KARYOLOGICAL, MORPHOLOGICAL, AND PHYSIOLOGICAL INVESTIGATION OF THE CELLS OF HUMAN TRANSPLANTABLE LINE I-96 AND CLONE LINES L-98 AND L-56

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UDC 616-006.446.018.1

During a study of the natural changes in the sensitivity of cells to a virus within the limits of one cell population in vitro, tests were carried out on the sensitivity of 30 clone lines isolated by cultivation of different cells of human leukemia line I-96 to the cytopathogenic action of poliomyelitis virus. Besides cell lines characterized by a degree of sensitivity typical of the parent cells, clones were found with altered sensitivity: some more sensitive, some more resistant. For example, line L-98 was more resistant and L-56 more sensitive than the maternal I-96 cells.

The sensitivity to the cytopathogenic action of poliomyelitis virus was specific. Coxsackie B3 and vaccinia viruses had the same action on all three cell lines. The observed difference in sensitivity persisted throughout the period of observation (60 passages, approximately 240 cell generations).

Data reported in the literature show that subcultures differing in their sensitivity to a particular virus from the cells of the parent line are often characterized by different morphological [2, 5, 13, 14] and karyological properties [1, 6, 9, 10, 12].

In the present investigation an attempt was made to discover the possible connection between the changes in sensitivity and the karyotype, and also between the changes in certain morphological and physiological signs of the cells of lines I-96, L-98, and L-56.

EXPERIMENTAL METHOD

The cells used for the morphological investigations were grown on mica sheets, fixed with Bouin's fluid, and stained with hematoxylin-eosin. To study the growth curve, cells grown in tubes were removed daily with versene solution containing neutral red (4 tubes each time) and counted in a Goryaev's chamber. The mitotic activity of the culture was determined by counting the mitotic figures in 2000 nuclei.

To determine the duration of the mitotic cycle, the colchicine method was used [3]. Colchicine solution was added to the vessels with the growing cultures in the period of their logarithmic growth to give a final concentration of 0.0002% for 5 h. Control mica sheets were fixed before the addition of colchicine to determine the mitotic activity of the cultures.

The duration of mitosis was calculated from the formula [7], $t_m = (MI \cdot A)/MI_{col}$, where t_m is the duration of mitosis, MI the mitotic index of the particular culture, MI_{col} the number of mitoses after treatment with colchicine, and A is the time of action of the colchicine.

To determine the duration of the mitotic cycle the formula was used, $T = (0.693 \cdot t_m)/MI$, where T is the duration of the mitotic cycle, t_m the duration of mitosis, MI the mitotic index of the particular culture, and 0.693 a constant.

The karyological analysis was carried out on preparations made by the method of Pogosyants and co-workers [4]. Colchicine was added to the culture for 5 h in a concentration of 0.0002% after treatment with hypotonic solution (1% solution of sodium citrate) for 30 min at 37°. The cell suspension was treated with the fixative (1 part glacial acetic acid and 3 parts absolute methyl alcohol) and centrifuged. A drop of the suspension was placed on glass slides, and dried, stained with aceto-orcein, and mounted in balsam. The chromosomes were counted in the

Laboratory of Viral Cytopathology, Moscow Research Institute of Viral Preparations, Ministry of Health of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR G. V. Vygodchikov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 62, No. 7, pp. 86-89, July, 1966. Original article submitted June 5, 1965.

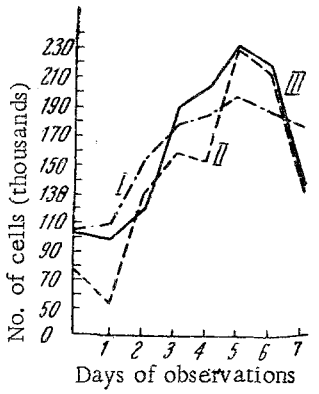

Fig. 1. Growth curves of lines I-96 (I), L-98 (II), and L-56 (III).

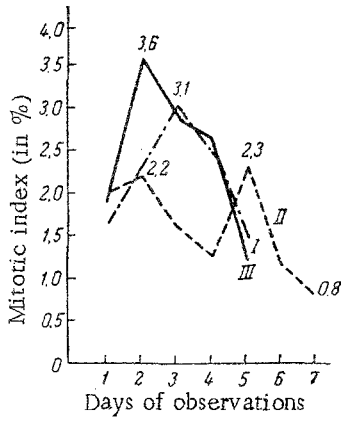

Fig. 2. Curves of mitotic activity of cells of lines I-96 (I), L-98 (II), and L-56 (III).

metaphase plates with a minimal number of overlapping chromosomes. For each cell line 100 metaphase plates were counted.

EXPERIMENTAL RESULTS

The cells of all three lines were polygonal in shape with round nuclei, and some cells had processes. In contrast to the maternal cells, the cells of the clone lines L-98 and L-56 were smaller in size. Some degree of granularity and vacuolation of the cytoplasm was observed. Often multipolar mitoses and lagging behind of the chromosomes were found. In all the cultures syncytia were present, possibly as a result of imperfect mitotic divisions.

