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Activation of the endogenous metalloproteinase, gelatinase, by triggered human neutrophils

(phagocytes/hypochlorous acid/oxidative activation/chronic granulomatous disease)

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ABSTRACT **Triggered human neutrophils degraded denatured type I collagen (gelatin) by releasing and activating the latent metalloenzyme, gelatinase. The ability of the neutrophil to activate this enzyme was significantly, but not completely, inhibited by agents known to inhibit or scavenge chlorinated oxidants generated by the H_2O_2/myeloperoxidase/chloride system. A direct role for chlorinated oxidants in this process was confirmed by the ability of reagent HOCl to activate the latent enzyme in either the cell-free supernatant or in a highly purified state. Gelatinase activity was also expressed by triggered neutrophils isolated from patients with chronic granulomatous disease. The amount of gelatinolytic activity expressed by the patients' cells was similar to that released by normal neutrophils that were triggered in the presence of antioxidants. Thus, human neutrophils have the ability to activate gelatinase by either an HOCl-dependent process or an uncharacterized oxygen-independent process. The ability of the neutrophil to directly regulate this enzyme suggests an important role for the metalloproteinase in physiologic and pathophysiologic connective tissue metabolism.**

Human neutrophils can be triggered to release the collagenolytic metalloenzyme, gelatinase, which is stored in a unique secretory particle compartment (1, 2). The purified enzyme has been shown to directly attack denatured collagens (i.e., gelatin), to solubilize native type IV and type V collagens, as well as to potentiate the activity of neutrophil interstitial collagenase (3–6). Based on the substrate specificity of the isolated enzyme, an increasing number of studies have postulated potential roles for gelatinase in diapedesis, chemotaxis, inflammation, and wound repair (1–4). However, neutrophil gelatinase, like many other collagenolytic metalloenzymes, is synthesized in a latent form and must be activated before it can catalyze substrate degradation (3–6). At present, it is clear that neutrophils can release latent gelatinase (1, 2), but the potential significance of the enzyme's action *in vivo* is blunted by the fact that the ability of the phagocyte to activate gelatinase is unknown.

In addition to gelatinase, neutrophils contain a second latent metalloenzyme, collagenase, whose substrate specificity is limited to the interstitial collagens—i.e., types I, II, and III collagens (3, 4). Recently, we demonstrated that latent collagenase is activated by triggered neutrophils via an unusual process dependent on the cell's ability to generate the highly reactive oxygen metabolite, hypochlorous acid (HOCl; ref. 7). Despite the fact that both collagenase and gelatinase are latent metalloenzymes, the proteinases are structurally distinct, can be released independently, and possess little or no overlap in substrate specificity (1–6). In this study, we demonstrate that triggered neutrophils are able to activate a portion of their released latent gelatinase, but by two distinct processes; one that is directly mediated by HOCl and a second that is independent of respiratory burst reactions.

MATERIALS AND METHODS

Cell Preparation. Neutrophils were isolated from normal volunteers or from two males with well-characterized chronic granulomatous disease (CGD; ref. 8) by Ficoll-Hypaque density centrifugation and dextran sedimentation as described (9). Cells were suspended in Hanks' balanced salt solution (pH 7.4; GIBCO).

Incubation Conditions. Neutrophils (5×10^6 cells per ml) were incubated alone, in the presence of 30 ng of phorbol 12-myristate 13-acetate (PMA; Consolidated Midland, Brewster, NY) or with 1.25 mg of opsonized zymosan particles (Sigma) prepared as described (9) in a final vol of 1 ml. Other additions to the neutrophil incubation mixtures included superoxide dismutase (3000 units/mg; Boehringer Mannheim), catalase (88,000 units/mg; Cooper Biomedicals, Malvern, PA), α-1-proteinase inhibitor (PI; Cooper Biomedicals), glucose oxidase, sodium azide, or L-methionine (Sigma). The mixtures were incubated for 15 min at 37°C in a 5% CO_2/95% air atmosphere, and the cell-free supernatants were recovered by centrifugation ($1250 \times g$ for 5 min). In selected experiments, the cell-free supernatant (500 μl) or the purified neutrophil gelatinase (see below) was incubated with sodium hypochlorite (Fisher) before being assayed. All supernatants were then treated with an equal vol of 2 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$) and 10 μg of PI.

