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Research Article

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Neutrophils Degrade Subendothelial Matrices in the Presence of Alpha-1-Proteinase Inhibitor

Cooperative Use of Lysosomal Proteinases and Oxygen Metabolites

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Abstract. Triggered neutrophils rapidly degraded labeled matrices secreted by cultured, venous endothelial cells via a process dependent on elastase but not oxygen metabolites. In the presence of high concentrations of alpha-1-proteinase inhibitor, the ability of the stimulated neutrophil to solubilize the matrix was impaired. However, at lower concentrations of alpha-1-proteinase inhibitor the neutrophil could enhance the degradative potential of its released elastase by a H_2O_2-dependent process. Coincident with this increase in matrix damage, the stimulated neutrophil destroyed the elastase inhibitory activity of the alpha-1-proteinase inhibitor via a catalase-inhibitable process. The ability of the triggered neutrophil to solubilize the matrix in the presence of alpha-1-proteinase inhibitor was unaffected by superoxide dismutase or hydroxyl radical scavengers but was markedly impaired by catalase, azide, or hypochlorous acid scavengers. We conclude that neutrophils can cooperatively use an oxidant with characteristics similar, if not identical, to hypochlorous acid and the lysosomal proteinase elastase to negate the protective effects of alpha-1-proteinase inhibitor in order to attack the subendothelial matrix.

Introduction

Neutrophils migrating into an inflammatory site have the potential to mediate tissue injury by either generating cytotoxic oxygen metabolites or releasing lysosomal proteinases (1, 2). Defense mechanisms designed to control the steady state concentration of released oxygen metabolites are primarily confined to intracellular sites, while proteinase activities are regulated by a complex system of extracellular antiproteinases (1, 2). Thus, antiproteinases localized at an inflammatory site are exposed to a relatively uncontrolled oxidizing environment into which the target proteinases are released.

Recent studies have demonstrated that alpha-1-proteinase inhibitor (α-1-PI),[1] the primary regulator of neutrophil elastase, can be inactivated by a variety of oxidants (for examples and references see 3–8). Indeed, exposure of α-1-PI to phagocyte-generated oxidants effectively destroys its ability to bind or inhibit the activity of exogenous pancreatic or neutrophil elastase (3–6). This has led to the proposal that triggered neutrophils may have the ability to mediate elastase-dependent damage in the presence of α-1-PI by altering the proteinase-antiproteinase balance (3–7). Although this is an attractive hypothesis, no studies have successfully demonstrated that intact phagocytes can suppress the elastase inhibitory capacity of α-1-PI at a rate that allows their coincidentally released elastase to degrade biologically relevant substrates. In this report we have examined the ability of triggered human neutrophils to degrade naked, radiolabeled matrices secreted by cultured endothelial cells in the absence or presence of α-1-PI. Our results indicate that neutrophils can use oxygen metabolites to potentiate the ability of their released elastase to solubilize a subendothelial basement membrane in the presence of α-1-PI.

Methods

Neutrophil preparation. Neutrophils were isolated from the peripheral venous blood of volunteers by Ficoll-Hypaque density centrifugation and dextran sedimentation as previously described (9). Cells were sus-

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1. *Abbreviations used in this paper:* α-1-PI, alpha-1-proteinase inhibitor; GLPCK, benzyloxycarbonyl-glycine-leucine-phenylalanine-chloromethyl ketone; HOCl, hypochlorous acid; LDH, lactate dehydrogenase; AAPVCK, methoxysuccinyl-alanine-alanine-proline-valine-chloromethyl ketone; PMA, phorbol myristate acetate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SOD, superoxide dismutase.

pended in Dulbecco's phosphate-buffered saline (pH 7.4) supplemented with 1 mg/ml glucose (Gibco Laboratories, Grand Island, NY).

