Alpha-1-Antitrypsin–Human Leukocyte Elastase Complexes in Blood: Quantification by an Enzyme-Linked Differential Antibody Immunosorbent Assay and Comparison With Alpha-2-Plasmin Inhibitor–Plasmin Complexes

By Mark S. Brower and Peter C. Harpel

An enzyme-linked immunosorbent assay (ELISA) has been developed for the quantification of α1-antitrypsin–human leukocyte elastase (α1-AT-E) complexes. In the ELISA, the α1-AT-E complex is bound to a surface by rabbit antileukocyte elastase antibody, and the inhibitor–proteinase complex is quantified by a second antibody, rabbit anti-α1-antitrypsin F(ab')2, labeled with alkaline phosphatase. α1-AT-E complexes were detected when a final concentration of 2.2 nmole/liter of leukocyte elastase was added to plasma. The concentration of these complexes increased with additional elastase. In clotting blood, α1-AT-E complexes were generated in parallel with the conversion of 125I-fibrinogen to fibrin, whereas α2-plasmin inhibitor–plasmin (α2Pl-P) complexes were not formed. The concentration of α1-AT-E complexes in 19 of 21 controls was less than 2.2 nmole/liter. Patients with laboratory evidence for disseminated intravascular coagulation (DIC) demonstrated elevated α2Pl-P complexes with either increased or normal concentrations of α1-AT-E complexes. Patients without evidence for DIC, but who demonstrated prolonged reptilase clotting times, were studied. This group had increased α1-AT-E but normal α2Pl-P complex levels, raising the possibility that elastase release in vivo may be accompanied by limited degradation of fibrinogen. These assays thus serve as useful probes for the study of leukocyte activation and of the interactions between cellular and plasma proteolytic enzyme systems.

The response of blood or of other tissues to injury frequently involves the activation of protein-cleaving enzymes. The direct measurement of the activity of these proteinases in blood currently is not feasible because these enzymes rapidly become bound to cell surfaces or to plasma protease inhibitors. Recently, a methodology was developed for the detection and quantification of α2-plasmin inhibitor–plasmin and α2-macroglobulin–plasmin complexes in purified systems as well as in the plasma of patients with disseminated intravascular coagulation. This technique employs an enzyme-linked immunosorbent assay utilizing two different antibodies to specifically recognize both the proteinase inhibitor and the proteinase within the inhibitor–enzyme complex.

In the present study, this method has been applied to the quantification of α1-antitrypsin–human leukocyte elastase complexes. Human polymorphonuclear leukocytes (PMN), during the process of phagocytosis, or when activated by other stimuli such as soluble immune complexes, C5a, or endotoxin, release several different lysosomal proteinases active at neutral pH. Leukocyte elastase appears to be the major proteinase elaborated under these circumstances. Leukocyte extract has been shown to contain 1–12 μg elastase/107 PMN, and as much as 40 μg of elastase/107 PMN can be released from stimulated leukocytes. The studies of Ohlsson indicate that α1-antitrypsin is the predominant inhibitory protein in plasma, as it binds 92% of the elastase added to plasma, the remainder binding to α2-macroglobulin.

Several investigators have suggested that the decreased concentration of coagulation factors in the blood of patients with acute leukemia or sepsis may be due to proteolytic degradation by leukocyte elastase, since this enzyme, either in purified systems or when added to plasma, decreases the activity of human coagulation factors, II, V, VIII, XII, and XI. Leukocyte elastase also has been found to inactivate human antithrombin-III and to destroy the plasmin-inhibiting capacity of α1-plasmin inhibitor and C1 inactivator by limited proteolytic cleavage.

