Spontaneous Esophageal Carcinoma and Epithelial Cell Line of an Adult Rhesus Monkey

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ABSTRACT

A continuous epithelial cell line, 816A, was established from a lymph node of an adult rhesus monkey with metastatic esophageal carcinoma. These cells are characterized by the presence of desmosomes and a markedly heteroploid karyotype. At a relatively early culture age, electron microscopy showed both budding and extracellular type C virus. Antigen reactive with antisera to Mason-Pfizer monkey virus was observed by complement-fixation. The level of this antigen decreased with increased culture age. To our knowledge, the 816A cells represent the only established simian or human cell line of esophageal carcinoma origin.

INTRODUCTION

Several cases of spontaneous tumors of the gastrointestinal tract have been reported in rhesus monkeys, but few of these have been esophageal in origin (10). This is in contrast to humans, where esophageal carcinoma is a relatively common tumor (14). No cell culture lines representative of spontaneous esophageal carcinomas of humans or simians have been reported. In this paper we report on a case of esophageal carcinoma of an adult rhesus monkey (Macaca mulatta) and on a continuous line of epithelial cells established from a lymph node with metastatic tumor.

MATERIALS AND METHODS

Cell Culture. Tissue was taken at necropsy from a primary tumor in the esophagus and from pancreatic lymph nodes. Tissues from the 2 sources were separately cultured in McCoy's Medium 5A supplemented with 10% fetal bovine serum, sodium pyruvate (200 µg/ml), insulin (10 µg/ml), penicillin (50 units/ml), and streptomycin (50 µg/ml). Tests for possible Mycoplasma contamination were performed by Richard Del Giudice of the Frederick Cancer Research Center, Frederick, Md.

Cytogenetic Analyses. Cytogenetic analyses were performed on conventional Giemsa-stained preparations. Further analyses were kindly performed on trypsin:Giemsa-banded preparations by Dr. Ward D. Peterson, Jr. and Dr. William F. Simpson of the Child Research Center of Michigan, Detroit, Mich.

Tests for Viral Antigen and Polymerase Activity. Assays for the presence of antigens cross-reactive with those of mammalian retroviruses were performed by CF3 with 1.8 to 2.2 units of complement in the presence of 4 units of antibody (12). In an attempt to enhance virus expression, the cells were stimulated with IdUrd by treating with IdUrd (30 µg/ml) for 24 hr, washing with balanced salt solution, and refeeding with complete medium. Cells were tested for increased antigen on Days 1 through 5 after removal of drug (11). Assays for antigens cross-reactive with Epstein-Barr virus and Herpesvirus saimiri were performed by indirect immunofluorescence. Immunofluorescence tests for the papovavirus major structural antigen were generously performed by Dr. Keerti V. Shah of Johns Hopkins University, Baltimore, Md. (16). Assays for RDDP activity in culture fluids were performed with the synthetic templates poly(rA)-d(pT)n and poly(dA)-d(pT)n. Both Mg2+ and Mn2+ were tested as the divalent cation for enzyme activity.

Electron Microscopy. Cell culture monolayers were rinsed with 1.25% glutaraldehyde, scraped, and sedimented by slow-speed centrifugation. Post-fixation, en bloc staining, dehydration, and embedding in Epon 812 were as described (8). Thin sections were cut on an LKB Ultratome III with a diamond knife. Sections were mounted naked on 300 mesh copper grids and stained with uranyl acetate followed by Reynolds lead citrate. Observations and micrographs were made with an Hitachi HU-11E microscope operated at 75 kV.

Nude Mouse Inoculations. Five-week-old homozygous nude NIH Swiss athymic nude mice were obtained from the animal production area at the Frederick Cancer Research Center. Mice were given injections of 3.5 × 106 cells in 0.2 ml s.c. or with 3.5 × 108 cells in 0.02 ml i.c. (4).

