Behavior of Lymphoid Cell Population, Cell Nuclei and Nucleoli in Periodic Disease and Leukemia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 2, pp. 162-166, February, 2008
Original article submitted September 5, 2006

Similar behavior of lymphoid cells, their nuclei and nucleoli in periodic disease and leukemia attest to nonspecific reaction of the immune system to these diseases, but the intensity of this reaction and mechanisms of the population recovery are different. DNA hyperreplication plays an important role in this process: in periodic disease it is realized via gene amplification, which manifests by the formation of H2c nuclei and increase in the number of nucleoli, while in leukemia bone marrow lymphoblasts double the DNA content during S phase, maturate during G_2 phase, and then divide. We called this mechanism "reserve lymphopoiesis" by analogy with reserve erythropoiesis discovered previously by us.

Key Words: *periodic disease; leukemia; lymphocytes; nuclei; DNA*

Lymphocytes traditionally attract the attention of scientists due to their role in immunogenesis and capacity to antibody production. These characteristics are explained by specific functions of their genetic apparatus, its activity presumably being reflected in parameters of their nuclei and nucleoli directly involved in the synthesis of nucleic acids. This determined the aim of this work: to study the behavior of lymphocytes, their nuclei, and nucleoli at different stages of periodic disease (PD) and leukemia and to compare changes in their parameters by analytical microscopy, quantitative cytochemistry, cytophotometry, and cytomorphometry.

MATERIALS AND METHODS

Material for the study was obtained from R. O. Eolyan Center of Hematology. Peripheral blood smears from clinically healthy donors (n=5) and patients with PD (5 during remissions, 13 during attacks, and 4 with PD complicated by amyloidosis), peripheral blood smears and bone marrow impressions from patients with acute leukemia (n=20) and 6 patients with remissions were fixed and stained for DNA detection by our variant of Feulgen reaction [2]. The amount of DNA (λ=575 nm) in the nuclei and nucleoli (perinucleolar chromatin, PNC), areas and perimeters of the nuclei and nucleoli were measured and summary weight of DNA, volume and surface areas of the nucleoli for each nucleus were evaluated by television scanning (100/1.30 objective) using image analyzer on the base of SMP-05 photometer microscope (Opton) and UPIAM-2000 (Universal Program of Image Analysis of Microobjects) software, created at our Department. Stages of lymphoid cell development were determined by the size of nuclei, number of nucleoli, and degree of chromatin condensation using Feulgen reaction. Multiparameter analysis of donor lymphocyte nuclei (images were digitally processed after scanning) was carried out using previously proposed by us coefficients of relief, texture, and mutual disposition of particles (estimation formulas

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were presented previously [1,2,4]). Cytomorphological studies were carried out on non-fixed preparation stained by Pappenheim's method in the May—Grunwald and Giemsa solutions [5]. Population composition, mean number of nucleoli in the nuclei, and nuclear distribution by the number of nucleoli were evaluated. The volumes ($V=4/3\pi R^3$) and complete surface areas ($P=4\pi R^2$) of nuclei and nucleoli were estimated by the cytometry data, because these parameters objectively reflect the possibility of metabolite and information exchange between the nucleus and cytoplasm. At least 100 nuclei were studied in each case. The arithmetic and weighted means were calculated [6]. The significance of data (p) and sufficiency of the sample volume were evaluated.

RESULTS

Multiparameter analysis of donor peripheral blood lymphocyte nuclei (Table 1) showed differences between the parameters in men and women: DNA weight in lymphocyte nuclei of women was 10.6% higher than in men ($p<0.0001$), while the coefficients of relief and mutual disposition of particles were 17.5 and 11.2% lower ($p<0.0001$). These unexpected results, particularly DNA weight, showed usefulness of preliminary analysis of donor lymphocyte nuclei, and further studies were carried out only in men.

Differences between donors and PD patients were detected at the level of the lymphoid population composition. Blast forms were detected in the blood of patients (4-20%; $p<0.0001$), but not in donors. Blasts were rare in remission (4-6%; $p<0.0001$), their content increasing during an attack (6-10%, $p<0.0001$) and reaching the peak in amyloidosis (16-20%; $p<0.001$). Immature forms in the blood reflect the immune reaction to disease and indicate the need of rapid replenishing of the lymphocyte population. High reactivity of lymphocytes manifests at the level of subcellular structures as well. The content of DNA in the nuclei increases, particularly in amyloidosis.