Radiolabeled subendothelial matrices. Human umbilical vein endothelial cells were cultured in 24-well, gelatin-coated tissue culture plates (Costar 3424, 16-mm well diam, Data Packaging, Cambridge, MA) according to the methods of Maciag et al. (10), except that 20% human serum was substituted for fetal calf serum. After the monolayers were confluent, the media was changed to proline-free M199 (Gibco Laboratories) to which [^{3}H]proline (1.5 μCi/ml; New England Nuclear, Boston, MA) was added along with daily additions of ascorbic acid (20 μg/ml). The cells were cultured for an additional 7–10 d with media changes every 3 d. Endothelial cell monolayers were lysed with 25 mM NH_4OH (30 min at 4°C); and the adherent matrix was washed five times with phosphate-buffered saline and used immediately (11). Total radioactivity incorporated into each matrix was determined after overnight digestion with 2 N NaOH as described in reference 11. In 45 plates (24 wells each), the total counts per minute incorporated into each individual matrix ranged from 18.5 to 42.6 $\times$ 10^3 (31.4±7.3 $\times$ 10^3 cpm/matrix; mean±1 SD). Within individual plates, the total counts per minute per matrix varied by <10% while plate-to-plate variation from a single pool of endothelial cells also varied by <10%. Because large differences in the total counts per minute incorporated into each matrix existed between plates obtained from different endothelial cell preparations, duplicates were always arranged within a single plate or on plates from a single endothelial cell preparation.

Matrix degradation. Matrices were incubated alone or with neutrophils in the presence or absence of phorbol myristate acetate (PMA; Consolidated Midland Corp., Brewster, NY) prepared as previously described (9). Duplicate mixtures (1 ml final volume) were incubated at 37°C in an air incubator. At selected times, the plates were centrifuged (50 *g* for 5 min); and 0.1-ml aliquots were removed for scintillation counting. At the end of the experiment each reaction mixture was removed, the residual matrix digested, and the total counts per minute per matrix determined for each test sample. Results are expressed as the percent radioactivity released and duplicates varied by <10%.

Other additions to the matrix system included superoxide dismutase (SOD; 3,400 U/mg; Boehringer Mannheim Biochemicals, Indianapolis, IN), catalase (100,000 U/mg; Worthington Biochemical Corp., Freehold, NJ), human albumin (fatty acid free), azide, taurine, L-methionine, mannitol (Sigma Chemical Co., St. Louis, MO) and ethanol. Methoxysuccinyl-alanine-alanine-proline-valine-chloromethyl ketone (AAPVCK) and benzyloxycarbonyl-glycine-leucine-phenyl-alanine-chloromethyl ketone (GLPCK) were obtained from Enzyme Systems Products, Livermore, CA. Human α-1-PI was purchased from Calbiochem-Behring Corp. (American Hoechst Corp., San Diego, CA). Greater than 95% of this preparation migrated as a single 52,000-mol wt band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and 96% of the original material was identified as α-1-PI on radial immunodiffusion plates (Calbiochem-Behring Corp.). The α-1-PI had ~80% of ideal functional activity when assayed in either a porcine pancreatic elastase or neutrophil elastase binding assay (kindly performed by Dr. P. Stone, Boston University). In selected experiments, highly purified α-1-PI (provided by Dr. N. Matheson and J. Travis, University of Georgia) was substituted for the commercial preparation.

SDS-PAGE. [^{3}H]proline-labeled material released from the matrix by neutrophils was analyzed by SDS-PAGE and fluorography. Matrices were incubated alone, with neutrophils, or with PMA-stimulated neutrophils for 60 min. The supernatants were then removed from triplicate wells, pooled, immediately cooled to 0°C, precipitated with ethanol (12 vol of 95% ethanol, 0°C, 16 h), and centrifuged (10,000 *g*, 30 min). The pellets were solubilized by heating (100°C, 5 min) in 2% SDS, 5% β-mercaptoethanol, 0.001% bromophenol blue, and 62.5 mM Tris-HCl, pH 6.8. Samples were electrophoresed by using discontinuous slab gel electrophoresis with a 3% polyacrylamide stacking gel (pH 6.8) and a 5% polyacrylamide resolving gel (pH 8.8) as described in reference 12. Electrophoresis was performed at 25 mA/slab until the sample entered the resolving gel, then the current was increased to 50 mA/slab. After fixation in 50% trichloroacetic acid, treatment with EN^{3}HANCE (New England Nuclear), and drying, gels were visualized by fluorography. High molecular weight, ^{14}C-labeled standards were obtained from New England Nuclear.