RESULTS

Necropsy Findings. Rhesus Monkey 816A was a mature male that had been in the Litton Bionetics, Inc., Kensington, Md. colony for almost 11 years prior to his death. Clinical examination had shown chronic pneumonia associated with intractable vomiting. Gross examination at death revealed that the distal half of the esophageal wall...
was thickened, rough, ulcerated, and firm. A few small nodules of firm, whitish-gray tissue were attached to the serosa. The pancreatic and right renal lymph nodes were enlarged and firm. Firm, whitish, raised tissue areas were found between and involving several ribs on the right and left sides. Microscopic examination revealed squamous cell carcinoma involving the distal half of the esophagus with submucous lymphatic and vascular spread and mural infiltration beyond the sites of mucosal ulceration. The regional lymph nodes were involved, and there was infiltration of the muscular wall of the stomach at the cardia with focal extension into the submucosa and mucosa. The pancreatic lymph nodes were enlarged and filled with tumor (Fig. 1). The ribs showed tumor invasion of skeletal muscle, peristeam, and bone.

Establishment of Cultures. Fresh tissue was collected at necropsy from esophagus and pancreatic lymph nodes. Tissues were kept separate, finely minced, and cultured. The most evident indication of outgrowth occurred from explants of lymph node. This initial outgrowth consisted morphologically (Fig. 2a) of large, polygonal, vacuolated cells that slowly grew into colonies. Subculture was first carried out approximately 2 months after initial cultivation. By 5 months after initial cultivation, the cells were smaller, less vacuolated, and growing more rapidly. Tests for the presence of Mycoplasma were performed at 9 months and gave negative results. By 10 months, the cultures were growing well enough to be subcultured by trypsinization at weekly intervals at a ratio of 1:2. The cells are currently at a passage level >54 and appear as polygonal cells with decreased vacuolization (Fig. 2b). Although mostly adherent to the substrate, many cells detach and float in the medium. The detached cells stain with trypan blue and are incapable of initiating new cultures. Fibroblast growth in these cultures was minimal and failed to persist.

Cytogenetic Analysis. Analysis was first performed at passage 11. The rhesus monkey diploid karyotype 2n = 42, was nearly obliterated due to chromosome aberrations that consisted of telocentric chromosomes (not common to the karyotype), chromosome and chromatid fragments, and multiple rearrangements. Each metaphase was essentially unique. The marker chromosome for the cercopithecidea (chromosome 20) was evident. There was a broad range in chromosome counts of 48 to 60 with a modal number of 59. Subsequent analyses performed 3 and 5 months later confirmed the persistence of aberrations and the high chromosome number. The majority of metaphase spreads examined had >50 chromosomes. In addition, it was possible to identify the rhesus monkey marker chromosome, chromosome 2 (9), in most banded metaphases where it generally occurred as a single homolog.

Electron Microscopic Examination. Cells were first examined in passage four, 9 months after initial cultivation. Examination revealed the presence of numerous desmosomes, further indicating the epithelial nature of the cells, and low levels of budding and extracellular type C virus (6) (Fig. 3). The appearance of budding forms, immature extracellular virus, and mature extracellular virus is shown in Fig. 3, a to d. Mature particles measured 110 nm in diameter. The cells were examined again in passage 11, 2 months later. In this assay, cultures had been treated with 10^-8 M dexamethasone (8) in an effort to enhance virus replication. Cultures showed a low level of immature type C virus. Further observations were carried out at passages 21 and 22, 13 months after initial cultivation. Cells at passage 21 had been treated with IdUrd and then examined on Days 1 through 5 after removal of the drug. No virus forms were seen in control or IdUrd-treated cells, nor was virus observed in cells of passage 22. Intracellular type A particles were not observed at any time in the 816A cells. In cultures examined at passages >50, cells revealed abundant tonofilaments as well as numerous desmosomes.

Viral Antigens. Cell packs prepared from 816A cultures were tested for the presence of oncornavirus antigens by CF at 10.5 months after initiation of the culture. A positive reaction was found with a caprine anti-MPMV serum to an antigen titer of 1:128. No positive reactions were observed with antisera to a series of other oncornaviruses, including baboon endogenous virus, gibbon ape virus, and type C viruses of hamster, rat, mouse, and cat origins. When the cells were tested again 3.5 months later, the antigen titer had dropped to 1:8. No cross-reactions to the viruses previously tested were seen; additionally, antisera to simian sarcoma virus and mouse mammary tumor virus were negative. The antigen titer had fallen still further to 1:2 when the cells were tested again at about 14.5 months of culture age. Treatment with IdUrd at this time increased antigen level to 1:16 by the third day after removal of the drug.

