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α_2-Macroglobulin
chronic pancreatitis
cystic fibrosis
proteinases
trypsin-binding Ig

Binding of ^{125}I-Labeled Proteinases to Plasma Proteins in Cystic Fibrosis

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Summary

Samples of plasma or serum from 53 cystic fibrosis (CF) patients, 90 relatives of CF patients, and 159 controls have been incubated with porcine or bovine ^{125}I-trypsin, electrophoresed on polyacrylamide gel, and autoradiographed. In these individuals, the main binding protein for ^{125}I-trypsin has been shown to be α_2-macroglobulin (α_2M). Using this method of analysis, no difference in electrophoretic migration of ^{125}I-trypsin-α_2M complexes has been observed between CF and control individuals. However, trypsin binding to IgG has been observed in 80% of CF patients, 30% of their mothers, 3% of controls, and in two patients affected with pancreatitis. These trypsin binding immunoglobulins are called TbIg, and specifically, TbIgG when referring to the G class.

Experimental evidence indicates that binding of trypsin to IgG occurs through the Fab portion of the molecule. TbIgG must be antibodies most probably induced by the exogenous trypsin ingested daily by most CF patients (and by patients affected with chronic pancreatitis).

Antibodies against porcine pancreatic elastase have been observed using the same analysis, but not as frequently as TbIg.

Speculation

Antibodies against trypsin are known to have an inhibitory capacity on its enzymatic activity: is TbIg responsible, at least in part, for the decrease in arginine esterase activity observed by some authors in plasma of CF patients and of some heterozygotes?

The presence of TbIgG in CF patients raises again the interesting question of an autoimmune process in CF, directed not only against structural pancreatic proteins but also against human pancreatic enzymes.

In spite of extensive investigation, the primary molecular abnormality of CF remains unknown. Among other findings, it has been reported that an α_2M-protease complex is missing in most CF patients (26, 32) and that binding of trypsin and other proteinases to α_2M is decreased in plasma of CF patients with respect to normal controls (25, 32). In addition, a deficiency of arginine esterase activity has been reported in CF patients (22). In interpreting these observations, it is important to establish their relevance to the pathogenesis of CF, a disease in which a host of secondary phenomena are known to occur.

Trypsin binding Ig is present in most CF patients and in some CF relatives, as already reported by the authors (23). This immune response to porcine and, possibly, human trypsin might aid in interpreting some of the current observations on decreased arginine esterase activity.

MATERIALS AND METHODS

Venous blood was collected from CF patients, CF relatives, and normal controls, some of whom were matched for age with the CF patients and their parents (36). All samples were collected at Stanford Children's Hospital and processed immediately. ACD (trisodium citrate 22 g/liter, citric acid 8 g/liter, dextrose 24.5 g/liter) in the ratio of 0.15 ml/ml blood was used for the collection of plasma. Samples were kept at 4° for not more than 4 hr and stored in aliquots of 20 and 400 μl in plastic microfuge tubes at −70°.

RADIOLABELING OF PROTEINASES

Among the proteinases studied were the following: porcine trypsin (Miles), bovine trypsin (Worthington, Sigma), papain (Worthington), bovine thrombin (Sigma), and porcine elastase (Worthington). The proteinases were radioiodinated using the chloramine T method (14) and free ^{125}I was removed by Sephadex G-25 or Dowex anion exchange chromatography. Stock solutions of ^{125}I-trypsin were stored at −70° for periods of up to 3 months in 1 mM HCl containing 1 mg/ml of bovine serum albumin. Specific activities of 20 mCi/mg were usual; preparations of higher specific activity were found to be less useful due to extensive breakdown. When further purification of commercially available trypsin was performed before iodination, this was achieved using column chromatography on SP-Sephadex (24) or gel filtration on Sephadex G-75. Carrier free $^{54}MnCl_2$ and ^{125}I were purchased from NEN and Amersham, respectively.

SAMPLE ANALYSIS

To 20 μl aliquots of serum or plasma, 5 μl of ^{125}I-proteinase (approximately 30,000 dpm) were added and the mixture was incubated at 37° for 30 min. Preliminary experiments indicated that binding to plasma proteins was complete at this time. Storage of plasma and serum samples at 4° overnight or at −70° for a period up to 8 months did not show any effect on binding. Because manganese has been reported to be selectively bound by α_2M (12), in another series of experiments, 20 μl aliquots of serum or plasma were incubated either at 37° or at 4° with 5 μl of ^{54} Mn containing 5000 dpm.

Samples were analyzed by discontinuous alkaline polyacrylamide gel electrophoresis (PAGE) at 4° followed by autoradiography, as previously described (4). A 4% acrylamide separating gel (5% crosslinking) was found to give the best resolution of the plasma proteins of interest. To allow the sample to migrate through the stacking gel a voltage of 6 V/cm was applied; separation was accomplished using a potential difference of 12 V/cm. Electrophoresis was considered complete when a bromophenol blue tracer dye reached the end of the gel. After drying, the gels were exposed to autoradiographic film (Kodak XR-5) for approximately 5 days.