This article describes a sensitive and specific enzyme-linked differential antibody immunosorbent assay that measures α1-antitrypsin–leukocyte elastase complexes. The development of this assay and its utilization in conjunction with our previously described immunosorbent assay for α2-plasmin inhibitor–plasmin complexes has made it possible to study the release of leukocyte elastase and activation of plasmin both in vitro and in vivo.
ELISA OF ANTITRYPSIN-ELASTASE COMPLEXES

MATERIALS AND METHODS

Blood Samples

Venous blood was obtained in plastic syringes from normal volunteers or patients and added to 3.8% sodium citrate (9:1 v/v). Samples were maintained at 4°C and centrifuged at 17,000 g for 20 min within 30 min of collection. The plasma was harvested so as not to disturb the buffy coat, and then frozen in plastic tubes at −70°C. Specimen collection and informed consent procedures were approved by the institution’s Human Rights Committee.

Human Polymorphonuclear Leukocyte Elastase

Human polymorphonuclear leukocyte elastase was purified from outdated white blood cell concentrates, as we have previously described. The concentration of elastase was determined by its extinction coefficient of 9.85. The purified leukocyte elastase demonstrated one protein-staining band when analyzed by SDS-PAGE following reduction with dithiothreitol, performed as described. Antisera prepared in rabbits against the leukocyte elastase produced one precipitin arc when diffused against the elastase preparation and no reactivity against normal human plasma. The specific activity of the purified leukocyte elastase was 92% as established by active site titration using the tritrat Ac-Ala-Ala-Anval-ONp (a gift of Dr. J. C. Powers), as described by Powers and Gunton.

Alpha-1-Antitrypsin

α1-Antitrypsin was purified from plasma as previously described and its concentration determined by its extinction coefficient of 5.3. The final inhibitor preparation was free of other contaminating plasma protease inhibitors as analyzed by double diffusion in agarose gels with specific antibodies against α1-macroglobulin, CI inactivator, antithrombin-III, inter-α-trypsin inhibitor, or chymotrypsin inhibitor obtained from Calbiochem-Behring Corp., San Diego, CA.

Preparation of Antisera and Immunoglobulin Fractions

Rabbits were immunized intradermally with elastase or α1-antitrypsin in equal volumes of complete Freund’s adjuvant. The IgG of the antisera or of normal rabbit serum were isolated, after dialysis against 0.02 M K2HPO4 buffer, pH 8.0, by chromatography on DE-52 cellulose (H. Reeve Angel & Co., Inc. Clifton, NJ) to which cibacron-blue F 3GA (Polysciences, Inc., Warrington, PA) was coupled. F(ab’)2 fragments were prepared from the rabbit anti-α1-antitrypsin IgG and subsequently labeled with alkaline phosphatase as described previously.

ELISA

The enzyme-linked differential antibody immunosorbent assay for human leukocyte elastase–α1-antitrypsin complexes was performed by modifications of the methods detailed for the assay of α2-plasmin inhibitor-plasmin complexes. Micrertitation plates (Linbro, E.I.A. Microtitration Plate, Flow Laboratories, Inc., Hamden, CT) were coated with the IgG fraction of normal rabbit serum or of the antisera against leukocyte elastase. IgG, 10 μg/ml in bicarbonate coating buffer, was incubated in 0.2-ml portions overnight at 4°C in a humid chamber. Contents of the micrertitation plates were removed and the wells washed for 3 min each in 0.15 M phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20. The solutions containing the α1-antitrypsin–elastase complexes were diluted in PBS-Tween buffer, added (0.2 ml) in duplicate to coated wells, and incubated for 4 hr at room temperature. The washing procedure was repeated, and the alkaline-phosphatase-labeled anti-α1-antitrypsin F(ab’)2, prepared as detailed by Voller et al., using Sigma type VII-S calf mucosa alkaline phosphatase, was added for an 18-hr incubation in a humid chamber at 4°C. After washing the wells, the substrate (0.2 ml) p-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO) (1 mg/ml in 10% diethanolamine buffer, pH 9.8) was added. Color development at 405 nm was followed at 10-min intervals in a Titertek Multiscan photometer (Flow Laboratories, Inc., Rockville, MD). The change in absorbance per min of the wells coated with normal rabbit IgG was subtracted from the values obtained in the wells coated with anti-elastase IgG. Plots of the color development with time demonstrated a linear relationship.

Preparation of the reagents and performance of the enzyme-linked differential antibody immunosorbent assay for α1-antiplasmin inhibitor-plasmin complexes were as detailed by Harpel.

