The Establishment of a Cell Strain (MAC-21) from a Mucoid Adenocarcinoma of the Human Lung*

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SUMMARY

A cell strain MAC-21 has been established from adult human lung tumor tissue. The tumor was identified as a well differentiated mucoid adenocarcinoma.

A relatively small clump of cells (200-300) was the source of the outgrowth that occurred about 1 month after initial cultivation in vitro. No fibroblasts were ever seen, and no alterations, transformations, or lag phase of growth were observed once the initial outgrowth appeared. The cells were epithelial in appearance and had a mean chromosome number of 79.

Viral infectivity tests, with the use of herpes simplex, adenovirus Type 8, vaccinia, and rabbit myxoma on both MAC-21 and HeLa cells gave similar results with both cell lines.

In the past few years the number of established cell strains in tissue culture has grown prodigiously. However, few strains have been established directly from adult human lung or bronchial tissue (1, 10). Most attempts to produce cell lines directly in tissue culture from human carcinomatous lung tissue have been unsuccessful. While our own attempts proved fruitless for more than 3 years (4), a tissue culture strain from a mucoid adenocarcinoma of the lung (MAC-21) has recently been established and has been growing for over a year.

A lower left lobectomy was performed January 13, 1959, on a 53-year-old male. The excised tumor had a well defined irregular outline, was 5 cm. in diameter, peripherally white, and extremely mucoid. The tumor was diagnosed as a well differentiated mucoid adenocarcinoma, probably primary in the bronchus. Comprising the tumor were glandlike structures, frequently situated “back to back,” separated from one another by a single layer of stromal cells (Fig. 1).

MATERIALS AND METHODS

A 1 X 2 X 2-cm. segment of the tumor, handled under sterile conditions, was placed in medium 199 containing 10 per cent calf serum, 0.5 per cent peptone solution, and antibiotics (100 units of penicillin and 100 µg of streptomycin/ml). After being rinsed in several changes of Tyrode’s solution containing the above antibiotics, it was trimmed and cut into small pieces about 2 mm. square and ½-1 mm. thick.

A modification of the organ culture technic of Fell (6) was used. The pieces were explanted directly onto clots composed of 0.6 ml. human cord serum, 0.3 ml. human placental extract30, 1 drop of human fibrinogen solution containing 30 mg/ml, and one drop of bovine thrombin solution containing 100 units/ml. After 2 days the clots showed an acid pH, lysis, and peripheral sloughing of the explant. The tissue was, therefore, transferred to fresh clots. However, in three cultures, several pinhead-sized pieces of tissue or groups of cells had settled to the bottom of the clot. Microscopic examination of this material revealed strands of healthy-appearing epithelial cells, two or several layers thick (Fig. 2). These were transferred to a T-15 flask into a liquid medium containing 15 per cent fresh human cord serum, 15 per cent human placental extract30, 70 per cent medium 199 (with added antibiotics).

Most of these cells did not attach to the glass surface of the flask and were poured off when two-thirds of the medium was changed on the 4th day. Where two or three clusters did adhere to the
glass, they were sharply circumscribed, encapsulated, and peripherally presented a smooth border of epithelial cells. No fibroblasts were noted then or later, either as outgrowths from these clumps or as isolated cells. After a total of 3 weeks' cultivation with two additional fluid changes, one macroscopic clump remained. When viewed under the microscope this area was completely visible in the low power field (100X) and consisted of approximately 200-300 cells. After another week, during which time the flask remained untouched, some acidification of the medium was noted. Microscopic examination showed that this large encapsulated clump had burst open at one end and several cells had migrated out. This occurred exactly 1 month after the original explantation of the tissue. The migratory cells were quite similar in appearance to that of other vigorously growing

tables from established epithelial-like strains carried in this laboratory.

In the next few days three additional growth areas were observed which showed similar actively proliferating cells. Centers of two of these clumps were detached from the glass, transferred to Carrel flasks, and grown in a medium composed of 15 per cent fetal calf serum, 70 per cent medium 199, and 15 per cent Tyrode's solution plus antibiotics (50 units penicillin and 50 μg streptomycin/ml of medium). The cells in all flasks remained epithelial-like in appearance and continued to proliferate vigorously.

**RESULTS**

The cells have been in continuous cultivation more than a year and have undergone over 45 passages at dilutions of 1:5 up to 1:50. They have been grown easily, in several laboratories in various T-sized flasks, bottles, and Leighton tubes. At the third passage, and at intervals thereafter, cover-slips were placed in T-flasks and removed and stained after a few days' growth of the cells. All cover slips examined showed actively multiplying cells with a fair number of abnormal mitotic figures (Figs. 3, 4). Chromosome counts, made on cells cultured for 6 months and in their 14th passage, gave a mean value of 79 chromosomes (Fig. 5).

Optimal growth was observed in the fetal calf serum medium described above. Good growth was also obtained with 10-20 per cent human adult or cord serum, or 10 per cent calf serum replacing the fetal calf serum in the medium. Equally good results were noted with a medium composed of 40 per cent human ascitic fluid, 50 per cent medium 199, 5 per cent fetal calf serum, and 5 per cent of a solution of lactalbumin hydrolysate.

