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Mucus-Stimulating Activity in the Sera of Patients with Cystic Fibrosis: Demonstration and Preliminary Fractionation

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Summary

The ciliated, mucus-secreting urn cell complex (UCC) is found swimming in the coelomic cavity of the marine invertebrate *Sipunculus nudus*. This cell complex, which can be maintained in suspension cultures, responds to various stimuli by hypersecreting mucus in the form of a cohesive mucus "tail." This tail can be measured and expressed as a multiple of "urn cell diameters." Using this bioassay, heated sera from 35 patients with cystic fibrosis (CF), 29 patients who were obligate heterozygotes for the CF gene, and 42 controls with a variety of diseases were tested. Control sera yielded a mean (± S.D.) mucus tail length of 2.5 (± 2.3); CF sera yielded a mean mucus tail length of 7.5 (± 2.9), ($P < 0.0005$), and obligate heterozygote sera yielded a mucus tail length of 6.2 (± 2.1), ($P < 0.0005$). These responses were reproducible with different UCC suspensions from the same *Sipunculus*, as well as from different *Sipunculi*.

In addition, sera from 3 CF patients and 3 controls were chromatographed on protein A-Sepharose. The bound IgG fraction was then washed with 8 M urea and subsequently eluted with 1 M acetic acid. Pooled dialyzed, lyophilized fractions were assayed as coded samples in the UCC assay. Mucus-stimulating activity as measured by mucus tail length per mg protein was greatest in the fractions eluted with 8 M urea. The 8 M urea fractions from 3 CF sera were 2.8 to 5.5 times as active as fractions from 3 control sera.

The UCC assay can quantitatively measure mucus-stimulating activity in CF serum. This activity appears to be associated with a serum fraction which can be dissociated from IgG.

Speculation

The urn cell complex assay can quantitatively demonstrate the presence of a mucus stimulating substance(s) in the sera of patients who are homozygous or heterozygous for the cystic fibrosis gene(s). Such a bioassay may prove useful in the purification and characterization of this substance(s).

Cystic fibrosis (CF) is the most common potentially lethal genetic disease occurring in the Caucasian population (16). Although the basic defect is unknown, evidence suggests that CF is transmitted in an autosomal recessive manner. Inasmuch as Farber (17) first observed the sticky nature of the mucus secretions of the respiratory and gastrointestinal tracts of patients with CF, it has appeared that the abnormal physicochemical behavior of these mucus secretions is the pathophysiologic basis for many of the clinical manifestations of this disease.

In 1967, Spock *et al.* (23) reported the presence of a serum factor in patients with CF which disrupted normal ciliary movement in rabbit tracheal explants. Subsequently, the ability of CF serum to alter mucus production and ciliary action in other *in vitro* mucociliary systems was noted (7, 11). More recent investigations have indicated that this dyskinetic effect upon ciliary function may be secondary to an increased discharge of mucus (15, 18). All of these assay systems are bioassays and qualitative or semiquantitative in nature. As such, they are subject to the inherent variability of any bioassay (10). A more quantitative assay would be useful for the isolation and characterization of this serum factor (or factors).

One such potential system is the ciliated, mucus-secreting "urn cell complex" (UCC) found free swimming in the coelomic cavity of the marine invertebrate *Sipunculus nudus*. This cell complex serves a host defense function by hypersecreting mucus which differs in rate, quality, and quantity in response to specific stimuli. The mucus secretion can be measured because it is produced in the form of a cohesive mucus "tail." This unique cell complex was first described in 1928 (14). More recently, Bang and Bang (1–4) have further characterized this cell and demonstrated that it will respond to certain human biological fluids *in vitro* by secreting mucus (2). In addition, they have demonstrated that this free-swimming mucociliated cell complex may prove useful in the study of the regulation of mucus secretion (2).