Detection of α1-Antitrypsin

Purified human α1-antitrypsin diluted from 0.5 to 1600 ng/ml (0.009–29 nM) in carbonate-coating buffer was absorbed in duplicate (0.2-ml portions) to the wells of microtitration plates, 18 hr at 4°C in a humid chamber. The wells were washed 3 times with PBS-Tween, and the alkaline-phosphatase-labeled anti-α1-antitrypsin F(ab’)2 antibody added for an additional 18 hr at 4°C. The washing step was repeated, the p-nitrophenyl phosphate substrate added, and the change in absorbance monitored.

Generation of α1-Antitrypsin–Leukocyte Elastase Complexes by the Addition of Leukocyte Elastase to Plasma

Elastase diluted in PBS was added to plasma in final concentrations from 0.016 μg/ml to 8.33 μg/ml (0.55–290 nmole/liter) and incubated for 10 min at 23°C. These incubation mixtures were diluted 1:80 in PBS-Tween buffer and added to microtitration plate wells previously coated with normal rabbit IgG or with rabbit antileukocyte elastase IgG. The assay then was performed as described above.

Generation of α1-Antitrypsin–Elastase Complexes in Clotting Blood

Venous blood was collected in plastic syringes and allowed to clot in polystyrene tubes at 37°C. Alternately, blood was added to tubes containing (A) 3.8% sodium citrate (9:1 v/v), (B) sodium heparin (125 U/ml), or (C) 3.8% sodium citrate (9:1 v/v) containing sodium heparin (125 U/ml) and soybean trypsin inhibitor (SBTI, 0.45 mg/ml) and incubated at 37°C. To each tube, 14 μg 125I-fibrinogen (Amersham Corp., Arlington Heights, IL) was added to quantify the fibrinogen-to-fibrin conversion. Portions (1.0 ml) were removed at various times and citrate-heparin-SBTI anticoagulant was added to those samples not previously anticoagulated. The plasma or serum was harvested after centrifugation, and detection of α1-antitrypsin–elastase and α1-plasmin inhibitor-plasmin complexes were performed as detailed above. 125I activity was quantified by counting samples of plasma or serum in a Searle 1185 gamma counter.

Quantification of Circulating α1-Antitrypsin–Leukocyte Elastase Complexes by the Enzyme-Linked Differential Antibody Immunosorbent Assay

Plasma samples from normal individuals or from hospitalized patients were diluted 1:80 in PBS-Tween buffer. Portions (0.2-ml) in duplicate were added to microtitration plate wells coated with normal rabbit IgG or anti-elastase IgG and processed as detailed above. The change in absorbance per minute of the plasma sample...
added to the well coated with normal rabbit IgG was subtracted from the value that resulted when the sample was added to the wells coated with antibody directed against elastase. This value was then used to calculate concentration of α1-antitrypsin–elastase complexes in plasma using a standard curve run simultaneously generated by the addition of elastase to pooled normal plasma as detailed above.

Fibrin Degradation Products

Fibrin degradation products were assayed using the Thrombo-Wellcotest, Wellcome Diagnostics, Temple Hill, Dartford, England.

Patient Selection

Patients selected for the study of circulating α1-antitrypsin–elastase complexes included the following: (1) patients referred to the Blood Coagulation Laboratory whose studies demonstrated disseminated intravascular coagulation with elevated levels of fibrin degradation products; (2) plasma specimens from individuals undergoing hypertonic saline-induced abortions with resultant disseminated intravascular coagulation at the peak elevation of fibrin degradation products and thrombin-increasable fibrinopeptide-B levels (samples generously provided by Dr. H. Nossel); and (3) patients without elevation of fibrin degradation products who demonstrated elevated reptilase clotting times and elevated thrombin times.

Statistical Analysis

Statistical analysis was performed on an HP 9815A calculator (Hewlett-Packard Co., Palo Alto, CA) using prepared programs in volume I of the HP Statistics Library. Data were analyzed using the four-parameter logistic method of Rodbard and Hutt. The minimal detectable concentration was determined by the method of Rodbard.

