Differences in Myeloperoxidase Activity From Neutrophil Polymorphonuclear Leukocytes of Differing Density: Relationship to Selective Exocytosis of Distinct Forms of the Enzyme

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**MYELOPEROXIDASE** (MPO) is present in large amounts in the azurophilic granules of polymorphonuclear neutrophils (PMN) and is a component of the oxygen-dependent microbialicidal system of the cell. Biochemical studies have indicated a molecular heterogeneity of MPO from PMN from a number of sources. In addition, peroxidase activity is also found in cells of the monocyte/macrophage series. Peroxidase in these two cell lineages may be identical, although possible cell-specific differences may also exist. Differences that have been reported for putative macrophage peroxidase include increased sensitivity to inhibition by 3-amino-1,2,4-triazole (AT) and less activity in the oxidation of chloride when compared to the activity of neutrophil MPO. Similar differences, however, have also been observed between multiple forms of MPO isolated from murine leukocytes and human PMN.

To investigate further the nature of the cellular specificity of these peroxidase activities as well as possible structure–function relationships, we have used Percoll density gradient centrifugation to obtain purified cell populations from elicited murine peritoneal exudates. As reported in the accompanying article, we observed that elicited PMN could be separated into two density populations operationally referred to as intermediate density PMN (ID-PMN) and high density PMN (HD-PMN). Both of these PMN populations could be resolved from lower density mononuclear cells and appeared to represent PMN in “activated” and “resting” states, respectively. It seemed that possible structure–function differences among distinct forms of MPO might be related to PMN activation and might be reflected in the peroxidase activity associated with elicited peritoneal PMN of differing density (ID-PMN, HD-PMN).

In this article we report that MPO activity associated with ID-PMN was more sensitive to inhibition by AT and had a lower intrinsic activity in the oxidation of chloride and iodide than the corresponding MPO activity from HD-PMN. In addition, we present the results of in vitro studies using murine and human PMN, which support a possible functional relationship between degranulation and changes in the enzymatic characteristics of cell-associated MPO activity. Finally, using human PMN, we show that the basis for such differences in the enzymatic characteristics of MPO activity can be related to the selective exocytosis of chromatographically and enzymatically distinct forms of the enzyme.

**MATERIALS AND METHODS**

**Mice**

C57BL/6J mice of both sexes and male BALB/c mice were obtained from our own breeding colonies. Male CF-1 mice were...
from Charles River Laboratories, Boston, MA. Mice were 2–5 mo old when used.

Preparation and Purification of Cells
Elicited murine peritoneal exudates were prepared and fractionated by Percoll density gradient centrifugation, as described in detail previously. Briefly, leukocyte-rich peritoneal exudate cells (PEC) were elicited by injection (i.p.) of 2 ml sterile 1% glycogen or 0.4% caseinate in saline, and were harvested at the times indicated in the text. PEC were initially pelleted by centrifugation and washed once with deficient Hank's balanced salt solution (BSS; Ca²⁺,Mg²⁺-free). After lysis of contaminating erythrocytes, cells were suspended in Dulbecco's phosphate-buffered saline (PBS; Ca²⁺,Mg²⁺-free). To remove residual debris and obtain a single cell suspension, the cells were passed through a 200-mesh stainless steel screen. Three milliliters of cell suspension (6–7 x 10⁶ cells/ml) were mixed with 6 ml of pH-adjusted (7.5), isotonic Percoll. The mixture was centrifuged in acid-washed siliconized glass tubes (15 ml, Correx) in a Sorvall RC2-B centrifuge using a SS-34 fixed angle rotor for 25 min at 18,000 rpm (10² g·min). High density (HD) PMN were obtained at 89% purity. Intermediate density (ID) PMN, isolated from BALB/c or C57BL/6 mice, were obtained at 90% purity. Recovery of the cells from the gradient was 75%–80% of total cells. Viability of the cells as determined by trypan blue exclusion was >90% for PMN and 80%–90% for macrophages. Murine peripheral blood cells were obtained by peribuccal sinus puncture; EDTA (10%) was used to inhibit coagulation. Murine and human peripheral blood PMN were isolated by dextran sedimentation and purified by Ficoll-Hypaque density centrifugation as described previously.