The purpose of this study was two-fold: (1) to determine the feasibility of using the UCC assay to quantitatively demonstrate the presence of a serum factor(s) in CF serum which induce and/or alter mucus production; and (2) to evaluate the usefulness of this system in the isolation and characterization of CF serum factor(s). It was found that this UCC assay could quantitatively differentiate control sera which generated a small amount of mucus from sera from either patients with CF or obligate heterozygotes which generated more mucus. Furthermore, this mucus stimulating substance(s) could be dissociated *in situ* from IgG bound to protein A-Sepharose by 8 M urea with subsequent elution of bound IgG by 1 M acetic acid. This procedure is a new method to obtain UCC mucus-stimulating factors.

MATERIALS AND METHODS

SERUM SAMPLES

After obtaining informed consent, blood was obtained by venipuncture and placed immediately in a polypropylene test tube (Corning No. 25330, Corning, NJ, or Falcon No. 2059, Oxnard, CA) and refrigerated at 4°C for 6 to 18 hr and then centrifuged at $1000 \times g$ for 20 min at 4°C. Serum was then transferred with a siliconcoated glass pipette to plastic aliquot tubes (Falcon No. 2063) and stored at −70°C. Each aliquot was then thawed and stored in an ice-water bath immediately before use.

Stored samples of sera were obtained from 35 patients with CF, 29 adults who were obligate heterozygotes for the CF gene, and 42 controls who were either healthy or had an illness which mimicked a clinical aspect of CF. Table 1 lists the diseases which were represented in the control group. Table 2 compares the control and CF groups for age, sex, and race.

URN CELL COMPLEX SUSPENSION

Urn cell complex suspensions were obtained by Dr. and Mrs. Bang following the procedure described in Bang and Bang (1, 3). The suspensions were stored in capped plastic test tubes (Cole Scientific No. MC-10, Calagasas, CA) at 4°C in an upright position to allow heavy sediment and cells to settle. Only the supernatant containing the UCC was used for experiments.

SEAWATER

A stock supply of seawater was obtained from the Marine Biology Laboratory, Woods Hole, MA. On the day of assay, seawater was boiled for 3 to 5 min and passed through a 0.20 μm filter (Nagle Sybron Corp., Rochester, NY) and stored in a sterile container in an ice-water bath. This is called boiled, filtered seawater (BFSW) (pH 7.9) at 4°C.

ASSAY PROCEDURE

Nine hundred microliters BFSW was mixed in a polypropylene test tube (Falcon No. 2063) with 100 μl of thawed test serum. The test tube was capped, and the mixture was heated in a water bath at 85°C for 10 min. The sample was then removed and immediately placed in an ice-water bath for 10 min. After cooling, 40 μl of this assay mixture (pH 7.88 at 4°C) were placed in the well of a depression microscope slide, and 10 μl of UCC suspension were added. This mixture was allowed to incubate at room temperature in a humidified setting for 10 min. The slide was examined at × 100 under a light microscope. The mucus tail lengths of 10 to 20 cell complexes were measured and divided by the diameter of the globular cell of the UCC. Mucus tail length was expressed as a multiple of urn diameter. This mean tail length was then computed along with the standard deviation for each test sample.

Table 1. *Control group (42)*[1]

Normal (17)
Pulmonary disease (14)
Upper respiratory infection
Acute pneumonia
Recurrent pneumonia
Asthma
Bronchitis
Atelectasis
Gastrointestinal disease (6)
Gluten sensitive enteropathy
Crohn's disease
Gastroenteritis
Alpha-1-antitrypsin deficiency
Pancreatic insufficiency (Shwachman's)
Neonatal cirrhosis
Immunodeficiency (3)
Common variable
IgA deficiency
Mucocutaneous candidiasis
Other (8)
Congenital heart disease
Failure to thrive (maternal neglect)
Sinusitis
Allergies
Leukemia

[1] Some patients are included in multiple categories.

