Leukocyte Elastase Release during Blood Coagulation

A POTENTIAL MECHANISM FOR ACTIVATION OF THE ALTERNATIVE FIBRINOLYTIC PATHWAY

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ABSTRACT Immunological detection of elastase, an enzyme present within leukocyte granules, has been used as a marker for polymorphonuclear leukocyte activation. Polymorphonuclear leukocytes contained 4.6 μg/10^7 cells, whereas erythrocytes, mononuclear cells, and platelets contained < 1% of this level. In plasma that was separated from blood cells after 1 h at 22°C, the mean level of elastase-related antigen in seven normal donors was 25±6 ng/ml. This level was unaltered by immediate separation of the plasma from the cells, by inclusion of protease inhibitors, or by anticoagulation of the plasma with either EDTA or acid-citrate-dextrose (the level in heparinized plasma was approximately threefold higher). In serum, the level of elastase-related antigen was 288±125 ng/ml, representing an 11.5-fold increase above plasma levels. The antigen detected in serum was immunologically indistinguishable from the leukocyte enzyme. Release of elastase was observed when isolated polymorphonuclear leukocytes were added to nonanticoagulated platelet-rich or platelet-poor plasma, recalcified plasma, or to serum. Addition of a chelating agent to serum prevented elastase release, but calcium or magnesium did not induce release in the absence of plasma. Coagulation induced by addition of thrombin to plasma also failed to induce release. In whole blood or in anticoagulated plasma reconstituted with polymorphonuclear leukocytes and then recalcified, initial release of elastase occurred concomitantly with or slightly after clotting and reached maximal levels within 20–40 min after clot formation. The data indicates that early events in coagulation or other pathways that occur in parallel with coagulation induce leukocyte release. The release of elastase, a major fibrinolytic protease of leukocytes, from the cells provides a mechanism for this enzyme or other granule proteases to participate in physiological events.

INTRODUCTION

Dissolution of the fibrin clot by enzymes with fibrinolytic activity is required to restore integrity to intravascular and extravascular sites of thrombus formation. Plasmin is the primary fibrinolytic pathway of plasma, but cells including platelets (1) and neutrophils (2–5) also contain fibrinolytic proteases. Numerous studies dating from the observations of Rulot (6) in 1904 and Opie (7) in 1907 have demonstrated that leukocytes are capable of degrading fibrin and fibrinogen substrates. Evidence consistent with the participation of leukocytes in physiologic fibrinolysis includes: (a) the preferential accumulation of leukocytes within thrombi relative to their blood concentration (8); (b) the ability of the cells to readily penetrate preformed thrombi (9); and (c) the direct interaction with and internalization of fibrin by leukocytes as visualized by immunofluorescence and electron microscopy (10). The fibrinolytic proteases of the leukocyte reside primarily within secretory granules (4), and cell lysis, phagocytosis, and/or secretion provide potential mechanisms for activation of the leukocyte fibrinolytic pathway.

The fibrinolytic activity of leukocyte extracts is independent of the plasminogen system as plasmin (ogen) inhibitors and activators fail to inhibit or augment fibrinolytic activity (4, 11) and fibrinogen degradation products generated by leukocyte proteases are structurally and immunologically distinguishable from plasmin-derived degradation products (4, 12, 13). A variety of neutral proteases (5, 14–18) and acidic cathepsins (3) of neutrophils are capable of degrading fibrin (ogen). Within leukocyte granules, elastase has been identified as one of the major fibrinolytic proteases active at neutral pH (19). In this study, a specific radioimmunoassay for leukocyte elastase has been de-
veloped, and the detection of this antigen has been used as a marker for leukocyte activation. The data demonstrate that this enzyme is released from the cell in association with blood coagulation.