Attempts to produce tumors in the cheek pouch of the conditioned hamster were partially successful. The animals used weighed between 35 and 80 gm. and were treated with cortisone according to the technic of Toolan (18). The tissue culture cells used for the inoculations were in their 4th to 20th subculture. The total number of cells injected was estimated to range between $1 \times 10^6$ and $1 \times 10^7$ per cheek pouch. Usually only one cheek pouch was used.

Out of twenty animals given injections, only three showed nodules which were not reabsorbed within a 2-3-week period. Two of these nodules, excised after 14 and 18 days, respectively, showed slight vascularization, considerable necrosis, and a small area of undifferentiated carcinoma. Injected into both cheek pouches of the third hamster were cells which had grown for 4 months in vitro and were in their 13th subculture. Nodules the size of a small lentil were present in each pouch, 5 weeks after injection, and appeared slightly vascularized. At this time they were removed, and one-third of each was fixed in Bouin's for sectioning (Fig. 6) and the remainder cut into small pieces. The pieces inoculated into Carrel flasks and a T-30 flask remained quiescent for nearly 1 month, showing only some slight fibroblastic outgrowth which soon degenerated. At this time the characteristic epithelial-like cells of the original tissue culture grew out from the explants. They have subsequently been maintained and subcultured just as well as the original cell line.

Viral studies were carried out comparing the relative susceptibilities of MAC-21 and HeLa cells to inoculations with herpes simplex, adenovirus type 8, and vaccinia. The results (shown in Table 1) demonstrate comparable titers for the two cell strains. Attempts to obtain signs of cytopatho-
genicity in either MAC-21 or HeLa cells with inoculations of rabbit myxoma virus were negative.

DISCUSSION

Previous studies from this laboratory (4) have stressed the difficulty in obtaining cell strains from human adult lung tumor. Normal bronchial mucosa is present within the explanted tumor tissue in many instances. These normal cells often multiply or survive for longer periods than the preponderant tumor tissue which may surround them. They have been responsible for some outgrowths observed in "organ-type" tissue cultures and may be suspect in other types of tissue cultures.

Because two rather unusual conditions combined to lead to the establishment of cell strain MAC-21, we believe these cells to be definitely derived from a mucoid adenocarcinoma of the lung.

First, the original tumor was highly differentiated and composed of delicate, pretzel-like strands of malignant cells. Fragments of these strands separated easily from the explanted pieces and showed no apparent connective tissue stroma (Fig. 2). Secondly, the tissue that gave rise to the established cell line was relatively minute, had been examined under the microscope frequently, and seemed homogeneous in character with no fibroblasts or other migratory cells visible either prior to or following the initial "breaking out" of the epithelial-like cells from the original clump.

Of the established cell lines of human bronchial origin, the normal bronchial epithelial strain of Hoch-Ligeti and Hobbs (10) and the Maben cell of Frisch et al. (9) were obtained without going through transitional stages. Baron and Rabson (1) also obtained a human epithelial strain from an adenocarcinoma of the lung; however, fibroblasts predominated for the first 11 weeks of culture. Then colonies of epithelial-like cells emerged and became the progenitors of the established strain. The Detroit 6 cell strain of Berman and Stulberg (3) obtained from the sternal marrow of a patient with carcinoma of the lung, went through several phases before showing the epithelial plaques which gave rise to the cell line 51 days after explantation.

Parker, Castor, and McCulloch (14) have discussed in detail the topic of "altered cell strains" and present a general review of the subject. Most workers who have carefully described their isolation of cell strains notice transitional stages, alterations in appearance, or lag periods (2, 5, 8, 12, 15–17, 19, 20).

Whether the establishment of a stable cell strain is coincidental with an abnormal (near tetraploid) chromosome number is not yet clear. A careful study by Levan and Biesele (11) as well as the data of Fernandes (7) and the unpublished results obtained by Zitec indicate that chromosomal variations may occur almost as soon as "in vitro" conditions exist.

Chromosome counts, made on strain MAC-21 after several months of culture, gave a mean value of 79. However, abnormal mitotic figures were noted as early as the third subculture, about 7 weeks after the tissue was explanted. Since the primary tumor was highly differentiated and the emergent cell line arose from a small clump of cells, the presence of abnormal numbers of chromosomes in the original explant is quite possible. This may account for the ease with which the strain was established.

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Fig. 1.—Histological section of the freshly excised tumor showing well differentiated glandlike structures lined with a single to double layer of epithelial tumor cells. Small amounts of faintly staining mucoid coagulum are present in the lumina. H & E stain. X300.

Fig. 2.—Peripheral edge of fresh tumor explant grown 4 days on a clot composed of human cord serum, placental extract, fibrinogen, and thrombin. Note the pretzel-like double strands of cells and the absence of fibroblasts or connective tissue. H & E stain. X300.

Fig. 3.—Appearance after 4 days' growth in the third subculture on cord serum medium. Note active mitosis with some abnormal mitotic figures and multinucleate giant cells. H & E stain. X110.

Fig. 4.—Same slide as Fig. 3, only higher magnification showing tripolar mitosis. X250.

Fig. 5.—Preparation showing hypotetraploidy (79 chromosomes). Cultures were in their 14th passage and had been growing for 6 months in vitro. Orcein stain, phase contrast microscopy. X850.

Fig. 6.—Section of tumor after 5 weeks' growth in the hamster cheek pouch produced from an inoculum of cells which had been growing in vitro 44 months. A diffuse rather than acinar pattern is evident. H & E stain. X850.
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