CHROMATOGRAPHIC SPECIFICATIONS

Columns (1.6 × 20 cm), adaptors, Protein A-Sepharose CL-4B (lot CD 0252) were purchased from Pharmacia Fine Chemicals (Piscataway, NJ). Fractionation procedures and dialysis were carried out in a cold room maintained at 4°C. The pH of all buffers and solutions (always prepared fresh in deionized water) was determined at 4°C against a standard at 4°C. Column fractions were collected in polypropylene tubes (Falcon) using an LKB 7000 fraction collector with an in-line LKB Uvicord II 8300 (percentage of transmission) and LKB strip chart recorder (LKB, Rockville, MD). The absorbance of column samples at 280 nm was measured on a Beckman Model 25 spectrophotometer (Palo Alto, CA) equipped with the Return Sipper Accessory using plastic tubing and a microcuvette. A Duostaltic pump (Buchler Instruments, Fort Lee, NJ) was used to pump samples and buffers on columns. Samples were dialyzed in Spectrapor 3 tubing (Spectrum Medical Industries, Los Angeles, CA) with a molecular weight cut off of 3500.

CHROMATOGRAPHY ON PROTEIN A-SEPHAROSE

Dry protein A-Sepharose was swollen in deionized water for 2.5 hr at room temperature followed by washing three times with 0.010 M sodium phosphate (Fisher Certified ACS, Pittsburgh, PA) pH 7.4 at room temperature. The swollen gel was allowed to equilibrate overnight in this buffer in the cold room. Two columns were packed and equilibrated with the 0.010 M sodium phosphate (pH 7.4) buffer. One column was used only for control sera, and the other was used only for sera from CF patients. The ratio of 1 ml of serum per 2 ml of swollen gel was maintained in the fractionations. Thawed serum was pumped on to the column (1.6 cm in diameter) at a flow rate of 25 to 30 ml/hr. The bound IgG was washed with the sodium phosphate buffer until the absorbance returned to baseline, at which time 8 M urea (electrophoresis purity; Bio-Rad Laboratories, Richmond, CA) in the sodium phosphate buffer (pH 7.4) was started (the pH was adjusted to a measured pH of 7.4 with a small amount of 0.1 N HCl after addition of urea). When the absorbance returned to baseline, 1 M acetic acid (pH 2.8) was initiated to elute the bound IgG. Pools of the flush through, 8 M urea, and eluted IgG fractions were made on the basis of absorbance at 280 nm. Each pool was dialyzed for 36 hr against 3 × 4000 ml of 5 mM ammonium bicarbonate (Fisher Scientific certified, Pittsburgh, PA). The pH was 7.82 at 4°C. Samples were lyophilized and reconstituted in 0.010 M sodium phosphate (pH 7.4) for testing in the UCC assay. All column samples were tested as coded samples. The protein A-Sepharose was regenerated with sodium phosphate buffer and reused about six times. A serum sample was held in the cold room during the fractionation procedures and tested in the bioassay.

PROTEIN DETERMINATIONS

Lyophilized samples were weighed and reconstituted in 0.010 M sodium phosphate (pH 7.4) buffer. Protein was also determined

Table 2. *Characteristics of patients*

	Age		Sex		Race	
	Mean (yr)	Range	Female	Male	White	Black
Control	14.8	2 mos.–43 yr	17	25	31	11
CF	9.3	1 mo.–29 yr	13	22	32	3

by the method of Lowry *et al.* (21) with bovine serum albumin (Sigma A04503; Sigma Chemical Co., St. Louis, MO) in 0.85% (w/v) saline (Fisher Scientific Biological Grade) as a standard as outlined elsewhere (13).

IMMUNOLOGIC TECHNIQUES

Antisera to human IgG (H and L chain specific, prepared in horses) and whole human serum (prepared in rabbits) was purchased from Behring Diagnostics (Somerville, NJ). Ouchterlony double diffusion (22) and immunoelectrophoresis (19) were set up as detailed elsewhere (6).

RESULTS

EFFECTS OF SERUM CONCENTRATION

Sera from a normal control and a patient with CF were diluted in BFSW at varying dilutions from 1:5 to 1:50. These dilutions were then assayed as outlined in "Materials and Methods." As can be seen in Figure 1, dose-response curves resulted for both normal and CF sera. In the case of the CF serum, a linear relationship was noted between increasing serum concentrations, and the mucus tail length produced up to a dilution of 1:10 at which point the mean mucus tail length was 9.4. At a dilution of 1:5, the amount of coagulated material prevented any meaningful determination of the mucus tail length. In the case of the normal serum, the mean mucus tail length leveled off at 1.6 and remained constant over a range of increasing serum concentration.

