POSSIBLE EFFECT OF SOME DISPERSING AGENTS (TRYPSIN, EDTA) ON THE CELL COMPOSITION OF ZAJDELA'S HEPATOMA

E. S. Valkina and N. S. Kiseleva*

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Prolonged (for 10 to 16 generations respectively) transplantation of Zajdela's ascites hepatoma with metastatic and ascites cells, coupled with repeated application of cell dispersing agents (trypsin and EDTA), led to the selective accumulation in the composition of both variants of the strain ("metastatic" and "ascites") compared with cells characterized by a nucleus of smaller diameter than that of the ascites cells of the original strain. The distribution of the cell nuclei of the ascites cells of both variants of the strain by diameter was the same as in the cell population of a metastasis of the original strain in the paratracheal lymph gland. The study of the distribution of the cells of these populations among complexes of both variants of the strain, produced with the aid of treatment by dispersing agents, revealed a shift toward the numerical predominance of single tumor cells in the population. This shift was absent in the cell population of the metastasis of the original strain in the lymph gland.

KEY WORDS: Zajdela's hepatoma; cellular dispersing agents; diameter of nuclei; cell complexes.

The concept of the heterogeneity of the population structure of most transplantable (especially ascites) tumors, based primarily on the results of cytogenetic investigations, is not disputed at the present time. Very probably the genotypically dissimilar cells of the original tumor also possess dissimilar biological properties (proliferative activity, ability to invade and to metastasize, and so on). In that case it must be possible, in principle, to isolate tumor cells selected for these biological properties. One result of this selection would be the "unification" of the original heterogeneous structure of the tumor cell population with respect to a number of interconnected or apparently independent parameters.

In the investigation described below, an attempt was made to compare the distribution of cells of a transplantable rat ascites hepatoma (Zajdela's hepatoma), the metastases of this tumor in the paratracheal lymph glands, and also "metastatic" (M) and "ascites" (A) variants of this tumor [2] with respect to the diameter of the cell nuclei and the number of tumor cells in the cell complexes (islets) of different sizes.

It might be supposed that prolonged transplantation of a tumor by metastatic cells would lead to changes in the population characteristics of the M-variant of the original strain with respect to one or both of the parameters named, whereas transplantation of the strain by ascites cells, not susceptible to the action of dispersing agents (original strain) or dispersed by the same method as cells of the metastasis (A-variant), would not bring about similar changes.

*Deceased.

Laboratory for the Study of Metastasization of Tumors, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Blokhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 11, pp. 95-98, November, 1974. Original article submitted March 14, 1974.

TABLE 1. Comparative Characteristics of Different Variants of Cell Populations of Zajdela's Ascites Hepatoma

№	Object	Mean diameter of cell nuclei (in μ)	Fraction of cells with diameter of nuclei less than 80 μ (% of total no.)	Mean number of cells in complex	Fraction of single cells (% of total number)
I	Ascites cells of original strain (rat 1)	116,1±1,86	5,0±2,20	5,1±0,34	5,8±0,74
II	Ascites cells of original strain (rat 2)	110,2±2,85	12,0±3,25	2,4±0,14	24,0±1,35
III	Cells of metastasis (rat 2)	78,2±2,60	68,9±4,49	2,7±0,20	15,5±1,15
IV	Ascites cells of A-variant (rat 3)	79,8±2,72	57,0±4,95	1,4±0,04	61,0±1,54
V	Ascites cells of M-variant (rat 4)	85,4±2,54	52,0±5,00	1,6±0,05	45,7±1,58