RESULTS

Detection and Quantification of Purified α1-Antitrypsin

After absorption to the plastic microtitration plate, α1-antitrypsin was detected by the alkaline-phosphatase-labeled anti-α1-antitrypsin F(ab′)2 at concentrations above 0.1 n mole/liter (Fig. 1). The hydrolysis of the alkaline phosphatase substrate, p-nitrophenyl phosphate, was proportional to the concentration of α1-antitrypsin in the range of 0.8–8 n mole/liter.

Generation of α1-Antitrypsin–Leukocyte Elastase Complexes in Plasma and in Clotting Blood

Increasing quantities of leukocyte elastase were added to plasma to generate α1-antitrypsin–leukocyte elastase (α1AT-E) complexes (Fig. 2). The minimal detectable concentration of these complexes was 2.2 n mole/liter. The coefficient of variation for “within” and “between” assay precision was 14% and 16%, respectively. The concentration of the complex increased linearly at final elastase concentrations of 9–40 n mole/liter. Simultaneous measurement of α2-plasmin inhibitor–plasmin complexes after addition of the elastase demonstrated that no plasmin complexes were generated.

α1-Antitrypsin–leukocyte elastase complexes were found to be generated in clotting blood (Fig. 3). Whole human blood was allowed to clot at 37°C in the presence of a trace amount of 125I-fibrinogen. Portions removed at various intervals were added to tubes containing sodium citrate, heparin, and SBTI. An apparent increase in 125I-fibrinogen-associated radioactivity occurred in the 45 min before clotting commenced. This appeared to be due to the sedimentation of red blood cells, causing a progressive increase in the amount of plasma sampled at each interval. Detection of α1AT-E complexes preceded the visible formation of fibrin clot and the decrease in fluid phase 125I-fibrinogen. A peak of α1AT-E complex formation...
Fig. 2. Generation of α₁-antitrypsin–elastase complexes by the addition of leukocyte elastase to plasma, as measured by the enzyme-linked differential antibody immunosorbent assay. Increasing amounts of elastase were added to plasma in final concentrations from 0.55 to 290 nM. After incubation for 10 min at 23°C, the plasma was diluted 80-fold with PBS-Tween buffer, and samples were added to microtitration plates coated with rabbit anti-elastase IgG or normal rabbit IgG and assayed as detailed in Materials and Methods.

Fig. 3. Measurement of α₁-antitrypsin–elastase (α₁-AT-E) and α₂-plasmin inhibitor–plasmin (α₂PI-P) complexes in clotting blood. Whole blood was collected in citrate, heparin, and soybean trypsin inhibitor or was allowed to clot in polystyrene tubes at 37°C after addition of ^125^I-fibrinogen. Portions removed at times indicated in the figure were added to tubes containing citrate, heparin, and soybean trypsin inhibitor if not previously anticoagulated. Samples were then assayed for α₁-AT-E and α₂PI-P complexes and for ^125^I activity. The concentration of α₁AT-E (---) and α₂PI-P complexes (O-O) measured in clotting blood or of α₁AT-E complexes (Δ-Δ) in anticoagulated blood is expressed in nM derived from a standard curve produced by adding increasing concentrations of leukocyte elastase to normal plasma, as detailed in Fig. 2. ^125^I-fibrinogen (Δ---Δ) in clotting blood is expressed as cpm/0.1 ml sample.

occurred 40 min following visible clotting, when a total of 67 n mole/liter of complex was generated, representing the release of 7.0 μg elastase/10⁷ PMN. An additional elevation of α₁AT-E complex occurred at 3 hr following visible clotting, when a cumulative total of 140 n mole/liter of complex was detected. In experiments not shown, only trivial increases in the level of α₁AT-E complexes were detected in the remaining 24 hr after clotting. α₂-Plasmin inhibitor–plasmin (α₂PI-P) complexes were not detected during clotting. In the control samples collected in citrate, heparin, and SBTI, and to which a trace amount of ^125^I-fibrinogen was added, there was a gradual increase in α₁AT-E complexes first detectable at 90 min, and increasing linearly between 2 and 4 hr. A maximum of 27 n mole/liter of complex was generated. No decrease in ^125^I-fibrinogen was detected. Similar results were observed in blood collected in citrate alone or in heparin.

