A STUDY OF SOME BIOCHEMICAL AND BIOPHYSICAL CHARACTERISTICS OF A SUBPOPULATION OF RAT THYMOCYTES ISOLATED IN A FICOLL GRADIENT

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The lymphocytes of the thymus are highly heterogeneous with respect to many parameters and they constitute different stages of maturation of the precursors of T-lymphocytes [1]. Accordingly, when the biological properties of thymocytes under normal conditions and also their changes under the influence of ionizing radiation are studied, one task which arises is how to isolate more or less homogeneous subpopulations from the total mass of cell forms. To solve this problem the differentiation of fractions of thymocytes on the basis of buoyant density (the method of isodensity sedimentation [7]), on cell size (isovelocity sedimentation [9]), and the surface charge (electrophoresis [3]), and so on, have been used.

The object of this investigation was to isolate subpopulations of rat thymocytes by sedimenting the cells in a stepwise Ficoll gradient and to study some biochemical and biophysical characteristics of the resulting fractions.

EXPERIMENTAL METHOD

A suspension of thymocytes in Ringer's solution, pH 7.3, was obtained from noninbred male albino rats weighing 100-110 g. To form a gradient, layers of Ficoll-400 (from Pharmacia, Sweden) in physiological saline with densities of 1.055, 1.065, 1.071, 1.077, and 1.095 g/ml^3 were prepared. The density of the layers was measured by means of an areometer at 20°C and verified by the relative refractive index. The gradient was prepared in a 25-ml centrifuge tube by successive layering of 2-ml portions of solutions in order of diminishing density. The suspension of thymocytes was suspended in the last layer of the gradient (1.055 g/ml^3, 2×10^8 cells). Sedimentation was carried out at 300g and at 20°C for 10-40 min. Five fractions corresponding to different densities of Ficoll (from A to E) were then withdrawn by means of a syringe. Fraction F consisted of cells in the residue. The suspensions thus obtained were washed twice in Ringer's solution, pH 7.3, at 300g for 10 min. (The washing was repeated 5 times when the protein concentration was determined.)

The protein concentration in the cells was determined by Lowry's method [11]. The intensity of intrinsic UV fluorescence (UVF) of the thymocytes was measured on an apparatus based on a UV-fluorescence microscope [4]. The relative mobility of the nucleoid was detected by the method in [8] using the modification in [2]. The cell diameter was measured with an ocular micrometer. The number of cells was determined in a Goryaev's chamber and the viability of the thymocytes was assessed by staining with trypan blue.

Cells of the various fractions were cultured for 3 h in medium No. 199 with 10% rat serum at 37°C. The cell suspensions were irradiated with γ rays in a dose of 5 gy on the Luch-1 apparatus, with a dose rate of 0.73 gy/min, at 20°C. Additional sedimentation of the thymocytes was carried out in Ficoll–Pak medium (from Pharmacia, Sweden) with a density of 1.077 g/ml^3 at 300g and at 20°C for 25 min. The results were subjected to statistical analysis by Student's and Wilcoxon's tests.

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TABLE 1. Characteristics of Thymocyte Fractions (M ± m)

Fraction	Percent of viable cells	Percent of cells in fractions	Radius of cell, μ	UVF intensity, conventionals units	Protein in content, pg
	(n=4)	(n=14)	(n=5)	(n=5)	(n=5)
A	89±2	8±2	2,66±0,02	12,2±1,0	52±4
B	84±1	12±1	2,70±0,03	12,4±0,6	42±3
C	83±5	16±1	2,75±0,03	14,7±1,3	40±3
D	80±1	26±3	2,86±0,02	18,7±0,3	39±3
E	82±1	25±2	3,04±0,04	22,3±0,9	40±3
F	85±1	13±2	3,04±0.04	20,9±1,0	49±4

Legend. n) Number of experiments.

EXPERIMENTAL RESULTS

The study of dependence of the thymocyte distribution by fractions on the sedimentation time showed that during sedimentation for 40 min the cells continued to migrate toward the denser layers of the gradient. During sedimentation for 10 min the maximum of the distribution corresponded to fraction C, for 25 min to fraction D, and for 40 min to fraction F. Staining with trypan blue showed that in the last case about 50% of the cells were damaged. During sedimentation for 25 min, however, the fraction of nonviable cells was small, only 10-20% (Table 1). Since these conditions were not sustained, the corresponding distribution of the cells by fractions was evidently not isodensity, but isovelocity. In the latter case the cells as a rule are distributed by size: The cells of larger size, possessing higher sedimentation rates, are found in the lower fractions [7].

