Long-Term Organ Culture of Human Bronchial Epithelium

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SUMMARY

Human bronchial epithelium has been maintained in organ culture in serum-supplemented medium for 4 months. After 4 to 6 weeks in culture, various changes in morphology were apparent. There was an increase in autophagic vacuoles in mucous, ciliated, and basal cells, a reduction in the height of the columnar cells, a decrease in the number of goblet mucous cells, and an increase in cells with small mucous granules. After 3 months in culture, the basal lamina was frequently covered by 2 or 3 layers of epithelial cells consisting of nonkeratinizing squamous cells with short microvilli and small mucous granules. Less frequently, keratinizing squamous cells were seen.

Differentiated epithelium incorporated precursors into macromolecules in serum-free medium, supplemented with vitamin A, at 1 week of culture. These explants exhibited changed epithelium by 2 weeks, similar to that described for epithelium in serum-supplemented medium after 4 to 6 weeks.

INTRODUCTION

This paper represents part of a larger study designed to study the effects of carcinogens on human bronchial epithelium in vitro (6, 7, 23). This and the following paper (6) demonstrate the potential usefulness of an in vitro method in elucidating the biological and metabolic effects of chemicals on human bronchial epithelium in carcinogenesis studies. In this paper we describe conditions for the organ culture of human bronchial epithelium obtained from either surgically resected lungs or from “immediate autopsies” (24). The results indicate that human bronchial epithelium can be cultured for at least 4 months and that the epithelial cells can be studied with respect to carcinogen metabolism.

Tracheas from hamsters (15), rats (5, 8, 17), and other animals (25) have been used extensively in organ culture. Lasnitzki (9) is credited with being the first to maintain human respiratory epithelium in organ culture. Since then, others (3, 4, 13, 16, 20) have maintained human bronchus in organ culture for up to 21 days in vitro. Human bronchial epithelial cells have been cultured for 4 months (21).

MATERIALS AND METHODS

Organ Culture. The bronchus was dissected from the lung by aseptic technique and placed in cold Leibowitz Medium L-15 (11) for transportation to the organ culture laboratory. The bronchus was further trimmed of lung tissue and cut into flat 2-sq cm pieces. In all cases, bronchus that was not grossly involved with tumor was used. Pieces of bronchus were placed, with the epithelium uppermost, into 60-mm plastic Petri dishes. The medium used was CMRL-1066 (Grand Island Biological Co., Grand Island, N. Y.) containing 0.1 μg hydrocortisone hemisuccinate per ml (Schwarz/Mann, Rockville, Md.), 1 μg bovine recrystallized insulin per ml (Schwarz/Mann), 100 units penicillin per ml, 100 μg streptomycin per ml (Grand Island Biological), 1 μg amphotericin B per ml (Grand Island Biological), 2 mM L-glutamine (Grand Island Biological) and 5% heat-inactivated (30 min at 56°C) fetal calf serum (Grand Island Biological). In selected short-term culture experiments, 0.1 μg β -retinyl acetate per ml (Hoffman-LaRoche, Nutley, N. J.) was added, and the inactivated fetal calf serum and amphotericin B were omitted. The culture dishes were placed in a controlled atmosphere chamber (Belco Glass Co., Vineland, N. J.) (Fig. 1) and gassed with 45% O2, 50% N2, and 5% CO2 for approximately 5 min. The chamber was then placed on a rocker platform which rocked at 10 cycles/min causing the media to flow intermittently over the epithelial surface. The explants were incubated at 36.5°C. The media and atmosphere were replaced 3 times each week.

Electron Microscopy. Explants were fixed in 4% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.4) at room temperature for 4 to 6 hr just prior to and after intervals of culture in medium with serum. In most cases, the samples were sliced perpendicular to the epithelium, prior to postfixation with OsO4 using a Smith-Farquhar tissue chopper so as to give sections that were 100 μm thick. Subsequent to glutaraldehyde fixation, the tissue sections were washed in 0.1 M sodium cacodylate buffer, postfixed in 1% OsO4 buffered with 0.1 M s-collidine, and stained en bloc with uranyl acetate. The tissues were dehydrated in a graded ethanol series and embedded in Epon 812. Areas of epithelium were chosen for thin sectioning by examining 0.5-μm sections stained with toluidine blue. Thin sections were double-stained on the grids with uranyl magnesium acetate and lead citrate and examined in a JEOL 100B electron microscope.

Radioautography. After 1 week in culture in a serum-free medium, bronchial pieces were incubated for 6 hr with either: (a) [3H]uridine (15 μCi/ml; specific activity, 27.8 Ci/m mole; New England Nuclear, Boston, Mass.); (b)
RESULTS

Morphology of Bronchial Epithelium Fixed at Zero Time.

For the most part, the epithelium consisted of a pseudostratified columnar epithelium with goblet cells, ciliated cells, basal cells, small granule or neurosecretory cells, and cells with small mucous granules that were tall and columnar with a dark cytoplasm and short microvilli; they possessed small mucous granules (Fig. 2). These will be termed SMGC.

As described by Auerbach et al. (1), areas of abnormal epithelium were sometimes encountered in the specimens fixed prior to culture, referred to as the zero time samples. These areas showed focal abnormalities, including squamous metaplasia and dysplasia in scattered areas. The most common types of focal abnormality consisted of additional layers of basal or intermediate cells, decrease in goblet cells, shortening of columnar cells, both ciliated and mucous types, and at times loss of cilia and squamous metaplasia, both keratinizing and nonkeratinizing.

The age, sex, and smoking habits of the patients and the type of tumor seen in the cases used in this study are shown in Table 1.