Gelatinase Isolation. Neutrophil gelatinase was isolated from the cell-free supernatant of triggered phagocytes according to Hibbs *et al.* (5), except that Ficoll-Hypaque-purified neutrophils were used in place of dextran-sedimented blood to decrease the likelihood that contaminating platelets would release metalloproteinase inhibitors (10), and azide (0.1 mM) was included in the incubation mixture to prevent oxidative activation of gelatinase (see *Results*). After the incubation period, the cell-free supernatant was removed and treated with $PhMeSO_2F$ and PI. The released gelatinase was purified by a combination of DE52 (Whatman) and gelatin-Sepharose (Pharmacia) affinity chromatography as described by Hibbs *et al.* (5). $NaDodSO_4$/PAGE of the isolated enzyme was performed according to the method of Laemmli (11). Gelatinase activity was visualized following $NaDodSO_4$/PAGE in $NaDodSO_4$/substrate gels containing 2 mg of gelatin per ml (5).

Gelatinase Assays. Cell-free supernatants (250 μl) prepared as described above or purified gelatinase were incubated for 3 or 6 hr at 37°C with 50–100 μg of native or heat-denatured ^{3}H-acetylated type I collagen (≈1.2×10^6 cpm/mg) prepared

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Abbreviations: PI, α-1-proteinase inhibitor; CGD, chronic granulomatous disease; PMA, phorbol 12-myristate 13-acetate.

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from rat tails (7). Total gelatinase activity (i.e., latent and active) was assessed in 50 μl of supernatant in the presence of 1 mM *p*-aminophenylmercuric acetate (Sigma; refs. 1–5). Gelatin degradation was determined by the release of trichloroacetic acid-soluble (15%, wt/vol) radioactivity (12) or by $NaDodSO_4$/PAGE performed on reduced heat-denatured samples that were electrophoresed in a 3% stacking and 8.5% resolving gel. Protein bands were visualized with Coomassie brilliant blue (Sigma).

HOCl Generation. Neutrophils (5×10^6) were incubated as described above in the presence of 15 mM taurine (Sigma) for 15 min at 37°C. The HOCl trapped as *N*-chlorotaurine was quantitated as described by Weiss *et al.* (9).

RESULTS

Release of Gelatinolytic Activity from Triggered Neutrophils. As shown in Table 1, supernatants recovered from resting neutrophils degraded only small amounts of gelatin during a 3-hr incubation period. However, if neutrophils were incubated with the soluble stimulus, PMA, or with opsonized zymosan particles for 15 min, gelatinolytic activity significantly increased ≈14-fold and ≈5-fold, respectively.

In the gelatinolytic assay, substrate degradation is only quantitated if the radiolabeled fragments remain soluble in 15% trichloroacetic acid. Because only those gelatin polypeptides of $M_r \leq 5000$ are detected under these conditions (12), proteolysis was directly assessed by $NaDodSO_4$/PAGE (Fig. 1). As expected, supernatants from resting neutrophils mediated barely discernable gelatin degradation after a 3-hr (lane 2) or even a 6-hr incubation (lane 3). In the presence of the metalloproteinase inhibitor EDTA (10 mM), no degradation was observed (lane 4). In marked contrast, supernatants from either PMA (lane 5) or zymosan-triggered cells (lane 8) extensively degraded the gelatin substrate during a 3-hr incubation. After 6 hr, the α, β, and γ bands of gelatin were almost completely degraded by the triggered cell supernatants (lanes 6 and 9) via an EDTA-inhibitable process (lanes 7 and 10).

