PREPARATION OF CLONES OF EHRLICH'S ASCITES CARCINOMA IN VIVO

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When a single tumor cell is introduced into an animal, it does not become well established in more than 12% of the cases [4]; the very highest values reported are 33% [6] and 46% [5]. The method which has proved most reliable for increasing the effectiveness of cloning in vivo is to increase the percentage of solitary cells which can be transplanted. However, it would appear possible and very tempting to introduce into the bloodstream a few dozen completely isolated cells. With a moderate number, the chance of them settling in close proximity so that a fusion of clone nodes occurs is extremely small. These two principles (of a clone from one cell, or of many clones from many cells) have been studied independently for cloning in vitro by Sanford [12] and Puck [10].

We have carried out experiments on cloning by injecting mice with one or many cells of Ehrlich's ascites carcinoma.

Clone from a Single Cell

The cells were injected with a 0.5% hydrolysate of lactalbumin in Hank's solution and 10% horse or calf serum into the peritoneal cavity of young mice less than two days old. At the start, the usual method was employed – diluting the ascites fluid many times to obtain a suspension in which one drop contained a single cell. However, after the cells had been in the medium for as little as half-an-hour, they frequently appeared swollen. Subsequently, therefore the cells were taken from undiluted ascites fluid. A drop of this fluid was placed on an object glass, and beside it a drop of nutritive fluid. A glass fiber was placed in the first drop and a small number of cells were picked up on it and immediately transferred by the fiber into the drop of medium. This drop was taken up into a pipette and squirted or broken up into about ten small droplets. These droplets were then examined in a moist chamber. One which contained only a single complete cell was selected.

In half of the experiments the isolated cell was taken up into a micropipette under microscopic observation and then injected intraperitoneally [4]. In some of the experiments the cell was drawn up into a Pasteur pipette having a finely drawn out end, but it was always sucked up from a drop which contained only a single cell, and always under the microscope. A mark was made with India ink next to the drop chosen under the microscope, and the object glass was turned over. Previously 0.015-0.02 ml of medium had been drawn into the Pasteur pipette; part of this medium was introduced under pressure into the drop in order to wash the cell away from the glass; the whole drop including the medium was than drawn into the pipette. On injection the upper portion of medium, which had not contacted the drop, washed the channel of the pipette, thus guaranteeing that the cell was inserted into the abdominal cavity of the mouse. So that the injected liquid would not be ejected, the puncture was made between the ribs. Although no microscopic observation was made to ensure that the cell was taken up, this very simple method is sufficiently reliable, because a cell can almost always be found in a drop blown out of such a pipette.

The same method could be applied to solid tumors. The end of a glass fiber was inserted into the tumor and the contents immediately immersed into a drop of nutritive medium. To separate the cells, the drop was sucked through a pipette which was then used to distribute fine droplets on an object glass, some of the droplets containing one cell. In this way we were able to avoid the action of trypsin which is harmful to cloning [10] and which sometimes occurs when clones are obtained in vivo [2]; we also avoided any prolonged period in the medium during the dilution [7].

Of the 53 mice which received a single cell into the peritoneal cavity, 38 remained alive until the ascites developed. Ascitic tumors developed in 15 mice. They died with strongly developed ascites on the 23-49th day after the injection. Adult mice receiving 0.5 ml of ascitic fluid introduced in the normal way survived for 15-23 days. We did not make a detailed comparison of the clones, but by ordinary microscopic investigation of the ascitic fluid no immediately apparent difference between the cells of the different clones could be perceived. Ascites from one mouse which was killed by accident were given in the usual way to three adult mice which then died from ascites on the 14-17th day.

Production of Clones by the Injection of Numerous Cells

The injection was made with pipettes from square capillary tubing into the tail vein of newborn mice [1]. The end of the pipette was drawn out to a point; at the other end, which was also slightly drawn out, a thin polyethylthene tube was connected to a dispensing syringe. The latter consisted of a syringe filled to the 0.5 ml mark with 0.5% lactalbumin hydrolysate in Hank's solution and a dosage device integral with the syringe. Both parts were fixed to a wooden board. The rack of the dosage device was connected to the piston of the syringe; the piston was held against the rack by a rubber band, so that it accurately followed the movement of the rack, enabling accurate dosage of the fluid to be obtained.

