Phagocytic Activity of Neutrophils and Lymphocytes at Various Periods of Storage in the State of Cold Anabiosis (-40°C)

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We studied changes in phagocytic activity of neutrophils and lymphocytes subjected to cold anabiosis. The proposed effective method for introduction of human blood leukocytes into cold anabiosis at moderately low temperature (-40°C) in the presence of a cryopreserving agent does not require washout of the biological material after defrosting.

Key Words: *neutrophils; lymphocytes; phagocytic activity; phagocytic index; cold anabiosis*

Phagocytosis is a complex and energy-consuming process that depends on cell energy reserves, surface charge, reaction to foreign agents, and activity of proteolytic enzymes. Various cells in the state of anabiosis are characterized by reversible arrest of metabolic and physiological processes [4]. The entry into anabiosis is determined by the temperature, morphological and functional characteristics of cells, physicochemical changes in a medium at the water-ice phase transition, and other factors. The depth of anabiosis depends on the degree of cell dehydration. The greater is the amount of non-freezing fractions of free and bound water, the higher is the survival rate of cells exposed to low temperature. The fraction of free water serves as a medium for transport of substances (ions and metabolites) and regulates the intensity of metabolic and physiological processes. The fraction of bound water maintains the structure and function of catalytic and transport intracellular proteins [1]. Binding of extra- and intracellular water prevents its transition to ice, hyperconcentration of salts, and cell destruction. It can be achieved by addition a water-stabilizing cryopreserving agents.

In the present work phagocytic activity (PA) of human blood neutrophils and lymphocytes was studied during various periods of cold anabiosis (CA) at -40°C in the presence of a cryopreserving agent. This method does not require washout of the biological material after defrosting.

MATERIALS AND METHODS

Donor leukocyte concentrates (LC) were obtained from the whole blood by cytopheresis with a preserving agent CDF at 2500*g* for 5 min (Sorvell). A mixed-action cryopreserving agent served for cryoprotection. This preparation contributes to supercooling of the intracellular medium and its transition to amorphous ice consisting of small crystals. It excludes the possibility of damage to cell membranes and organelles, prevents hyperconcentration of salts, and maintains cell energy reserves during storage.

The cells were introduced into the state of CA. LC (15-25 ml) were mixed 1:1 with a cryoprotective solution using a Kompoplast 300 plastic box. The mixture was maintained at room temperature for 20 min (equilibration). The box with the biological material was placed in a 4000-ml bath with 96% ethyl alcohol (co-

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oling agent). The chamber of a Kriostat electric freezer was cooled to -28°C. The time of exposure was 15 min. The biological material was maintained in freezer at -40°C for 4-5 min. Thermograms of exponential freezing were recorded: 7-8°/min, eutectic point -2.6°C; 1-2°/min, eutectic point -28°C (stage I); 3-4°/min, eutectic point -40°C (stage II). LC were stored at -40°C for 1, 20, 30, 40, 50, and 60 days.

LC were rapidly defrosted in a water bath (20 liters) at 38°C for 45-60 sec, which depended on the volume of biological material. The box was shaken at a rate of 3-4 oscillations per 1 sec.

The number of leukocytes and white blood count were estimated by standard clinical and laboratory methods for calculating neutrophils and lymphocytes in the medium.

PA of neutrophils and lymphocytes was determined by the ability of cells to engulf inert latex particles [4]. We estimated the percentage of phagocytic cells (relative to the total number of cells) and phagocytic index (number of engulfed latex particles with a diameter of 1.05 μ).

The results were analyzed by Student's *t* test.

RESULTS

The absolute number of leukocytes significantly decreased after storage for 40-60 days ($p<0.05$, Table 1). Neutrophils are characterized by a complex structure and intensive metabolism. These cells were least resistant to the adverse effect of CA. Lymphocytes had the highest resistance to CA (Table 2), which is probably associated with their evolutionarily determined resistance to various factors.

The main function of neutrophils is phagocytosis. Phagosomes with engulfed latex particles were formed in various periods of low-temperature storage of the biological material. However, the percentage of phagocytic cells significantly decreased after CA for 40 days ($p<0.05$, Table 3). The phagocytic index of neutrophils progressively decreased over 50-day storage of the biological material at -40°C ($p<0.05$, Table 3). Probably, bound intracellular water did not entirely freeze in the state of CA at -40°C. Slow-rate metabolic processes continued under these conditions [1]. Long-term storage at 40°C is accompanied by exhaustion of cell energy reserves and inhibition of metabolic processes. Destruction and death of cells occur by the end of CA.

