NUCLEOLAR APPARATUS OF AKR MOUSE LYMPHOID CELLS AT DIFFERENT STAGES OF LEUKEMIA DEVELOPMENT

N. V. Levitan, M. I. Loseva,
G. N. Shorina, and G. S. Yakobson

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Difficulties arising in the detection of the early signs of leukemia in man in the absence of any clinical manifestations justify the use of spontaneous and induced leukemias of animals as an experimental model [7, 8]. The high frequency of discovery of clinical features of leukemia and the homogeneity of the hematologic features characterize mice of the AKR line as a convenient object with which to study the time course of development of the lymphoproliferative process [1].

The nucleolar apparatus is a morphologically labile structure which undergoes considerable changes during malignant transformation of tissue [5, 10]. Meanwhile, the character of reorganization of the nucleolar apparatus in different stages of leukemia development has not been adequately studied.

The aim of this investigation was to study the morphological features of the nucleolar apparatus of lymphoid cells of AKR mice in periods preceding the development of the disease and in the stage of complete clinical manifestation of leukemia.

EXPERIMENTAL METHOD

Experiments were carried out on AKR mice aged 2 months (10), 6 months (12), and 12 months (12). Considering data [7] indicating a more uniform manifestation of the clinical features of leukemia in AKR females, only AKR mice of that sex were chosen for the experiments. The control group consisted of noninbred female mice aged 2, 6, and 12 months, 10 animals of each age. The level of functional activity of the nucleolar apparatus of the lymphoid cells of the hematopoietic organs of the mice was assessed on the basis of the following morphological criteria: 1) the classical "Smetana" nucleolar test [10], namely the nucleolar coefficient, and ratio between different morphological nucleolar types; 2) impregnation of the nucleoli with silver nitrate and counting the number of Ag/+/ zones in the nucleus [10]; 3) new nucleolar tests used for the first time to assess the level of functional activity of the nucleolar apparatus of lymphoid cells of AKR mice: the PNZ (perinucleolar zone of the nucleus) test, the LCPNZ (lipid component of the boundary of the perinucleolar zone of the nucleus) test, the NLC (nucleolar lipid component) test. The PNZ test takes account of the relative percentage of nucleoli possessing structural elements of the perinucleolar zone (Fig. 3), the LCPNZ test records those nucleoli the boundary of whose perinucleolar zone countains a lipid component, detectable on staining with fluorescent dyes: benzo(a)pyrene 3-methoxybenzanthrone, and rhodamine B. On staining with rhodamine B, a light-optical version of visualization of the lipid component also was used. The NLC test revealed nucleoli containing a lipid component actually in the nucleolus and on the boundary of the perinucleolar zone [2, 3]. To study proliferative activity we used the method in [4], according to which the relative numbers of cells in stages G_0, G_1, S, and G_2/M of the cell cycle was determined on the basis of the spontaneous optical density and the induced optical density of nuclei stained by the Feulgen method. For the cytophotometric analysis we used the LYUMAM PM-11 instrument, coupled with a desk-top Élektronika 60 M computer.

EXPERIMENTAL RESULTS

Clinical features of leukemia (a tumor of the thymus, enlargement of the spleen, the liver, and all groups of peripheral lymph nodes) were found in all the AKR mice aged 12

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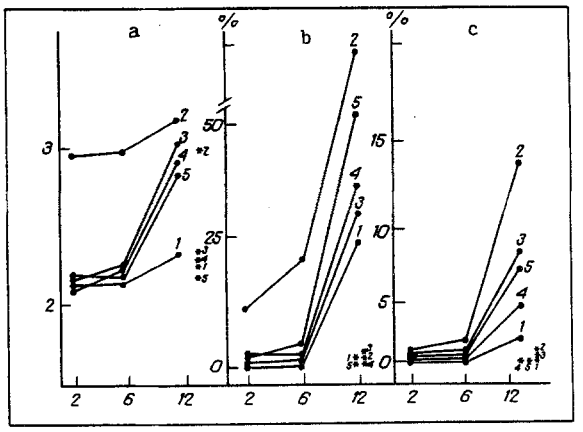

