THE EFFECTS OF PROTEINS SECRETED BY FIBROBLASTS FROM PATIENTS WITH CYSTIC FIBROSIS ON HAMSTER TRACHEAL EXPLANTS

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SUMMARY

Hamster tracheal explants have been used to assay for mucosecretory activity in media taken from cultures of fibroblasts isolated from patients with cystic fibrosis (CF). Cystic fibrosis and normal sera were first used to establish optimal conditions for mucus release in the hamster tracheal ring assay. Unless protein levels were maintained at 5% serum concentration or greater there was loss of cilia, nonspecific mucus accumulation, and extensive epithelial damage to the luminal surface. Likewise, it was shown that exposure of the explants to unconcentrated conditioned media from CF (GM 770, 768, 1348, 142) or normal (GM 3349, 38) cultured fibroblasts for 1, 6, or 12 h resulted in the same type of damage and this was due to low protein levels. When the protein concentration of the conditioned media was increased with fetal bovine serum, the morphological integrity of the explants was maintained, demonstrating that there was no apparent difference between CF and normal fibroblast-secreted proteins in ability to induce mucus release.

The ciliary inhibitory capacity of CF serum-derived or fibroblast-derived factor had been reported to require IgG for activity. However, addition of IgG to high molecular weight (VoP10) or low molecular weight (VeP10) secreted proteins had no apparent effect on stimulating secretion. In conclusion, it is possible that CF fibroblasts do not secrete a protein that has the mucostimulatory effect and thus these cells may not be suitable for studying the CF-related activity.

Key words: cystic fibrosis; fibroblasts; mucus; secretion.

INTRODUCTION

Cystic fibrosis (CF) is a disease with multiple clinical aberrations including: ion transport abnormalities, pulmonary disease, and digestive disorders. Clinical manifestations vary among affected subjects and may include one or all of these symptoms. It has been proposed that humoral protein(s) is (are) responsible for these widespread symptoms (10,11,18,25). Using bioassays that measure ciliary dyskinesis and ciliotoxicity, circulating proteins have been detected in the serum (3,4,7,12,15,19), saliva (13), and urine (17) of CF patients. Furthermore, the serum protein has been shown by several investigators (3,4,7,15,19) to induce mucus release from the luminal surface of rodent tracheal ring explants. However, it is not known whether these humoral proteins are identical, nor is it clear whether any one of them is responsible for both stimulating secretion and inducing ciliotoxicity. Recent work with sera from CF patients indicates that first, the mucus-stimulating response may be separable from the activity that affects ciliary move-

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a prerequisite for the ciliary inhibition activity of the CF fibroblast protein (2) whereas others do not find this to be the case (1,5,26). Furthermore, it has been assumed that the fibroblast protein has many properties in common with the serum counterpart, but in fact the documentation is insufficient to either support or refute this assumption.

One problem in doing research with the CF fibroblast factor is lack of a reliable method to assay for its presence and activity. Tracheal explants from rabbit, hamster, and guinea pig have been used in conjunction with the light microscope to detect CF serum protein by looking both for changes in ciliary movement and for hypersecretion (3,19,26,27). We have demonstrated in a preliminary report that hamster tracheal explants do seem to respond to CF sera by releasing mucus as observed by scanning electron microscopy (22). However, the optimal requirements for use of explants to detect the CF fibroblast factor or the serum protein or both in such bioassays to minimize false positive results have not been reported in the literature. Thus, the first objective of this study was to determine the optimal conditions under which the hamster tracheal ring bioassay might be used as a qualitative system to observe morphological changes, specifically mucus release, induced by a presumptive CF serum protein. This type of defined system could then be used as a relatively reliable way to assay for CF protein during its purification. The second objective of the present study was to use the defined hamster ring assay to determine if CF fibroblasts produce and secrete protein(s) that will induce mucus release from epithelial tissues in a manner analogous to that found with CF serum. If it were demonstrated that CF fibroblasts produce a protein that stimulates mucus release, then they could provide an ideal in vitro system for the study of the production of these molecules.

MATERIALS AND METHODS

Chemicals. Ham's F12 medium was purchased from K.C. Biological, Lenexa, KA; fetal bovine serum (FBS), normal human pooled serum, and trypsin/EDTA were from GIBCO Laboratories, Grand Island, NY. Hoechst reagent 33258 was obtained from Aldrich Chemical Co., Inc., Milwaukee, WI; [4,5-F73H]L-leucine (55 Ci/mmol) was from ICN, Irvine, CA; BioGel P10 (100 to 200 mesh) and DEAE Affi-gel blue came from BioRad Laboratories, Richmond, CA; and ENHANCE and Aquasol were purchased from New England Nuclear, Boston, MA. Sheep antihuman IgG was from Cappell Laboratories, Richmond, CA; and ENHANCE and Aquasol were purchased from New England Nuclear, Boston, MA. Hoechst reagent (20) All column chromatography was performed at 4°C.

Hamster tracheal explants. Outbred male golden Syrian hamsters, strain CR R6H (SYR), were obtained from Harlan Sprague Dawley, Inc., Madison, WI. Only hamsters of less than 4 mo. of age were used as tracheal donors. Hamster tracheas were removed surgically under sterile conditions, as described by Collier (9). After excision the tracheas were placed in phosphate buffered saline plus glucose (PBS-glc: 8.0 g NaCl, 0.2 g KCl, 1.15 g Na2HPO4 + 2.0 g glucose/l) and excess connective tissue was removed gently with forceps. The cleaned tracheas were then placed into Ham's F12 medium, cut into rings, and transferred to F12 plus 10% FBS followed by incubation at 37°C in a humidified 95% air:5% CO2 atmosphere for a period of 24 h, before experimental treatment. All rings were examined microscopically for cytomegocysis before use in experiments and only those that seemed healthy as judged by lack of mucus accumulation and by having active ciliary activity were employed. For experimentation, rings were placed in 35-mm culture dishes or, in some instances, single rings were placed into separate sterile Eppendorf microfuge tubes with Ham's F12 medium and test protein. If Eppendorf tubes were used, the ring was at an air-liquid interphase to prevent asphyxiation and the tube was only loosely capped to allow the air-CO2 mixture in the incubator to reach the ring. Samples were taken with time and processed for observation with the scanning electron microscope.

Fibroblast cell culture. Human skin fibroblast strains were obtained from the Human Genetic Mutant Cell Repository, Camden, NJ, and are designated as follows: GM3349 and GM38 are normal human fibroblasts; GM1348, GM142, GM768, and GM770 are CF fibroblasts from...