Cultural Characteristics of a Cell Line Derived from an Equine Sarcoid

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A cell line, derived from a spontaneous equine connective tissue tumor (equine sarcoid), has been established. The morphological and growth characteristics indicative of malignant transformation of the cells include a disoriented, rapid growth and loss of contact inhibition. Further evidence of transformation is the agglutination of these cells by concanavalin A and their ability to divide in semisolid media.

Equine sarcoïds are spontaneous connective tissue tumors which occur on the skin of horses (6, 7). Reported studies concerning this tumor have not included the establishment of a cell line and the consequent description thereof. This report deals with cultural characteristics of one of the several equine sarcoïds presently being cultured in this laboratory.

MATERIALS AND METHODS

Tumor removal. An equine sarcoïd, measuring 4 by 4 by 3 cm, was removed aseptically from the right front fetlock of a 3-year-old male quarterhorse. The tumor tissue for culture was placed in Dulbecco saline solution (5) for approximately 1 hr while being transported to the laboratory. Samples for histopathological diagnosis were submitted to Department of Pathology, Colorado State University.

Culturing the tumor. After 1 hr in Dulbecco solution, the tumor tissue was debrided and washed thoroughly in Hanks balanced salt solution (5) containing 100 µg of streptomycin sulfate per ml and 100 units of potassium penicillin G per ml. The deepest area of the tumor mass was removed for use in tissue culturing. The tissue was cut into pieces approximately 2 mm³ in size and allowed to soak in Hanks balanced salt solution with antibiotics for an additional hour. The tumor pieces were trypsinized overnight at room temperature using 0.5% trypsin in Dulbecco saline solution.

After trypsinization, the cells (designated "Mc1" cells) were washed three times in phosphate-buffered saline, pH 7.2, solution and inoculated into 250-ml plastic tissue culture flasks (Falcon Plastics, Oxnard, Calif.). The flasks were seeded with 3 x 10⁶ to 5 x 10⁴ cells/ml in Eagle minimal essential medium with Earle basal salt solution (MEM) containing 20% equine serum and incubated at 37 C. After 24 hr of incubation the medium was removed and centrifuged at 200 x g for 5 min to remove all nonviable cells. At confluency the cells were subcultured by pouring off the media and placing 0.05% trypsin plus 0.025% ethylenediaminetetraacetic acid in saline A (5) into the tissue culture flask. This solution was allowed to stand at room temperature for 2 to 5 min, during which time the cells became detached from the surface of the flasks. The cells were then washed twice in PBS and seeded (10⁵ cells/ml) into new flasks containing MEM plus 10% equine serum. Subsequent subcultures were conducted in the same manner.

Morphological examinations. The cells were examined daily with an inverted tissue culture microscope to record morphological characteristics and to determine when to subculture the cells. Periodically, Leighton tube cultures (5) were stained with Giemsa and Papanicolaou stains for further morphological studies.

Agar suspension cultures. Mc1 cells were seeded in agar suspension culture according to a modification of the method of Macpherson (4). Eight milliliters of a 0.6% solution of Noble agar in MEM and 10% equine serum was pipetted into plastic petri dishes (60 by 15 mm) and allowed to harden at room temperature. The cells were trypsinized off the flasks (in the same manner as for subculturing) and suspended in MEM to give 1.2 x 10⁴ cells/ml. One-fourth milliliter (3 x 10⁴ cells) of this individual cell suspension was mixed with 2.5 ml of the agar medium and gently pipetted onto the surface of the hardened agar base. The cultures were then incubated at 37 C in an 80% humidified atmosphere of 5% CO₂ in air and examined daily. Control cultures consisted of an equine dermis cell strain (obtained from the American Type Culture Collection, Rockville, Md.).

Concanavalin A agglutination assays. Mc1 cells were tested for agglutination with concanavalin A (Con A) by the method of Inbar et al. (3). Cells were suspended in Con A (200 µg/ml) at a concentration of 10⁴ cells/ml. They were examined with an inverted tissue culture microscope for presence and time of agglutination.