METHODS

Cell isolation. Human leukocytes were isolated from citrated blood by differential sedimentation in 1% dextran followed by brief hypotonic or NHCl lysis of residual erythrocytes (4, 20). The isolated cells consisted of 75–85% polymorphonuclear leukocytes (PMN) and 10–20% mononuclear cells. Other cell types and purer preparations of PMN were isolated by placing 10-ml aliquots of heparinized blood onto 3 ml of Ficoll-Hypaque (density 1.074 g/cm³) and centrifuging at 2,260 g for 6 min at 20°C. Mononuclear cells were removed from the interface and washed three to five times in PBS. Erythrocytes and PMN were recovered from the pellets of the Ficoll-Hypaque gradient and separated by dextran sedimentation and NHCl lysis. The purity of the isolated cells determined by neutral red and Giemsa staining was as follows: the mononuclear cells contained 80–85% lymphocytes, 10–20% monocytes, <2% PMN; PMN contained ≤3% mononuclear cells, and ≤0.1% erythrocytes; the erythrocytes contained ≤0.05% PMN. Platelets were prepared by differential centrifugation followed by gel filtration (21) and contained ≤0.05% PMN.

Isolation of leukocyte elastase. Leukocyte granules were isolated by differential ultracentrifugation (22) and disrupted by repeated freezing and thawing. The extracts were applied to a 2.5 × 40-cm column of CM-Sephadex C50 (Pharmacia Fine Chemicals, Piscataway, N. J.) equilibrated with 0.1 M NaCl, 0.05 M sodium phosphate, pH 7.2, and a linear gradient was developed with a final buffer of 1.0 M NaCl, 0.05 M sodium phosphate, pH 7.2. Fractions containing elastase activity, identified by hydrolysis of the N-tBOC-L-alanine-p-nitro-phenylester (23) were concentrated by ammonia sulfite precipitation at 80% saturation, and applied to a 1.5 × 20-cm column of elastin-Sepharose (20 mg elastin/ml Sepharose) at 4°C (24). After extensive washing with buffers containing 0.15 M and 1.0 M NaCl, the purified elastase was eluted with 8.0 M urea and dialyzed against 0.15 M NaCl, 0.035 β-alanine, 0.025M sodium pyrophosphate, pH 5.9.

Radioimmunoassay for leukocyte elastase. Leukocyte elastase was radioiodinated by a micromole chloramine-T procedure (25). Elastase, 15 μg in 100 μl, 50 μl of 0.2 M sodium phosphate buffer, pH 7.2, 2 mCi carrier-free Na125I (1 mCi/μg 125I, Amersham Corp., Arlington Heights, Ill.) and 20 μg chloramine-T in 10 μl PBS were incubated for 5 min at 22°C. The reaction was stopped by addition of 23 μg sodium metabisulfite in 10 μl, and 100 μl of 1% KI and 100 μl of 1% bovine serum albumin were added. Phenylmethylsulfonylfluoride or diisopropylfluorophosphate (DFP) were added to final concentrations of 2 mM; and after 30 min at 22°C, free 125I was removed by extensive dialysis or gel filtration of Sephadex G25. The radioactivity of 125I-elastase was 92% precipitable in 10% trichloroacetic acid, and its sp act was 3–10 μCi/μg. Antiserum to leukocyte elastase was prepared in rabbits by injecting 100-μg doses in complete Freund's adjuvant at multiple intradermal sites on a weekly basis. Serum was collected 1 wk after the fourth booster injection. The radioimmunoassay for leukocyte elastase was of the double-antibody type (26). 125I-elastase was added at 15 ng/ml in a 0.25-ml vol, followed by the test sample in a 1.0-ml addition and 0.25 ml of diluted antielastase. The buffer system was 0.6 M NaCl, 0.05 M sodium borate buffer, pH 8.3, containing 2 mM EDTA, 20 U aprotinin and heparin/ml, and 2% heat-inactivated normal rabbit serum. Nonspecific precipitation of 125I-elastase by the second antibody was <5%, and results were routinely corrected for the precipitability of the ligand in 10% trichloroacetic acid.