Immunofluorescence assays performed on acetone-fixed cells at 8 months of culture age for Epstein-Barr virus, Herpesvirus saimiri, and papovavirus antigens were all negative. Culture fluids were tested at irregular intervals throughout the cultivation period of the cells for RDDP activity with both Mg2+ and Mn2+. All assays were negative. At 8 months, 816A culture fluids were inoculated into cultures of rhesus monkey fibroblasts in attempts at isolation of cytopathogenic agents such as simian foamy viruses. Inoculated cultures were held for 4 to 6 weeks. No tumors developed at either site of inoculation.

Nude Mouse Inoculations. At 13 to 14 months after initiation of the culture line, cells were tested in nude mice for tumor production. Ten mice were each inoculated with 3.5 x 10^6 cells by the s.c. route, and 10 mice were each inoculated with 3.5 x 10^6 cells by the i.c. route. Mice were held for 4 to 6 weeks. No tumors developed at either site of inoculation.

DISCUSSION

Although esophageal carcinomas are not uncommon in humans, there are no reports of cell lines established from such human tumors. A cell line designated Det. 562 (13) has been listed as being of esophageal origin (7), but it actually arose from pleural fluid of an adult female with metastatic adenocarcinoma that originated in the upper pharynx (W. D. Petersen, Jr., personal communication).

The spontaneous rhesus monkey tumor of greatest virological interest is the mammary carcinoma originally de-
scribed by Chopra and Mason (5). This tumor was found to contain a type D retrovirus, termed MPMV. Subsequently, MPMV was isolated from cell cultures initiated from lactating breast tissue and placenta (1). MPMV is considered to be a horizontally transmitted virus of rhesus monkeys. A cell culture line was not established from the original breast tumor.

In the 816A culture, we found morphological evidence for the presence of type C virus and serological evidence for the presence of antigen related to MPMV. The presence of virus morphologically similar to type C virus has been reported in rhesus monkey fetal cells cocultivated with Mason-Pfizer tumor cells (cultivated monkey mammary tumor cells) and in lymphoid cells infected with virus from cultivated monkey mammary tumor cells (2). These cells showed the presence of MPMV as well as type C virus. Type C virus has also been seen in rhesus monkey placental cells by electron microscopy (15). Rhesus monkey tissue has been reported to contain an antigen that cross-reacts by radioimmunoassay with the major structural antigen of endogenous baboon type C virus (17). Over the period of a few months, the 816A cells lost all morphological evidence of virus expression and showed a marked reduction in amount of MPMV-related antigen. In regard to loss of virus expression, Ahmed et al. (3) found that levels of MPMV expression varied greatly among clones of MPMV-infected rhesus monkey cells with 1 clone failing to release infectious virus. The failure to detect virus-associated RDDP activity is probably a reflection of the low and decreasing levels of virus expression in the 816A cells.

That the antigen measured by CF and the virus seen by electron microscopy might represent the same agent is a possibility that must be considered. It has been reported that the major envelope glycoprotein of MPMV cross-reacts with a similar antigen of endogenous baboon type C virus (18). The anti-PMPV serum in CF assays of 816A cells was prepared against partially purified viral antigens and undoubtedly has antibodies to several different viral antigens. In addition, it has been observed that infection of equine cells with MPMV results in the appearance of type C virus by electron microscopy. Antigenically, however, the infected cells show only MPMV-related antigens (D. Fine, personal communication). The situation is similar to that found in the 816A cells. The exact nature of the type C viruses observed in 816A and other rhesus monkey cells must await the isolation of a rhesus monkey type C virus and the preparation of appropriate immunological reagents to it. Efforts to isolate such a virus have so far been unsuccessful, but a type C virus of a related species, *Macaca arctoides*, has recently been isolated (19). Efforts are in progress to recover virus from 816A cells by a variety of cocultivation procedures.

In view of the frequency of esophageal carcinoma in humans and the lack of cell lines established from such tumors, the 816A cell line that is of primate origin may prove useful in the study of this disease.

REFERENCES

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