Organ Culture. The normal appearance of the epithelium was maintained for only 1 week when cultured in serum-free medium. After 2 weeks, there was a decrease in number and height of ciliated cells and a decrease in goblet cells, but an increase in number of dark SMGC, which were cuboidal or squamous and lined the lumen. Because of this, culture was not made for longer than 2 weeks in serum-free medium. After 1 week, incorporation of precursors into macromolecules could be demonstrated. This included incorporation of labeled uridine (Fig. 3), leucine (Fig. 4), and thymidine (Figs. 5 and 6). Labeling was seen in various cells of the epithelium. Labeled thymidine incorporation occurred in basal cells and in SMGC (Fig. 5) but was not observed in ciliated cells. Sometimes thymidine incorporation was seen in many of the cells at the edge of the culture which appeared to be growing around the edge of the explants (Fig. 6).

Explants cultured in medium containing serum maintained differentiation longer and the epithelium consisted of ciliated columnar cells, with a reduced number of goblet cells, up to 1 month. This was observed most consistently in explants from patients exhibiting minimally changed areas at the start of culture (Fig. 7). As seen in zero-time samples, microvilli and luminal surface of the apical membrane of the goblet cells were always coated more thickly with colloidal iron than either the cilia or the microvilli of the ciliated cells (Fig. 7, inset). Early during culture there was an increase in the number and size of autophagic vacuoles in some mucous, ciliated, and basal cells (Fig. 8). Acid phosphatase activity was demonstrated in these vacuoles (Fig. 9). During the 1st 2 months of culture, there tended to be a reduction in the height of columnar cells (Fig. 8, inset). The Golgi apparatus was very prominent in these cuboidal cells and the lamellae of the maturing face were stained with PTA3 (Fig. 7, inset). Early during culture there was an increase in the number and size of autophagic vacuoles in some mucous, ciliated, and basal cells (Fig. 8). Acid phosphatase activity was demonstrated in these vacuoles (Fig. 9). During the 1st 2 months of culture, there tended to be a reduction in the height of columnar cells (Fig. 8, inset). The Golgi apparatus was very prominent in these cuboidal cells and the lamellae of the maturing face were stained with PTA; cell surface, mucous granules, and lysosomes were also stained (Fig. 10). Cuboidal SMGC with short microvilli and a prominent glycoalyx (Fig. 11, inset) were frequently encountered after 2 months in culture, and mucus was present only as small granules at the luminal surface (Fig. 11). Ciliated and goblet cells were rare, although in 2 cases they were relatively numerous after 3 months. Cuboidal cells containing both cilia and mucus were seen more frequently than in zero-time samples (Fig. 12).

After 3 months in culture, the basal lamina was often covered by 2 or 3 layers of cells. The surface cells in such areas consisted mainly of flattened nonkeratinizing squamous cells with short microvilli and small mucous droplets. A few of these cells showed early keratinization. The cytoplasm contained mitochondria, profiles of endoplasmic re-

[3] The abbreviations used are: PTA, phosphotungstic acid; SMGC, small mucous granule cells.
Table 1
Age, sex, diagnoses, and smoking histories of patients used in this study

<table>
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<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Sex</th>
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<td>SCC</td>
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<td>M</td>
<td>Abscesses and fibrosis</td>
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<td>10 cigarettes/day for 40 yr</td>
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<td>Smoked cigarettes for many years</td>
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* SCC, squamous cell carcinoma; AC, adenocarcinoma; LCC, large cell carcinoma; HI, head injury.
* Smoking habits were obtained from a retrospective review of the patient's medical record in hospital charts.

SMGC that had short microvilli and a prominent glycocalyx. An important factor in the interpretation of the changes that occur under these conditions is the nature of the epithelium at the beginning of the culture period. As is well known from the work of Auerbach (1), the bronchial epithelium of human patients with smoking histories, especially those with bronchogenic squamous cell carcinomas, show variable numbers of abnormal areas including metaplasia, dysplasia, and other changes. Although such changes were, indeed, present in the starting material used in this study, careful evaluation of all 50 cases so far selected indicated that focal areas of abnormality were noted in about 10 to 20% of the area of the surface epithelium. The morphology of the epithelium was variable throughout the 1st 2 months of culture. This may have been due at least in part to the variable state of the epithelium at the start of culture. After 2 months of culture, increasing occurrence of nonkeratinizing (SMGC) and keratinizing squamous cells suggests that the culture conditions, although maintaining viability after that time, could not maintain normal differentiation. Although the mechanism of this morphological change is not presently known, it is conceivable that it involves the vitamin A content (27) which, in the present study, only consisted of that present in the fetal calf serum added. Furthermore, since the fetal calf serum was heat inactivated, this would tend to further decrease the vitamin A content.

It is important to note, however, that the maintenance of viable human epithelium in organ culture for 17 weeks and of differentiated epithelium for 2 months is considerably longer than has been previously reported, although others...
(21) have maintained explanted bronchial epithelial cells, some with cilia, for 4 months without additional vitamin A. Modifications of the culture medium such as adding vitamin A could perhaps result in even better maintenance. The principal changes occurring during the early time intervals consisted of decreased numbers of goblet cells and increased numbers of autophagic vacuoles. The former is thought to represent effective discharge of the mucus and the latter is a common accompaniment of organ culture (22).

REFERENCES


Organ Culture of Human Bronchial Epithelium

1. Photograph of the culture apparatus.
2. Micrograph showing the organ culture.
3. Higher magnification of the epithelial cells.
4. Detailed view of the cellular structure.
5. Sectional view of the epithelium.
6. Close-up of a specific region of interest.

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