To determine whether unactivated latent enzyme was also present in the triggered cell supernatant, total gelatinolytic activity (i.e., latent and active) was unmasked by the organomercurial activating agent 4-aminophenylmercuric acetate (1–5). Under these conditions, the gelatinolytic activity in supernatants recovered from PMA or zymosan-triggered cells increased ≈6-fold and ≈8-fold, respectively. In six experiments, PMA-stimulated cells activated 18.2% ± 1.9% (mean ± 1 SD) of their released gelatinolytic potential while zymosan-stimulated cells activated 12.3% ± 1.0%.

Linkage of Gelatinase Activation to Oxygen Metabolite Generation. We recently proposed that reactive oxidants generated by neutrophils could act as endogenous activating

Table 1. Release of gelatinolytic activity from neutrophils

Supernatants obtained from	Gelatin degraded, μg per 5×10^6 cells per 3 hr	*n*
Resting neutrophils	24 ± 13	11
PMA-triggered neutrophils	328 ± 51	22
Zymosan-triggered neutrophils	122 ± 18	6

Neutrophils were incubated as described and supernatants were assayed for gelatinolytic activity. Results are expressed as the mean ± 1 SD of the indicated number of experiments. The amount of trichloroacetic acid-soluble radioactivity released from buffer controls averaged 8.3% ± 3.3% ($n = 18$) of the total radioactivity added, and this value was subtracted from all test mixtures.

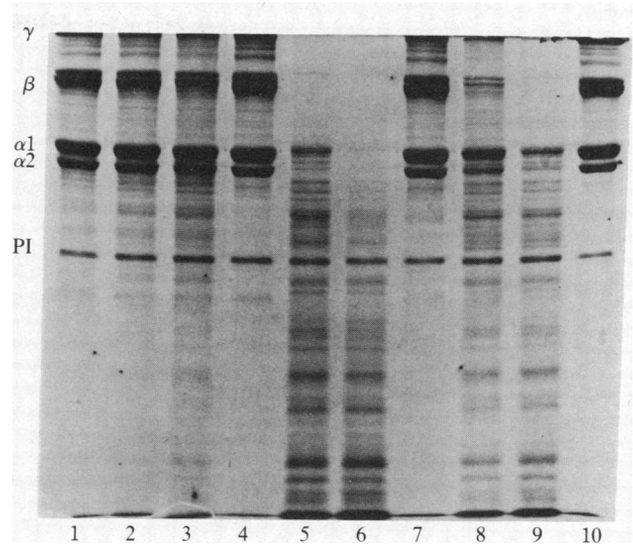

FIG. 1. Expression of gelatinolytic activity by neutrophils. Supernatants from resting or triggered neutrophils were incubated with 85 μg of gelatin at 37°C as indicated and analyzed by $NaDodSO_4$/PAGE. Gelatin was incubated alone (lane 1) or with supernatants from resting neutrophils for 3 hr (lane 2) or 6 hr (lane 3) and with 10 mM EDTA for 6 hr (lane 4). Supernatants from PMA-triggered neutrophils were incubated with gelatin for 3 hr (lane 5) or 6 hr (lane 6) or with EDTA for 6 hr (lane 7). Supernatants from neutrophils triggered with zymosan were incubated with gelatin for 3 hr (lane 8) or 6 hr (lane 9) and with EDTA for 6 hr (lane 10).

agents for collagenase (7), and we sought to determine whether these species also played a role in gelatinase activation. Thus, neutrophils were triggered with PMA in the presence of agents known to reduce $O_2^{\cdot-}$ (superoxide dismutase), degrade H_2O_2 (catalase), inhibit myeloperoxidase (azide), or scavenge chlorinated oxidants (methionine), and gelatinolytic activity was determined. As shown in Table 2, catalase (but not heat-inactivated catalase), azide, or methionine significantly inhibited gelatin degradation by ≈75%. The addition of 4-aminophenylmercuric acetate to each of the supernatants increased gelatin degradation comparably. However, it should be noted that total activity was slightly, but consistently, lower in supernatants recovered from neutrophils triggered in the presence of catalase, azide, or methionine. Similar results were obtained with zymosan-triggered neutrophils (data not shown).