The volume of the nuclei changes: in patients this parameter 1.5-fold surpasses that in donors even during remission, remains elevated during attacks, and sharply increases in amyloidosis (Table 2). Nuclear surface area directly correlates with the nuclear volume. Changes in the parameters of the nuclei are reflected in the nucleolar activity. Normally not all nuclei have nucleoli: less than one nucleolus per nucleus on average. In patients, all nuclei have nucleoli, more than one even in remission, the volume and surface area being greater than in donors, particularly in amyloidosis.

The distribution of the nuclei by the number of nucleoli also varied. During remission 80-90% cells had 1-2 nucleoli ($p<0.0001$), 10-14% cells had 3 nucleoli ($p<0.0001$), and some patients had nuclei with 4 and more nucleoli ($p<0.001$). The number of mono- and binucleated nuclei sharply decreased in amyloidosis, while the number of cells with nuclei containing 3-6 and even 8 nucleoli increased (Fig. 1).

Lymphoblasts were detected in the blood of patients with acute leukemia (20%; $p<0.0001$), similarly as in PD; the greater part of the population were prolymphocytes (48%; $p<0.0001$) and lymphocytes (32%; $p<0.0001$). The percent of lymphoblasts in the bone marrow reached 42-50% ($p<0.0001$) of the population, of prolymphocytes 28-42% ($p<0.0001$), and of lymphocytes 16-22% ($p<0.0001$). During remission, the proportion of blood cells changed towards lymphocytes (46%; $p<0.0001$), the percent of prolymphocytes remained unchanged (50%; $p<0.0001$), while the percent of lymphoblasts decreased to 4% ($p<0.001$). The content of prolymphocytes and lymphoblasts in the bone marrow decreased to 22 and 34%, respectively ($p<0.0001$), while the content of lymphocytes reached 44% ($p<0.0001$), leveling with their blood content. The appearance of immature forms in the blood in leukemia can be explained by the need (similarly as in PD) of rapid recovery of blood cell count. Comparison of lymphoid populations in PD and leukemia showed similar features in their behavior, though significant differences were also detected. The nu-

TABLE 1. Multiparameter Characteristics of Peripheral Blood Lymphocyte Nuclei in Donors ($\bar{x}\pm S_{\bar{x}}$)

Parameter	DNA weight, arb. units	Optical density of DNA-fuchsin, D	Arithmetic mean deviation of particles	Coefficient of relief	Texture coefficient	Coefficient of mutual disposition of particles	Sphericity
Men	55.3±0.8*	0.27±0.01	0.07±0.01	4.7±0.1	0.930±0.003	1.29±0.01	1.29±0.01
Women	61.2±0.6	0.23±0.01	0.07±0.01	4.0±0.1	0.90±0.01	1.16±0.01	1.29±0.01

Note. Here and in Tables 2, 3: weighted means are presented.

TABLE 2. Characteristics of Nuclei and Nucleoli in Peripheral Blood Lymphocytes of Donors and Patients with Different Stages of PD ($\bar{x}\pm S_{\bar{x}}$)

Group	Nuclei			Nucleoli (summary values)		
	DNA weight, arb. units	volume, μ^3	surface area, μ^2	volume, μ^3	surface area, μ^2	mean number per nucleus
Donors	56.9±0.5	34.5±0.2	51.2±0.3	0.47±0.08	2.92±0.03	0.74±0.03
Patients with PD						
in remission	54.6±0.5	49.5±0.4	65.2±0.2	0.55±0.02	3.25±0.02	1.44±0.02
during attack	59.7±0.3	58.9±0.5	73.2±0.3	0.64±0.01	3.59±0.01	1.53±0.01
in amyloidosis	74.5±0.4	149.5±2.0	136.2±0.8	1.03±0.02	4.93±0.03	4.23±0.10

