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Antibodies Cross-Reacting with Porcine and Human Trypsin in Cystic Fibrosis

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Summary

A solid-phase radioimmunoassay has been developed for the titration of TbIgG (trypsin binding IgG) in sera of CF patients. Using this assay system it has been found that TbIgG are primarily directed against porcine trypsin and cross-react with human trypsin. A specific subpopulation of anti-human trypsin antibodies has not been found, making unlikely the occurrence of an autoimmune process in CF, at least against human pancreatic enzymes.

Speculation

It is known that anti-proteinase antibodies modify the turnover in the circulation of free proteinases, which are usually bound and rapidly sequestered by α_2-macroglobulin (α_2M). TbIgG might create the conditions by which porcine trypsin, and possibly human activated trypsinogen, could be present in the circulation in the form of immunocomplexes.

Introduction

Trypsin binding immunoglobulins, especially of the IgG class (TbIgG), have been shown in sera of cystic fibrosis (CF) patients (8). The antibody nature of TbIgG is supported by the demonstration that trypsin binds to the Fab portion of the IgG molecule and their production seems to be elicited by the porcine trypsin ingested daily by most CF patients (8,9). Recently it has been reported that high levels of immunoreactive human trypsin are present in blood of CF patients during the first few months of life (1). It was therefore of interest to investigate whether CF patients produce antibodies primarily directed against human trypsin. Using a solid-phase radioimmunoassay we have found that antibodies made by CF patients against porcine trypsin cross-react with human cationic trypsin, and that these patients do not produce a specific subpopulation of antibodies directed against the human enzyme.

PATIENTS AND METHODS

Written informed consent from CF patients or their parents was obtained before drawing blood samples for this investigation. The protocol was approved by the Stanford University Medical Committee for the Protection of Human Subjects in Research. Human cationic trypsin was kindly provided by Dr. C. Largman (Veterans Administration Hospital, Martinez, California) and porcine trypsin was purchased from Worthington. Both enzymes were inactivated by incubation for 1h at 37° with N-α-p-tosyl-L-lysine-chloromethylketone HCL (TLCK) in phosphate buffered saline (PBS) pH 7.4, using a molar ratio of trypsin to TLCK of 20:1. After extensive dialysis against PBS at 4°, the concentration of the enzyme was calculated from the extinction coefficient $E_{1\%}^{1cm}$ = 15.4 at 280 nm (5). Trypsin antibodies were assayed by a solid-phase radioimmunoassay (RIA) in microtiter plates (Cooke Lab. Products) following a described procedure (13). Aliquots of 50 μl containing 20 μl/ml of TLCK-inhibited trypsin or bovine serum albumin (BSA) in control wells were left for 1h to coat the plates. After washing 3 times with PBS containing 1% BSA and 5% normal goat serum (RIA special buffer), serial dilutions of 40 μl of sera from CF patients and controls were incubated for 1h at room temperature in the trypsin-coated wells. Finally, after washing again 3 times with RIA special beffer, 30000 c.p.m. of ^{125}I-labelled goat anti-human IgG (γ-chain specific) prepared as described (13) were added to each well and the radioactivity bound was measured in a γ-counter. When rabbit antiserum against human cationic trypsin (kindly provided by Dr. C. Largman) was assayed by solid-phase RIA, goat anti-rabbit IgG, labelled with ^{125}I in the same way, was used.

An affinity column for anti-porcine trypsin antibodies was prepared by coupling TLCK-inhibited porcine trypsin, dialyzed against borate buffer 0.1M pH 8.0, to Sepharose beads, following a standard procedure (7). Aliquots of 1 ml of patient or control sera were loaded on a 5 ml affinity column and, after 10', elution with PBS and collection of 1 ml fractions were started. A pool of the 3 void volume fractions with the highest O.D.[illegible] reading was passed again over the same column. A final pool of the fractions having the highest O.D.[illegible] was assayed for antibodies against trypsin not bound by the affinity column. The same assay was performed on the serum sample loaded on the column, diluted in PBS to the protein concentration present in the second pool, as jusged by O.D.[illegible] reading.

