors and patients with the initial stage and peak of TBE.

Studies of neutrophilic granulocytes and monocytes from the peripheral blood of patients with TBE revealed activation of neutrophils determining nonspecific resistance of the organism. Monocytes playing a role in the antiinfectious protection were not stimulated. Bactericidal activity of granulocytes was suppressed in various stages of TBE. However, bactericidal activity of monocytes returned to normal.

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