Intracisternal Type A Particles and Properties of a Continuous Cell Line Originating from a Gerbil Fibroma[1] (35439)

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Tumor tissue from a gerbil (*Meriones unguiculatus*) was explanted to study the properties and behavior of such cells *in vitro*. The cell line (IMR-33) originating from this tumor has been propagated more than 70 passages and now is considered a continuous line. Some of the properties of IMR-33 are described below.

Methods and Materials. Origin and cultivation. A portion of spontaneously occurring fibroma[2] measuring about 1 cm in diameter and located on the dorsal part of the paw of a 14-month-old male gerbil was excised. The tumor tissue was minced into fine fragments, and the fragments, suspended in a growth medium consisting of 80% medium 199 and 20% fetal bovine serum and containing penicillin (200 units/ml) and streptomycin (200 μg/ml), were placed in prescription bottles (Brockway Glass Co., Brockway, Pa.). With the first renewal of medium and subsequently, growth medium contained one half the initial concentration of each antibiotic.

The growth medium was renewed twice weekly usually; temperatures for cultivation were between 35.5 and 37°. Cells were subcultivated, using 0.25% trypsin (trypsin and medium components, except antibiotics, from Hyland Labs., Inc., Los Angeles, Calif.) in Hanks' balanced salt solution (HBSS), at a 1:2 ratio each time. Cultures were subcultivated every 2 or 3 weeks until the sixth subculture and then were subcultivated at approximately weekly intervals.

Preparation of cytotoxic antisera. A 20% suspension of gerbil organs and tissues in HBSS was homogenized in a vessel submerged in ice, frozen and thawed quickly 3 times, filtered through coarse gauge, and centrifuged 5 min at 350*g*. The supernatant extract was divided into 3 vol and placed in storage at —70°.

Eight- to 10-lb albino rabbits were inoculated intraperitoneally 3 times at weekly intervals with 3.5 to 4.0 ml of the extract each time. Four days after the final injection, blood was taken by cardiac puncture, and sera from 3 rabbits were pooled.

Electron microscopy. Cells, removed from glass by scraping, were fixed doubly with 3% glutaraldehyde and with 1% osmium tetroxide in phosphate buffered saline or with osmium tetroxide alone. Ultrathin sections of fixed cells embedded in Epon 812 were stained with lead citrate (1) and with 2% uranyl acetate in distilled water. Sections were examined with the RCA model EMU3G electron microscope (RCA, Camden, N.J.).

Viral titrations. The susceptibility of IMR-33 cells to various DNA and to RNA viruses was determined. Each of 3 or 4 confluent roller tube cultures, maintained in 95% Eagle's minimum essential medium with nonessential amino acids in HBSS and 5% agamma newborn calf serum and containing penicillin (100 units/ml) and streptomycin (100 μg/ml), was inoculated with 0.2 ml of 10-fold dilutions of virus. Depending on the rapidity of development of cytopathic effects (CPE), cultures were maintained 1 to 3 weeks. Fresh medium was added once weekly to cultures maintained more than 1 week. WI-38 cultures served to monitor viral CPE.

Results. Growth characteristics. Cell line IMR-33 consists of fibroblast-like cells and has grown vigorously from its inception to

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[2] Pathology of original tumor and of nodules in hamsters was evaluated by Dr. J. H. Vickers of Lederle Laboratories, Pearl River, N.Y.

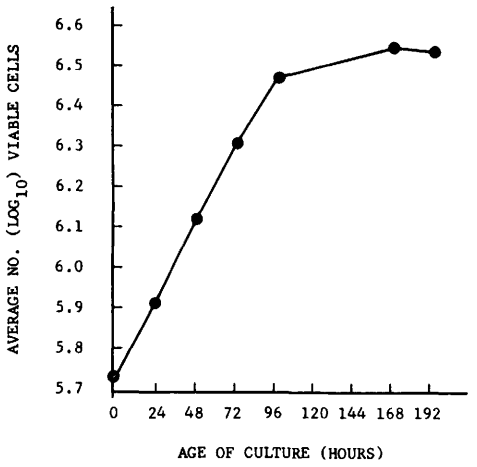


FIG. 1. Average population doubling time of IMR-33 cultures.

the present with no obvious period of growth crisis. Characteristically, cells can remain adherent to glass and viable even when medium is not renewed for several weeks. The fibroblast-like cells initially grew in parallel arrays generally, but such orientation gradually diminished giving way to a completely disoriented mode of alignment and growth.