The effect of these agents on the expression of gelatinolytic activity was confirmed by $NaDodSO_4$/PAGE (Fig. 2). Neutrophils triggered with PMA alone released large amounts of

Table 2. Regulation of gelatinolytic activity released from neutrophils

Supernatants obtained from neutrophils triggered with	Gelatin degraded, μg per 5×10^6 cells per 3 hr		*n*
	− APMA	+ APMA	
PMA	309 ± 46	1688 ± 112	5
PMA + superoxide dismutase (10 μg)	358 ± 36	1772 ± 144	5
PMA + catalase (25 μg)	75 ± 19	1400 ± 248	5
PMA + heat-inactivated catalase (25 μg)	290	1542	2
PMA + azide (1 mM)	75 ± 13	1380 ± 216	5
PMA + methionine (5 mM)	67 ± 16	1320 ± 264	5

Neutrophils were incubated as described. Results are expressed as the mean ± 1 SD of the indicated number of experiments performed in the absence or presence of 4-aminophenylmercuric acetate (APMA).

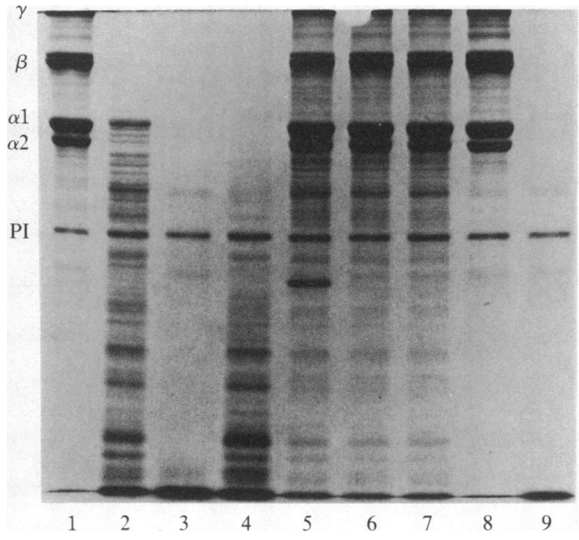

FIG. 2. Effect of antioxidants on gelatinolytic activity released by neutrophils. Neutrophils were triggered as indicated, and the supernatants were removed, incubated with 85 μg of gelatin for 3 hr at 37°C, and analyzed by $NaDodSO_4$/PAGE. Gelatin incubated alone (lane 1) or with the supernatant from neutrophils incubated with PMA (lane 2), PMA followed by treatment with 4-aminophenylmercuric acetate (lane 3), PMA and 10 μg of superoxide dismutase (lane 4), PMA and 25 μg of catalase (lane 5), PMA and 1 mM azide (lane 6), PMA and 5 mM methionine (lane 7), PMA and methionine followed by 10 mM EDTA (lane 8), and PMA and methionine followed by 4-aminophenylmercuric acetate (lane 9).

gelatinolytic activity (lane 2), and this was slightly increased if cells were stimulated in the presence of superoxide dismutase (lane 4). In contrast, gelatinolytic activity was significantly depressed in those supernatants recovered from cells stimulated in the presence of catalase (lane 5), azide (lane 6), or methionine (lane 7). However, as described in the quantitative assay, residual EDTA-sensitive gelatinolytic activity could still be detected (compare lane 1 to lanes 5–8). As expected, the addition of 4-aminophenylmercuric acetate to supernatants obtained from cells triggered in the absence (lane 3) or presence of antioxidants (lane 9) resulted in the complete degradation of the gelatin substrate.