Control experiments consisted of treating normal equine dermis cells with Con A in the same manner as described for the Mc1 cells. In addition, spontaneous
agglutination was determined by incubating Mc1 and equine dermis cells in distilled water.

RESULTS

Diagnosis. The histopathological diagnosis was consistent with the clinical diagnosis of equine sarcoid.

Cultural characteristics. The Mc1 cell line has been in continuous culture for 8 months involving over 70 passages. Several phases of growth patterns were observed during the culturing of these cells.

In the first stage, prior to the 10th passage, the cells appeared very fibroblastic and grew in parallel arrays. Without staining it was often difficult to recognize individual cell membranes. In addition, the cells exhibited classical contact inhibition. During the 10th to 12th subcultures a second type of growth characteristic and morphology developed (Fig. 1). There appeared to be distinctly different clones of cells intermingled among the original cell type. These cells were highly refractile and more rounded than the neighboring cells. Although they appeared spindle-shaped while growing at low cell densities, they became more rounded as the cell density increased. At their first occurrence they were seen in definite foci scattered throughout the culture flask. The cells in these foci grew more rapidly and in a more disoriented manner than the surrounding cells, and by the 15th passage were the predominant cell type in the culture. After the 18th subculture a third change of growth pattern was detected. Foci of "piled-up" cells (Fig. 2 and 3) were frequently seen, suggestive of loss of contact inhibition. These foci have been selectively detached from the surface of the flask and subcultured; they grew in distinct foci immediately after passing, but eventually formed a confluency of cells several layers deep. These have been passed every two days and yield a fourfold increase in cells from seeding to confluency.

Agar suspension cultures. Immediately after seeding, no cell aggregates were detected. Three to four days after seeding, an increase in the number of cells was detected, giving evidence of cell growth. After 7 to 10 days incubation the average colony contained 15 to 30 cells (Fig. 4a). An uncountable cell increase was detected after 14 to 16 days of incubation (Fig. 4b). The equine dermis cell culture controls did not multiply in agar suspension cultures.

Con A agglutination assays. Individual Mc1 cells began to agglutinate almost immediately after addition of Con A. The size of the aggregates increased steadily for about 15 min at which time there were over 100 cells per aggregate.

Although early passages of these cells agglutinated, subcultures after the 20th to 25th agglutinated more readily and formed larger aggregates. Slight agglutination (less than 20 cells per aggregate) of normal equine dermis cell controls was detected only after 10 to 15

Fig. 1. Mc1 cells in their 10th subpassage. A focus of morphologically transformed cells is seen among the original cell type. ×100, bright field.
min of incubation. Neither the Mc1 or the equine dermis cells agglutinated in distilled water.

DISCUSSION
Insight to neoplasia is often enhanced by the development of tissue culture cell lines derived from tumors. In an attempt to study various aspects of equine sarcoids, we have established a cell line derived from this tumor. The establishment of an equine sarcoid cell line has not been reported previously. This report describes the establishment of this cell line and some of the cultural characteristics thereof.

Many of the morphological and growth patterns of the Mc1 cells are suggestive of a
malignant transformation. This evidence has been further substantiated by the use of specific in vitro tests which are known to detect transformed cells.

Specifically, the Mc1 cells exhibited loss of contact inhibition in the early passages. This type of disoriented, uncontrolled growth is known to be a characteristic of transformed cells. In addition, the change in morphology which accompanied the loss of contact inhibition gives additional evidence that the Mc1 cells were transformed.

These growth and morphological changes, which were detected, led to two experiments which have been reported to assay for transformation: namely, agar suspension cultures and Con A agglutination. Macpherson (4) reported that normal cells cease to divide in semisolid media, whereas transformed cells continue to divide. The Mc1 cells grew well in agar suspen-
sion cultures giving direct evidence for transformation. The second assay system involved the use of Con A which has been reported to have the ability to agglutinate transformed cells but not normal cells (1–3). The Mc1 cells agglutinated very rapidly and formed large aggregates in the presence of Con A.

Further characteristics of the Mc1 cells which are being investigated include tumor antigen detection, karyotyping, and electron microscopy studies. Cultural characteristics of cell lines derived from additional equine sarcoïds are also being examined.

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