The clotting blood of four additional subjects was also assayed for α₁AT-E complexes. The pattern of complex formation was similar to that illustrated in Fig. 3. The amount of complex generated in the initial peak ranged from 14 to 67 n mole/liter (1.3–6.9 μg/10⁷ PMN) and in the second elevation, 28–140 n mole/liter (3.2–15.3 μg/10⁷ PMN).

**Measurement of α₁-Antitrypsin–Leukocyte Elastase and α₂-Plasmin Inhibitor–Plasmin Complexes in Patients’ Plasma**

α₁-Antitrypsin–leukocyte elastase complex levels were less than the minimal detectable concentration of 2.2 n mole/liter in 19 of 21 normal individuals (6 females and 15 males ages 21–46 yr, with a mean of 31
yr), with a maximum of 3.3 nmole/liter. The mean and standard deviation for the concentration of α2PI-P complexes was 7.5 ± 6.4 nmole/liter. When plasma concentrations of both types of inhibitor–enzyme complexes were compared in a selected patient population, elevation in the plasma concentration in either one or both types of inhibitor–enzyme complexes were observed (Fig. 4). Sixteen patients had elevations in both α1AT-E and α2PI-P levels (Fig. 4A). These patients all had disseminated intravascular coagulation as documented by laboratory tests, including elevated levels of fibrin degradation products. The diagnoses in these patients included thrombophlebitis associated with carcinoma (2 patients), acute promyelocytic leukemia (2), sepsis (2), carcinoma (4), and one each with hemorrhagic shock, cirrhosis with thrombocytopenia, postoperative retroperitoneal hematoma, purulent pericarditis, refractory anemia with systemic lupus erythematosus, and factor-IX-deficiency receiving replacement therapy after hip surgery.

Elevations of α2PI-P complexes with normal concentrations of α1AT-E complexes were demonstrated in six individuals during hypertonic saline-induced abortion (Fig. 4B). Samples from these patients were obtained 4 hr after initiation of the saline infusion, when the peak of fibrin degradation products and thrombin-increasable fibrinopeptide-B levels had occurred.26 Before intrauterine infusion of saline, levels of α2PI-P complexes were normal.

Ten patients had elevated circulating α1AT-E complexes with normal α2PI-P complex concentrations (Fig. 4C). The diverse clinical diagnoses and the results of selected coagulation studies are detailed in Table 1. Fibrin degradation product levels were less than 10 μg/ml in these patients. These patients all had elevated thrombin and reptilase clotting times, indicating an abnormality in the conversion of fibrinogen to fibrin.

DISCUSSION

These studies detail a method for the detection and quantification of nanogram amounts of α1-antitrypsin–leukocyte elastase complexes in plasma based on the enzyme-linked differential antibody immunosorbent assay previously described for the measurement of α2-plasmin inhibitor–plasmin complexes.2 The latter complexes were first identified when 6.7 nmole/liter of plasmin was added to plasma, whereas α1AT-E complexes were detected with 2.2 nmole/liter of elastase added to plasma. Thus, the enzyme-linked immunosorbent assay for α1AT-E complexes has proven to be about threefold as sensitive as that previously demonstrated for α2PI-P complexes. This variation is not due to the different distribution of the two proteases between their plasma inhibitors, since approximately 90% of these enzymes bind to their major inhibitor when added to plasma, α1-antitrypsin for elastase4 and α2-plasmin inhibitor for plasmin.27-29 Although relative antibody titers can influence the sensitivity of the two assays, it is likely that the decrease in sensitivity in the detection of the α2PI-P as compared to the α1AT-E complexes is due to the competition between the free α2-plasmin inhibitor and the α2-plasmin inhibitor–plasmin complex in plasma for the rabbit anti-α2-plasmin inhibitor antibody bound to the microtitration plate. In contrast, there appears to be little leukocyte elastase in plasma to compete with the binding of the α1-antitrypsin–elastase complex to the rabbit anti-elastase antibody on the microtitration plate.

