Dear Editor:

At the Southwest Foundation for Biomedical Research, a premature baboon (Papio cynocephalus) model has been developed and successfully used to study the etiology, pathobiology, and therapy of bronchopulmonary dysplasia (BPD) (1,2). These infants display a multitude of clinical problems including fibrosis and metaplasia of the bronchiolar and bronchial epithelium. Recent evidence suggests that lung fibroblasts from premature, ventilated baboons secrete a growth factor(s) which may impact on the changes in cell populations seen in BPD (5). In this letter, we report on our initial efforts to culture lung epithelial cells from premature baboon infants and on findings that indicate that these cells secrete a trypsin-sensitive factor(s) which stimulates fibroblast proliferation.

Fetal baboon lung epithelial (FBLE) cells were isolated from lung tissue obtained from two female baboons delivered prematurely by hysterotomy at 140 days (full-term gestation is 180 days). The deliveries were 7 weeks apart. One animal (1Y89) was intubated and ventilated for 24 hr with minimal oxygen to maintain blood gasses. At the time of sacrifice approximately one-third of the upper left lobe of the lung (0.69 g) devoid of extrapulmonary airways was obtained and placed in cold Hanks’ balanced salt solution (HBSS) supplemented with antibiotics. The second animal (2R89) was sacrificed at delivery and a portion of the upper left lobe (0.61 g) was obtained. Specifics of the management and study of these premature animals have been described (3). The lung tissue was finely minced with scissors and treated at 37°C with 20 ml F12 medium containing 0.25% protease XIV (Sigma Chemical). After 30 min, tissue clumps were allowed to settle and the supernatant, containing unsettled cells, was collected with a pipette. These cells were pelleted from the supernatant by centrifugation (300 × g/5 min) after adding fetal bovine serum (FBS) to a 10% concentration. Cell pellets were resuspended in 10% FBS/M199 medium containing penicillin, streptomycin and fungizone and incubated in a 775 cm² flask in a 5% CO₂:95% air atmosphere (37°C). After 2 hr, the culture medium was removed from flasks, passed through a 46 μm nylon mesh (Tetko, Inc.) and centrifuged to pellet unattached cells. Culture of cells that attached to flasks during the 2 hr interval was continued in the serum-supplemented medium. Pellets of the unattached cells were resuspended in a 1:1 mix of F12/M199 medium containing 20 ng/ml epidermal growth factor (Collaborative Research), 5 μg/ml insulin (Sigma Chemical), 10 μg/ml transferrin (Sigma), 75 μg/ml hypoxanthine extract, 3 × 10⁻⁷ M hydrocortisone (Sigma), 50 ng/ml cholera toxin (Sigma), and 10 ng/ml all-trans retinol (a gift from Hoffmann-LaRoche) (4,6). The cells were seeded in 35 mm dia. culture dishes and refed at 2–3 day intervals.

In primary cultures in the growth-factor-supplemented medium, FBLE cells were initially observed as small colonies which frequently contained beating, ciliated cells (Fig. 1 A). After 2 weeks, enlarged epithelial colonies were present surrounded by fibroblasts (Fig. 1 B,C). At this point, cloning rings (6 mm i.d.) were used with 0.25% trypsin to preferentially subculture the FBLE cells. Passage 1 FBLE cells proliferated rapidly in the growth-factor-supplemented medium with few fibroblasts apparent. For both isolations, subsequent passages at 1/4 split ratios were judged free of fibroblasts (Fig. 1 E,F). FBLE cell proliferation continued for 8–10 subculturings, then slowed, and stopped altogether between passages 11–13. Continued culture of cells in the 2 hr attachment flasks in 10% FBS/M199 medium yielded pulmonary fibroblasts which were readily subcultured and later used in growth assays (Fig. 1 D).

FBLE cells in culture were examined ultrastructurally following fixation in 1.2% glutaraldehyde and post fixation in 1% Zetquist’s buffered osmium tetroxide. Fixed monolayers were embedded in polybed 812 and sections stained with uranyl acetate and lead citrate. For both isolations, cells proliferated with a columnar to cuboidal morphology and possessed other distinctive epithelial features such as surface microvilli (Fig. 2 A,B). Positive cytokeratin staining with purified anti-cytokeratin (human) antibody (Becton-Dickinson, CAM 5.2) also confirmed the epithelial nature of the FBLE cells (data not shown).

Conditioned medium (CM) was prepared from both isolations of FBLE cells at Passage 5 and assayed for growth stimulating activity using pulmonary fibroblasts. As shown in Fig. 3 A, CM significantly stimulated the growth of pulmonary fibroblasts when used at levels constituting 10–33% of a basal 1% FBS/M199 medium in comparison to controls exposed only to basal medium (Fig. 3 A, none). If CM was pretreated at 37°C for 2.5 h with 10 μg/ml trypsin as previously described (5), the CM-stimulated growth of pulmonary fibroblasts was significantly reduced (Fig. 3 B, 33% + trypsin). In contrast, CM simultaneously pretreated with trypsin and 20 μg/ml soybean trypsin inhibitor (STI) for the 2.5 h retained the ability to stimulate pulmonary fibroblast proliferation (Fig. 3 B, 33% + trypsin + STI). CM similarly stimulated the growth of 3T3 fibroblasts (3T3-L1, CCL 92.1, American Type Culture Collection) (data not shown).

In summary, a procedure is outlined for the isolation of fetal primate lung epithelial cells from small amounts of lung tissue. Initial findings here also indicate that these cells produce an extracellular, trypsin-sensitive factor(s) capable of stimulating the growth of lung fibroblasts which should be of interest to pulmonary cell biologists.
Fig. 1. FBLE cells in culture. A, epithelial colony in primary culture 5 days. ×25. B, epithelial colony surrounded by fibroblasts in 16 days. ×25. C, same as B. ×50. D, pulmonary fibroblasts in a 2 hr attachment flask. ×25. E, FBLE cells, passage 2. ×25. F, FBLE cells, passage 3. ×50.