Fig. 1. Dynamics of nucleolar coefficient (a), and relative percentages of homogeneous nucleoli (b) and proliferative activity (c) in lymphoid cells from peripheral blood (1), thymus (2), bone marrow (3), cervical lymph nodes (4), and spleen (5) in AKR mice aged 2, 6, and 12 months. Circles denote AKR mice; asterisks control animals. Since in the control animals values for mice aged 2, 6, and 12 months did not differ statistically significantly ($p > 0.05$), averaged data are given on the graph.

months. On microscopic study of the bone marrow total infiltration by lymphoblasts was found in all animals aged 12 months. The mean peripheral blood leukocyte count was 106.3 ± 9.3^9 per liter [sic], including up to 95% of balst cells (on average 92.5 ± 3.7%). Thus on the basis of all the clinical and hematological manifestations, lymphatic leukemia in a generalized stage could be diagnosed in all AKR mice aged 12 months. According to data in the literature [1, 6-8] the state of the AKR mice at the age of 6 months can be characterized as preleukemic. Accordingly, all 12 mice of this age, even though no clinical manifestations of leukemia were found in nine of them, were combined into a single experimental group. In the 10 AKR mice aged 2 months no clinical or hematological manifestations of lymphatic leukemia were observed and these animals formed a group used to search for early preclinical signs of leukemia.

The dynamics of changes in the nucleolar coefficient for lymphoid cells of hematopoietic organs of AKR mice of the three age groups is shown in Fig. 1a and the relative content of different morphological types of nucleoli in Fig. 1b. The experiments showed the lymphoid cells of hematopoietic organs in the dynamics of development of lymphatic leukemia to be characterized by a progressive increase in the fraction of homogeneous nucleoli at the expense of inactive morphological types (circular and micronucleoli), evidence of an increase in functional activity of the nucleolar apparatus of these cells in the late stages of the disease.

The number of Ag/+/ zones in the nucleoli of the lymphoid cells of leukemic mice aged 12 months was 2-2.5 times greater than the value of this index in healthy AKR mice aged 2 months and the control animals, and when calculated per nucleus, for peripheral blood as a whole it was 2.5 ± 0.3, for the thymus 4.1 ± 0.2, for cervical lymph nodes 3.0 ± 0.2, bone marrow 2.9 ± 0.2, and spleen 2.8 ± 0.1.

To determine the prognostic value of the above-mentioned nucleolar markers, a comparative analysis was made of the data for animals aged 2 and 6 months. A significant increase in activity of the nucleolar apparatus was recorded only for lymphoid cells in the thymus of preleukemic mice aged 6 months, and which was expressed as an increase in the relative percentage of homogeneous nucleoli (from 13.2 ± 1.4 to 20.1 ± 1.7%) and in the number of Ag/+/ zones (from 2.5 ± 0.1 to 3.3 ± 0.2). These data are evidence that tests such as the relative percentage of homogeneous nucleoli and the number of Ag/+/ zones, used to determine levels of activity of the nucleolar apparatus of the lymphoid organs of an organ which is the substrate of the tumor process (in this case the thymus [6, 7]), can be characterized as prognostic.

Besides morphological changes in the nucleolus in the course of development of leukemia in the AKR mice there was substantial reorganization of the perinucleolar space in the lymphoid cells. A study of the process of leukemia development showed that a characteristic feature of malignantly transformed cells is the formation of a typical perinucleolar zone, containing radial cords, diverging from the body of the nucleolus, and boundary structures (Fig. 2). In the peripheral blood of AKR mice aged 12 months the fraction of these cells was 44 ± 2.3%, in the thymus it was 54.2 ± 3.1%, in the bone marrow 21.3 ± 1.4%, in the cervical lymph nodes 25.9 ± 1.3%, and in the spleen 26.8 ± 2.2% (Fig. 2). Values of the LCGPNZ test were close to those of the PNZ test shown above (Fig. 3b).