Enzyme assays. Lactate dehydrogenase (LDH) was assayed according to Buetler (13). The elastase inhibitory capacity of α-1-PI was assayed with porcine pancreatic elastase (Worthington Biochemical Corp.) and *N*-succinyl-L-alanyl-L-alanyl-L-alanine-*p*-nitroanilide (Sigma Chemical Co.) as described (14).

All results are expressed as the mean±1 SD.

Results

Matrix degradation by neutrophils. Neutrophils incubated alone (1.25 $\times$ 10^5 cells/well) or in the presence of PMA (30 ng/ml) solubilized significant amounts of the matrix (Fig. 1) while control matrices incubated with buffer or PMA alone released only small amounts of radioactivity. After a 60-min incubation, PMA-stimulated neutrophils degraded ~4.5 times more matrix than neutrophils alone. LDH release by PMA-stimulated cells increased by <5% during this incubation period. 65% of the radioactivity released by triggered neutrophils at 60 min was precipitable with 10% trichloroacetic acid. If the radioactive material(s) released from the matrix by triggered neutrophils was examined by SDS-PAGE and fluorography, at least seven bands could be clearly identified (Fig. 2). The polypeptides ranged in M_r from ~157,000 to 69,000, while the remainder of the radioactivity was localized at the tracking dye front ($M_r \leq 55{,}000$).

Role of oxygen metabolites in solubilization of matrix components. To determine if oxygen metabolites play a role in matrix degradation by triggered neutrophils, experiments were performed in the presence of agents designed to (*a*) lower the concentration of O_2^- (superoxide dismutase) or H_2O_2 (catalase), (*b*) inhibit myeloperoxidase (azide), (*c*) scavenge the hydroxyl radical

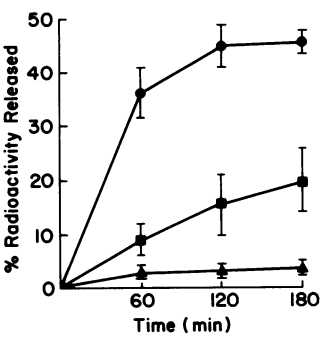

Figure 1. The effect of neutrophils on the digestion of [^{3}H]proline labeled matrices. Matrices were incubated with buffer or PMA alone (▲), 1.25 $\times$ 10^5 neutrophils (■), or 1.25 $\times$ 10^5 neutrophils and 30 ng/ml PMA (●). Aliquots (0.1 ml) were removed from duplicate samples at 60, 120, and 180 min and the released radioactivity was determined. Results are expressed as the mean percent radioactivity released ±1 SD of six experiments.