Values of the mean radius of the cells in the various fractions (Table 1) and the distribution of cells by size or the different fractions (Fig. 1) actually reveal a steady increase in the radius of the thymocytes from the upper fractions to the lower. This confirms the isovelocity character of the gradient. Conversely, with isodensity sedimentation of the thymocytes the cells of the lower layers of the gradient are smaller than those of the upper, less dense fractions [6].

Typical results of one experiment to determine mobility of the nucleoid in the various fractions (relative to mobility of the nucleoid in the total thymocyte population), given in Fig. 2, show that thymocytes of the middle fractions had the greatest nucleoid mobility.

It will be clear from Table 1 that the level of intrinsic UVF of the cells due to luminescence of the aromatic residue of proteins follows a similar course to changes in cell size. Meanwhile the distribution of the cell protein content among the fractions differed in character: The minimal protein content was found in thymocytes of the middle fractions. The reason may be that the UVF signal is formed not by all the cell proteins, but only by proteins of the surface membrane.

To characterize thymocytes in the fractions by their buoyant density, additional sedimentation of the cells in Ficoll–Pak medium (1.077 g/ml^3) was used. In this case no increase in the number of nonviable cells could be detected in the subpopulations. The ratio of the number of cells which passed through the layer with density 1.077 g/ml^3 to that in the interphase was evidently proportional to the density of the thymocyges (Fig. 3). As Fig. 3 shows, thymocytes of the middle fractions had the lowest density (curve 1), a result which correlates with the lowest protein content and the smallest mean size of these cells (Table 1). Incubation of the cells for 3 h led to an increase in density of the thymocytes in all fractions, especially in the middle fractions (Fig. 3, curve 2). This may be connected with cytodifferentiation and "terminalization" of part of the population [13]. The even more marked increase in density of the thymocytes after irradiation (Fig. 3, curve 3) confirms this hypothesis. An increase in the density of thymocytes dying after irradiation has been reported in the literature [14].

Isovelocity sedimentation of a population of continuously dividing cells can be used to separate them by phases of the cell cycle [10]. Since some thymocytes are permanently in the mitotic cycle, it can be postulated that the same distribution also was obtained in the present investigation. This is confirmed by data on nucleoid mobility. The relative mobil-

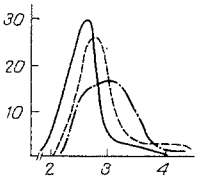

Fig. 1

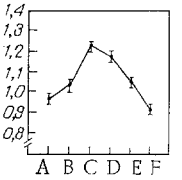

Fig. 2

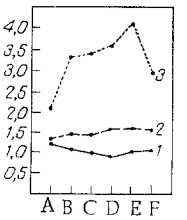

Fig. 3

Fig. 1. Distributions of thymocytes by size for fractions B, D, and F. Abscissa, radius of cell (in μ); ordinate, fractions of cells of the given size (in % of total number).

Fig. 2. Mobility of nucleoid of thymocytes in different fractions. Abscissa, designation of fractions, A) top fraction; ordinate, mobility of nucleoid relative to mobility of nucleoid of total thymocyte population (in relative unts).

Fig. 3. Ratio of number of thymocytes passing through density layer 1.077 g/m^3 to that in interphase, proportional to density of cells for thymocytes of different fractions. Abscissa, designation of fractions, A) top fraction; ordinate, ratio of number of thymocytes passing through layer with density 1.077 g/ml^3 to that in interphase. 1) Normal, 2) incubation of thymocytes for 3 h, 3) irradiaiton of thymocytes.

ity of the nucleoid is known [12] to increase in the G_1 and S phases of the mitotic cycle, in connection with changes in the number of superterms of the superhelical structure of the nucleoid. The greater mobility of the nucleoid, and the smaller size and lower protein content of the thymocytes of the middle fractions compared with those of the lower fractions can be explained by enrichment of the middle fraction- by thymocytes in the G_1 + S phases and of the lower fraction by those in the G_2 phase. However, the protein-rich, small thymocytes of the top fraction are difficult to classify on the basis of phase of the cycle. Thymocytes of different fractions also differ somewhat in density (Fig. 3), whereas it has been shown that the density of the cells remains unchanged during passage through the mitotic cycle [5].

It can be tentatively suggested that as a result of enrichment with thymocytes in different phases of cell cycle, the resulting fractions contain thymocyte subpopulations at different stages of differentation.

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