IDENTIFICATION OF BANDS

The identification of proteins which bind trypsin was performed using various immunologic methods. Antisera (Behring and Atlantic Antibodies) against individual human plasma proteins were tested for monospecificity against human serum before use. Immunoelectrophoresis on agar gel (20) was performed on aliquots

of samples previously incubated with ^{125}I-trypsin and after the appearance of a precipitin line, the slides were dried and autoradiographed. The presence of a radioactive arc corresponding to the precipitin line was considered evidence of trypsin binding.

A modified immunoelectrophoresis technique was used to identify bands on polyacrylamide gel. After PAGE, portions of the gel adjacent to the sample were removed and agar was poured around the gel strip. Troughs were cut in the agar and monospecific antiserum was applied. Use of agar was necessary because the diffusion of large molecules in acrylamide gel is quite slow. Resulting arcs specified the location of the protein after PAGE. The arcs were examined for radioactivity by autoradiography.

Laurell antigen-antibody crossed immunoelectrophoresis (16) was also performed. PAGE was the first dimension and agarose gel containing monospecific antiserum was the second. The first dimension was run as described earlier and the second was performed at 4° using a potential difference of 3 V/cm overnight. Gels were rinsed, dried, and stained with Coomassie Blue R-250. The presence of a rocket indicated the location and the quantity of the protein in the polyacrylamide gel.

Haptoglobin was stained with O-dianisidine (21) after electrophoresis on a 4.0 or 7.5% polyacrylamide gel. Polyacrylamide gels were stained for protein with Coomassie Blue R-250 as described in LKB Application Sheet RB423.

PAPAIN DIGESTION OF IGG

To obtain specific cleavage of the IgG molecule, serum of CF patients and normal controls was precipitated by the addition of an equal volume of saturated $(NH_4)_2SO_4$, redissolved in phosphate buffered saline and incubated with papain using modifications of published procedures (27).

The resuspended precipitate (10 mg/ml protein concentration) was mixed with an equal volume of 0.1M phosphate buffer, pH 6.7, containing 4 mM Na_2EDTA and 0.02% Na Azide. 2-Mercaptoethanol was added to a final concentration of 0.02 M. Papain (Worthington PAP, Lot #37J611) was added to reach 1% of the protein concentration (w/w) and this mixture was incubated for 16 hr at 37°C. The digestion was stopped by the addition of 1 M iodacetamide in 1 M Tris-HCl pH 7.4 to 10% excess of the 2-mercaptoethanol and the mixture was dialyzed overnight against 0.01 M Tris-HCl pH 7.4.

Digest samples of 20 μl were incubated with 5 μl of ^{125}I-trypsin (50,000 dpm) and 4 μl aliquots were loaded on 1% agar gel slides. Electrophoresis was run for 90 min at 4 V/cm. Antisera (Atlantic Antibodies) against human IgG, Fab fragment, and Fc fragment were applied in the troughs. After the appearance of a precipitin line, the slides were wrapped in damp bibulous paper (VWR Scientific), dried, and exposed for one wk to autoradiographic film (Kodak XR-5). The presence of a radioactive arc corresponding to the precipitin line was considered evidence of trypsin binding.

RESULTS

IDENTIFICATION OF ^{125}I-TRYPSIN BANDS ON PAGE

Samples of plasma or serum from CF patients, presumed heterozygotes, and controls were incubated with porcine or bovine ^{125}I-trypsin, electrophoresed on PAGE, and autoradiographed as described under *Methods*. The band patterns visible after autoradiography (Fig. 1) were as follows: 1) The most anodal radioactive band shown in Figure 1 migrated with the tracking dye, bromphenol blue, and was present in all samples as well as in trypsin alone (well no. 19). In gels of higher concentration of acrylamide, this band was resolved into two components, the slower of which was seen in samples of ^{125}I-trypsin mixed with serum or plasma, but not in samples of trypsin alone. This slower band contained little radioactivity and was identified by antigen-antibody-crossed immunoelectrophoresis as α_1-antitrypsin (Fig. 2). The faster component was always present, and represents nonaggregated trypsin and/or trypsin fragments, as indicated in the next paragraph. 2) Next, a series of radioactive bands was present in samples of trypsin incubated without serum or plasma. Commercial preparations of trypsin are known to contain a mixture of different forms of this enzyme: α, β, and pseudotrypsin (15), differing in one or more specific peptide cleavages resulting from autocatalytic digestion. However, the mobilities of pure α and β trypsin (33) after radioiodination were not distinguishable from those produced by commercially available trypsin.