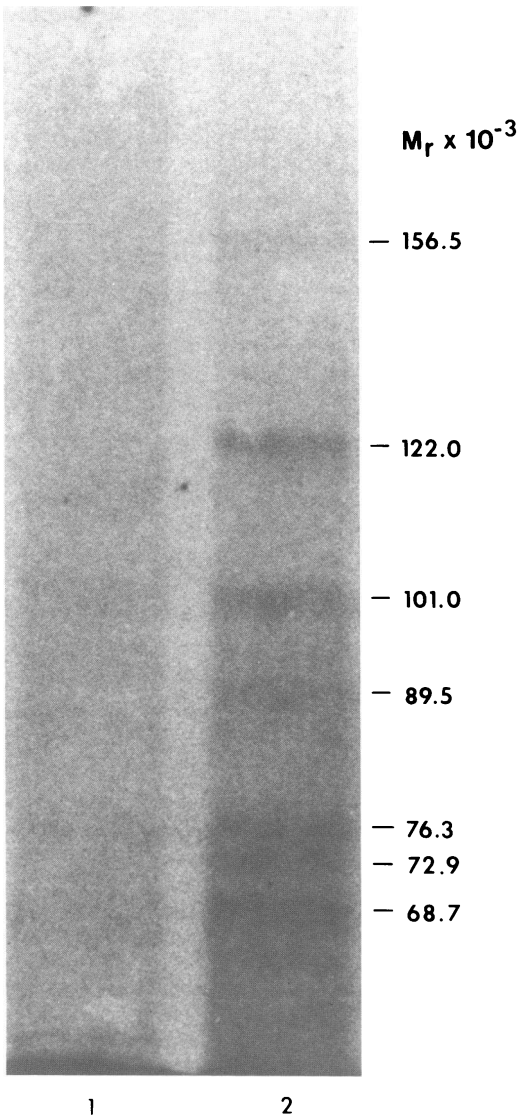

Figure 2. [^{3}H]proline-labeled material released from matrices by neutrophils. Neutrophils (1.25×10^5/ml) were incubated alone (lane *1*) or with 30 ng/ml PMA (lane *2*) for 60 min on labeled matrices. Supernates were removed and processed as described in Methods. Autofluorograms were developed after 21 d.

(ethanol, mannitol), or (*d*) intercept hypochlorous acid (taurine, methionine; for references see 1). As shown in Table I, none of these agents affected matrix degradation by stimulated neutrophils.

Role of elastase in neutrophil-mediated degradation. If released lysosomal proteinases are responsible for matrix degradation, then cell-free supernates obtained from triggered neutrophils should degrade the matrix comparably to whole cells. Thus, supernates were recovered from PMA-stimulated neutrophils (1.25×10^5/ml) that were incubated in tubes for 60 min and then added to the matrices. In six paired experiments, 27.7±5.4% of the matrix-associated radioactivity was solubilized by the supernates during a 60-min incubation as compared with 35.9±5.3% degraded by the intact cells. The addition of the specific neutrophil elastase inhibitor AAPVCK (20 μM; reference 2) to the supernate almost completely inhibited matrix degradation (97.7±2.0% inhibition; $n = 6$) while the neutrophil cathepsin inhibitor GLPCK (20 μM; 2) had a moderate but variable inhibitory effect (18.6±16.9% inhibition; $n = 5$). If elastase is primarily responsible for matrix degradation, then α-1-PI should also inhibit solubilization. Indeed, as little as 0.25 μg of α-1-PI completely (>99%) inhibited the supernate's ability to solubilize the matrix.

Inhibitory effect of α-1-PI on matrix degradation by neutrophils. The ability of exogenous α-1-PI to inhibit matrix degradation by PMA-stimulated neutrophils incubated directly on the matrix for a 60-min period is illustrated in Fig. 3. High concentrations of α-1-PI (250 μg/ml) inhibited matrix degradation by ~90%, while lower doses that were clearly in excess of those required to inhibit the activity in cell-free supernates were much less effective. For example, 6.25 μg of α-1-PI inhibited matrix degradation by 1.25×10^5 triggered neutrophils by only 25% (Fig. 3), while 0.25 μg of α-1-PI completely inhibited the activity in the corresponding cell-free supernates (see above). Albumin (6.25–250 μg/ml) had no inhibitory effect on matrix solubilization by stimulated neutrophils (data not shown). At the lowest dose tested (6.25 μg/ml), α-1-PI completely inhibited matrix degradation by the resting neutrophils ($n = 10$).