Activation of Neutrophil Gelatinase by Hypochlorous Acid. Triggered neutrophils are known to generate H_2O_2 and to release the lysosomal heme–enzyme, myeloperoxidase, which can catalyze the peroxidation of chloride to hypochlorous acid (see refs. 9, 13–15). The inhibitory effect of catalase, azide, and methionine on gelatinolysis suggested that HOCl played an important role in the activation of gelatinase. To directly determine the ability of HOCl to increase gelatinolytic activity, reagent HOCl was added to cell-free supernatants obtained from mixtures of neutrophils triggered in the presence of catalase. The addition of HOCl at 50 nmol/ml, an amount similar to that generated by 5×10^6 PMA-triggered neutrophils in 15 min (44.5 ± 15.8 nmol; n = 4), increased gelatinolytic activity from 107 ± 43 μg degraded (mean ± 1 SD; n = 6) to 412 ± 82 μg degraded (n = 6). Gelatinase activity was slightly increased at 100 nmol of HOCl per ml (477 μg ± 69 μg degraded; n = 6), but then decreased at higher HOCl concentrations (data not shown). To determine the effect of HOCl on the total gelatinase activity (i.e., both active and latent gelatinase) in the untreated and HOCl-treated supernatants, the samples were incubated with 4-aminophenylmercuric acetate. Supernatants recovered from neutrophils triggered in the presence of catalase increased their gelatinolytic activity from 107 ± 43 μg of gelatin degraded to 1343 ± 303 μg degraded (n = 6) following activation with 4-aminophenylmercuric acetate. Interestingly, the addition of the organomercurial to the HOCl-treated supernatants (50 nmol/ml) resulted in the expression of even larger amounts of gelatinolytic activity (2121 ± 551 μg; n = 6). Thus, the addition of HOCl to the cell-free supernatants not only activated gelatinolysis, but also increased the amount of gelatinolytic activity that could be detected after organomercurial activation. Nonetheless, reagent HOCl activated gelatinase at a dose and to an extent almost identical to that produced by the intact cell.

Although HOCl might mediate its enhancing effect by acting directly, it could act by first reacting with endogenous amines to form long-lived *N*-chloroamines (RNCl; see refs. 13–15). The major RNCl generated by the neutrophil is *N*-chlorotaurine (13–15), and the ability of this oxidant to increase gelatinase activity was determined. Whereas HOCl at 100 nmol/ml optimally increased gelatinolytic activity from 107 ± 43 μg degraded to 477 ± 69 μg, *N*-chlorotaurine (100 nmol/ml) was slightly less effective and increased activity to 336 ± 81 μg degraded (n = 4).

To further elucidate the mechanism underlying gelatinase activation, the latent enzyme was purified and the effect of reagent HOCl on its activity was determined. As recently described (5), purified gelatinase migrated as three distinct bands on $NaDodSO_4$/PAGE of M_r ≈225,000, 130,000, and 92,000 (Fig. 3*A*). After reduction, gelatinase migrated as a single band with a M_r of 92,000 (Fig. 3*A*). All three bands expressed gelatinolytic activity after activation in gelatin-containing $NaDodSO_4$ gels (Fig. 3*B*). The purified gelatinase degraded only small amounts of gelatin in the standard 3-hr assay system (9.9 ± 1.0 μg; n = 5), but after the addition of 4-aminophenylmercuric acetate, 149.9 ± 10.5 μg (n = 5) of substrate was degraded. (To conserve enzyme, the amount of purified gelatinase activity used in these experiments was 1/10th that detected in the neutrophil supernatants.) Thus, the purified enzyme existed almost entirely in a latent state while remaining sensitive to organomercurial activation. The effect of increasing doses of reagent HOCl on the purified