EFFECT OF HEATING SERUM

Sera from normal controls and patients with CF were diluted to a concentration of 1:10 in BFSW. Each of these samples was then divided into four aliquots. Each aliquot was then incubated at one of the following temperatures for 10 min: 0, 56, 85, and 100°C. The assay was then performed as indicated in "Materials and Methods." No mucus tails were secreted by the urn cells in response to either sera from normal controls or CF patients at 0, 56, or 100°C. At 85°C, characteristic mean mucus tails were produced by the normal and CF sera.

EFFECT OF SERUM-PROCESSING TIME

Samples of blood from patients were placed in polypropylene test tubes and allowed to clot for 6 to 18 hr at 4°C. To evaluate the effect of this range of processing times, blood from a patient with CF was centrifuged at one and 18 hr after venipuncture. These samples were then aliquoted and frozen at −70°C. These sera were then tested in the bioassay. Sera processed at one hr produced a mean mucus tail length of 8.4 (± 3.0), whereas sera processed at 18 hr produced a mean mucus tail length of 10 (± 1.4). Thus, the processing time did not seem to significantly alter the length of the mucus tail produced by a particular serum sample.

CONSISTENCY OF UCC RESPONSE

An UCC suspension obtained from a given *S. nudus* remained viable for approximately one month in a test tube at 4°C. Subsequently, a second *Sipunculus* was bled to obtain another UCC suspension. In Figure 2, one can see the consecutive responses elicited by control and CF sera retested with UCC from the same culture over the period of a month. As can be seen, the mean mucus tail responses are reproducible within the range of standard deviation of each assay.

Aliquots of serum from each of 3 patients, obtained from single venipunctures, were stored and subsequently tested in different urn suspensions from different *Sipunculi* over the period of many months. As shown in Figure 3, the mucus responses are consistent and reproducible.

STUDY OF PATIENT SAMPLES

Figure 4 summarizes the data of this study. The mean mucus tail lengths produced by each group were as follows: CF (7.5 ± 2.9), controls (2.5 ± 2.3), and obligate heterozygotes (6.2 ± 2.1). Both sera from the group of CF patients and sera from obligate heterozygotes produced significantly greater mucus responses than did sera from the control group ($P < 0.0005$). However, there was no statistically significant difference between the CF and obligate heterozygote groups. Nine of the CF patients in Figure 4 had not previously been treated with pancreatic enzymes, antibiotics, or other medications common in the treatment of CF. Most of these patients were newly diagnosed cases. As can be seen from Figure 4, their ability to stimulate mucus secretion spanned the range of responses seen in the CF group.

CHROMATOGRAPHY ON PROTEIN A-SEPHAROSE

Sera (in amounts of 1.2 to 2.1 ml) from three controls and from three patients with CF were chromatographed on protein A-Sepharose. A quantity of control serum and an equal volume of

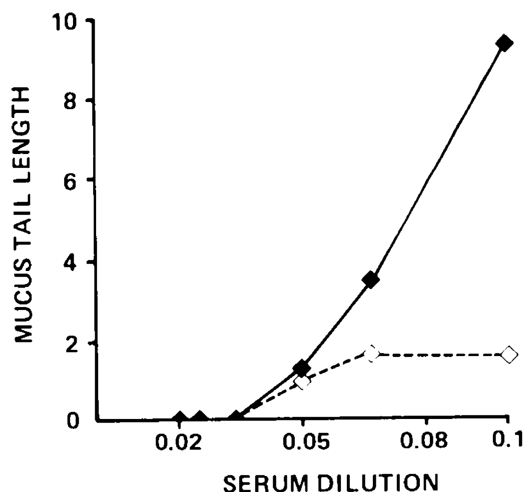

Fig. 1. The relationship between mucus-secretion ability and serum dilution with BFSW for serum from a patient with CF (◆) and a normal control (◇).