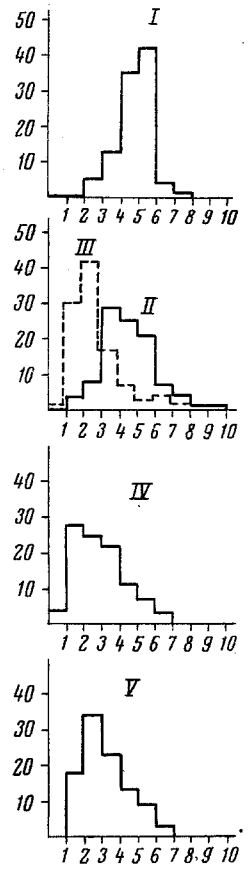

Fig. 1

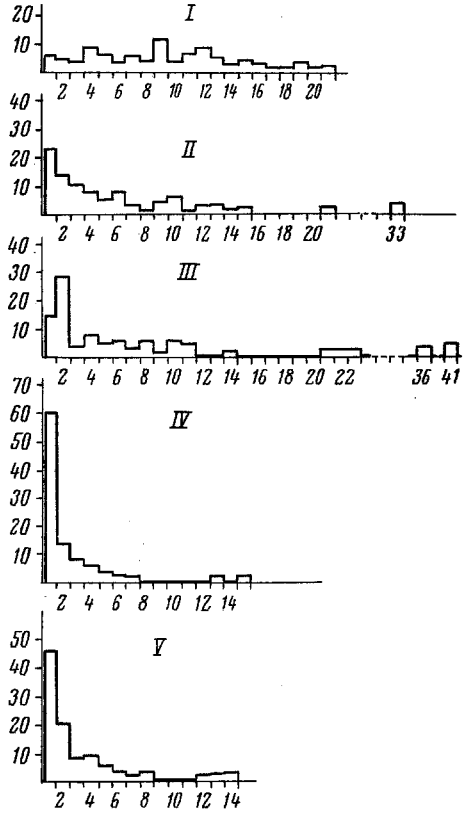

Fig. 2

Fig. 1. Distribution of cells of compared populations by diameter of nuclei. Roman numerals denote test objects in the same order as in Table 1. Abscissa, diameter of cell nuclei in conventional units (interval between classes 19 μ; class 1 includes cells with a nucleus from 38 to 43 μ in diameter; class 10, cells with a nucleus from 197 to 206 μ in diameter); ordinate, percentage of cells in each class.

Fig. 2. Distribution of cells of compared populations by complexes. Abscissa, number of cells in complex; ordinate, percentage of cells in each type of complex. Remainder of legend as in Fig. 1.

EXPERIMENTAL METHOD

The A- and M-variants of the strain were obtained by the method described by Kiseleva et al. [2].* Dispersion of the cell complexes in order to obtain a suspension of single cells for use in the transplantation of both variants was carried out by treatment with a mixture of equal volumes of 0.01% trypsin solution and 0.02% EDTA solution on a magnetic mixer at 37°C for 7 min. The cells of the original strain were not treated in any way, and undiluted ascites fluid was used for the inoculations. The inoculating dose in every case was $1 \cdot 10^6$–$3 \cdot 10^6$ tumor cells.

The diameter of the nuclei of the tumor cells was measured in films of the freshly obtained ascites fluid and in squash preparations from the tissue of the metastatically changed paratracheal lymph glands, stained by Leishman's method, by means of an ocular micrometer (MBI-3 microscope, magnification 90 × 10 × 1.5, oil immersion). The diameter of the nucleus of each cell was measured in two mutually perpendicular directions, the mean diameter of the nucleus was then calculated in conventional units of the ocular micrometer scale, and the values thus obtained were converted into microns with the aid of an objective micrometer gauge.

In each of the objects for study a 100 cell nuclei were measured, excluding complexes containing more than 25 cells from the measurement (because of frequent superpositions of one nucleus over another).

The distribution of the tumor cells among the complexes was calculated in the same films. Altogether 100 cells were counted in each film.

The objects investigated included: 1) ascites cells of two different generations of the original strain; 2) cells of a metastasis in a paratracheal lymph gland, obtained from the same rat as the ascites cells of the second test generation of the original strain; 3) ascites cells of A- and M-variants of the strain (at the 16th and 10th generations respectively). For statistical analysis of the results, Student's criterion and the λ criterion were used (differences between the distributions were assessed by the Kolmogorov–Smirnov method).

EXPERIMENTAL RESULTS

Data reflecting the distribution of the cells of the test objects by the diameter of the cell nuclei and by complexes are given in Table 1 and Figs. 1 and 2.