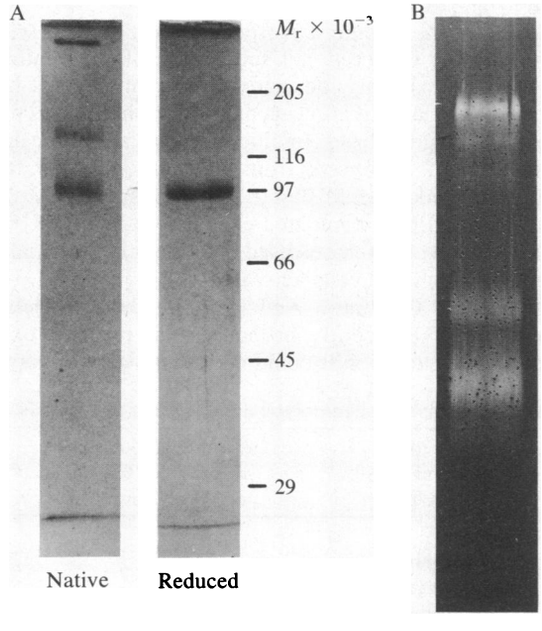

FIG. 3. (*A*) $NaDodSO_4$/PAGE of purified neutrophil gelatinase. Purified neutrophil gelatinase (≈5 μg) was electrophoresed in the absence or presence of 2-mercaptoethanol (8.5% polyacrylamide gel). Molecular weight standards are indicated to the right. (*B*) Gelatin-degrading activity of purified neutrophil gelatinase. Purified gelatinase was electrophoresed on a 6% polyacrylamide gel that contained gelatin and was processed as described. Negative staining indicates zones of gelatinolysis.

enzyme is depicted in Fig. 4. [Although gelatinase is purified in Tris buffers, the enzyme must be dialyzed free of Tris for the HOCl experiments. HOCl rapidly reacts with Tris—i.e., Tris(hydroxyaminomethane) to form the corresponding *N*-chloroamine.] Peak activity was obtained with 0.5 nmol of HOCl wherein gelatinase degraded 45.2 ± 6.3 μg of gelatin (n = 5). Increasing the dose of HOCl resulted in a rapid loss in gelatinolytic activity (Fig. 4). In data not shown, less effective activation could also be obtained with either *N*-chlorotaurine or *N*-chloroamine (13–15). In contrast to the results obtained when cell-free supernatants were treated with reagent HOCl, the addition of 4-aminophenylmercuric acetate to mixtures of purified gelatinase that had been incubated with HOCl did not result in the expression of more gelatinolytic activity than that detected in the activated gelatinase control. That is, gelatinase incubated with 4-aminophenylmercuric acetate degraded ≈150 μg of gelatin per 3 hr, while latent gelatinase treated with 0.25, 0.5, 0.7, 1.0, and 5.0 nmol of HOCl and then activated with the organomercurial degraded 153 ± 13 μg (n = 3), 137 ± 11 μg (n = 5), 128 μg (n = 2), 108 μg (n = 2), and 0 μg (n = 1), respectively. Relative to the organomercurial activator, HOCl maximally activated 30% of the total gelatinolytic potential of the proteinase.

Oxygen-Independent Gelatinase Activation. Although HOCl played a primary role in the expression of gelatinolytic activity by the intact neutrophil, the inability of catalase, azide, or methionine to completely inhibit gelatin degradation suggested that a portion of the activation process was not linked to oxidative metabolism. The residual gelatinolytic activity was inhibitable by EDTA, and the gelatin polypeptides generated appeared identical to those generated by the oxidatively linked gelatinase (see Fig. 2), but these data alone do not allow us to rule out the possibility that an additional gelatinolytic metalloenzyme was responsible for this activity. Because either collagenase (16) or stromelysin (17) could potentially participate in gelatin degradation, cell-free supernatants were recovered from neutrophils that were triggered in the absence or presence of azide, electrophoresed on $NaDodSO_4$/gelatin gels, and examined for bands of gelatinolysis. As shown in Fig. 5, electrophoresis of an aliquot of the triggered neutrophil supernatant equal to that used in the trichloroacetic acid assay revealed three bands of gelatinolytic activity identical to those observed with the purified gelatinase. If the amount of activity applied to the gel was increased 8-fold, these results were unchanged whether the cells were triggered in the absence (lane 2) or presence of azide (lane 3). Thus, gelatinase was the only metalloenzyme detected in the supernatants that was capable of degrading gelatin.