clear volume and surface area are significantly greater in acute leukemia than in PD (Tables 2, 3), and, as these parameters characterize the possibility of metabolite and information exchange between the nucleus and cytoplasm, presumably, activity of this exchange is several-fold higher in acute leukemia than in PD. In leukemia, the mean number of nucleoli per nucleus is less than 1 (Table 3), which might indicate their lower activity in comparison with that in PD, but the nucleolar (and nuclear) volume and surface area are significantly greater than in PD. Considering these facts, the data on the time course of DNA content in the lymphoid cell nuclei in PD and leukemia are particularly interesting. Hyperreplication (extra synthesis) of DNA, realized by different ways, was observed in cell nuclei in various diseases [3]. Aliquant 2c increase of DNA volume in the nuclei was detected in PD during attacks and in amyloidosis (Table 2). Parallel increase in the number of nucleoli suggests that this can be a manifestation of MEFV gene locus amplification; this gene is responsible for the synthesis

TABLE 3. Surface Characteristics of Peripheral Blood and Bone Marrow Lymphocyte Nuclei and Nucleoli in Patients with Acute Leukemia ($\bar{x}\pm S_{\bar{x}}$)

Parameter	Nuclei			Nucleoli			
	DNA weight, arb. units	volume, μ^3	surface area, μ^2	DNA volume (PNC), arb. units	volume, μ^3	surface area, μ^2	mean number per nucleus
Peripheral blood							
lymphoblasts	77.1±6.9	238.5±2.7	186.0±0.6	4.8±0.8	4.3±0.6	12.8±0.6	1.3
prolymphocytes	57.5±2.6	138.1±0.9	127.2±0.4	3.2±0.2	2.1±0.2	8.0±0.3	0.9
lymphocytes	52.1±1.4	90.7±0.9	97.6±0.4	—	—	—	—
Bone marrow							
lymphoblasts	122.4±5.7	182.1±2.9	186.4±0.6	8.0±0.7	4.9±0.3	13.8±0.4	1.6
prolymphocytes	82.4±2.3	110.3±1.8	111.0±0.4	4.2±0.9	1.8±0.3	7.3±0.4	0.8
lymphocytes	65.3±1.9	58.5±1.2	72.8±0.4	—	—	—	—
Remission. Peripheral blood							
lymphoblasts	59.5±0.9	183.2±5.7	156.0±3.2	3.5±1.1	3.5±1.0	3.5±0.2	1.0
prolymphocytes	55.2±0.9	113.4±0.9	112.6±0.3	3.3±0.5	1.7±0.3	6.8±0.2	0.4
lymphocytes	47.7±0.7	49.1±0.6	64.8±0.3	—	—	—	—
Remission. Bone marrow							
lymphoblasts	107.9±9.0	243.4±2.8	188.2±0.7	7.3±1.0	5.0±0.4	12.8±0.5	1.5
prolymphocytes	77.1±6.3	110.0±1.5	111.8±0.6	4.3±0.4	1.7±0.2	6.8±0.2	0.7
lymphocytes	55.9±2.7	41.4±0.6	57.8±0.3	—	—	—	—