The main function of lymphocytes is realization of specific cellular and humoral immune reactions. We revealed that phagocytic activity of lymphocytes decreased by 8.5 times 1-60 days after CA. It was probably due to cold stress-induced activation of several lymphocyte populations. The phagocytic index of lymphocytes stored over various periods of time did not differ from that of intact cells not exposed to CA (Table 3).

The proposed method for introduction of leukocytes into CA at -40°C suggests the use of a cryoprotective solution, does not require washout of the biological material after defrosting, and allows us to maintain the optimal level of PA in neutrophils (30 days) and lymphocytes (various periods of storage). This me-

TABLE 1. Absolute Number of Leukocytes Cryopreserved at -40°C for Various Periods ($n=7$, $M\pm m$)

Duration of CA, days	Absolute number of leukocytes in 1 μl LC	
	before CA	after CA
1	10560.0±581.4	9890.0±950.3
20	7040±1698	6584±1300
30	11530±2176	10170.0±981.1
40	19700±2000	15910.0±634.3*
50	8050±1472	6420.0±887.1*
60	18720±2207	14990±1867*

Note. Here and in Tables 2 and 3: *$p<0.05$ compared to the parameter recorded before CA.

TABLE 2. Relative Number of Neutrophils and Lymphocytes Cryopreserved at -40°C for Various Periods ($n=7$, $M\pm m$)

Duration of CA, days	Relative number of cells (white blood count, %)			
	neutrophils		lymphocytes	
	before CA	after CA	before CA	after CA
1	32.14±3.29	29.00±2.83	65.86±3.29	68.5±1.0
20	32.20±1.64	29.60±3.13	65.80±1.64	68.40±3.13
30	32.67±6.80	26.5±5.2	64.83±5.60	71.00±4.76
40	32.0±5.8	18.71±5.82*	66.00±4.83	79.14±5.34*
50	42.86±9.94	19.86±2.07*	55.43±8.83	79.86±4.26*
60	35.71±6.82	11.80±2.39*	61.43±6.16	82.14±7.84*

TABLE 3. PA of Neutrophils and Lymphocytes Cryopreserved at -40°C for Various Periods (n=7, $M\pm m$)

Duration of CA, days	PA, %		Absolute number of phagocytic cells in 1 μl LC		Phagocytic index, %	
	before CA	after CA	before CA	after CA	before CA	after CA
Neutrophils						
1	40.00±2.08	39.33±1.51	1105.00±61.13	1106.0±71.9	4.43±0.53	4.57±0.98
20	35.86±4.56	31.29±5.22	557±0	461.40±26.99	4.57±1.40	4.43±1.27
30	48.00±4.47	47.25±2.22	1332.0±382.3	1129.0±96.4	2.86±0.69	3.43±0.98
40	38.00±1.41	20.14±1.86*	1306.0±167.4	509.00±99.11*	3.29±1.38	3.00±0.82
50	33.86±1.07	15.43±2.07*	1244.0±132.2	201.7±22.1*	3.29±0.95	1.57±0.53*
60	36.43±5.38	13.67±3.27*	2405.0±280.1	366.3±27.3*	3.25±0.96	1.29±0.49*
Lymphocytes						
1	1.57±0.98	12.57±2.37*	69.80±8.49	1127.0±175.3*	1.29±0.49	1.86±0.69
20	1.29±0.49	8.43±1.62*	44.60±7.67	290.00±23.52*	1.71±0.76	2.00±0.82
30	1.14±0.38	9.25±1.26*	71.5±17.0	667.00±93.63*	1.43±0.53	1.71±0.76
40	1.17±0.41	12.83±3.37*	132.5±13.0	1256±215*	1.43±0.53	2.00±0.82
50	0.86±0.38	10.86±2.85*	42.33±11.36	638.60±99.74*	1.86±0.69	1.29±0.49
60	1±0	7.83±1.94*	133.00±12.12	1165±172*	1.43±0.53	1.14±0.38

thodically simple and reliable method holds much promise for scientific, medical, and biological studies.

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