Finally, to determine whether gelatinase could be activated by a process independent of a respiratory burst, CGD neutrophils were examined for their ability to express gelatinolytic activity. Triggered CGD neutrophils degraded the gelatin substrate (136 ± 35 μg; n = 4) comparably to normal cells that were triggered in the presence of catalase (see Table 2). As described in studies with normal cells, the gelatinolytic activity released from the CGD neutrophil was almost completely inhibited in the presence of 10 mM EDTA (activity was reduced from 136 ± 35 μg degraded to 4.4 ± 2.1 μg; n = 3). If CGD cells were supplemented with exogenous H_2O_2 (glucose and 15 milliunits of glucose oxidase per ml), gelatinolytic activity increased to normal levels (349 ± 49 μg degraded; n = 4) in a catalase-sensitive manner (activity fell to 93 ± 23 μg degraded in the presence of 10 μg of catalase).

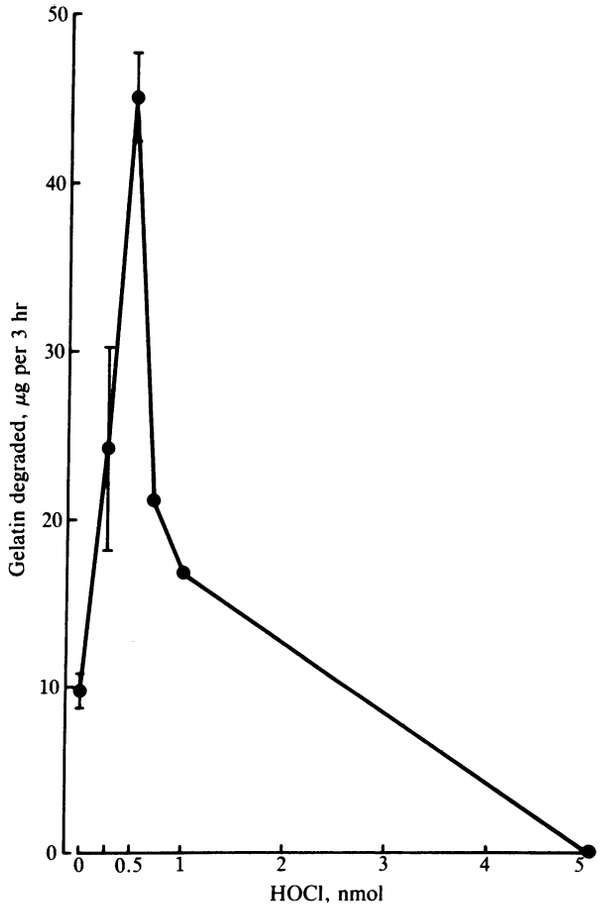

FIG. 4. Activation of latent gelatinase by HOCl. Purified gelatinase (100 ng) was incubated alone or with the indicated doses of HOCl in a final vol of 0.5 ml for 15 min at 37°C. Results are indicated as the mean ± 1 SEM.

DISCUSSION

We have demonstrated that triggered neutrophils possess endogenous activating systems capable of regulating latent gelatinase activity. The ability of exogenous organomercurials to activate latent collagenolytic metalloenzymes has focused attention on the potential role of a critical thiol or thiols in the regulation of the activity of the enzyme (18–20). HOCl can also react with thiols, and it is tempting to speculate that thiol oxidation underlies gelatinase activation. However, the common thiol reactivity of HOCl and organomercurials and their mutual ability to activate metalloproteinases may be fortuitous. Skin fibroblast procollagenase can be activated by a variety of organomercurials, but attempts to detect a free thiol group in the enzyme have been unsuccessful (21). Apparently, organomercurials act by penetrating and disrupting the inner domains of the zymogen

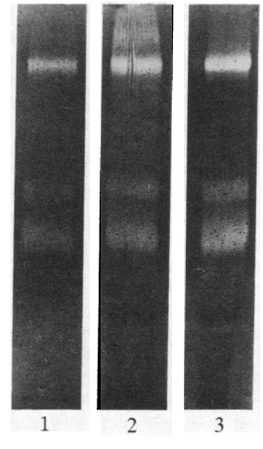

FIG. 5. Detection of gelatinolytic enzymes released by neutrophils. Neutrophil supernatants were prepared as described and electrophoresed on a 6% polyacrylamide gel that contained gelatin. The gel was processed as described in Fig. 3*B*. Lanes: 1, unconcentrated supernatant from PMA-triggered cells; 2, supernatant from PMA-triggered cells concentrated 8-fold; 3, supernatant from neutrophils triggered in the presence of azide and concentrated 8-fold.