Identification of PMN
PMN were distinguished by characteristic morphology using polychrome staining and the presence of lactoferrin, a specific protein of this cell type. Slides were prepared using a cytocoentrifuge, and light microscopy was performed using Wright's stain. Lactoferrin was identified immunochemically using the tetrahydrodiamidine-labeled F(ab')₂ portion of rabbit IgG directed against murine lactoferrin, as described previously. Peroxidase was demonstrated following fixation of cells in 10% formalin-ethanol (v/v) using 3,3-diaminobenzidine as described by Graham and Karnovsky, except that 10 mM sodium phosphate buffer, pH 7.0, was employed.

Degranulation of PMN Induced by Chemotactic Factors
Degranulation of murine PMN was induced by treatment of cells with endotoxin (lipopolysaccharide, S. typhosa) activated serum. Both control and activated serum were prepared as described in the accompanying paper. HD-PMN (5 x 10⁶ to 1 x 10⁷ cells) recovered after Percoll density gradient centrifugation of elicited peritoneal exudate cells (PEC) were incubated in 1 ml complete Hanks' balanced saline solution (BSS, containing Ca²⁺,Mg²⁺) buffered with HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 20 mM), pH 7.4, containing 20% activated or control serum. The cell suspensions were gently agitated in plastic tubes at 37°C in a metabolic shaker water bath for 30 min. Secretion of granule enzymes was induced by pretreatment of PMN with cytochalasin-B (5 µg/ml) for 10 min before the addition of endotoxin-activated serum. Human PMN (10⁷ cells/ml) were incubated in the presence of 10⁻⁸ M N-formyl-methionyl-leucyl-phenylalanine (FMLP) for 30 min at 37°C in complete BSS (containing Ca²⁺,Mg²⁺) buffered with 20 mM HEPES, pH 7.4. The peptide was removed by washing and cells were resuspended in the same medium and maintained at 4°C for 60 min. Secretion of granule protein was then induced by incubation of the cells with cytochalasin-B (5 µg/ml) for 5 min followed by reexposure of the cells to 10⁻⁷ M FMLP. After 5 min, cells were centrifuged (400 g for 5 min), and cell pellets and supernatants were saved for quantitation of released (supernatant) or residual (cell pellet) granule protein.

Solubilization of MPO From Human PMN Granules
PMN were suspended at a concentration of 2.5 x 10⁶ cells/ml in ice-cold 0.34 M sucrose containing 10 µg/ml each of phenylmethyl-sulfonylfluoride (PMSF), L-1-tosylamide-2-phenyl-ethylchloromethyl ketone (TPCK), and 1 mM EDTA. In some preparations, PMN were also treated with diisopropylfluorophosphatase (DFP), as described by Amrein and Stossel. Cell lysates were prepared by making continuous passes with a motor-driven Teflon-glass homogenizer (900 rpm for 5 min), centrifuged at 500 g for 5 min (4°C), and the supernatants retained. The pellets, containing unbroken cells, nuclei, and membranes, were rehomogenized, centrifuged, and 500 g supernatants collected. This process was repeated until pellets had lost most of their green color (usually 1–2 times). The pooled supernatants were centrifuged at 27,000 g for 15 min, and the resulting granule pellets were either used fresh or stored frozen at -20°C. Granule pellets were suspended in an ice-cold solution containing 10 mM sodium phosphate buffer, pH 7.0, 0.3% cetyltrimethylammonium bromide (CTAB), 10 µg/ml each of PMSF and TPCK, and 1 mM EDTA and were dispersed with a motor-driven Teflon-glass homogenizer (1,900 rpm, 15–30 sec) until well dispersed. After 30 min in an ice bath, the suspension was centrifuged at 27,000 g for 15 min. MPO was recovered in the supernatant.

Immunochemical Precipitation of Murine MPO
Immunochemical precipitation of MPO activity was carried out using rabbit antiserum prepared against purified murine MPO. The enzyme was purified using a modification of the method of Desser et al. Fluorochrome conjugates of this antibody stained PMN but did not stain eosinophils. Serum from immunized and nonimmunized rabbits was clarified by centrifugation at 20,000 g for 30 min at 0°C, and then was dialyzed against 10 mM sodium phosphate buffer, pH 7.5, containing 0.15 M NaCl. Cell lysates (180 µl, 10⁷ cells) were made using 0.1% Triton X-100 containing 0.2 M NaCl and were incubated in small tubes with various amounts of antisemum (5–30 µl) or nonimmune rabbit serum. Each reaction mixture was brought to a final volume of 0.26 ml with Dulbecco's phosphate-buffered saline (PBS), and EDTA was added to a final concentration of 0.1 mM. Incubation was carried out for 16 hr at 4°C, after which the tubes were centrifuged at 1,800 g for 15 min in a swinging bucket rotor. Supernatants and pellets were then assayed for MPO activity. Complete activity was recovered in each precipitate, except at the highest antisemum concentration used (80% recovery).

