FORMATION OF COLONIES OF P-388 LEUKEMIC CELLS IN SEMISOLID AGAR

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By alternate passages of P-388 mouse ascites leukemia cells in a primary agarized culture and intraperitoneally in mice a substrain P-388/A_2 adapted to culture in agar gel in the form of compact colonies without the use of special conditions of colony-stimulating factors was obtained. Reseeding efficiency and size of the colonies depended on the original density of the cell suspension. After the addition of 100 cells/ml seeding efficiency reached 20% and the number of cells in the colony by the 8th-10th day of culture was 13,000.

KEY WORDS: leukemic cells; cloning.

The formation of colonies of leukemic cells in semisolid agar has been described for cell lines of experimental leukemias L-1210, L-5178, etc., with a clonogenicity of 28-90% [14]. Hematopoietic and leukemic cells form colonies also in primary agarized cultures [3, 12], although in this case the clonogenicity is 0.05-5% [9], and cloning methods envisage the use of special conditioners, enriched nutrient media with the addition of colony-stimulating factors, feeders from suspensions of normal cells, and so on [5, 7]. The complexity of the methods described limits their experimental use, whereas the investigation of the colony-forming activity of tumor cells is a very valuable method of studying certain problems in experimental oncology.

For screening antitumor compounds the writers use primary suspension cultures of cells and employ substrains of transplantable ascites leukemias specially adapted for growth in primary cultures (P-388/A, L-5178/A, etc.). Adaptation is obtained by alternate passages of tumor cells in primary culture and intraperitoneally in animals [1, 2]. The present investigation was devoted to the obtaining of a new adapted substrain of P-388 mouse leukemia capable of growing under submerged conditions in primary agarized cultures as compact colonies without the use of special conditions or colony-stimulating factors.

EXPERIMENTAL METHOD

DVA/2 mice were infected intraperitoneally with substrain P-388/A of ascites lymphatic leukemia adapted for growth in primary suspension culture. The 5-day tumor ascites fluid formed was added to agarized medium, cooled to 45°C, containing 50 ml medium No. 199 or Eagle's medium, 10 ml native bovine serum, 10 units/ml streptomycin, and 0.3% agar (Difco), previously melted in boiling distilled water. The medium with the cells was quickly mixed and poured in volumes of 1 ml into Leighton or Widal tubes. Carbon dioxide was blown through the air space above the medium until the medium became tinted pinkish-organge, when the tubes were closed with rubber stoppers and incubated at 37°C for 10 days.

EXPERIMENTAL RESULTS

After the addition of 500-100,000 cells/ml to the medium on the 3rd-4th day of culture clusters consisting of 10 to 50 cells formed in the depth of the agar. With further culture they did not increase in size. It was therefore decided to select variants of leukemic cells capable of forming large colonies in agar. Selection was carried out by alternate passages of cells in primary agarized culture and intraperitoneally in animals. For this purpose, a 4-day agarized culture with clusters (1 ml) was injected intraperitoneally into mice. The as-

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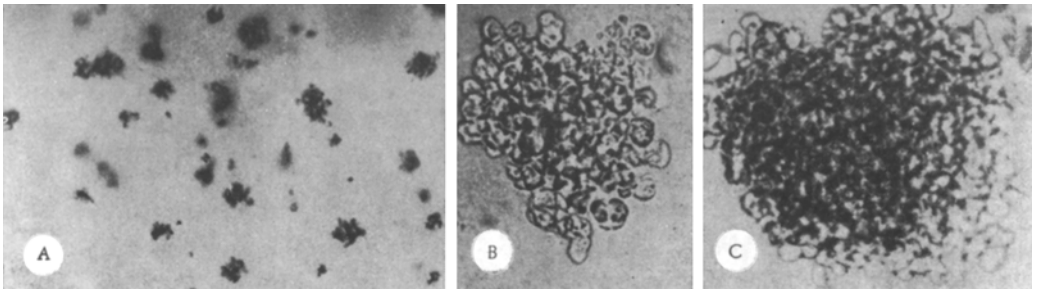

Fig. 1. Colonies of P-388/A_2 leukemic cells in semisolid agar. A) General appearance of agarized culture (20×); B) colony of P-388/A_2 cells in 5-day agar culture (200×); C) colony of P-388/A_2 cells in 10-day culture (200×).

TABLE 1. Effect of Initial Number of P-388/A_2 Cells in Agarized Culture on Seeding Efficiency and on Colony Size (10-day culture)

Initial number of cells in 1 ml medium	Number of colonies in 1 ml medium	Seeding efficiency, %	Number of cells in colony
20 000	1 100±650	5,5 1—10,5	50±13
10 000	650±90	6,5 5,6—7,4	330±23
1 000	80±40	8 4—12	1 530±251
100	13±11	13 8—20	13 000±4 550

cites fluid developing after 2-3 weeks was used to obtain clusters in an agarized culture. The times of keeping the cells in the agarized culture were gradually increased to 8-10 days. After five or six in vitro–in vivo passages the formation of compact colonies in which the number of cells varied from 50 to 10,000 was observed. The substrain adapted for the agarized medium was called P-388/A_2 (Fig. 1).

The rate of formation and the size of the colonies depended on the initial seeding density. With a density of 10,000 cells/ml, after 3-4 days in the agarized medium clusters formed which, by the 6th day, were converted into colonies containing up to 300 cells. With a seeding density of 100 cells/ml the latent period was increased to 6 days and the colonies attained their greatest size only after incubation for 9-10 days.

The initial density of the suspension also had a marked effect on the seeding efficiency, i.e., on the ratio between the number of colonies which grew and the number of cells added (Table 1). The considerable scatter of the data in Table 1 was due to the use of different batches of nutrient medium and serum in the experiments.

As regards the prospects for the experimental use of this model it is worth noting that the results of the colony-formation test are a reliable criterion of the survival of tumor cells treated with cytostatics at different phases of the cell cycle [4, 9] and, for that reason, it can be used to study the phase-specificity of action of antitumor agents when investigating relations between the G_0-phase and the mitotic cycle of tumor cells [6, 8, 10, 14, 15]. The model can be used to search for colony-stimulating factors facilitating the emergence of cells from the G_0-phase into the cell cycle [8, 11, 13], to obtain "pure lines" of tumor cells with assigned properties, such as high resistance to cytostatics, from a single cell, and so on. Tumor cells do not proliferate in the depth of a large colony (spheroid), just as within a tumor nodule in the host organism, for they are exposed to a deficiency of metabolites and oxygen. The spheroid model has therefore been suggested for the search for antitumor compounds active under conditions of anoxia [15]. Finally, good growth of P-338/A_2 cells both in primary liquid or agarized cultures, as well as intraperitoneally in animals, means that experiments in vivo and in vitro can be combined.

To maintain the clonogenic activity of substrain P-388/A_2 periodically repeated passages in agarized culture are essential: Afterthree ordinary passages of ascites cells from animal to animal, a passage leading to the production of formed colonies must be carried out.

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