RESULTS

Elastase was isolated from leukocyte granules by ion exchange and affinity chromatography. The isolated enzyme yielded a single band on SDS polyacrylamide gels under nonreducing conditions (Fig. 1), and a mol wt of 30,000 was estimated from its mobility relative to protein standards. When radioiodinated to a sp act

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1 Abbreviations used in this paper: PMN, polymorphonuclear leukocytes; ACD, acid-citrate-dextrose (1% dextrose, 0.063 M citrate buffer, pH 4.55); PBS, 0.15 M NaCl, 0.01 M phosphate buffer, pH 7.3; DFP, diisopropylfluorophosphate.
of 5 μCi/μg, >95% of the radioactivity migrated as a single peak on a SDS polyacrylamide gel with a mobility identical to that of the stained protein (Fig. 1A and B). Antiserum to leukocyte elastase, raised in rabbits, formed a single precipitin line with isolated elastase and with the extract of leukocyte granules, and these precipitin lines fused in a reaction of complete antigenic identity (Fig. 1C).

For quantitation of leukocyte elastase, a competitive inhibition radioimmunoassay of the double antibody type was developed. As shown in Fig. 2, nonlabeled elastase yielded a typical semilogarithmic inhibition profile producing complete competitive inhibition at 50 ng/ml and 50% relative inhibition at 3 ng/ml. During the course of this study, the limit of sensitivity of the radioimmunoassay (10% relative inhibition) was 0.47±0.36 ng/ml. A soluble extract of leukocyte granules also produced complete competitive inhibition and yielded an inhibition slope similar to that of the isolated enzyme. This antigenic identity suggested minimal immunoochemical alteration of the enzyme during isolation. Cathepsin G, purified from leukocyte granules, failed to produce inhibition at 1 μg/ml.

The specificity of leukocyte elastase as a marker for PMN and their activation was assessed with respect to: (a) restriction of elastase-related antigen to the PMN as contrasted to other cells, and (b) the capacity to detect secretion of elastase from stimulated leukocytes. Human peripheral blood cells were fractionated into PMN, platelet, erythrocyte, and mononuclear cell populations, and the elastase-related antigen in the detergent-solubilized extracts of the cells was quantitated by radioimmunoassay. As shown in Table I, the PMN extract contained at least 130-fold more elastase-related antigen than the other cell populations. Thus, the low levels of elastase-related antigen detected in other cell populations could be accounted for by less than a 1% PMN contamination. The level of 4.6 μg/10⁷ PMN is within the range of ~1–12 μg elastase/10⁷ PMN reported by others (16, 29).

The capacity to detect the nonlytic release of elastase-related antigen from PMN was assessed by using serum-activated zymosan particles to stimulate leukocyte secretion. Activated zymosan stimulated release of elastase-related antigen from the PMN of all five individual donors tested. Calculated relative to freeze-
The data indicated that low levels of elastase-related antigen were present in normal plasma and that when blood was permitted to clot, the level of elastase-related antigen in the resultant serum increased markedly. The basis for the elastase-related antigen in plasma was further investigated by examining the effects of anticoagulants and incubation time. Venous blood was drawn into ACD, EDTA, or heparin, as well as into the combination of these anticoagulants supplemented with the protease inhibitors, 6-aminohexanoic acid, aprotinin, and hirudin, and incubated 1 h at 22°C before separation of the plasma. In addition, blood drawn into the combination of anticoagulants and protease inhibitors was immediately centrifuged at 4°C (10 min required for plasma separation). The blood drawn into ACD, EDTA, or the combination of anticoagulants and inhibitors contained very similar levels of elastase-related antigen (Table II). The quantity of elastase-related antigen in heparinized plasma was approximately threefold higher than in the other anticoagulants. Immediate separation of the blood drawn into the combination of anticoagulants and inhibitors did not diminish the plasma elastase level. Thus, normal plasma appeared to contain a basal level of leukocyte elastase-related antigen that could not be attributed to in vitro liberation of the enzyme from leukocytes.