Particular attention was directed to the possibility of using the PNZ and LCGPNZ tests as diagnostic and prognostic criteria for recording early stages of the lymphoproliferative process. Thus high values of the PNZ and LCGPNZ tests were observed in the lymphoid cells

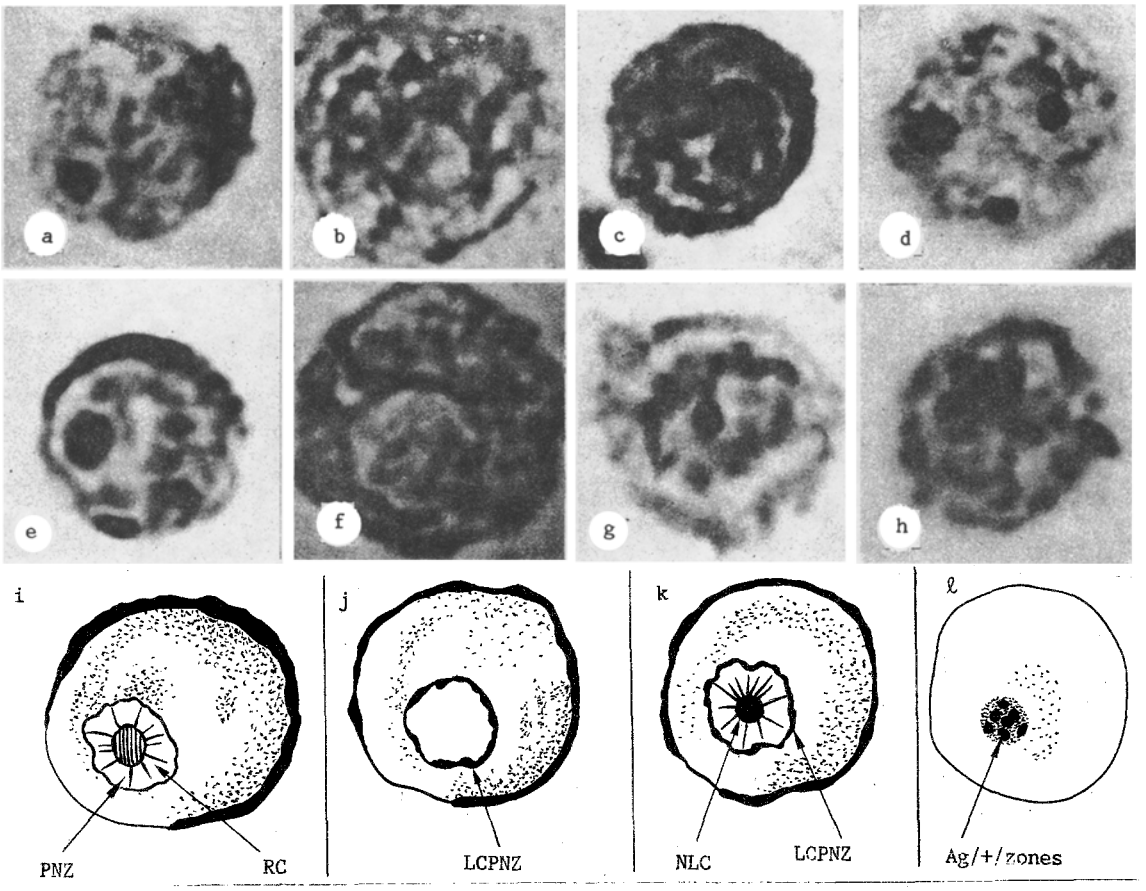

Fig. 2. Morphological types of nucleoli of leukemic cells from peripheral blood (a, b, c, d) and thymus (e, f, g, h) of AKR mice aged 12 months. Stained with toluidine blue (a, e, i) and rhodamine B (b, c, f, g, j, k). Impregnated with silver nitrate (d,h,ℓ), explanatory scheme (i-ℓ). Magnification 100. PNZ) perinucleolar zone of nucleus; RC) radial cords; LCPNZ) lipid component on boundary of perinucleolar zone of nucleus; NLC) nucleolar lipid component. Ag/+/ Argentophilic zones of nucleolus.