We have observed that concentrations of α1AT-E

![Fig. 4](image-url)
ELISA OF ANTITRYPSIN–ELASTASE COMPLEXES

Table 1. Patients With Elevated α1-Antitrypsin–Elastase (α1,AT-E) and Normal α2-Plasmin Inhibitor–Plasmin (α2PI-P) Complex Concentrations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>α1,AT-E (nM)</th>
<th>α2PI-P (nM)</th>
<th>FDP (μg/ml)</th>
<th>Thrombin Time (sec)</th>
<th>Reptilase Time (sec)</th>
<th>PT (sec)</th>
<th>Fibrinogen (mg/dl)</th>
<th>Platelets (per cu mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lymphoma, sepsis, respiratory distress*</td>
<td>15.8</td>
<td>19.7</td>
<td>&lt;10</td>
<td>30</td>
<td>18-21</td>
<td>21.3</td>
<td>22.5</td>
<td>10.8</td>
</tr>
<tr>
<td>2</td>
<td>Subdural hematoma on coumadin</td>
<td>16.6</td>
<td>13.5</td>
<td>&lt;10</td>
<td>27.9</td>
<td>27.4</td>
<td>39.5</td>
<td>15.1</td>
<td>410</td>
</tr>
<tr>
<td>3</td>
<td>Sepsis, cardiac arrest</td>
<td>36.2</td>
<td>19.6</td>
<td>&lt;10</td>
<td>26.7</td>
<td>28.5</td>
<td>48.4</td>
<td>13.4</td>
<td>600</td>
</tr>
<tr>
<td>4</td>
<td>ITP, splenectomy with abscess</td>
<td>33.8</td>
<td>0</td>
<td>&lt;10</td>
<td>25</td>
<td>23.2</td>
<td>25.6</td>
<td>13.2</td>
<td>500</td>
</tr>
<tr>
<td>5</td>
<td>Preleukemia, vasculitis, lupus</td>
<td>50.0</td>
<td>10.1</td>
<td>&lt;10</td>
<td>26.7</td>
<td>29</td>
<td>25.7</td>
<td>14.2</td>
<td>520</td>
</tr>
<tr>
<td>6</td>
<td>Pneumonia, hematuria</td>
<td>43.1</td>
<td>8.6</td>
<td>&lt;10</td>
<td>22.8</td>
<td>24.3</td>
<td>56.1</td>
<td>14.9</td>
<td>415</td>
</tr>
<tr>
<td>7</td>
<td>Massive pulmonary embolus†</td>
<td>32.4</td>
<td>11.7</td>
<td>&lt;10</td>
<td>&gt;3 min</td>
<td>28.7</td>
<td>&gt;150</td>
<td>&gt;60</td>
<td>550</td>
</tr>
<tr>
<td>8</td>
<td>Sepsis, jaundice, amyotrophic lateral sclerosis</td>
<td>25.9</td>
<td>11.9</td>
<td>&lt;10</td>
<td>66.4</td>
<td>42</td>
<td>46.9</td>
<td>19.1</td>
<td>95</td>
</tr>
<tr>
<td>9</td>
<td>Uremia, gastrointestinal bleed</td>
<td>7.9</td>
<td>8.2</td>
<td>&lt;10</td>
<td>28.5</td>
<td>22.7</td>
<td>32.9</td>
<td>13.6</td>
<td>150</td>
</tr>
<tr>
<td>10</td>
<td>Perforated carcinoma of the appendix</td>
<td>8.8</td>
<td>15.9</td>
<td>&lt;10</td>
<td>32.1</td>
<td>30.0</td>
<td>40.3</td>
<td>13.5</td>
<td>455</td>
</tr>
</tbody>
</table>

*Heparin used to flush Swan–Gans catheter.
†Patient anticoagulated on heparin.