The increase in serum elastase-related antigen was further characterized by experiments in which cell-depleted plasma samples were reconstituted with PMN. Isolated PMN were added to ACD plasma, nonanticoagulated plasma (both platelet-rich and platelet-poor plasma), serum, or serum to which ACD was

### Table I

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Elastase-related antigen ng/10^7 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMN</td>
<td>4,600</td>
</tr>
<tr>
<td>Platelets</td>
<td>&lt;0.08</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>1.5</td>
</tr>
<tr>
<td>Mononuclear cells</td>
<td>33.5</td>
</tr>
</tbody>
</table>

* Cells were extracted with 1.0% Triton-X100 and the solubilized elastase-related antigen was quantitated by radioimmunoassay.

### Table II

<table>
<thead>
<tr>
<th>Anticoagulant</th>
<th>Elastase ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (serum)</td>
<td>307±54</td>
</tr>
<tr>
<td>ACD</td>
<td>46±25</td>
</tr>
<tr>
<td>EDTA</td>
<td>44±29</td>
</tr>
<tr>
<td>Heparin</td>
<td>139±59</td>
</tr>
<tr>
<td>Inhibitors!</td>
<td>42±22</td>
</tr>
<tr>
<td>Inhibitors!</td>
<td>52±15</td>
</tr>
</tbody>
</table>

* Cells were separated from the blood samples after 30 min at 22°C with the exception of the last sample [inhibitors (no incubation)] in which the plasma was obtained by immediate centrifugation at 4°C

† These samples were drawn into plastic syringes containing ACD supplemented with 10 mM EDTA, 0.2 M 6-aminohexanoic acid, 100 U aprotinin/ml and 50 U hirudin/ml (final concentrations).
added. The elastase-related antigen within the resultant plasma or serum samples was determined after a 1-h incubation at 22°C. The data shown in Table III illustrates the following points: (a) PMN incubated in ACD plasma did not spontaneously liberate elastase-related antigen; the elastase level within the ACD plasma incubated with cells was only 1.8-fold greater than in the plasma without added cells. (b) In the absence of anticoagulant, the level of elastase-related antigen in the resultant serum increased markedly, and this increase was independent of the presence or absence of platelets within the plasma. (c) A similar marked increase was observed when PMN were added to serum, indicating that the factors responsible for the liberation of elastase from PMN were maintained in serum. (d) Addition of ACD to serum prevented the release of elastase, suggesting a potential role for divalent ions in liberation of the enzyme.

The relationship between divalent ions, plasma, and the release of elastase was further examined. Isolated PMN were suspended in Puck’s saline A, which lacks both calcium and magnesium. Calcium, magnesium, thrombin, plasma, or combinations of the agents were added to the cell suspensions, and elastase release was measured after 1 h at 22°C. As shown in Table IV, calcium, magnesium, or the combination of these divalent ions were in themselves not sufficient to induce elastase release. Similarly, thrombin alone or clotting induced by the combination of thrombin and plasma failed to liberate elastase-related antigen. Only when both plasma and calcium were added to the cells (which resulted in clot formation), was a significant increase of elastase in the soluble fraction observed.

Cell lysis or nonlytic secretion represent possible mechanisms for the coagulation-associated liberation of elastase from PMN. To evaluate cell lysis, 51Cr-labeled PMN were suspended in Puck’s saline A or ACD plasma in the presence or absence of calcium. After 1 h at 22°C, the samples were centrifuged, and the 51Cr in the supernatant was measured. As shown in Table V, only a low level of 51Cr was released in the presence or absence of calcium in either suspending medium. The 51Cr in the supernatants of the coagulated and noncoagulated plasma samples was 6.4 and 6.2%, respectively. Quantitation of elastase-related antigen in the supernatants of these samples verified that significant release occurred only in the clotted plasma. In addition, cell viability as assessed by trypan blue exclusion was >94% in all samples. Thus, cell lysis