Further purification of porcine trypsin by SP-Sephadex chromatography (24) before iodination did not alter the pattern of bands seen. However, in the presence of 1% BSA (or other protein), the slowest free trypsin band is absent. Chromatography of noninhibited ^{125}I-trypsin over BioGel A.5M at pH 7 reveals high molecular weight species (Fig. 3) not found in the unlabeled material or in the presence of the irreversible inhibitor tosyl-lysyl-chloromethyl-ketone (TLCK). This evidence suggests the formation of aggregates, probably resulting from the iodination procedure and autodigestion.

IDENTIFICATION OF ^{125}I-TRYPSIN-α_2M COMPLEXES

When ^{125}I-trypsin was incubated with serum or plasma, a series of slow moving bands appeared in the upper portion of the gel (Fig. 1). These were not present when the enzyme was preincubated with an irreversible inhibitor such as TLCK, nor were they found if the plasma was preincubated with monospecific antiserum against α_2M. They were further identified as α_2M-trypsin complexes by immunoelectrophoresis on PAGE using monospecific antiserum against human α_2M (Fig. 4). No difference was found between CF patients and controls with respect to the number and/or mobility of these bands.

Using the same system of electrophoretic and autoradiographic analysis, the authors investigated the binding between plasma proteins and $^{54}MnCl_2$. ^{54}Mn has been reported to be selectively bound by α_2M and transferrin in ovine and bovine serum (12). As shown in Fig. 5, serum and plasma from a normal control and a CF patient incubated with ^{54}Mn showed two bands, with the band of ^{54}Mn-labeled α_2M migrating on PAGE with the first of the multiple bands produced by interaction of α_2M and ^{125}I-trypsin. In our PAGE system, the mobility of ^{54}Mn labeled α_2M present in plasma of nine CF patients was identical with that of normal controls.

The multiple bands identified by monospecific antiserum as ^{125}I-trypsin α_2M complexes (Fig. 4) resemble those produced by haptoglobin (Hp) of individuals expressing the Hp^2 allele. In samples from Hp 1-2 and 2-2 individuals, the majority of Hp migrates faster than α_2M in our PAGE system, although higher order polymers may be present in the upper portion of the gel. The radioactive multiple bands did not react with monospecific antiserum against human Hp whereas they did with antihuman α_2M. Furthermore, samples from 5 individuals of Hp 1-1 phenotype, whose haptoglobin does not polymerize, clearly demonstrate the slow moving multiple radioactive bands when analyzed in this system.

IDENTIFICATION OF TRYPSIN BINDING IG (TBIG)

An unexpected difference between CF patients and controls was observed using the PAGE system of analysis. As can be seen in Figure 1, the fast moving bands of trypsin were clearly preserved in the presence of serum or plasma from normal individuals, but were missing when trypsin was mixed with sera or plasma of CF patients. In the latter samples, however, radioactive material appeared in the γ-globulin region, *i.e.*, toward the cathodal end of the gel. Immunoelectrophoresis in agar gel of ^{125}I-trypsin incubated with sera of control individuals and of CF patients, followed by autoradiography (Fig. 6a), clearly confirmed that the "missing" trypsin bands in CF samples are actually bound to IgG. In addition, two patients affected with chronic pancreatitis and some mothers of CF patients showed the same phenomenon. It was