of the thymus (43.6 ± 3.2 and 45.2 ± 2.3%, respectively), and also the peripheral blood (16.7 ± 1.1 and 19.1 ± 1.3%, respectively) in preleukemic AKR mice aged 6 months. Moreover, high values of the PNZ and LCGPNZ tests for lymphoid cells of the thymus were observed in healthy AKR mice aged 2 months (31.4 ± 1.6 and 32.7 ± 2.1%). The results of this investigation of the perinucleolar zone show that structural reorganization of the perinucleolar zone of lymphoid cells precedes changes in morphology of the nucleolus itself; these changes, moreover, affect all hematopoietic organs studied. Whereas the "Smetana" nucleolar tests and impregnation with silver nitrate can be used for the early diagnosis, by testing only thymus cells, the PNZ and LCPNZ tests record a statistically significant rise of the parameters in the peripheral blood and hematopoietic organs.

Figure 3 shows the course of accumulation of the nucleolar lipid component in the nucleoli of lymphoid cells of hematopoietic organis of AKR mice aged 2-12 months. It was shown that lymphoid cells of mice aged 12 months, in the peripheral blood (21.6 ± 1.4), the thymus (43.5 ± 2.3%), the cervical lymph nodes (27.9 ± 2.0), the bone marrow (23.7 ± 1.1%), and spleen (25.8 ± 1.2%) are characterized by the presence of a nucleolar lipid component; values of the NLC-test, moreover, differ significantly from the control. Consequently, accumulation of the nucleolar lipid component in lymphoid cells of the peripheral blood, thymus, lymph nodes, bone marrow, and spleen accompanies the development of leukemia, whereas the progressive rise in the parameters of the NLC test reflects processes taking place in lymphocytes during malignant transformation. A high (28,7 ± 1.3%) content of NLC-positive nucleoli was observed in the lymphoid cells of the thymus in preleukemic animals aged 6 months. These findings, when compared with the results of the "Smetana" nucleolar test, the number of Ag/+/ zones, and also with data on proliferative activity of the lymphoid cells (Fig. 1), are evidence of the leading role of the thymus in the development of spontaneous lymphatic leukemia in AKR mice, which is confirmed by the results of cytogenetic [6] and biochemical [8, 11] investigations.

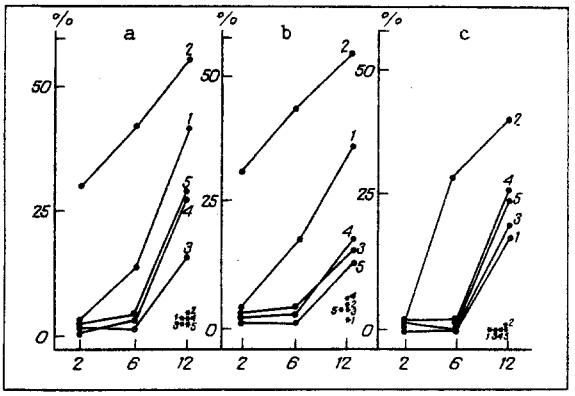

Fig. 3. New nucleolar markers of PNZ test (a), LCGPNZ test (b), and NLC test (c) during spontaneous lymphatic leukemia development in AKR mice aged 2-12 months. Legend as to Fig. 1.

Accumulation of the nucleolar lipid component in lymphoid cells in vitro was demonstrated by the writers previously during culture of phytohemagglutinin-stimulated human lymphocytes [3]; this process, moreover, also was closely interconnected with the increase in proliferative activity of the cells. The further study of this phenomenon is of definite interest for our understanding of the pathogenetic mechanisms of development of the lymphoproliferative process.

The investigations described above thus demonstrated the diagnostic and prognostic value of the new nucleolar markers – the PNZ, LCPNZ, and NLC tests, and the indications for their use in conjunction with existing methods of studying the nucleolar apparatus.

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