### Table III

**Liberation of Elastase-related Antigen from Isolated PMN Added to Plasma or Serum**

<table>
<thead>
<tr>
<th>Cells added to</th>
<th>Elastase-related antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticoagulated (ACD) plasma</td>
<td>47 ± 12</td>
</tr>
<tr>
<td>Platelet-rich plasma (no anticoagulant)</td>
<td>784 ± 86</td>
</tr>
<tr>
<td>Platelet-poor plasma (no anticoagulant)</td>
<td>1,176 ± 120</td>
</tr>
<tr>
<td>Serum</td>
<td>854 ± 48</td>
</tr>
<tr>
<td>Serum + ACD</td>
<td>88 ± 22</td>
</tr>
</tbody>
</table>

* Samples were reconstituted with 10⁷ leukocytes per milliliter. The plasma sample without added cells contained 26 ng elastase/ml. Elastase-related antigen in solution was quantitated after a 1 h incubation at 32°C. The serum was obtained from plasma clotted in the absence of cells.

### Table IV

**Effect of Divalent Ions and Thrombin on the Release of Elastase-related Antigen from PMN**

<table>
<thead>
<tr>
<th>Addition to PMN*</th>
<th>Elastase-related antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>14 ± 8</td>
</tr>
<tr>
<td>Calcium, 30 mM</td>
<td>38 ± 6</td>
</tr>
<tr>
<td>Magnesium, 30 mM</td>
<td>26 ± 6</td>
</tr>
<tr>
<td>ACD-plasma, 50%</td>
<td>21 ± 8</td>
</tr>
<tr>
<td>ACD-plasma, 50% + calcium, 30 mM</td>
<td>650 ± 23</td>
</tr>
<tr>
<td>Thrombin, 5 U/ml</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>Thrombin, 5 U/ml + ACD plasma, 50%</td>
<td>28 ± 12</td>
</tr>
</tbody>
</table>

* 1 × 10⁷ PMN/ml were suspended in Puck’s Saline A, which lacks calcium and magnesium. Additions were to the final concentrations indicated in parentheses, and release of elastase-related antigen was measured after 1 h at 22°C.

### Table V

**51Cr-Release from PMN in Clotted and Nonclotted Plasma**

<table>
<thead>
<tr>
<th>Suspending medium*</th>
<th>Calcium</th>
<th>51Cr-release</th>
<th>Elastase-related antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 mM</td>
<td>%</td>
<td>ng/ml</td>
<td></td>
</tr>
<tr>
<td>Puck’s Saline A</td>
<td>–</td>
<td>8.0</td>
<td>42</td>
</tr>
<tr>
<td>Puck’s Saline A +</td>
<td>7.8</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>ACD-plasma</td>
<td>–</td>
<td>6.2</td>
<td>28</td>
</tr>
<tr>
<td>ACD-plasma +</td>
<td>6.4</td>
<td>720</td>
<td></td>
</tr>
</tbody>
</table>

* 51Cr-PMN were reuspended at 1 × 10⁷ cells/ml in the indicated media with or without calcium. After 1 h at 22°C supernatant fractions were obtained by centrifugation. Percent 51Cr-release was calculated relative to the radioactivity solubilized by 1% Triton X100, and elastase-related antigen was quantitated by radioimmunoassay.

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and death could be reasonably excluded as the basis for elastase release.

The role of centrifugation in the induction of elastase release was evaluated. Two samples of PMN (10^7 cells/ml) were placed in ACD-plasma, and clotting was induced in one by recalcification. After 1 h at 37°C the clot was wrung and removed with a wooden applicator. Paraformaldehyde was added to a final concentration of 2% to both samples, and the cells were fixed for 1 h at 37°C. An equal volume of 0.2 M Tris, pH 7.2, was then added to neutralize excess paraformaldehyde, and the cells were then removed by centrifugation. The elastase-related antigen in recalcified and fixed samples was 390 ng/ml compared with 400 ng/ml in a sample treated in parallel but omitting the paraformaldehyde. In the nonclotted samples, these values were 32 and 28 ng/ml for the fixed and nonfixed samples, respectively. Fixation of the cells by paraformaldehyde-treated cells failed to release elastase-related antigen when stimulated with activated zymosan particles. Thus, the release of elastase from the cells in association with clotting was not dependent upon centrifugation of the cells.

