DETECTION OF HETEROGENEITY OF LYMPHOCYTE POPULATIONS BY MEASURING THE DIAMETER OF CELLS AND NUCLEI

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Certain parameters of distribution of lymphocytes and their nuclei by size in peripheral blood, bone marrow, thymus, and spleen of healthy rats were studied. Comparative analysis revealed homogeneity of the population for thymus and bone marrow lymphocytes, but a difference between them in their mean diameter. Mixing these types of cells appreciably altered the parameters of distribution of the resulting population, due to migration of lymphocytes of thymus origin, as shown by a model of the lymphoid reaction in the bone marrow to 5-fluorouracil. Preliminary thymectomy abolished migration, so that the homogeneity of the bone marrow lymphocytes for size was preserved.

KEY WORDS: lymphoid reaction; distribution by diameter; 5-fluorouracil.

In radiation sickness, after administration of antitumor agents, and during pathological processes in the bone marrow, lymphoid reactions due in some cases to redistribution and migration of lymphocytes from the lymphoid organs or to stimulation of proliferation of bone marrow lymphocytes can develop [1-6]. Morphological analysis does not enable lymphocytes from the thymus and bone marrow to be distinguished, and the use of modern functional methods is not always possible in clinical and laboratory practice, especially when retrospective analysis of bone marrow films is required.

EXPERIMENTAL METHOD

To detect thymocytes in a cell population giving the lymphoid reaction in bone marrow, in cases when the percentage of thymocytes was below 30, we have used a method of estimating the distribution of the cells and their nuclei by diameter, by calculating the coefficients of asymmetry (A_S) and excess (E_X);

$$A_s = \frac{\Sigma p a^3}{n\sigma^3}, \quad E_x = \frac{\Sigma p a^4}{n\sigma^4},$$

where p is the frequency of the variants; a the difference between the variant and mathematical expectancy; n the number of variants; σ the standard deviation.

Asymmetry is considered not to be significant if $A_S < 0.2$; if $A_S \geq 0.5$, the skewness of the distribution is strong. A positive value of the coefficient indicates right-sided asymmetry, a negative value left-sided. If the group studied is homogeneous and variants are concentrated in the central classes of the variation series, the value of the excess increases and the distribution is described as pointed. If $E_X \geq 0.5$, the excess is considered to be appreciable. Small negative excess indicates flattening of the peak, and if $E_X < -1$, the distribution is bimodal [3].

The diameters of the lymphocytes and their nuclei in films of peripheral blood and bone marrow and in squash preparations of the thymus and spleen of healthy rats were measured by means of an AM-9-2 ocular micrometer, by means of which measurements can be made with an accuracy of not more than 0.1 μm. The diameters of 2000 cells and nuclei in each tissue were measured. The mathematical analysis was carried out by the Nairi-K computer. The results are given in Table 1.

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TABLE 1. Mean Diameter and Parameters of Distribution of Lymphocytes and Their Nuclei by Diameter in Healthy Rats

Test object	Mean diameter, μm	Coefficient of asymmetry	Excess of distribution
Peripheral blood			
cell	8,94±0,11	0,263	—0,298
nucleus	8,18±0,11	0,235	—0,223
Bone marrow:			
cell	8,60±0,13	0,492	0,344
nucleus	7,92±0,10	0,397	0,364
Thymus			
cell	8,09±0,07	0,780	0,617
nucleus	7,58±0,09	0,581	0,387
Spleen			
cell	8,88±0,10	0,203	—0,370
nucleus	8,35±0,09	0,094	—0,480

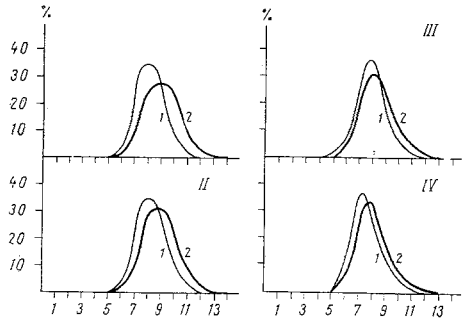

Fig. 1. Distribution of lymphocytes (2) and their nuclei (1) by diameter. I) Peripheral blood; II) spleen; III) bone marrow; IV) thymus.