The average population doubling time curve of IMR-33 cultures, based on data obtained between subcultivations 58 and 60, is shown in Fig. 1. Each point on the curve is an arithmetic mean value obtained from 3 to 4 different trials. Under conditions described above, the population doubling time was 36 to 38.5 hours; there was a 6.7-fold increase in cell number after 7 days without renewal of growth medium, when the initial concentration of cells in 2-oz prescription bottles was 5 $\times$ 10^5 in 10 ml of medium.

The cytotoxic antibody test of Green *et al.* (2) verified that cultures were of gerbil origin. The optimal dilution of antiserum prepared against gerbil tissue extracts destroyed 98% of cells, as determined by uptake of trypan blue dye, whereas only 3 to 10% of control cells were not viable. The antiserum was not cytotoxic for cells from human, mouse, or Syrian hamster lines cultivated in the laboratory at the same time as IMR-33.

Chromosomal analysis. Cells from subcultivations 5, 14, and 52 were prepared for analysis of chromosomal constitution as described by Nichols (3). Figure 2 shows the distribution of chromosomal complements in cells of subcultivations 5 and 52. The modal number in passage 5 was 44 with 22% of the total number of cells deviating from the modal number. By passage 52, all IMR-33 cells examined had become heteroploid; the chromosomal constitution of 62% of cells ranged within 1 unit around the hypotetraploid modal number of 84. Heteroploidy of IMR-33 populations was well advanced by the 14th passage, when 58% of cells were heteroploid, while 42% of cells still showed a diploid constitution (4) of 44 chromosomes.

Ultrastructure. Salient characteristics of IMR-33 cells were a well-developed, rough endoplasmic reticulum (RER) often exhibiting distended cisternae, intricately ruffled or lobed nuclear membrane observed in many established cells, and a particle (Figs. 3–5) similar to the intracisternal type A particle classified previously (5). The type A particles in IMR-33 cells average 74 mμ in diameter, ranging from 66 to 82 mμ; the inner more intensely staining ring averages 38 mμ in diameter. Type A particles were rare and were observed almost invariably singly and never in groups; budding particles were extremely rare. Cells from subcultiva-

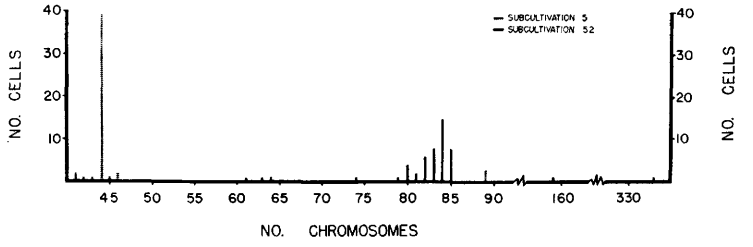
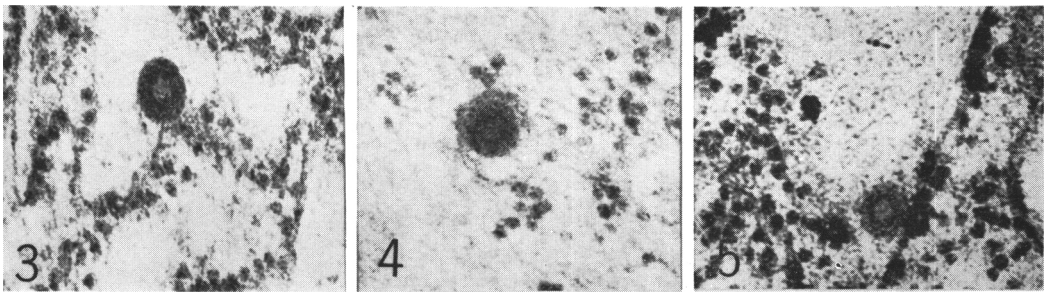


FIG. 2. Distribution of chromosomal complements in subculture 5 and in subculture 52 of IMR-33.



FIGS. 3-5. Intracisternal type A particles in cells of subcultivation 57. Fixed in glutaraldehyde and osmium.

FIG. 3. Type A particle within cisternum of RER. ×86,530.

FIG. 4. Type A particle within apparently spent portion of RER. ×86,440.

FIG. 5. Type A particle appearing to bud from surface of RER into cisternum, ×86,420.

tions 5, 26, and 57 contained the type A particle, however, exhaustive examination of the original tumor failed to reveal the type A particle.