The rate of elastase secretion from PMN in clotting whole blood or in plasma was assessed. The results shown in Fig. 3A were obtained by incubating blood in plastic tubes with heparin, ACD, or no anticoagulant at 37°C. In the absence of anticoagulant, clotting was detected at 19 min, and a concomitant rise in elastase-related antigen was observed in the serum after centrifugation. This increase reached a maximum level at 45 min and did not change for the next 45 min. The elastase-related antigen in the ACD plasma remained constant for the 90-min incubation, and the heparinized plasma exhibited a slight and progressive increase. Similar results were obtained with PMN added to plasma (Fig. 3B). With addition of calcium, clotting occurred at 8 min, and an increase in serum elastase was noted at 15 min. This increase again reached maximal levels at 45 min. In the absence of calcium, a slight increase in elastase-related antigen was detected between 5 and 15 min but then showed no subsequent change.

The interaction of elastase released from the PMN with plasma proteins such as protease inhibitors was evaluated in gel filtration experiments. A Sephadex G100 column (1.5 X 90 cm) was calibrated with free 125I-elastase (inactivated with DFP), with 125I-elastase added to serum before gel filtration, as well as from the elution pattern of serum proteins. As shown in Fig. 4A, free 125I-elastase eluted as a single peak beyond the elution position of albumin, consistent with its mol wt of 30,000. In contrast, when 125I-elastase was added to serum, no radioactivity was detected in the position of free elastase. Instead, a major peak of radioactivity eluted slightly before the albumin peak. The estimated mol wt of this peak at 80,000 is consistent with the formation of a 1:1 stoichiometric complex between elastase and its primary serum inhibitor, α₁-antitrypsin (29). In addition, a smaller proportion of the radioactivity eluted at the void volume of the column, perhaps arising from complex formation between elastase and a₂-macroglobulin, a second serum inhibitor of the enzyme. In Fig. 4B, serum derived 60 min after recalcification of PMN-rich plasma was applied to the column and the elastase-related antigen in the eluate was quantitated by radioimmunoassay. Two peaks of antigen were detected, and their elution positions were identical to those obtained when active 125I-elastase was added to serum. No elastase-related antigen was detected in the position of the free enzyme.

**DISCUSSION**

In this study, the detection of elastase-related antigen has been used as a marker for PMN activation. Other cells, including platelets (30) and macrophages (31), contain enzymes with elastolytic activity, but these enzymes appear to be immunologically unrelated to PMN elastase. The levels of elastase-related antigen detected in the extracts of other cell types could be...
radioimmunoassay. The elution profiles are from a 1.5 X 90-cm column of Sephadex G-100 equilibrated with 1.0 M NaCl, 0.05 M Tris, pH 7.4. Panel A shows the elution of the radioactivity of free 125I-elastase (△—△) that had been inactivated with DFP, and 125I-elastase incubated with serum for 30 min before application (●—●). The elution positions of albumin and transferrin were established from the serum. Panel B. Plasma containing 10^6 PMN/ml was recalcified and 0.3 ml of the serum removed 1 h after clotting was applied to the column. The elastase-related antigen was quantitated by radioimmunoassay.