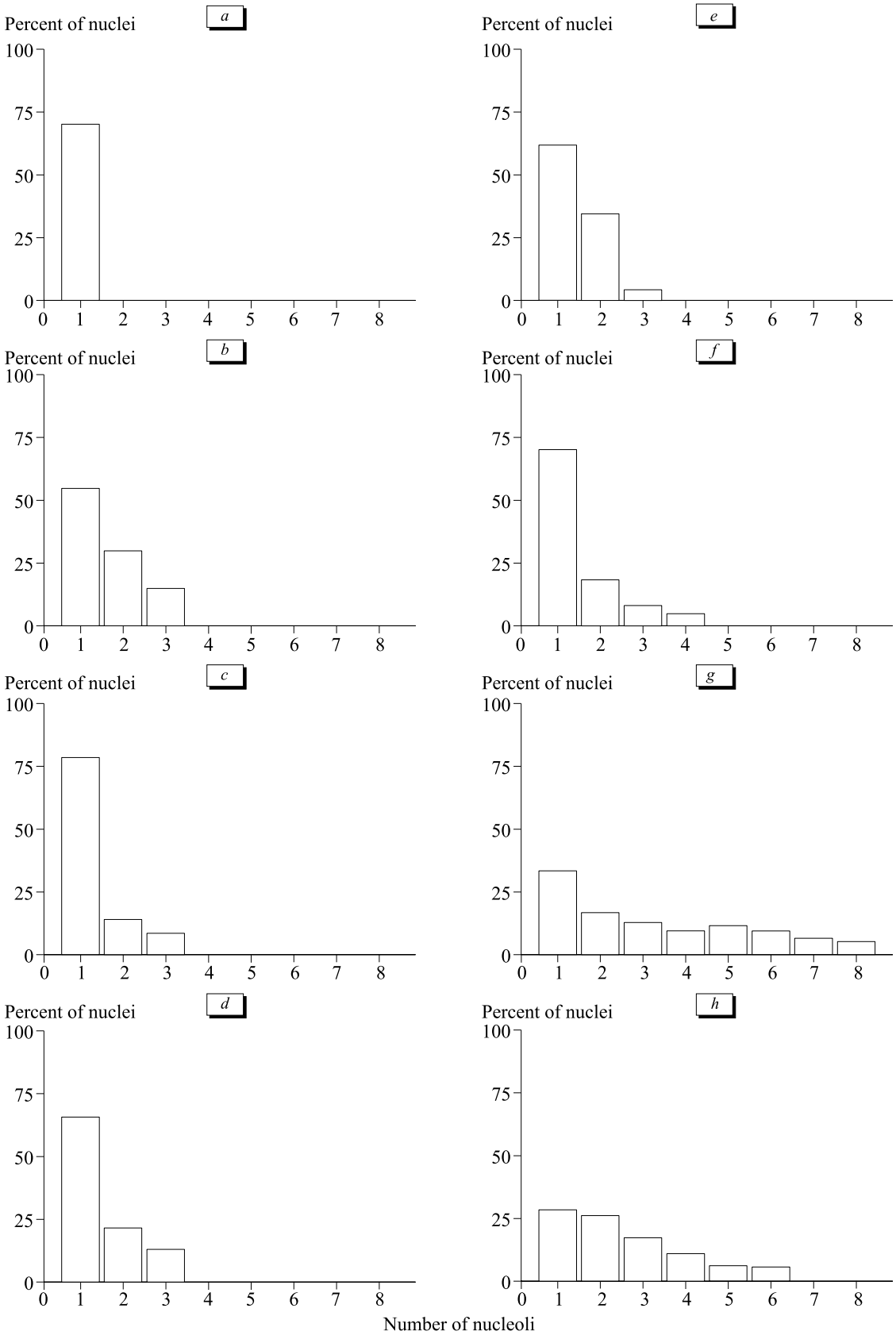

Fig. 1. Distribution of nuclei by the number of nucleoli: in donors (*a*), patients in remission (*b*), on days 1 (*c*), 2 (*d*), 3 (*e*), and 4 (*f*) of attack, in amyloidosis (*g*), and in a patient with undetected amyloidosis (*h*).

of specific protein pyrine (marenostrine), the expression of which determines transition to amyloidosis or results from disease transformation into amyloidosis [7]. Another mechanism of hyperreplication is realized in acute leukemia. The DNA weight in bone marrow lymphoblasts is 2-fold greater than in lymphocyte nuclei (Table 3). This means that lymphoblasts, after accumulating 4c DNA in the cell cycle S phase, are provisionally blocked in the G_2 phase, after which they divide (the DNA weight in their nuclei drops) and transform into mature cells (DNA weight in lymphocytes is almost equal to that in donor nuclei — 2c). We discovered this mechanism of rapid replenishing of blood cells (erythrocytes in acute anemia) rather long ago and called it reserve erythropoiesis [3]. By analogy, we called the above-described process "reserve" lymphopoiesis.

Hence, comparative analysis revealed similar features in the behavior of lymphoid cells, their nuclei and nucleoli in PD and leukemia. This indicates nonspecificity of the immune system reaction to diseases, but the intensity of the reaction and mechanisms of lymphoid population regeneration are different. Hyperreplication of DNA in different manifestations plays an important role in this process. Our data on DNA synthesis in lymphoid cell nuclei in PD and leukemia and on proliferation of these cells suggest that under extreme conditions (for example, in disease) lymphocyte can be activated and produce precursor cells.

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