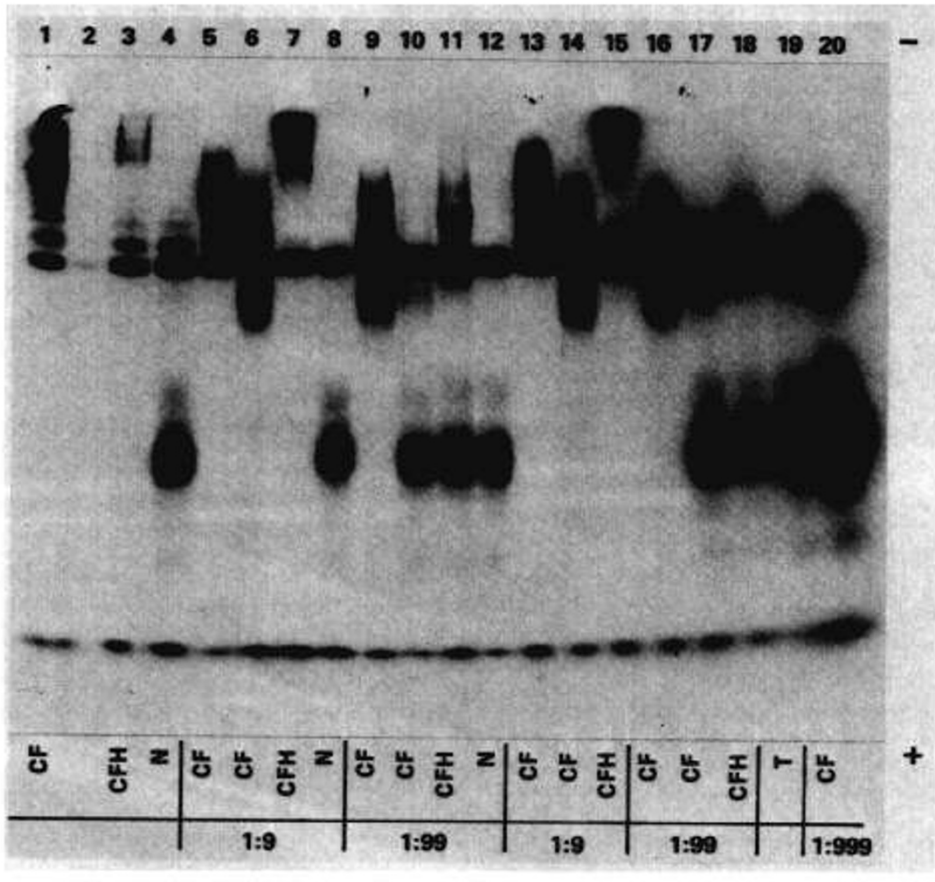

Fig. 1. Analysis of ^{125}I-trypsin binding to plasma proteins of two CF patients, a CF heterozygote, and a normal control by PAGE and autoradiography. Titration of TbIgG was performed by serially diluting plasma of two CF patients and a CF heterozygote in saline or in plasma of a normal control followed by incubation with ^{125}I-trypsin. Wells 1, 3, 4 contained undiluted plasma of patient CF 865, CF heterozygote (CFH) 914, and a normal control, respectively. Wells 5–8 contained plasma of CF 865, CF 862, CFH 914, and a normal control diluted 1:9 with saline. Wells 9–11, and 12 contained plasma of CF 865, CF 862, CFH 914, and a normal control diluted 1:99 with saline. Wells 13–15 contained plasma of CF 865, CF 862 and CFH 914 diluted 1:9 with normal plasma. Wells 16–18 contained plasma of CF 865, CF 862, and CFH 914 diluted 1:99 with normal plasma. Well 19 contained only ^{125}I-trypsin in sucrose. Well 20 contained plasma of CF 865 diluted 1:999 with normal plasma. This technique, although not quantitative, indicates a very high titer of TbIgG in plasma of CF 865.

noted that, in some patients, IgA also binds trypsin, but less regularly than IgG. These immunoglobulins are called TbIg or trypsin binding Ig. The class of Ig that has been most extensively investigated for trypsin binding is the gamma or G class. When this class has been specifically identified it is designated as TbIgG. TbIgG can also bind trypsin inhibited by TLCK or soybean trypsin inhibitor (STI).

PAPAIN DIGESTION AND IMMUNOELECTROPHORESIS EXPERIMENTS

The experimental evidence which supports the antibody nature of TbIgG is based on the capacity of Fab fragments to bind trypsin. The monovalent Fab fragment (as well as Fc) was produced by papain digestion and identified by agar gel immunoelectrophoresis. When these were incubated with ^{125}I-trypsin, only the Fab fragment from CF patients retained the capacity to bind trypsin (Fig. 6b). Binding was also observed with STI inhibited trypsin.

TbIgG are nonprecipitating antibodies as shown by the following experiments. When serial dilutions of a CF serum were incubated with STI inhibited ^{125}I-trypsin, at concentrations ranging between 2×10^{-6}M and 2×10^{-8}M, no radioactive trypsin could be precipitated using the conditions of a standard radioimmunoassay (incubation for 3 hr at 37° and overnight at 4°, with subsequent centrifugation at 10,000 *g* for 30 min). Likewise, when serial dilutions of sera from 10 CF patients were tested against ^{125}I-trypsin (either free or inhibited by STI or TLCK) by Ouchterlony gel diffusion technique (20) and autoradiography, no radioactive precipitin line could be revealed.

SCREENING FOR TBIGG

The experiments just described indicate the presence of an immune response to trypsin in CF patients, in patients affected with chronic pancreatitis, and in some CF relatives. The PAGE-autoradiography system proved to be a sensitive method for detecting TbIgG by the experiment reported in Figure 1, in which serial dilutions of CF serum in saline or in serum of normal controls were performed. One of the two CF sera analyzed in this experiment had a very high titer of TbIgG, revealed by the reappearance of the free trypsin radioactive bands on PAGE only after 1000-fold dilution of the patient's serum.