The requirement for higher concentrations of α-1-PI to inhibit matrix degradation by PMA-stimulated cells suggested that α-1-PI in the fluid phase may have inadequate access to the

Table I. Role of Oxygen Metabolites in Matrix Degradation by Triggered Neutrophils

Additive*	% Radioactivity released‡
Neutrophils + PMA (complete system)	33.8±4.4 ($n = 5$)
Complete system + catalase (2.5 μg/ml)	32.0±5.5 ($n = 4$)
Complete system + SOD (10 μg/ml)	33.6±5.8 ($n = 4$)
Complete system + albumin (10 μg/ml)	34.1±5.3 ($n = 5$)
Complete system + azide (0.1 mM)	32.5±4.3 ($n = 4$)
Complete system + taurine (5 mM)	32.9±5.0 ($n = 4$)
Complete system + methionine (5 mM)	35.1±5.4 ($n = 3$)
Complete system + ethanol (40 mM)	31.4 ($n = 2$)
Complete system + mannitol (40 mM)	32.3 ($n = 2$)

* Complete system consisted of 1.25×10^5 neutrophils and 30 ng of PMA in a final volume of 1 ml.

‡ Results are expressed as the mean percent radioactivity released ±1 SD during a 60-min incubation. Percent release is calculated on the basis of total radioactivity associated with each individual matrix. In the absence of neutrophils, 1.5±0.3% of the total radioactivity was released spontaneously during the 60-min incubation.

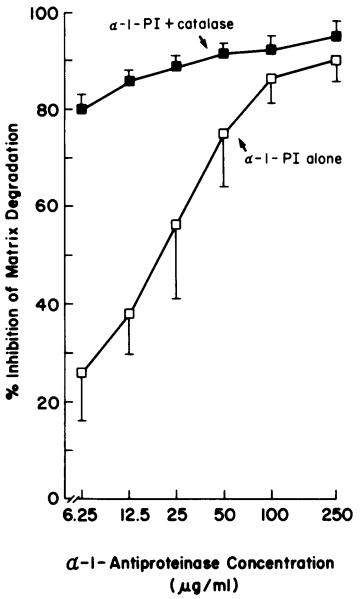

Figure 3. The effect of α-1-PI on matrix degradation by stimulated neutrophils. Neutrophils (1.25 × 10^5/ml) were incubated with PMA (30 ng/ml) in the presence of the indicated concentrations of α-1-PI without (□) or with (■) 2.5 μg/ml catalase. Duplicate mixtures were incubated for 60 min and the percent radioactivity released was determined. Results are expressed as the percent inhibition of matrix degradation relative to controls of PMA-stimulated neutrophils alone. Control values for the 11 experiments shown were 33.0±5.7% release.

neutrophil-matrix interface or that neutrophils were actively protecting their elastase from inactivation by α-1-PI. Because neutrophils are able to inactivate the elastase inhibitory capacity of α-1-PI by H_2O_2-dependent processes (3–6), we examined the ability of exogenous catalase to enhance the ability of α-1-PI to inhibit matrix degradation. In the presence of catalase (2.5 μg/ml), the inhibitory capacity of α-1-PI was dramatically increased (Fig. 3). Low concentrations of α-1-PI that was coincubated with catalase inhibited matrix degradation by stimulated neutrophils comparably to the highest doses of α-1-PI tested. For example, 12.5 μg/ml of α-1-PI inhibited neutrophil-mediated damage by 37.8±9.1%, while the addition of catalase increased inhibition 2.3-fold to 85.5±2.0% (n = 11). (Identical results were obtained with the highly purified α-1-PI [n = 2].)

To determine if the changes in the effectiveness of α-1-PI in the absence and presence of catalase correlated with its ability to inhibit pancreatic elastase activity, we directly examined the elastase inhibitory capacity in supernates recovered from test mixtures. Interestingly, when 12.5 μg/ml of α-1-PI were incubated with triggered neutrophils (1.25 × 10^5) on matrices for 60 min, there was a complete loss of the elastase inhibitory capacity (n = 5) despite the ability of this dose of α-1-PI to partially protect the matrix. (Identical results were obtained with the highly purified α-1-PI [n = 2].) Indeed, even if the α-1-PI concentration was raised to 25 μg/ml (a dose that inhibited matrix degradation by 57±16%), we could not detect any residual elastase inhibitory capacity after a 60-min incubation with 1.25 × 10^5 neutrophils under identical conditions. In either case, the addition of catalase (2.5 μg/ml) to the mixtures resulted in the recovery of >98% of the expected α-1-PI activity. These data indicate that triggered neutrophils (*a*) use an H_2O_2-dependent process to potentiate the matrix-degrading effects of released elastase in the presence of α-1-PI and (*b*) can destroy the elastase inhibitory capacity of large amounts of α-1-PI but not at rates that completely dissipate the protective effect of the antiproteinase.