About 20 cells were collected from a freshly prepared drop of the suspension. From the main mass of the medium filling the syringe, tube, and pipette, cells were separated with a small air bubble; it had been introduced before the cells were taken up. The cells were counted in the capillary itself (a procedure facilitated by the square section). The injection into the tail vein was made under stereomicroscopic observation with illumination from above. The tail was moistened to increase the visibility of the veins. The pipette was held in a cleft made in a rubber bung whose base was fixed by plasticine to the table of the microscope to allow some movement. Immediately after the cells a small amount of the fluid following after the bubble was also injected.

Injections were given to eight mice of one liter aged two days. Of these, seven survived. One died on the 14th day, and six nodular lesions measuring a few tenths of a millimeter to 1 mm were found in the lungs; another died on the 33rd day (18 nodular lesions from 0.5 to 1.5 mm), the third on the 38th day (12 nodules from 0.5 to 2 mm), and the other 4 animals were killed on the 39th day; in none of them were any nodules found. All the nodules were round, whitish, and had ill-defined edges but were quite isolated, except for a few mostly large nodules which had merged. The nodules were found only in the lungs. They were counted under a binocular loupe fixed to the forehead, and were examined under a stereoscopic microscope.

We carried out two further experiments: in one, to increase the percentage "takes" of single cells we injected tumor cells from the mother intraperitoneally; in the second experiment, to obtain clones in the greater circulation we injected cells into the hearts of newborn mice. These experiments gave no very clear results.

As a rule, when clones are formed in vivo, during the dilution of the cell suspension and the maintenance of the cells which had been isolated from each other, many minutes or even hours are spent by these cells in the artificial media [4, 9, 11]. Yet it is known that under these conditions tumors become less able to survive the transplantation even when normal methods are used. This consideration is all the more important in the case of clones. Attention should also be paid to the great dependence of survival of solitary cells on the constitution of the medium [3, 4, 6, 9]. But the most important goal is not the perfection of the medium but drastic reduction of the time spent by the cell in it. In our experiment the cells were in the medium for not more than a few minutes. It was evidently this circumstance which accounted for the high yield of the clones (15 out of 38) even in mice of impure lines.

In a pilot experiment with mass cloning produced by the injection of cells into the tail vein, the number of nodules in the lungs corresponded approximately to the number of cells injected. We deduced that each nodule arises from a single cell. It is true that these nodules cannot be considered pure clones, because the possibility of cells passing from one nodule to another cannot be discounted particularly in the later stages of development. But if sub-clones are grown from the nodules, particularly at the early stages, and broken up into separate cells, several tens of such cells can be introduced into the bloodstream; then with each transplantation we can presume that the purity of the clone will increase, and by the 2-3rd time a genetically pure population will have been established.

SUMMARY

Ehrlich's ascitic carcinoma cells were obtained directly from ascitic fluid without successive dilutions. After intraperitoneal injection of these cells into newborn mice, clones of Ehrlich's carcinoma were obtained in 15 out of

38 mice which survived. Mice with ascitic clones died on the 23rd-49th day. After injection of 20 ascitic cells into the tail vein of young mice isolated nodes developed in the lungs, and the number was nearly equal to the number of cells administered. It is suggested that these nodes were clones.

LITERATURE CITED

1. B. V. Perfil'ev, and D. R. Gabe, Capillary Methods of Study of Microorganisms [in Russian], AN SSSR (1961).
2. L. F. Sharlikova and U. Min', Byull. éksper. biol.,No. 3 (1961), p. 85.
3. J. Furth and M. C. Kahn, Am. J. Cancer, Vol. 31 (1937), p. 276.
4. T. S. Haushka and A. Levan, J. Nat Cancer Inst.,Vol. 21 (1958), p. 77.
5. K. Hosokawa, Gann, Vol. 41 (1950), p. 1.
6. K. Ishibashi, Gann, Vol. 41 (1950), p. 1.
7. E. Klein, Exp. Cell. Res.,Vol. 8 (1955), p. 213.
8. H. Lettré, Z. Krebsforsch.,Vol. 59 (1953), p. 287.
8. S. Makino and K. Kano, J. Nat. Cancer Inst.,Vol. 15 (1955), p. 1165.
10. T. T. Puck, Advances in biol. and med. Physics., New York (1957), p. 75.
11. H. Querner, Z. Krebsforsch.,Vol. 60 (1955), p. 307.
12. K. R. Sanford et al., Exp. Cell. Res.,Vol. 23 (1961), p. 361.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