A simple visual comparison of the histograms in Fig. 1 is sufficient to reveal the similarity between the distribution of the nuclei of the ascites cells of both investigated generations of the original strain on the basis of their diameter (histograms I and II, $\lambda = 1.63$, limiting value of the criterion 1.96). The shift to the left of the distribution of the metastatic cells compared with the ascites cells of the same rat (histograms II and III, $\lambda = 4.09$) and also the considerable similarity of the distributions characterizing the ascites cells of the A- (histogram IV) and the M- (histogram V) variants with each other and with the distribution of the metastatic cells of the original strain will be noted. It can be concluded from the assessment by the λ criterion that distributions III, IV, and V can be interpreted as samples from one general population (λ for these cases does not exceed 0.9–1.2). In other words, cells of a solid metastasis of the original strain, unexposed during transplantations to any special procedures, are distributed by diameter of their nuclei in the same way as the ascites cells of both (A and M) variants of the strain obtained as a result of regular treatment of the tumor cells with dispersing agents—trypsin and EDTA. The extraordinary closeness of the distributions IV and V to each other ($\lambda = 1.0$) suggests that the use of the M-variants of the metastatic cells for inoculations (for the period of 10 consecutive generations) cannot be regarded as the cause of the differences appearing between the original strain (distributions I-II) and the M-variant (distribution V, $\lambda = 4.3$ and 3.2 respectively). It is natural to suggest that the similarity observed above between distributions IV and V (characterizing the A- and M-variants of the strain) was due to the repeated and consecutive application of the cellular dispersing agents during the obtaining of the two variants.

It will be clear from Table 1 that the differences detected between the distributions probably consisted essentially of an increase in the proportion of cells with a nucleus of small (less than 70 μ) diameter among the ascites cells of the A- and M-variants, for this would be reflected in changes in the mean diameter of the nuclei and a shift of the histograms in Fig. 1 to the left. Similar changes characterized the cell population of the metastasis of the original strain.

*Inoculations of both variants and of the original strain were carried out by A. I. Pavlotskii.

In the comparative study of the distribution of the ascites and metastatic cells of Zajdela's hepatoma with respect to the DNA content in the nuclei [1], the cells of the metastasis were characterized by a lower mean level of ploidy and a shift of the histogram to the left, similar to that shown in Fig. 1. These two groups, irrespective of the findings obtained (the smaller mean diameter and the lower content of DNA in the cell nuclei of the metastasis), possibly had a common basis.

Prolonged inoculation (for 16 and 10 generations respectively) of A- and M-variants of the strain, coupled with the repeated application of cellular dispersing agents (trypsin and EDTA), thus led to the selective accumulation of cells characterized by a significantly smaller diameter of their nuclei and the cells of the original strain in the composition of both populations. Presumably the factor causing this unifying selection of "parvonuclear" cells was the repeated use of trypsin and EDTA during the preparation of A- and M-variants of the strain. Cells with "larger" nuclei were more sensitive to the harmful action of the dispersing agents. It is curious that a similar type of selection evidently takes place during the formation of the cell population of the metastasis of the original strain in the paratracheal lymph gland, and, in this case, it was brought about in the course of a very short period equal to the lifespan of the carrier of the original tumor.

The study of the distribution of the cells of the compared populations by complexes (Fig. 2) clearly revealed a shift toward numerical predominance of single cells over cells in the composition of the complexes, characteristic of the A- and M-variants. This shift was slight in degree in the cell population of the metastasis of the original strain.

According to some investigations, single cells of transplantable hepatomas are more resistant to the dispersing action of trypsin than cells forming complexes [4]. If the accumulation of single cells in the A- and M-variants of Zajdela's hepatoma is based on their higher resistance to the destructive action of the dispersing agents, increased mitotic activity of the ascites cells of the A- and M-variants would be expected by comparison with the ascites cells of the original strain, for it has been shown [4] that the single cells in fact constitute the proliferative pool of certain transplantable mouse hepatomas. The extremely high mitotic activity of the fraction of single tumor cells in Zajdela's ascites hepatoma [3] is in full agreement with this conclusion. However, it must be noted that after intraperitoneal inoculation of intact recipients with equal numbers of ascites tumor cells of the A- and M-variants only the A-variant exceeded the original strain in its rate of growth [2].

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