In order to investigate the correlation between TbIgG and positive history of porcine trypsin intake, a screening was carried out of 53 CF patients (Table 1). This correlation is quite strong. All the patients who showed a positive PAGE test had taken pancreatic extracts, suggesting that their ingestion is necessary for the production of TbIgG. The average age of patients that are negative by this test is higher than that of the remaining patients, and the Schwachman clinical scores (7) indicate they are more mildly affected. Using the same system, 159 normal controls were screened (some of whom were matched for age with our patients) and four positive individuals were found: two of these had been in close contact with CF patients, one being a nurse who works with CF children and the other the husband of a CF woman. Two

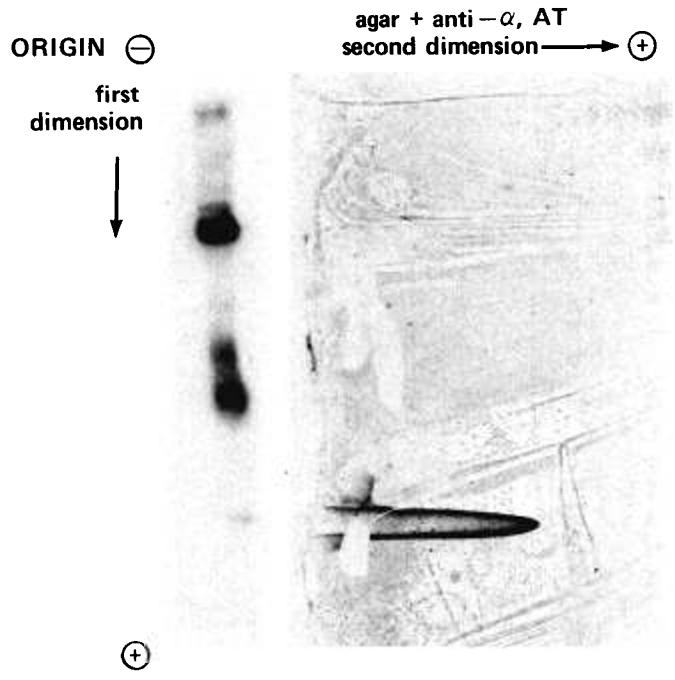

Fig. 2. Identification by Laurell antigen-antibody crossed immunoelectrophoresis of complexes formed by ^{125}I-trypsin and α_1-antitrypsin. The first dimension electrophoresis was run on PAGE as described under *Methods*. The agarose used for the second dimension electrophoresis contained anti-α_1-antitrypsin antiserum (Atlantic Antibodies) at 40 μl/ml.

additional adult controls were tested for TbIg because they were affected with chronic pancreatitis and had been taking pancreatic extracts for more than 1 yr (35). They were both positive by PAGE and by immunoelectrophoresis. Also tested by PAGE were 90 parents and sibs of CF patients (Table 2): of 41 mothers, 11 were positive and 30 negative, whereas the 30 fathers and 19 sibs were all negative. Because powdered pancreatic extracts have been reported to cause allergic responses in parents of CF children (8, 30) exposure of relatives of CF patients to such preparations was investigated. Of 11 PAGE-positive mothers, only 6 recollected being exposed to powdered pancreatic extracts and of the 20 PAGE-negative ones who were tested, 10 indicated exposure. Thus, among 16 mothers who declared to have been exposed to trypsin, 6 are PAGE-positive and among the 15 not so exposed, 5 were PAGE-positive. The difference between 5/16 and 5/15 is clearly negligible. The possibility remains that immunization occurs not so much because of exposure to powdered pancreatic extracts, but because of handling uncoated tablets of pancreatin.

In addition to trypsin, radioactively labeled papain, bovine thrombin, and porcine elastase were tested with plasma or sera from CF patients and controls. No difference was found between CF patients and controls in binding to papain or thrombin. The binding of α_2M to pancreatic elastase gave a pattern of multiple bands similar to that found with trypsin (Fig. 7). If the absence of a free elastase band is taken to indicate the presence of antielastase antibodies (a criterion which was found to be fully reliable for trypsin, as explained before) only 3 of 15 patients and none of the relatives or controls have detectable antielastase antibodies. All the 15 patients were positive for TbIgG. The differences noted between 15 CF patients, 28 controls, and 17 CF relatives are summarized in Table 3.

DISCUSSION

The correlation between presence of TbIgG in CF patients and a history of intake of pancreatic extracts is strongly suggestive of an immunization process against trypsin. The antibody nature of TbIgG is supported by the capacity of Fab fragments of the IgG molecule from CF patients to bind radioactive trypsin.