Ion-Exchange Chromatography
Detergent-solubilized MPO was analyzed for its characteristic chromatographic forms by ion-exchange chromatography on carboxymethylcellulose. Granule lysates were dialyzed against 25 mM sodium acetate, 0.2 M NaCl, pH 4.7, and then adsorbed onto a column (0.9 cm x 20 cm) equilibrated in the same buffer. Protein in the column effluent was followed at 280 nm with a flow-through absorbance monitor. After protein absorbance had returned to baseline, a linear salt gradient (250 ml, 0.2 M NaCl to 1.0 M NaCl) was used to elute the enzyme. Flow rate was maintained at 5.5 ml/hr. Enzyme recovered from cell incubation media after degranu-
lation was chromatographically analyzed in the same manner after first being dialyzed against column buffer.

Assays

Unless otherwise stated, all assays of total cellular enzymes from murine cells were performed on cell lysates prepared in 0.25 M sucrose by making 10 hand-held passes with a glass-Teflon homogenizer. For assay of MPO, the homogenate was brought to a final concentration of 0.3% CETAB by the addition of 1% CETAB; this concentration was determined to be optimal for solubilization of enzyme activity. In the case of human PMN, total cellular MPO concentration was determined to be optimal for solubilization of concentration of 0.3% CETAB by the addition of 1% CETAB; this during the course of the reaction. The reaction mixture contained 25 mM, 4°C. Activity was expressed as total mmole CO2 released/hr.

MPO was assayed by measuring the increase in absorbance at 470 nm due to the formation of tetraguaiacol. The reaction mixture (3 ml) consisted of 10 mM sodium phosphate buffer, pH 7.0, 13 mM guaiacol, 0.33 mM H2O2, and 0.1% CETAB.14 One unit of activity in murine studies was defined as 1 nmole tetraguaiacol formed/min using 26.6 mM 1 cm as the extinction coefficient of tetraguaiacol.29 In human studies, 1 unit was 1 μmole tetraguaiacol formed/min. The hydrogen peroxide concentration used in the studies was determined to be optimal for the range of enzyme concentrations used in the comparative studies reported on below. Initial slopes were used in calculating activity. In experiments where AT was used as an inhibitor, it was premixed directly with the reaction mixture. Decarboxylation of l-alanine was measured by the method of Migler et al. using 1-14C-l-alanine.21 The reaction was carried out in capped Teflon tubes inserted into scintillation vials. Gas diffusion holes in the tubes were covered by the rim of the scintillation vial during the course of the reaction. The reaction mixture contained 25 mM sodium acetate, pH 5.5, 1.67 mM l-alanine, 83.3 nCi l-14C-l-alanine (specific activity 1 mCi/mmmole), 0.1 M KCl, and 0.28 mM H2O2 in a final volume of 3 ml, and was allowed to proceed at 37°C for 1 hr. The reaction was stopped by the addition of 1 ml of 5% trichloroacetic acid. The tubes were then depressed below the rim of the counting vials, exposing the diffusion holes, and the 14CO2 released was collected in 0.5 ml of Hyamine hydroxide in the bottom of the vial. After collecting the CO2 for 45 min at 37°C, 10 ml of scintillation cocktail (Omnifluor, New England Nuclear, Boston, MA) were added, and the vials were counted in a liquid scintillation spectrometer (Beckman, model LS 7000) at an efficiency of 75% for 14C. Activity was expressed as total μmole CO2 released/hr.

The oxidation of iodide by MPO was measured spectrophotometrically by the method of Bos et al.,4 and enzyme activity was calculated as described by Hosoya.25 The 3 ml reaction mixture contained 20 mM KI, 200 mM Na2SO3, 50 mM sodium acetate buffer, pH 5.5, and 0.33 mM H2O2. One unit of activity was that amount of enzyme oxidizing 1 μmole iodide/min.