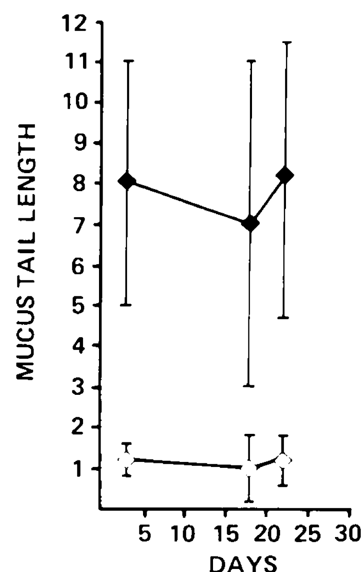

Fig. 2. The consistency of mucus-stimulating responses in the same urn cell suspension from one *S. nudus* over the period of one month. Serum from a patient with CF (◆) and a normal control (◇) are compared. *Vertical lines*, 1 S.D.

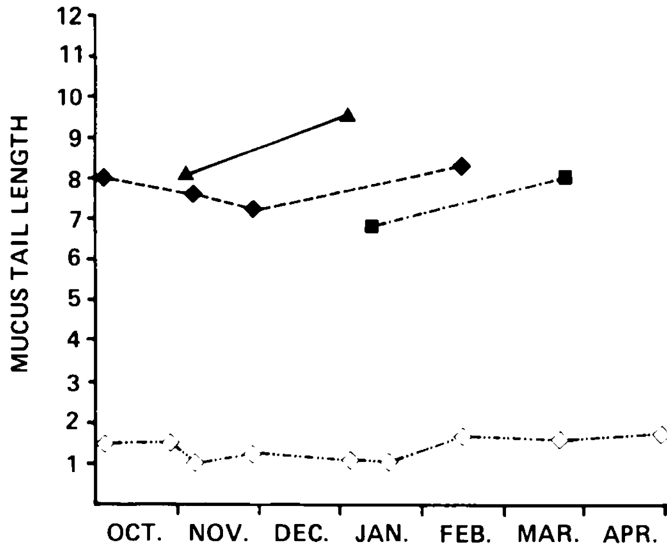

Fig. 3. The consistency of mucus-stimulating responses for individual stored serum samples in multiple urn cell suspensions from different *Sipunculi.* Sera from 3 individual CF patients are shown (◆, ▲, ■) as well as one control patient (◇).

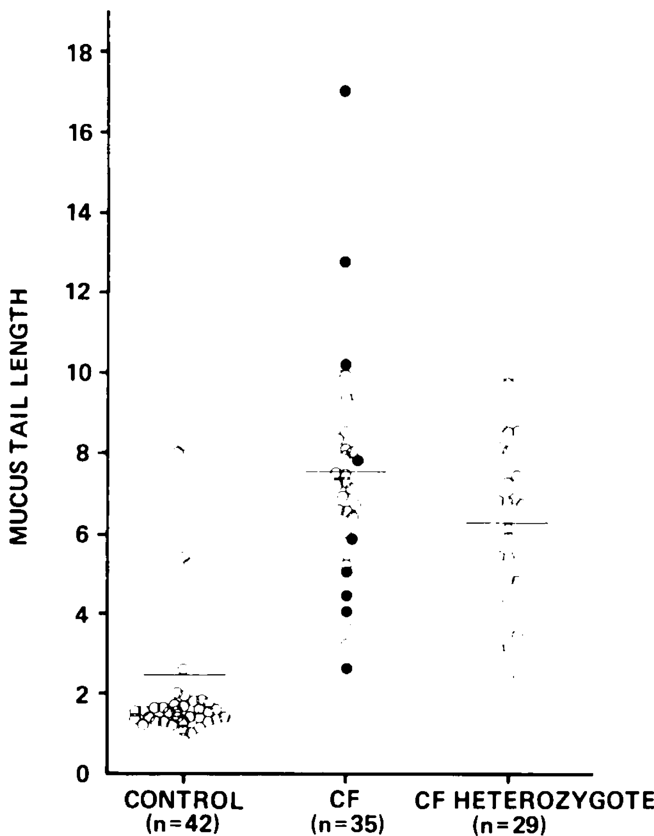

Fig. 4. Mucus-stimulating responses of control groups, patients with CF, and patients heterozygous for CF gene. *Horizontal line,* mean for each group. In the column of CF patients, ●, patients who have not been treated with medications common in CF. Most are newly diagnosed patients.