Role of H_2O_2 in the enhancement of elastase-mediated damage. Our data suggest that H_2O_2 plays a pivotal role in protecting elastase from inhibition by α-1-PI. Thus, we next determined whether this effect was mediated by H_2O_2 alone or via the use of H_2O_2 as a precursor for the generation of a more reactive species (1, 15). As demonstrated above, α-1-PI alone (12.5 μg/ml) partially inhibited matrix degradation but its ability to protect the matrix from the triggered neutrophil was enhanced by catalase but not by heat-inactivated catalase (Table II). SOD slightly enhanced the ability of α-1-PI to inhibit matrix destruction but this effect was no greater than that observed with human serum albumin. Although H_2O_2 may be involved in hydroxyl radical generation (1), neither ethanol nor mannitol impaired matrix degradation in the presence of α-1-PI. Neutrophils can use H_2O_2 in the myeloperoxidase system to generate a chlorinating species with characteristics similar if not identical to hypochlorous acid (HOCl) (9, 16, 17). Thus, we examined the ability of the myeloperoxidase inhibitor azide and the HOCl scavengers taurine and methionine to impair the ability of the triggered neutrophil to degrade the matrix in the presence of α-1-PI. As shown in Table II, all three agents enhanced the effectiveness of α-1-PI and decreased the ability of the neutrophil to attack the matrix to a level comparable to that observed with catalase.

Table II. Role of Oxygen Metabolites in Matrix Degradation by PMA-stimulated Neutrophils in the Presence of α-1-PI

Additive*	% Radioactivity released‡	% Inhibition of matrix degradation relative to stimulated neutrophils and α-1-PI§
Neutrophils + PMA	35.7±5.5 (n = 8)	—
Neutrophils + PMA + α-1-PI (complete system)	22.5±4.8 (n = 8)	—
Complete system + catalase	5.0±0.8 (n = 8)	77.2±3.7
Complete system + heat inactivated catalase	17.7±1.8 (n = 3)	10.7±8.5
Complete system + SOD	17.7±6.9 (n = 8)	9.0±11.1
Complete system + albumin	18.1±2.5 (n = 3)	9.3±8.3
Complete system + ethanol	19.8±3.4 (n = 3)	5.0±8.6
Complete system + mannitol	18.9±1.7 (n = 3)	5.0±2.6
Complete system + azide	4.4±1.1 (n = 7)	79.9±2.9
Complete system + taurine	5.7±1.1 (n = 4)	70.3±4.7
Complete system + methionine	6.6±2.1 (n = 8)	70.5±6.5

* 1.25 × 10^5 neutrophils stimulated with 30 ng PMA in a final volume of 1 ml. The concentration of α-1-PI was 12.5 μg/ml and the amounts of the other proteins and inhibitors were as shown in Table I. ‡ Percent radioactivity released is expressed as the mean percent±1 SD radioactivity released during a 60-min incubation based on the total radioactivity associated with each individual matrix. In the eight experiments shown the mean counts per minute released by stimulated neutrophils during the 60-min incubation were 11,345±1176 cpm. § Percent inhibition of matrix degradation relative to α-1-PI was calculated for paired experiments as percent radioactivity released by stimulated neutrophils in the presence of α-1-PI and tested oxygen metabolite inhibitor/percent radioactivity released by stimulated neutrophils in the presence of α-1-PI alone.