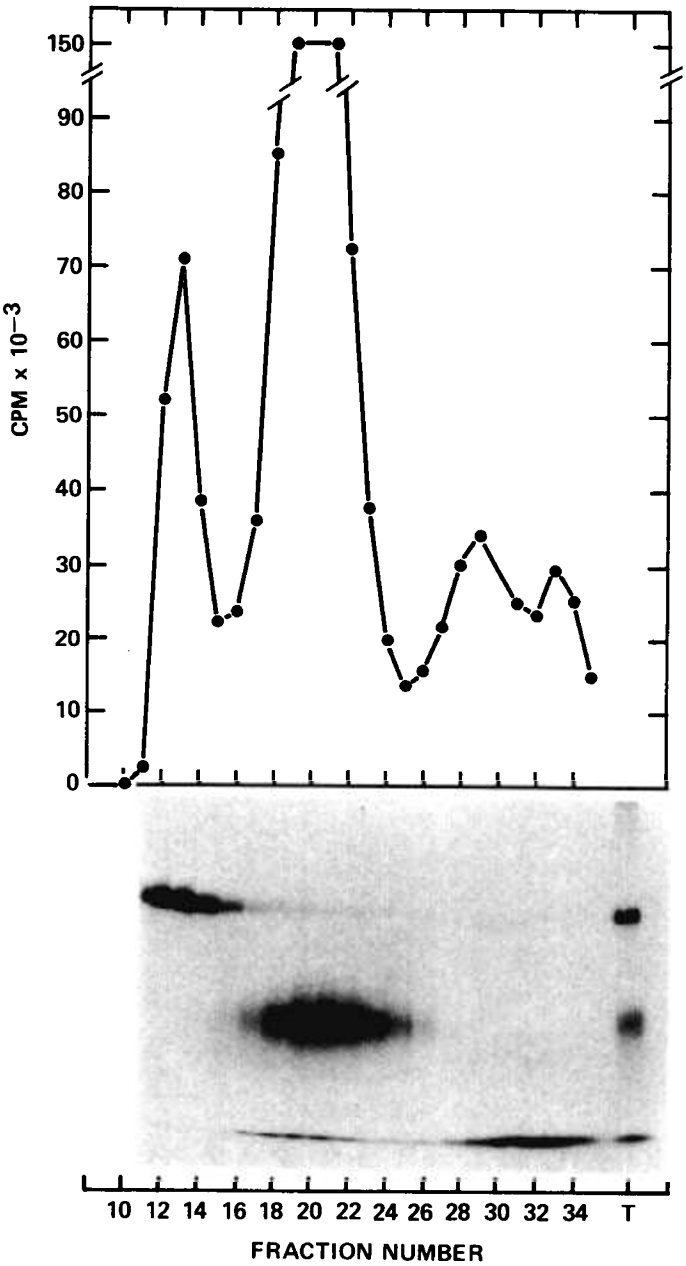

Fig. 3. Biogel A.5M fractionation of ^{125}I-porcine trypsin. ^{125}I-porcine trypsin in 1 mM HCl was diluted with 50 mm tris-HCl pH 7.2 and chromatographed over Biogel A.5M equilibrated in the same buffer containing 0.5 mg/ml BSA. The 30 × 1.2 cm column was run at 6 ml/hr and 10 min fractions were taken. The graph shows the amount of radioactivity contained in each fraction and the autoradiogram shows the pattern obtained after PAGE electrophoresis of these same fractions. As described in *Methods*, well "T" contain the unfractionated ^{125}I-trypsin. Ferritin (m.w. 540,000) elutes at fraction 11 and cytochrome C (m.w. 12,500) at fraction 33 using the same column.

Several questions relevant to present research on CF arise from our observations. It has been demonstrated that rabbit antibodies against trypsin inhibit the activity of the enzyme toward both high and low molecular weight substrates (1). Their affinity for trypsin increases with prolonged immunization and is proportional to their inhibitory capacity (10). The level of immunoreactive trypsin, which cannot be distinguished from trypsinogen by radioimmunoassay (2, 11), is considerably decreased in plasma of patients affected with chronic pancreatitis (9). As suggested by finding TbIgG in two such patients, this might be due to the presence of antibodies, which bind circulating trypsin and trypsinogen *in vivo*.

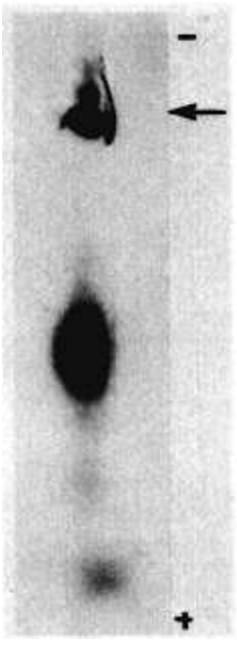

Fig. 4. Identification of α_2-macroglobulin-^{125}I-trypsin complexes after PAGE and autoradiography. Normal plasma was incubated with ^{125}I-trypsin, electrophoresed by PAGE, and the α_2-macroglobulin complexes were identified by monospecific antiserum applied in the trough to the right of the sample as described in *Methods.*