CF serum were each processed concurrently to make comparisons of results from the UCC assay. In addition, a serum sample of each was maintained at 4°C during the fractionation procedures. The urn tail lengths of these sera were similar to values obtained from freshly thawed aliquots stored continuously at −70°C. The absorbance of 280 nm plotted against fraction number from serum chromatographed on Protein A-Sepharose is shown in Figure 5. Similar patterns were obtained for the three CF sera and three control sera with the exception that the amount of protein eluted with 8 M urea varied (but in no consistent manner) in the small number of sera tested. Specific activity was defined as the urn tail length per mg of protein. Table 3 shows the specific activity data obtained from a set of coded samples in a typical urn cell assay. In this particular assay, the BFSW alone generated zero tail length per urn (the usual value), and the 0.010 M sodium phosphate (pH 7.4) generated 1.0 tail length per urn (usual value is zero to 0.5). The ratio of the CF 8 M urea specific activities to control 8 M urea specific activities were 2.8:1, 3.8:1, 5.5:1 (shown in Table 3 in the three paired experiments).

IMMUNOLOGIC STUDIES

The 8 M urea pools from the protein A-Sepharose columns (after dialysis, lyophilization, and reconstitution in buffer) were studied by Ouchterlony double diffusion experiments and immunoelectrophoresis. Both control and CF 8 M urea fractions generally revealed the presence of three to four arcs when tested

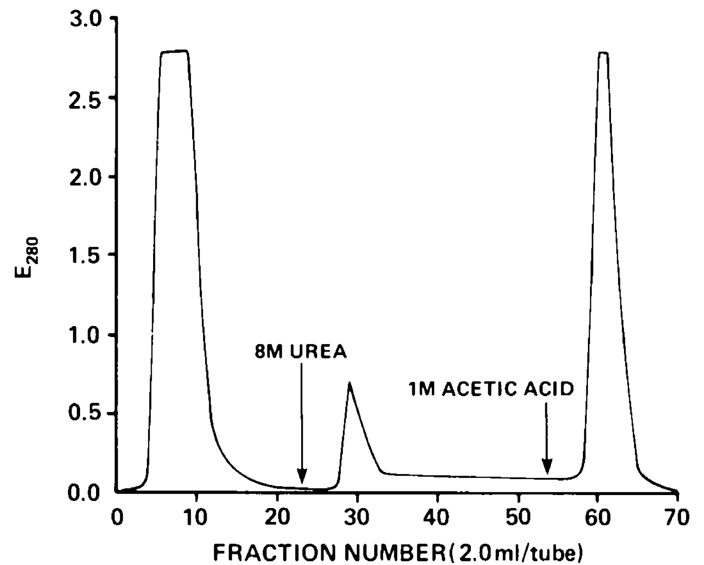

Fig. 5. Chromatography on protein A-Sepharose. Thawed control serum (2.1 ml) was pumped on to the protein A-Sepharose column (1.6 × 2.5 cm) at a flow rate of 30 ml/hr. The bound material was washed with 0.010 M sodium phosphate (pH 7.4) until the absorbance at 280 nm (——) returned to baseline at which time 8 M urea (↓) in the phosphate buffer at pH 7.4 was started. When the absorbance returned to baseline, 1 M acetic acid (pH 2.8) (↓) was started to elute bound IgG. Pools (based on the absorbance at 280 nm) were made as follows: flush through fractions 4 to 13; 8 M urea fractions 28 to 35; and eluted IgG fractions 58 to 65.