Discussion

In this study we have examined the ability of triggered neutrophils to solubilize subendothelial matrices in the absence and presence of α-1-PI. In an antiproteinase-free environment, triggered neutrophils solubilized the matrix by a process that was primarily dependent on elastase. Although we have not identified the specific matrix components attacked by the neutrophil, the subendothelial matrix contains a number of elastase-sensitive targets including glycoproteins (e.g., fibronectin, laminin [18, 19]), proteoglycans (20), and collagens (types I, III, and IV [20–23]). We found no evidence to suggest a direct or synergistic role for oxygen metabolites in matrix degradation but cannot exclude oxidative alterations of matrix components that do not yield soluble fragments.

For neutrophils to mediate elastase-dependent damage in vivo they must contend with high concentrations of α-1-PI in the circulation and tissue spaces. It has been proposed that phagocytes could use oxygen metabolites to inactivate α-1-PI and thus protect their released elastase (3–7). However, this supposition is based on the demonstrated ability of phagocytes to inactivate α-1-PI in noncompetitive systems wherein the antiproteinase is exposed to oxidants and then exogenous elastase is added (3–7). Campbell et al. (24) have correctly argued that for this process to operate in situ, the phagocyte must oxidatively inactivate α-1-PI before the antiproteinase complexes with and inhibits elastase. In our model the final outcome of the competitive interplay between elastase, α-1-PI, and oxygen metabolites can be readily monitored. The potential ability of the neutrophil to shield its released elastase was first suggested by the striking differences in the α-1-PI concentration required to inhibit comparable matrix degradation by the intact cell and its corresponding supernatant. Matrix degradation by the supernates obtained from stimulated cells could be completely inhibited by as little as 0.25 μg of α-1-PI, while a 1,000-fold increase in the antiproteinase concentration (i.e., 250 μg) failed to completely block solubilization by the intact, triggered neutrophil. As the α-1-PI concentration was lowered in the intact cell system to doses ranging from 50 to 6.25 μg/ml (200 to 40 times higher than the dose needed to inhibit the cell-free supernatants) the protective effect of the antiproteinase rapidly decreased. Although a requirement for higher concentrations of α-1-PI in the intact cell system could solely reflect the inaccessibility of the fluid phase α-1-PI to the neutrophil-matrix interface, interventions designed to lower the concentration of oxygen metabolites dramatically enhanced the ability of α-1-PI to protect the matrix. Inhibitor studies suggest that neutrophils triggered in the presence of α-1-PI used an oxidant with characteristics similar if not identical to HOCl to potentiate the ability of the released elastase to attack the matrix in the presence of α-1-PI. Presently, our studies do not allow us to conclude whether the effect is mediated directly by HOCl or indirectly via the generation of endogenous *N*-chloroamines (15). Nonetheless, it appears that both elastase and oxidants are released into an environment accessible to a large portion of the fluid phase α-1-PI. The final outcome of this complex interaction will be dictated by both the timing of elastase release and HOCl generation and the competing rates of oxidative inactivation of α-1-PI, elastase inhibition by active α-1-PI, and elastase binding to sensitive target proteins. Because α-1-PI inhibits neutrophil elastase at extremely rapid rates ($K_{association} = 6.5 \times 10^7$ [25]) and oxidants cannot reactivate bound elastase (5), it seems likely that the elastase is protected from α-1-PI either before or during its release. Oxidative protection of released elastase is not the only process used by the neutrophil to attack subendothelial matrix components in the presence of α-1-PI. Triggered neutrophils were able to solubilize the matrix in the presence of large amounts of α-1-PI even while the concentration of oxygen metabolites was reduced. Indeed, we have found that neutrophils isolated from a patient with chronic granulomatous disease could also slowly degrade the matrix in the presence of α-1-PI (S. J. Weiss, unpublished observation). Whether this process represents the inability of α-1-PI to gain access to the microenvironment between the neutrophil and matrix or represents solubilization of the matrix by α-1-PI-insensitive proteinases remains to be determined. A similar oxygen-independent process of matrix degradation has been suggested in a recent report by Campbell et al. (24). In their study, triggered neutrophils degraded ^{125}I-fibronectin in the presence of α-1-PI by an apparent elastase-dependent process. However, in contrast to our findings, oxidative inactivation of α-1-PI did not play an important role in potentiating elastase-mediated proteolysis. Direct comparisons of the two model systems will be required to fairly assess these differences.