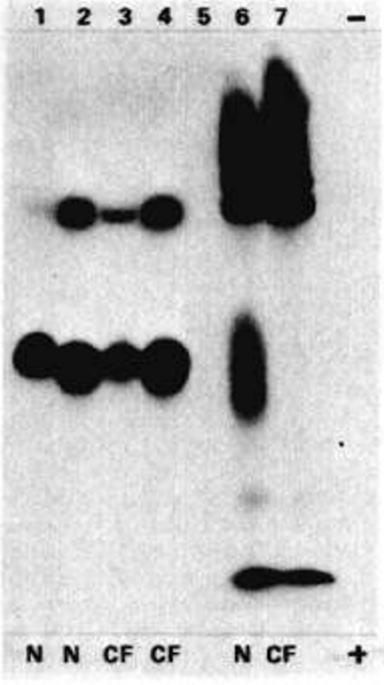

Fig. 5. Identification of α_2-macroglobulin migration by PAGE followed by autoradiography after incubation with $^{54}MnCl_2$ or ^{125}I-trypsin. Wells 1–4 contained sera or plasma incubated with $^{54}MnCl_2$ from a normal control or a CF patient. Wells 6 and 7 contained serum from either of the same individuals which had been incubated with ^{125}I-trypsin. Note the similarity in migration between the ^{54}Mn labeled-α_2-macroglobulin and the most anodal band of the ^{125}I-trypsin-α_2-macroglobulin complex.

Plasma proteinases are usually present in the circulation as enzymatically inactive precursors; these proteinases can be activated by a variety of treatments including chloroform and ellagic acid (22). A decrease of "trypsin-like" or arginine esterase activity

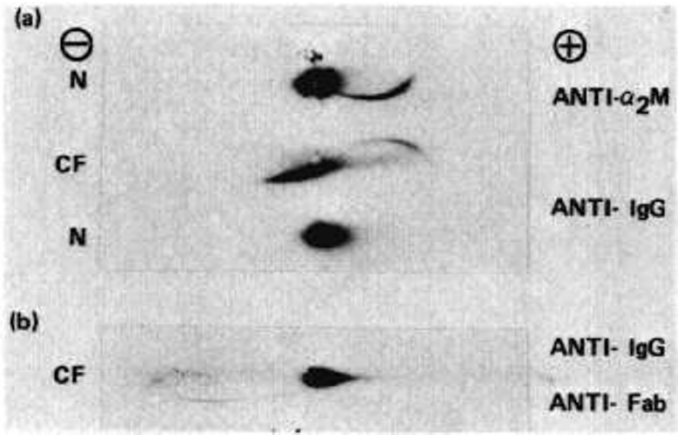

Fig. 6. *a*) Immunoelectrophoresis in agar gel of ^{125}I-trypsin incubated with sera of control individuals (*N*) and of CF patients. The upper trough contained antihuman α_2-macroglobulin antisera. The lower trough contained antihuman IgG antisera. Radioactive arcs indicate ^{125}I-trypsin bound to α_2-macroglobulin and in CF patients also to IgG. *b*) Immunoelectrophoresis in agar gel of ^{125}I-trypsin incubated with CF sera after papain digestion. Antihuman IgG (γ chain specific) was applied to the upper trough and antihuman Fab antisera to the lower trough. The radioactive arc indicates the Fab fragment's capability for binding ^{125}I-trypsin. Normal sera when treated in the same manner did not show binding of ^{125}I-trypsin.

Table 1. *PAGE test results in 53 CF patients*

	Total (%)	Mean Age (± SE)	History of trypsin intake[1] +	History of trypsin intake[1] −	Sex Male	Sex Female
Positive	42 (80)	15.0 (1.1)	42	0	22	20
Negative	11 (20)	22.9 (± 2.2)	6	5	4	7

[1] The association between history of trypsin intake and a positive PAGE test is highly significant ($P = 0.00016$) by Fisher's exact test.

Table 2. *Screening of CF relatives and controls by PAGE*

	CF relatives Fathers	Mothers	Sibs	Controls
Positive	0	11	0	4
Exposed to pancreas powder?		Yes 6		Yes 2
		No 5		No 2
Negative	30	30	19	155
Exposed to pancreas powder?				
Yes	13	10	5	5
No	9	10	10	66
Not Asked	8	10	4	84
Totals	30	41	19	159

which can be inhibited by STI has been described in plasma of CF patients and heterozygotes after proteinase activation (3, 22). Other authors, however, report no differences between CF and control arginine esterase activity (13, 17, 29). The picture is further complicated by the presence of antibodies to trypsin in CF plasma; such antibodies could bind and inhibit the enzyme yielding an apparent decrease in arginine esterase in activated plasma. This interpretation would be in agreement with the results obtained by active site "titration" of arginine esterase in CF plasma, which have led to the conclusion that the deficiency of this enzymatic activity might be due to a reduced number of functional molecules rather than to their decreased catalytic activity (31).