Table 3. *Fractionation of serum on protein A-Sepharose*

Sample	Specific activity	
	Tail length [mg protein (Lowry)]	Tail length [mg protein (dry)]
N-Flush through[1]	0	0
N-8 M urea[1]	132.6	134.6
N-Bound[1]	8.5	9.2
CF-flush through[1]	2.2	3.0
CF-8 M urea[1]	732.5	725.0
CF-bound[1]	30.2	34.7
N serum 4°C	4.5	ND[2]
CF serum 4°C	24.2	ND

[1] Pools were made on the basis of absorbance at 280 nm.
[2] Not determined.

against antibody to whole human serum: (1) in both, a fast α mobility arc was always present, and (2) two to three arcs with γ mobility were also present. At least one of the latter arcs was identified as IgG and/or IgG fragments by both immunoelectrophoresis and Ouchterlony double diffusion experiments against anti-human IgG.

The material which bound to the protein A-Sepharose and was eluted with 1 M HOAc was verified as IgG by immunoelectrophoresis. In each case, the bound IgG arcs exhibited complete fusion when tested against antibody to whole human serum in one trough and antibody to IgG in the other trough. Furthermore, each bound fraction showed only one arc of γ mobility when tested against anti-whole human serum.

DISCUSSION

A number of *in vitro* bioassays of mucociliary function have demonstrated the presence of an abnormal factor(s) in the serum of individuals who are homozygous or heterozygous for the CF gene (7, 11, 23). Further characterization of this serum factor suggests that it is a low-molecular-weight substance (less than 10,000), positively charged, and noncovalently associated with IgG (8, 9, 12). Nevertheless, the characterization of this factor has been hampered by the lack of a reliable, quantitative assay.

The free-swimming mucociliated urn cell complex responds to specific stimuli by secreting a cohesive mucus tail which can be observed and quantitatively measured. In the present study, the mucus-stimulating ability of sera from patients with CF, obligate heterozygotes for the CF gene, and a panel of controls was examined. The sera of CF patients and obligate heterozygotes produced significantly longer mucus tails than did a panel of control sera ($P < 0.0005$) (Fig. 4). However, the mucus-stimulating ability of sera from homozygotes and heterozygotes was indistinguishable. As can be seen in Figures 2 and 3, the assay produced a similar quantitative response to a particular serum whether tested over the course of a month in UCC suspensions from the same *Sipunculus* or over the course of many months in UCC suspensions obtained from different *Sipunculi.*

One intriguing aspect of the results in Figure 4 is that the sera of seven individuals in the control group elicited marked mucus secretion in the urn cell assay. Pertinent clinical data of these individuals is listed in Table 4. Four of these individuals had clinical signs suggestive of CF, but each had a sweat test(s) which was in the normal range. Three individuals did not manifest any signs suggestive of CF and did not have sweat tests performed. None of these individuals had a family history of CF. The meaning of these results is difficult to determine at this time with the small number of individuals studied. In a control population of 42, of which 31 are Caucasian, one would predict that one, and possibly two, might be heterozygous for the CF gene because the carrier rate in the Caucasian population is thought to be 1:20 (24). Clearly, all of these individuals are unlikely to be carriers of the CF gene. It is more likely that multiple substances may result in mucus-secreting activity in the urn cell assay. Further investigations may help to clarify these results.

Table 4. *High control group*

Patient	Diagnosis	Medications	Sweat test
2mo. W. M.	Cirrhosis	None	Normal
4mo. B. M.	RUL[1] atelectasis	Ampicillin	Normal
6mo. W. F.	Shwachman's syndrome bronchiolitis	Pancreatic Enzyme	Normal
8yr W. M.	Leukemia, remission	None	ND[2]
11yr W. M.	Recurrent bronchitis	Erythromycin	Normal
14yr W. F.	Leukemia, remission	None	ND
22yr W. F.	Normal	None	ND

[1] RUL, right upper lobe.
[2] ND, not determined.

In addition, many questions remain to be answered in regards to the nature of this bioassay. Future work will be devoted to the further refinement of the quantitative aspects of this bioassay as well as to clarification of its physiochemical basis. For instance, why is the incubation of serum at 85°C or the addition of seawater essential to elicit a mucus stimulating response?