accounted for by less than a 1% PMN contamination. The sensitivity of the radioimmunoassay used was ~0.5 ng/ml of elastase-related antigen, and this permitted ready quantitation of the elastase secreted from zymosan-stimulated PMN. In plasma, the mean level of elastase-related antigen detected was 25±6 ng/ml. Anticoagulation of the plasma with chelating agents, citrate and EDTA, did not alter this level, whereas heparinized plasma contained threefold higher levels of elastase-related antigen. Immediate separation of the cells from the plasma and addition of protease inhibitors failed to change the plasma levels of the antigen. These results would suggest that consistent low levels of elastase-related antigen may exist in blood in vivo. Of greater significance was the observation that serum levels of elastase were markedly increased above plasma levels. With each of the seven normal donors examined, serum levels were higher than plasma levels, and the mean increase was 11.5-fold. Purified leukocyte elastase and serum produced par-

allel inhibition slopes and both completely inhibited the radioimmunoassay, indicating that the leukocyte protease and the antigen detected in serum were immunochemically indistinguishable. Ohlson and Olsson (32) detected 135 ng/ml of granulocyte elastase in serum but performed no comparison of plasma and serum levels of the enzyme. The serum level of elastase-related antigen of 288±125 ng/ml detected in this study is in reasonable agreement with their report. Further evidence suggesting release of leukocyte constituents during coagulation may be derived from the study of Bennett and Mohla (33), which indicated that serum lactoferrin levels were higher than plasma levels and were thus unacceptable for clinical analyses.

Increased serum levels of elastase occurred when isolated PMN were added to nonanticoagulated plasma or to recalcified ACD plasma. This suggests that the increase is independent of other cell types, and the observation that elastase release was of a similar magnitude in coagulating platelet-rich and platelet-poor plasma supports this conclusion. Serum also supported the release of elastase from PMN, indicating a stability of the activating factor(s) following coagulation. Addition of citrate to the serum blocked elastase release. This is consistent with a role of calcium in PMN secretion (34, 35), but divalent ions were in themselves insufficient to induce elastase liberation in the absence of plasma or serum. Add tion of thrombin to PMN-rich plasma in the absence of calcium also failed to induce elastase release, indicating that direct formation of the clot by thrombin was in itself insufficient to liberate the elastase. This suggests that earlier events in coagulation or events that occur in parallel with coagulation are responsible for inducing elastase secretion. It is required that such other events occur during the conversion of plasma to serum and would be sensitive to heparin and divalent ion chelation. Active secretion is suggested because PMN lysis and decreased viability were not detected in clotted samples.

Our interest in measuring elastase release relates to its role as a fibrinolytic enzyme. Elastase release was found to occur in concert with or shortly after clot formation, and maximum release occurred within 20–40 min after clot formation. This time frame would permit the coagulative arrest of bleeding before its potential participation in fibrinolysis. Molecular exclusion chromatography indicated that the elastase-related antigen released from the PMN into serum ultimately formed high molecular weight complexes. α1-Antitrypsin and α2-macroglobulin are known inhibitors of leukocyte elastase (29) and the formation of these enzyme inhibitor complexes reasonably explains the gel filtration patterns observed. The ultimate formation of such complexes does not, however, pre-
clude a physiological role for the released enzyme. The interaction of elastase with elastin has been shown to protect the enzyme from α1-antitrypsin (36). The binding of elastase to fibrin(ogen) or other potential substrates could also prolong its half-life.

Elastase is an enzyme of broad proteolytic specificity. In addition to fibrinogen, other coagulation factors (5), complement (37), fibronectin (38), plasminogen (39), and immunoglobulins (40) have been shown to be substrates for elastase. Degradation by elastase of elastin within lungs has been implicated in the tissue damage associated with emphysema (41), and high concentrations of the enzyme are also found within rheumatoid synovial tissue (42). Furthermore, it is reasonable to assume that PMN release of elastase is not selective, so that other proteases within the leukocyte granules would also be secreted. Thus, leukocyte release associated with blood coagulation may have broad physiological and pathophysiological implications.

ACKNOWLEDGMENTS

The helpful comments of Dr. Mark Ginsberg are greatly appreciated. The technical assistance of Janet Plescia and the preparation of the manuscript by Roberta Novak-Buhman are gratefully acknowledged.

This work was supported by National Institutes of Health grant HL-17964.

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