Phagocytic Activity and State of Bactericidal Systems in Polymorphonuclear Leukocytes from Patients with Alzheimer's Disease

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Phagocytic activity of peripheral blood neutrophils underwent phase changes in patients with Alzheimer's disease. Neutrophils retained the ability to engulf microbes, but their digestive activity decreased at the early stage of Alzheimer's disease. At the late stage we observed a decrease in the count of phagocytizing neutrophils, reduction of myeloperoxidase activity, and increase in the content of cationic proteins.

Key Words: *Alzheimer's disease; neutrophils; phagocytosis; myeloperoxidase; cationic proteins*

Published data show that increased sensitivity to infections in patients with Alzheimer's disease (AD) [5,8] is probably related to disturbances in specific immunity and impairment of the nonspecific resistance. Immune disorders in patients with AD manifest in the formation of immune complexes and autoantibodies against structural elements of brain tissue [6, 10], $\beta_{1\text{-}42}$-amyloid peptide, and neuromediators [3,4], dysfunction of lymphocytes [1], and changes in the production of interleukin-1 [1] and interleukin-2 [5]. Recent studies showed that the count of peripheral blood leukocytes capable of engulfing infectious agents and average number of phagocytized microbes decrease in patients with AD and elderly people [5]. Here we studied phagocytic activity of polymorphonuclear leukocytes and state of bactericidal systems in the peripheral blood from patients with AD and healthy donors. To evaluate specific changes in the intensity of phagocytosis and activity of bactericidal systems in neutrophils during AD, we examined patients of the same age with atherosclerosis and vascular dementia (VD, control group). It was interesting to determine changes in phagocytic activity and the state of bactericidal systems in polymorphonuclear leukocytes of the peripheral blood at various stages of the disease.

MATERIALS AND METHODS

We examined 14 patients with AD (women, 75-90 years), 7 women of the same age with atherosclerosis and VD, and 26 healthy donors (50-65 years). The diagnosis of AD was confirmed by psychiatric, psychological, and neurologic examination. Some patients were subjected to computerized tomography of the brain. Clinical diagnosis was made by ADRDA and ICD-10 criteria (World Health Organization, 1994). We examined patients with AD and VD and without associated infectious diseases of the lungs and urogenital system. When the diagnosis was made, the blood was taken 1 time from the cubital vein.

For evaluation of phagocytic activity the blood (0.2 ml) stabilized with 4% sodium citrate was incubated with *Staphylococcus aureus* suspension (1-day culture, strain Zhaev, 0.2 ml, 5 million microbial bodies per 1 ml) at 37°C for 30 min. Smears were dried

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in air, fixed with methanol, and stained by the method of Romanovsky—Giemsa. The phagocytic number (count of active neutrophils phagocytizing microbes) and phagocytic index (average number of microbes engulfed by 1 neutrophil) were determined by microscopy. The blood was cultured with microbes for 60 min to evaluate completeness of phagocytosis. The index of complete phagocytosis was estimated during microscopic examination of smears. Viable and dead staphylococci were counted in cells engulfing microbes.

For detection of cationic proteins in neutrophils the blood smears were dried in air, fixed with 5% sulfosalicylic acid for 30 sec, stained with 0.1% primulin in 0.05 M borate buffer (pH 8.2) for 30 min, washed 3-fold with the same buffer, dried, and examined under a LYuMAM-I3 luminescence microscope equipped with an immersion objective. A total of 50 cells in each preparation were photometried. The intensity of cell fluorescence was determined by a galvanometer scale and expressed in arbitrary units [2].

Myeloperoxidase in peripheral blood neutrophilic leukocytes was visualized by the method of Sato (with benzidine). Blood smears were fixed with alcohol-formol, maintained in 0.5% copper sulfate for 1 min, thoroughly washed with distilled water, and placed in a freshly prepared solution of benzidine for 2 min. Neutrophil nuclei were poststained with safranin (1% aqueous solution). Myeloperoxidase activity was determined by the cytochemical index (Kaplow method).

The results were analyzed by Student's *t* test (PRIMER software).

RESULTS

In patients with AD and VD the count of active neutrophils capable of engulfing infectious agents decreased, but the number of microbes phagocytized by leukocytes increased (Table 1). The ability of cells from patients of both groups to digest microbes was lower than in healthy donors. Myeloperoxidase activity decreased, while the amount of cationic proteins increased in patients with AD. These changes were not observed in patients with VD.

For evaluation of phagocytic activity of peripheral blood neutrophils at various stages of the disease the patients with AD were divided into 2 groups. Groups 1 and 2 included patients with early (less than 5 years) and late stages of AD (more than 15 years), respectively. In group 1 patients the number of peripheral blood active neutrophils phagocytizing microbes did not differ from the control, but markedly surpassed the corresponding parameter in group 2 patients (p=0.007) and patients with VD (p=0.038). Digestive activity of cells in group 1 patients decreased more significantly than in group 2 patients. Myeloperoxidase activity in peripheral blood neutrophils decreased at the late stage of AD. The amount of cationic proteins in peripheral blood neutrophils from patients with early stages of AD did not differ from that in healthy donors and patients with VD. At the late stage of AD the amount of cationic proteins in neutrophils surpassed that observed in the early stage (p<0.001).

Our study revealed phasic changes in phagocytic activity of peripheral blood neutrophils in patients with AD. Neutrophils retained the ability to phagocytize infectious agents, but their digestive activity decreased at the early stage of AD. At the late stage we observed a decrease in the count of active phagocytizing neutrophils, reduction of myeloperoxidase activity, and increase in the content of cationic proteins. The number of phagocytizing neutrophils in the peripheral blood and digestive activity of cells decreased in patients with AD. On the one hand, phagocytic dysfunction of peripheral blood neutrophils was observed in patients with AD and VD and, therefore, could be related to age-related changes [5]. On the

TABLE 1. Phagocytic Activity and State of Bactericidal Systems in Peripheral Blood Neutrophils from Patients with AD and VD ($M \pm m$)

Parameter	Healthy donors (n=26)	Patients with AD			Patients with VD (n=7)
		total (n=14)	duration of the disease		
			<5 years (n=7)	>15 years (n=7)	
Phagocytic number, %	55.8±1.0	43.2±4.8***	55.0±5.9	31.4±4.3*	35.7±5.8*
Phagocytic index	1.6±0.1	2.2±0.1*	2.3±0.1*	2.1±0.1*	2.3±0.1*
Index of complete phagocytosis, %	49.7±0.9	41.4±3.9***	38.0±6.0*	44.9±5.1	44.1±3.4***
Myeloperoxidase activity, cytochemical index	2.6±0.1	2.1±0.1**	2.2±0.1	2.0±0.1**	2.35±0.20
Content of ationic protein, arb. units	199±12	246.4±8.1***	221.0±5.1	271.4±8.6**	237±22

Note. *p<0.001, **p<0.01, and ***p<0.05 compared to healthy donors.

other hand, disturbances in the bactericidal system of neutrophils manifested in decreased myeloperoxidase activity and increased amount of cationic proteins can be typical of the late stage of AD. Recent studies showed that myeloperoxidase activity in neutrophils is a new diagnostic criterion for noninfectious diseases [7]. The increase in the content of cationic proteins (inflammatory mediators) in peripheral blood neutrophils in patients with AD probably reflects inflammatory reaction in amyloid deposits of brain tissue [9].

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