TABLE 2. Mean Diameter and Parameters of Distribution of Lymphocytes and Their Nuclei by Diameter in Intact and Thymectomized Rats on 4th Day after Administration of 5-Fluorouracil (150 mg/kg)

Test object	Mean diameter, μm	Coefficient of asymmetry	Excess of distribution
Bone marrow of intact rats:			
cell	7,77±0,23	0,200	0,063
nucleus	7,37±0,14	—0,285	—0,415
Bone marrow of thymectomized rats			
cell	8,56±0,25	0,278	0,447
nucleus	7,98±0,27	—0,239	0,071

EXPERIMENTAL RESULTS

As Table 1 shows, peripheral blood lymphocytes formed a comparatively heterogeneous population (E_x = −0.298). The distribution of spleen cells by diameter was characterized by appreciable flattening of the peak (E_x = −0.370), in agreement with the view that the spleen is an organ in which lymphocytes of different origin can be found.

The parameters of distribution of the thymus cells indicate that the population was homogeneous for size (E_x = 0.617). The slight degree of asymmetry can be explained by the presence of lymphoblasts (2.4%) and medium-sized lymphocytes (7.2%). A similar distribution was obtained previously [8] as a result of measurement of the diameters of lymphocytes from sections through the thymus. Bone marrow lymphocytes also formed a population comparatively homogeneous for size (E_x = 0.344), differing from the thymocyte population only in the mean diameter of its cells and nuclei (Fig. 1).

These properties of the thymus and bone marrow cell populations suggested that if the cells are mixed the parameters of distribution of the resulting population would be different from the initial values and would indicate heterogeneity. To test this hypothesis, the lymphoid response of the bone marrow developing after depopulation under the influence of a large dose (150 mg/kg) of 5-fluorouracil was studied in 30 rats. During the period of maximal development of this response (the 4th day) the lymphocyte count was twice the normal level. After thymectomy (13 rats) this response was not observed and the number of lymphocytes in the bone marrow remained at the normal level. Spleenectomy had little effect on development of the lymphoid response. Removal of the lymphoid organs and mock operations by themselves did not change the number of lymphocytes in the bone marrow. Consequently, this lymphoid response was determined by migrating lymphocytes, chiefly from the thymus [4]. This is a convenient model with which to study lymphocyte migration, for 5-fluorouracil destroys only dividing lymphocytes and causes little damage to mature lymphocytes [7].

Measurement of the diameters of the lymphocytes and their nuclei in the bone marrow on the 4th day after administration of 5-fluorouracil, and also in the bone marrow of thymectomized rats at the same period after administration of the compound showed (Table 2) that the mean diameter of the lymphocytes ($7.77 \pm 0.23 \mu m$) in the bone marrow after the action of 5-fluorouracil was smaller than normally ($8.60 \pm 0.13 \mu m$) However, this was not the result of pycnosis of the cells under the influence of 5-fluorouracil, for the mean diameter of the bone marrow lymphocytes of the thymectomized rats after administration of the compound was unchanged ($8.56 \pm 0.25 \mu m$). The distribution of the cells and nuclei by diameters in the bone marrow of the thymectomized rats was the same as in intact animals. The presence of a lymphoid response in the intact rats changed the character of the distribution: considerable flattening of the curve was observed for the nuclei of the bone marrow lymphocytes ($E_x = -0.415$). This change in the distribution points to the appearance of a large group of cells in the bone marrow with a smaller diameter than that of the bone marrow lymphocytes, and the change in the character of asymmetry can be explained by disappearance of the large and medium-sized lymphocytes under the influence of the cytostatic.

The suggested method of comparing the parameters of distribution of lymphocytes under normal conditions and during development of the lymphoid response thus revealed the presence of thymocytes among lymphocytes in the bone marrow, without the need to resort to more complex laboratory procedures.

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