Labelling of TLCK trypsin with ^{125}I and immunoprecipitation assays for antibodies against trypsin were performed as already described (10). Similarly, immunoelectrophoresis and PAGE followed by autoradiography were performed using ^{125}I-labelled trypsin, as previously reported (9).

RESULTS

Using the solid-phase RIA described under Methods, high titers of antibodies against porcine trypsin were found in sera of CF patients, whereas the same antibodies were not present in CF patients previously shown to be negative for TbIgG or in normal controls (Fig. 1). By the same method of analysis antibodies against human cationic trypsin could be detected, although their titers usually corresponded to 1/4–1/5 of those found with porcine trypsin. On the contrary, when four CF patients (1027, 1103, 1111, 1124) were titrated for anti-human cationic trypsin antibodies using a previously described immunoprecipitation assay (10), a low titer of anti-human trypsin antibodies was detected only in one patient (1103), while the other three were negative by this assay.

Using immunoelectrophoresis we screened sera of nine CF patients for antibodies against radiolabelled human cationic and porcine trypsin (Table 1). Eight out of the nine sera had detectable antibodies against porcine trypsin. Of this eight, six had detectable antibodies against human trypsin. The remaining patient had no detectable antibody to either enzyme. A more extensive screening for the same antibodies, performed by PAGE and autoradiography on sera from 39 CF pateints and three presumed heterozygotes (data not shown), indicated again that antibodies against human trypsin could be detected only when the same sera had antibodies against porcine trypsin. In particular, a serum sample from a three month old baby, analyzed soon after the diagnosis of CF had been made, contained no antibodies against human or porcine trypsin.

Table 1. Screening for TbIgG by immunoelectrophoresis

	Antigen Used	
CF patients code	Human TLCK-typsin	Porcine TLCK-trypsin
CF 934	+	+
CF 1027	+	+
CF 1103	+	+
CF 1104	–	+
CF 1111	+	+
CF 1114	+	+
CF 1126	+	+
CF 1134	–	–
CF 1230	–	+

Human and porcine trypsin, inhibited by TLCK, were labelled with ^{125}I and immunoelectrophoresis, followed by autoradiography, was performed as described (9). Samples were considered positive for TbIgG when a radioactive precipitin line against the corresponding antigen was visible.

In order to discriminate whether the antibodies from CF patients reacting with human trypsin represent a subpopulation directed solely against this enzyme or a population of antibodies elicited by porcine trypsin and cross-reacting with the human enzyme, the following experiments were performed. An affinity column of porcine trypsin bound to Sepharose beads was prepared as described under Methods. When a rabbit antiserum raised against human cationic trypsin was passed through it, antibodies directed against porcine trypsin, as measured by solid-phase RIA, were efficiently removed. On the contrary, antibodies directed against human trypsin were only slightly reduced (Fig. 2). Finally, when serum samples from CF patients 1027, 1103, 1111, and 1124 were passed through the same column, both anti-human and anti-porcine antibodies were absorbed by the affinity beads (Fig. 3).

DISCUSSION

The results described in this paper indicate that human cationic trypsin shares some common antigenic determinants with porcine trypsin and that TbIgG of CF patients, which are primarily directed against porcine trypsin, cross-react with human trypsin. The first conclusion is supported by the results of the affinity chromatography experiments using the rabbit antiserum directed against human cationic trypsin. This antiserum reacted against porcine trypsin before but not after absorption on TLCK-porcine trypsin bound to Sepharose, indicating that the porcine enzyme has at least some common antigenic determinants with human cationic trypsin. Sera of CF patients contain such cross-reacting antibodies but not a subpopulation of antibodies directed solely against human trypsin as found in the rabbit antiserum. In addition, the titer of TbIgG measured with porcine trypsin is 4–5 times higher than that found with human cationic trypsin (Fig. 1).

Rabbit antisera against human trypsin have been reported to show little cross-reactivity with porcine trypsin using liquid-phase RIA in which increasing concentrations of the competing antigen were used (12). Our solid-phase RIA is expected to amplify the degree of cross-reactivity between human and porcine enzyme because the antibody titer is progressively increased in this system. The disappearance of the cross-reacting antibodies against human trypsin by immunoelectrophoresis and autoradiography constitute further proofs of this cross-reactivity.