complexes are elevated in serum as compared to plasma. This observation suggested that polymorphonuclear leukocytes release elastase during the process of blood coagulation in vitro. The immunosorbent assay has made it possible to document the evolution of α1,AT-E complexes in whole blood allowed to clot in plastic tubes at 37°C. These studies have demonstrated that the release of relatively large amounts of elastase occurs during the conversion of fibrinogen to fibrin in the blood coagulation process. A second elevation in the serum concentration of α1,AT-E complexes occurs 3 hr after clot formation. The mechanisms underlying this biphasic generation of α1,AT-E complexes are not known and are under study. Leukocyte elastase was released slowly in blood collected in citrate, heparin, and soybean trypsin inhibitor; however, about fivefold less α1,AT-E complexes were measured than in clotting blood. Slow release of elastase also occurred in blood collected in citrate alone or in heparin. The clotting process was not accompanied, however, by the generation of α2PI-P complexes.

The present study confirms and extends the recent findings of Plow, utilizing a radioimmunoassay for elastase, that leukocyte elastase is released in clotting blood. The maximum amount of elastase released at 30 min following clot formation as assayed by the elastase RIA was 1 μg/10⁷ PMN, and in our study of clotting blood, the values at a similar time (peak 1) were 1.3–6.9 μg/10⁷ PMN. This indicates that the two different methods produce similar results. Our assay, however, specifically measures the α1,AT-E complex, whereas the RIA may measure free elastase, inactive elastase, as well as complexes between elastase and several of its plasma inhibitors.

Our previous study has documented a 16–35-fold increase in the concentration of α2PI-P complexes in the plasma of 6 patients with laboratory evidence for disseminated intravascular coagulation. To explore the potential clinical utility of the present assay, the plasma concentration of α1,AT-E was measured and compared to α2PI-P complex levels in several different groups of patients. Our findings indicate that increased levels in either one or both types of complexes may be present simultaneously in the plasma in vivo. Sixteen patients with laboratory evidence for disseminated intravascular coagulation, including elevated levels of fibrin degradation products, had increased α1,AT-E complexes that ranged from 3 to 21 times the maximum detected concentration in normals. In this same group, α2PI-P complex levels ranged from 3 to 60 times the normal mean. That an elevated α1,AT-E concentration is not an invariable accompaniment of intravascular coagulation is demonstrated by the finding of an elevation of α2PI-P complexes in six patients following hypertonic saline-induced abortions in the absence of increased α1,AT-E complexes. Thus, disseminated intravascular coagulation differs from clot formation in the test tube, where leukocyte elastase release accompanies coagulation.

In contrast to that situation in the abortion patients, a group of ten patients has been delineated with normal plasma α2PI-P levels but with elevated concen-
We report here the discovery of a potential mechanism for the activation of the alternative pathway of complement, utilizing the enzyme-linked immunosorbent assay (ELISA) method developed in this study. Our data demonstrate that the enzyme-linked assay is sensitive and specific for the quantification of human plasma fibrinogen degradation products (FPD) not detectable by the latex agglutination assay. Proof of this hypothesis requires further experimental and clinical studies.

The demonstration that one type of inhibitor–enzyme complex may be elevated, in the absence of abnormalities in the other, raises the possibility that in vivo release of leukocyte elastase and plasminogen activation are independent events. Given the recent findings of Gonias, Fuchs, and Pizzo that human α1-plasmin inhibitor–plasmin complexes and α1-antitrypsin–trypsin complexes are cleared independently in a mouse model, it is also possible that the variations in concentration of complexes observed in our study reflect altered rates of clearance.

Our data thus demonstrate that the enzyme-linked differential antibody assay described in this study is a sensitive and specific probe for quantifying α1-antitrypsin–leukocyte elastase complexes in complex biologic fluids such as plasma. This assay, combined with our ELISA for α1-plasmin inhibitor–plasmin complexes, provides a new approach for the detection and quantification of intravascular proteases that may further delineate the role of cellular and plasma proteolytic enzyme systems.

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Alpha-1-antitrypsin-human leukocyte elastase complexes in blood: quantification by an enzyme-linked differential antibody immunosorbent assay and comparison with alpha-2-plasmin inhibitor-plasmin complexes

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