Viral spectrum. The susceptibility of IMR-33 cells to different viruses (Table I) was determined between subcultivations 61 and 69. IMR-33 proved insusceptible or virtually insusceptible to 3 human enteroviruses (lowest dilution 1:10) but susceptible to other RNA- and to DNA-containing viruses.

Attempts to isolate mycoplasma were carried out as described previously (6). No mycoplasma were isolated during any of 5 attempts in as many different subcultivations.

Hamster inoculation. IMR-33 cells from subcultures 64 and 69 showed no potential for progressive tumor development, when inoculated into the cheek pouches of unconditioned hamsters between 5 and 6 weeks old. Hamsters inoculated with 3×10^6 cells developed nodules averaging about 5 mm in diameter in one experiment and about 3 mm in another experiment. Nodules regressed within 7 to 15 days. Examination of histologic sections of 4 nodules still present at day 15 confirmed gross observations of the regressing state of nodules. Hamsters inoculated with 1×10^5 cells did not develop nodules.

TABLE I. Viral Susceptibility of IMR-33 Cells.

Virus	CPE
Echo-11	—
Polio-1	—
Coxsackie-B3	±
Vesicular stomatitis	+
Reo-3	+
Herpes simplex	+
Adeno-12	+
Vaccinia	+
Parainfluenza-1 (Sendai)	+

Discussion. Cells derived from a gerbil fibroma became adapted to continuous growth *in vitro* without any period of growth crisis. Whether this would be commonplace with attempts to explant gerbil tissues, neoplastic or normal, cannot be predicted from this first reported experience with cultivation of gerbil cells for an extended time. Either the heteroploid cells predominating at or about subculture 14 were present in the tumor, providing the "seed" population for the continuous cell line, or the diploid cells of the tumor tissue altered, assuming a hypotetraploid state most compatible with continuous survival under the stated conditions. Retrospectively, despite the potential of IMR-33 cells for continuous growth in a hypotetraploid state, the percentage of cells deviating from the $2N$ number of 44 at the fifth subcultivation seemingly was not significantly greater than the percentage of deviant cells counted by Pakes (7) in bone marrow populations from 2 of 5 presumably normal gerbil females and from 1 of 5 males.

The intracisternal type A particle has been observed in a variety of mouse tumors (8–10), and an intracisternal particle associated with guinea pig leukemia was by Opler (11) and by Nadel *et al.* (12). Based on observations in IMR-33 cells, gerbil tissues may contain an intracisternal type A particle. As in the mouse, the biological significance of an intracisternal type A particle in gerbil cells is obscure, and no conclusions can be drawn about its causal relationship to the original

fibroma. Whether or not the type A particle interferes with viral replication is not known, however, IMR-33 cells are susceptible to DNA and to RNA viruses.

Summary. Cells from a spontaneous fibroma of the Mongolian gerbil could be cultivated continuously, becoming an established cell line. The modal number of chromosomes exhibited by the stemline constituting early cultures of this cell line was 44, the *2N* chromosome number of *Meriones unguiculatus*. The continuing survivors in IMR-33 populations are predominantly hypotetraploid. With this report, the gerbil is included among those species which may harbor a structural entity resembling the intracisternal type A particle.

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1. Reynolds, E. S., J. Cell Biol. **17**, 208 (1963).

2. Greene, A. E., Coriell, L. L., and Charney, J., J. Nat. Cancer Inst. **32**, 779 (1964).

3. Nichols, W. W., Hereditas **50**, 53 (1963).

4. Nadler, C. F., and Lay, D. M., Z. Säugetierk **32**, 285 (1967).

5. Suggestions for the Classification of Oncogenic Viruses, J. Nat. Cancer Inst. **37**, 395 (1966).

6. Tumilowicz, J. J., Nichols, W. W., Cholon, J. J., and Greene, A. E., Cancer Res. **30**, 2110 (1970).

7. Pakes, S. P., Lab. Anim. Care **19**, 857 (1969).

8. Dalton, A. J., Potter, M., and Merwin, R., J. Nat. Cancer Inst. **26**, 1221 (1961).

9. Smith, G. H., Andervont, H. B., and Dunn, T. B., J. Nat. Cancer Inst. **44**, 657 (1970).

10. Kakefuda, T., Roberts, E., and Suntzeff, V., Cancer Res. **30**, 1011 (1970).

11. Opler, S. R., J. Nat. Cancer Inst. **38**, 797 (1967).

12. Nadel, E., Banfield, W., Burstein, S., and Tousimis, A. J., J. Nat. Cancer Inst. **38**, 979 (1967).

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