(21). HOCl can oxidize thiols and thioethers, chlorinate amines or aromatics, as well as rupture peptide bonds (13–15), but the manner in which it activates gelatinase remains unknown.

Despite the fact that purified gelatinase could be activated by HOCl, the oxidative regulation of gelatinolytic activity in the intact cell appeared more complex. The total gelatinolytic activity detected in supernatants recovered from neutrophils triggered in the presence of catalase, azide, or methionine was consistently lower after organomercurial activation than in the supernatants obtained from cells that were triggered in the absence of antioxidants. A requirement for HOCl in the maximal expression of total gelatinase activity was likewise noted in experiments designed to monitor the effects of reagent HOCl on gelatinase activity in the cell-free supernatant. This additional requirement for HOCl was not a property intrinsic to latent gelatinase, because the purified enzyme that was incubated with HOCl and then treated with 4-aminophenylmercuric acetate did not express increased total activity. These results would be most readily explained by the presence of either an endogenous HOCl-labile gelatinase inhibitor or an HOCl-dependent promoter of gelatinase activity, but mixing experiments have failed to detect either type of activity (data not shown). At present, we conclude that HOCl not only activated latent gelatinase, but also allowed for the maximal detection of gelatinolytic activity after organomercurial activation.

HOCl played a pivotal role in gelatinase activation, but we were intrigued to note that the oxidant failed to activate >30% of the total gelatinolytic potential in the intact cell, the cell-free supernatant, or in the purified enzyme system. The inability of HOCl to activate greater amounts of gelatinase is unclear, but neutrophils were triggered in suspension where gelatinase and HOCl would be rapidly diluted as they were released into a relatively large extracellular volume. Under more physiologic conditions, adherent neutrophils might release gelatinase and HOCl into a sequestered microenvironment and activation may be more efficiently regulated. In addition, neutrophils were triggered in the absence of a targeted substrate. Other latent enzymes are known to be activated by forming stoichiometric complexes with nonprotease proteins (22) and full activation of gelatinase may only occur when the enzyme is released in a more physiologic environment. Finally, only a portion of the released gelatinase may be sensitive to oxidative activation. In any case, our data indicate that triggered neutrophils can use endogenously generated HOCl to activate significant amounts of released gelatinase.

Neutrophils primarily relied on HOCl for the activation of gelatinase, but both normal cells triggered in the presence of antioxidants and CGD cells were able to express substantial gelatinolytic activity. The mechanism of non-oxidatively linked gelatinase activation was not determined, but we cannot rule out the possibility that gelatinase may undergo autoactivation (23).

In conjunction with our earlier studies on collagenase (7), it is clear that neutrophils exposed to triggering agents that elicit lysosomal enzyme release and an oxidative burst are able to release active collagenase and gelatinase. In concert, these metalloenzymes could arm the triggered neutrophil with the ability to degrade native as well as denatured types I–V collagen. However, gelatinase is stored in a highly responsive secretory compartment and it can also be released by stimuli that do not provoke a strong oxidative burst (1). Dewald *et al.* proposed that gelatinase might play an important role in neutrophil diapedesis and chemotaxis (1). Oxidative activation of gelatinase could allow normal neutrophils to traverse connective tissue barriers, but CGD cells would not be able to activate gelatinase by this route. Nonetheless, CGD neutrophils do not express a gross chemotactic defect *in vivo* (24). The fact that both normal and CGD neutrophils can comparably activate gelatinase by an HOCl-independent process suggests that a careful assessment of the enzyme's potential role in chemotaxis is in order. Further analyses of the neutrophil's ability to autoregulate gelatinase should provide new insights into the physiologic and pathophysiologic regulation of connective tissue metabolism.

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