The cells of line L-98 never formed a continuous sheet but grew in groups, forming curious shapes. The culture had a tendency to grow in a stratified form.

The growth curves (Fig. 1) show that the maximal number of cells for all lines was observed on the 5th day of cultivation. The number of I-96 cells increased gradually, and then fell gradually as the culture aged, whereas in the clone lines the number of cells fell sharply in the aging period.

Determination of the mitotic index revealed that the peak of mitotic activity appeared in the culture of the I-96 cells on the 3rd day of cultivation, whereas in the clone lines the maximal number of mitotic figures occurred on the 2nd day. In the culture of L-98 cells a second peak of mitoses occurred on the 4th day of cultivation. Mitoses were still present (0.9%) on the 7th day in the same culture, whereas none were seen in the cells of the I-96 and L-56 cultures. The highest mitotic activity (3.6%) was shown by the cells of line L-56, the lowest (2.2%) by the cells of line L-98 (Fig. 2).

TABLE 1. Evaluation of Significance of Differences in Mitotic Activity of Lines L-98, L-56 and I-96.

Line	Mitotic index	t
L-56 L-98	0,036 0,022	3,5
L-56 I-96	0,036 0,03	2,0
L-98 I-96	0,022 0,03	1,7

As Table 1 shows, the difference between the mitotic activity of lines L-56 and L-98 was statistically significant ($t = 3.5$). The difference between the mitotic activity of the cultures L-56 and I-96, and I-96 and L-98, although not significant, indicates a definite tendency towards a decrease in mitotic activity with an increase in resistance. The longest mitotic cycle was observed in the I-96 cells, and the shortest in the cells of the clone line L-56 (Table 2).

TABLE 2. Duration of Mitosis and of Mitotic Cycle in I-96, L-98, and L-56 Cells

Culture	MI	MI_{col}	A	t_M	T
I-96	0,03	4,2%	3	2,1	43
L-56	0,038	6,3%	3	1,8	28
L-98	0,025	6,1%	3	1,2	30

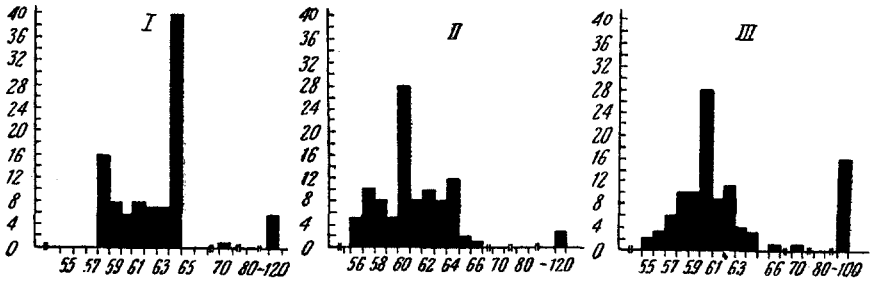

Fig. 3. Histogram of cells of lines I-96 (I), L-98 (II), and L-56 (III). Along the axis of abscissas—number of chromosomes; along the axis of ordinates—number of investigated metaphases, in %.

The results of the karyological investigation showed that the modal number of chromosomes for the I-96 cells was 64, in agreement with previous findings [1]. In both the sensitive and the resistant clones it was equal to 60 chromosomes. The line L-56 was characterized by a high content (15%) of giant nuclei with a chromosome number of between 100 and 200 (Fig. 3).

These results thus show that the cell lines studied differed not only in their activity to the cytopathogenic action of poliomyelitis virus, but also in a number of other characteristics.

However, it cannot be concluded from these results which morphological properties determine the greater or lesser sensitivity to the action of the virus. There are reports in the literature showing that fibroblast-like cells are more resistant to the virus than epithelium-like [13]. This correlation was not present in the present experiments and the cells of all three lines were epithelium-like.

In this particular case a connection was present between the sensitivity and the degree of mitotic activity of the cultures. The L-56 cells, with their greater sensitivity to poliomyelitis virus, were mitotically more active than the comparatively resistant L-98 cells; line I-96 occupied an intermediate position in regard both to sensitivity and to intensity of mitotic activity.

As pointed out above, the sign of altered sensitivity in the clone lines persisted throughout the period of observation (240 cell generations). Such stability in a sign suggests that it is hereditary in nature. However, the reduction in the modal number of chromosomes by comparison with the parent I-96 line in the clones with both increased and decreased sensitivity shows that the changes in sensitivity are based not on numerical but, possibly, on structural changes in the chromosomes or on point mutations.

SUMMARY

A morphologic study of leukemic cells I-96 and of two clones, L-6, L-56 and L-98, with modified susceptibility to poliomyelitis virus as compared to the parental line showed that cells of all three cultures were epithelium-like.

The modal number of chromosomes of both clonal sublines differed from that of the parental line I-96. There was a correlation between the degree of susceptibility to the virus action and the mitotic activity of the lines studied. The more susceptible L-56 clone had a higher mitotic index than the resistent L-98 clone. The difference was significant. The parental cells I-96 were intermediate both for their susceptibility and mitotic activity.

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