Circulating antibodies against human pancreas (6) or lung (11) have been described in CF, but the results presented in this paper do not support the hypothesis, already discussed (9), of an autoimmune process in CF directed against human pancreatic enzymes.

Finally, it is known that antiproteinase antibodies modify the turnover in the circulation of free proteinases, which are usually bound and rapidly sequestered by α_2M. In particular the clearance of radio-labelled subtilisin A, which is also inhibited by α_2M, is slower in rabbits immunized against this proteinase than in nonimmunized animals (2). We have already reported that antibodies against trypsin from CF patients, or from rabbits immunized against porcine trypsin, can effectively compete with α_2M for trypsin binding (10). Under normal conditions, human trypsin is not found as such in the circulation, but rather as its physiologic precursor, trypsinogen (4). The immunization process which takes place in CF patients against porcine trypsin might therefore create the conditions by which porcine trypsin, and possibly human activated trypsinogen, could be present in the circulation in the form of immunocomplexes. Since the inactivation of trypsin enzymatic activity in immunocomplexes is dependent upon the affinity of the corresponding antibodies (3), the question remains open whether trypsin bound to TbIgG of CF patients retains any esterolytic and proteolytic function, which in turn could affect the turnover and stability of many plasma proteins.

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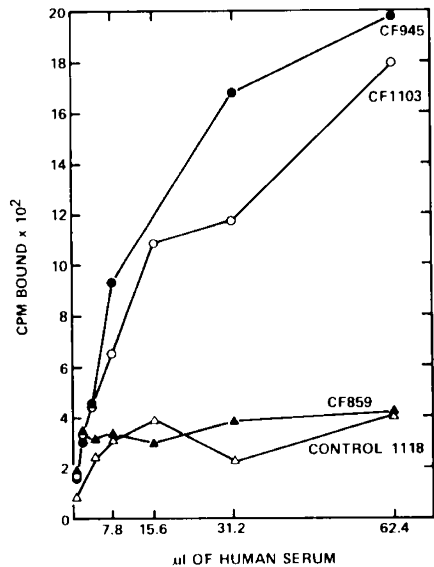

Fig. 1. Tibration by solid-phase RIA of TbIgG against porcine TLCK-trypsin in different CF and control serum samples. The TbIgG assay was negative for CF patient 859, who had no pancreatic insufficiency, and for controls.

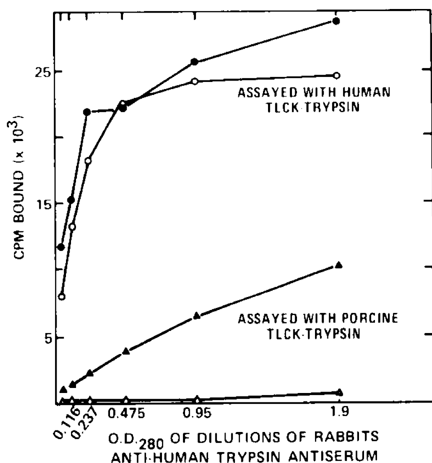

Fig. 2. Solid-phase RIA of antibodies against trypsin present in a rabbit antiserum raised against human trypsin, before and after affinity chromatography on a TLCK-porcine trypsin column, prepared as described under Methods. The amount of anti-porcine trypsin antibodies remaining in this antiserum after affinity chromatography was not significantly higher than background. The assay was performed with porcine trypsin (triangles) before (▲) and after (△) affinity chromatography and with human trypsin (circles) before (●) and after (o) affinity chromatography.

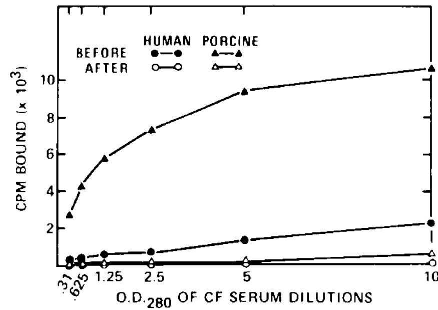

Fig. 3. Solid-phase RIA of TbIgG in serum of CF patient 1027 before (closed circles) and after (open triangles) the same affinity chromatography step described in Fig. 2. The assay was performed using either human (circles) or porcine (triangles) trypsin, as already indicated in the legend of Fig. 2.

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