The results obtained from the UCC assay suggest that sera from patients who are either homozygous or heterozygous for the CF gene either contain a mucus-stimulating substance which is not found in most control sera or contain a nonspecific artifact which results in elevated mucus-stimulating activity. The following aspects of this study support the former hypothesis. The mucus-stimulating activity of CF sera is not dependent on the processing time of the sera. In addition, the CF group and control group were matched as closely as possible for age and sex although these is a preponderance of black patients in the control group as compared to CF group (Table 2). This was felt to be justifiable as the CF gene statistically occurs less commonly in the Black population (20). Perhaps, most importantly, this study was able to demonstrate a linear dose-response curve in CF sera (Fig. 1) suggesting the presence of a substance in this serum, whose mucus-producing activity could be diminished by dilution. In addition, this mucus-stimulating activity does not appear to be due to circulating factors which are secondary to the various therapeutic modalities used in CF, such as pancreatic enzymes, antibiotics, or mucolytic agents. Nine CF patients (Fig. 4) were newly diagnosed and had not previously received any therapy commonly used in CF. This point is further supported by examining the control population. Many of the patients with recurrent or chronic pneumonias (who had low mucus-stimulating activity in their serum) were treated with the same antibiotics used in the treatment of exacerbations of pulmonary disease in CF. In addition, two patients in the control group received pancreatic enzymes: one infant with Shwachman's syndrome whose sera resulted in marked mucus-stimulating activity (8, 12), and one infant who had been previously misdiagnosed with CF. Her serum showed low mucus-secreting activity (1.6).

Additional evidence for the presence of a specific mucus-stimulating substance in the sera of CF patients was provided by the second thrust of this study: to utilize the UCC assay to identify mucus-secreting activity during the process of fractionating serum to purify the mucus-secreting substance. Previous studies have demonstrated that the putative CF ciliary dyskinesia factor is aosassociated with IgG in serum and can, in fact, be dissociated, resulting in a low-molecular-weight substance (9). Preliminary experiments in our laboratory indicated that the mucus-stimulating activity in CF sera is associated with IgG (5). To proceed to the next step, serum was fractionated on protein A-Sepharose in a manner which has not been previously described. Protein A-Sepharose binds human IgG (subclasses 1, 2, and 4) through its Fc portion. We reasoned that it might be possible to "dissociate" the mucus-stimulating substance from bound IgG *in situ* with a reagent like 8 M urea.

Our preliminary results with sera from 3 CF patients and 3 controls would indicate that it is possible to dissociate a mucus-stimulating substance from IgG bound to Protein A-Sepharose with 8 M urea (see Table 3 and Fig. 5). The detection of IgG in the 8 M urea fraction by immunoelectrophoresis and Ouchterlony double diffusion experiments may be due to: (1) removal of heavy and/or light chains with or without the mucus-stimulating substance from the bound IgG by 8 M urea; or (2) removal of IgG with or without the mucus-stimulating substance(s) bound to IgG by the 8 M urea. Further characterization of these 8 M urea pools is in progress. The "flush-through" pools have little or no mucus-stimulating activity for both CF and control patients. The bound material may have some mucus-stimulating activity (from either incomplete dissociation or perhaps a different factor), whereas the majority of the mucus stimulating activity is associated with the 8 M urea pool from CF sera. The 8 M urea pool of control sera also has some mucus-stimulating activity. However, the specific activity of the CF 8 M urea pool is 2.8- to 5.5-fold greater than the specific activity of the control 8 M urea pool. These prelimi-

nary experiments suggest that the UCC assay can indeed be used to follow fractionation of mucus-stimulating substance(s).

CONCLUSION

In conclusion, the urn cell complex system seems to provide a quantitative and reproducible bioassay for the study of mucus-stimulating substances in general and in CF in particular. In addition, preliminary evidence has suggested that this assay may be useful in the fractionation of serum for the purposes of characterizing the mucus-stimulating substance(s) in CF. Data in the present study suggests that the physical properties of this substance may be similar to, if not identical to, the ciliary dyskinesia factor(s). Future collaborative studies may help to clarify this point.

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25. The risks of venipuncture to human subjects in this study were approved by the Joint Commission on Clinical Investigation of the Johns Hopkins University. In accordance with their guidelines, informed consent for venipuncture was obtained from the parents of all children and from adults who participated as subjects in this work.

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