Quantitative analysis of the residual elastase inhibitory capacity from mixtures of stimulated cells and α-1-PI revealed that the neutrophil could completely inactivate doses of α-1-PI that provided partial protection of the subendothelial matrix. However, measurements of the elastase inhibitory capacity were performed with porcine pancreatic elastase, which is incapable of binding to oxidized α-1-PI (25). This contrasts with the ability of oxidized α-1-PI to bind neutrophil elastase at a slower but still significant rate (6, 25). In addition, elastase inhibitory capacity measurements determine the overall activity of a homogeneous mixture of α-1-PI and not the local concentrations of native vs. oxidized α-1-PI surrounding the triggered neutrophil and its released elastase. Our results are consistent with either a partial inhibition of matrix degradation by high concentrations of oxidized α-1-PI or the inability of the neutrophil to inactivate α-1-PI at rates that completely prevent partial elastase inhibition. Finally, despite the obvious limitations of elastase inhibitory capacity measurements, these results reinforce the striking ability of triggered neutrophils to lower the effective antiproteinase concentration (5). On the basis of our taurine trapping technique, 1.25×10^5 PMA-triggered neutrophils can generate ~12.5 nmol of HOCl (9). Assuming that two to four methionyl residues may be oxidized on each α-1-PI molecule (7, 8), 1.25×10^5 neutrophils have the potential to inactivate 162.5–325 μg of the antiproteinase. (Alpha-1-PI will also be inactivated after complex formation with elastase but, 1.25×10^5 PMA-triggered neutrophils released only enough elastase to bind 0.25 μg of α-

1-PI. In this case, the neutrophil could inactivate far larger amounts of α-1-PI via oxidative attack than by releasing elastase.) Although high concentrations of α-1-PI may be able to prevent tissue damage by successfully complexing small amounts of released elastase in the face of an oxidative attack, the concentration of native α-1-PI will nonetheless fall. If a second wave of phagocytes then enters the inflammatory site, the oxidized α-1-PI would leave the tissue vulnerable to elastase-mediated damage.

In summary, we have demonstrated that neutrophils can negate the protective effects of α-1-PI in order to attack a subendothelial matrix via the generation of an oxidant with characteristics similar to HOCl and the release of elastase. Our results clearly indicate that triggered neutrophils can use both oxygen metabolites and proteinases in a cooperative fashion to mediate tissue damage. In these studies a nonphysiologic triggering agent, PMA, was used but physiologic stimuli likely to be encountered in vivo would be expected to mediate qualitatively similar effects. Triggering agents that might be generated in pathological states in vivo include soluble or fixed antigen-antibody complexes, opsonized particles or surfaces, high concentrations of chemotactic factors and activated complement components (1). Although these stimuli differ quantitatively in their ability to trigger an oxidative burst and lysosomal enzyme release, several studies have indicated that "weak" stimuli can elicit much greater responses depending on the physical presentation of both the triggering agent and the phagocyte (26–28). Despite the limitations of our in vitro model, it is especially interesting to note that the findings presented here bear close analogy to reports of inactive or oxidized α-1-PI and free elastase in pulmonary lavage fluids from both cigarette smokers (29) and patients with respiratory distress syndrome (30–32) and in rheumatoid synovial fluids (33). If HOCl and elastase are responsible for these in vivo findings, it seems likely that oxidized α-1-PI and free elastase activity will be found in a variety of neutrophil-dependent, pathological states. The model system used in this study should provide us with a powerful tool to further analyze the processes used by triggered phagocytes to mediate tissue damage and to design potential therapeutic interventions.

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