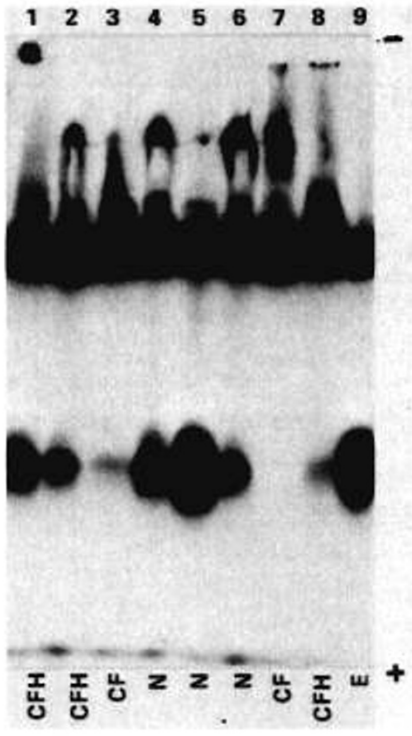

Fig. 7. ^{125}I-elastase binding to plasma proteins demonstrated by PAGE followed by autoradiography. Wells 3 and 7 contained serum of 2 CF patients. Wells 1, 2, and 8 contained serum of CF heterozygotes. Wells 4–6 contained sera from three normal controls. Well 9 contained ^{125}I-elastase in sucrose.

Table 3. *Screening of CF patients, CF relatives (fathers, mothers, and sibs), and controls by PAGE after incubation with ^{125}I-elastase*

Free elastase band	CF patients	CF relatives	Controls
Present	12	17	26
Absent	3	0	0

Whatever the role of TbIgG and other antiproteinase antibodies, one has to infer that an immunogenic stimulation occurs or has occurred in CF patients. A continuous immunization could be produced by the large amounts of pancreatine that is ingested after every meal by about 80% of these patients and by the endogenous proteinases reabsorbed from plugged pancreatic ducts. In either case, the amount of circulating digestive enzymes could exceed the binding capacities of α_2M and α_1-antitrypsin that under physiologic conditions remove them quite efficiently from the circulation (19). It is interesting to note that the clearance of radiolabeled subtilisin A, which also binds to α_2M, is significantly slower in rabbits immunized against this protease than in nonimmunized animals (6). Moreover, in the former group of animals, there is evidence in favor of competition between α_2M and antibodies for the binding of subtilisin A (6). It is possible that the turnover rate of antibody-bound proteinases is slower in CF patients than in control individuals. This hypothesis could also have various implications for research of CF "factors" (5).

Circulating antibodies against preparations of human pancreas have been demonstrated in sera of patients affected with chronic pancreatitis, carcinoma of the pancreas and CF (18), who, although not specified, might have all been taking porcine pancreatic extracts. In view of the results that show that TbIgG do not precipitate porcine trypsin (see *Results: Papain Digestion and Immunoelectrophoresis Experiments*), two possible interpretations should be considered: 1) the human trypsin antigen would precipitate TbIgG whereas porcine does not; 2) the observed precipitating antibodies are directed against other antigens of pancreatic tissue. In a later study (28), in which a different method of preparation of the human pancreatic extract has been used, the presence of circulating antibodies against pancreas in CF patients has not been confirmed, but antibodies against CF lung and pancreas have been demonstrated in bronchial mucus of CF patients. These antibodies, at least those against pancreas, could originate from tissue destruction and/or from ingestion of porcine pancreatic extracts that might be partially crossreactive with human pancreatic antigens. Additional research is needed to discriminate between these possibilities and to support further speculation regarding an autoimmune process in CF.

Hypersensitivity to pancreatic extracts mediated by IgE is frequent among parents of CF patients (8, 30) and, in comparison, is much rarer among CF patients (30). The presence of blocking antibodies against trypsin, (and against other pancreatic enzymes) in 80% of the CF patients, and only in 30% of their mothers may explain the higher incidence of hypersensitivity among CF parents.

Finally, it is obvious that our results on the electrophoretic mobility of α_2M trypsin or α_2M-^{54}Mn complexes, as revealed by our PAGE system, do not exclude the possibility that α_2M of CF patients might present altered properties when analyzed with other systems, as stated by others (26, 32).

The presence of multiple bands of α_2M, presumably produced upon interaction with trypsin in whole plasma, represents a novel interesting finding. Characterization of the molecular nature of these complexes is currently under way. Similar complexes of ^{125}I-elastase-α_2M are revealed by our PAGE system of analysis (Fig. 7). Using the same technique, antibodies against elastase have not been seen as frequently as TbIgG in CF patients. Further investigation is needed therefore to clarify the significance of TbIgG and of other antiproteinase antibodies in CF. In addition, the role of TbIg belonging to different classes, particularly IgA, warrants further study.

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