Lysozyme activity was measured turbidimetrically in murine cell lysates (0.1% Triton X-100, 0.2 M NaCl, 10 mM phosphate buffer, pH 7.0) by following the decrease in absorbance at 450 nm at 25°C using a modification of the method of Shugar.23 The reaction mixture contained 0.155 g/ml lysozyme substrate (Micrococcus lysodeikticus) in 38 mM sodium potassium phosphate buffer, pH 6.2, in a final volume of 3 ml. Egg white lysozyme was used as a standard.

Lactate dehydrogenase was assayed by a modification of the method of Bergmeyer and Bernt24 using PBS as the assay medium. One unit of enzyme activity was defined as a change in absorbance of 0.001/min. Beta-glucuronidase was measured at 37°C as described by Fishman, using phenolphthalein-β-glucuronide as substrate.25 Activity was expressed as μg phenolphthalein released/14 hr. The latter two enzymes were assayed in murine cell lysates prepared in 0.1% Triton X-100, 0.2 M NaCl, 10 mM phosphate buffer, pH 7.0.

Protein was assayed by the method of Lowry et al.,26 using bovine serum albumin as the standard. When CETAB was present, the reaction mixture was made 0.7% SDS, which prevented a precipitate from forming when the phenol reagent was added. Standards were run accordingly in the presence of the detergent.

Solutions and Chemicals

The BSS used with procedures involving murine cells was adjusted to an osmolality of 308 mosmole, as described previously.12 BSS of standard composition was used with human PMN.

Oyster glycogen (type II), egg white lysozyme, 3-amino-1,2,4-triazole, phenolphthalein-β-glucuronide, nicotinamide adenine dinucleotide, and guaiacol were purchased from Sigma Chemical Company, St. Louis, MO. Cetyltrimethylammonium bromide was purchased from Eastman Kodak, Rochester, NY. Lysozyme substrate (Micrococcus lysodeikticus) and lipopolysaccharide (S. typhosa) were purchased from Difco Laboratories, Detroit, MI. Percoll was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Wright's stain was manufactured by Harleco, Philadelphia, PA. Triton X-100 and hydrogen peroxide were purchased from Mallinkrodt, Paris, KY. Carboxymethylcellulose (CM-52) was obtained from Whatman Ltd., Springfield, Mill Maidstone, Kent, England. Diaminobenzidine was purchased from Baker Chemical Co., Phillipsburg, NJ. 1-14C-l-alanine was obtained from Radiochemical Center, Amersham, England. Hyamine hydroxide was from Packard, Downers Grove, IL. Omnifluor was purchased from New England Nuclear, Boston, MA. N-formyl-methionyl-leucyl-phenylalanine was obtained from Sigma Chemical Co. or Vega Chemical Co., Tucson, AZ. All other chemicals were of reagent grade purity or better.

RESULTS

Differences in MPO Activity in Murine PMN Fractions of Different Density

We previously observed11 that chromatographically distinct forms of MPO isolated from elicited murine PEC exhibited a differential sensitivity to inhibition by 3-amino-1,2,4-triazole (AT). For this reason, we examined the AT inhibition of MPO activity associated with high density (HD) and intermediate density (ID) PMN obtained by Percoll density gradient centrifugation of elicited PEC.12 The data of Fig. 1 show that cell-associated MPO activity was increasingly sensitive to inhibition by AT between the HD-PMN and ID-PMN regions of the gradient. In order to rule out the possibility that this effect was simply the result of differential sensitivity to a single concentration of AT, the concentration of the inhibitor was varied over a 40-fold range, using the same amount of activity (11 units) for each of the cell fractions tested. Under these conditions, there was a clear and different dose-response for the pooled HD- and ID-PMN fractions. Preincubation with the inhibitor for different periods of time (1–4 min) had no effect, indicating that observed differences in inhibition could not be explained on the basis of differences in access to, or rate of, reaction with AT.
A comparison of the oxidation of iodide and chloride by MPO from both HD- and ID-PMN showed that, per unit of activity based on the oxidation of guaiacol, the MPO of HD-PMN oxidized either halide approximately two times more effectively than did the MPO of ID-PMN (Fig. 2). Peripheral blood PMN showed iodide oxidation similar to that obtained for the HD-PMN. These results indicated a quantitative difference in the intrinsic enzymatic activity of MPO in the HD- and ID-PMN populations.

In light of the quantitative differences in MPO activity noted above, it was necessary to rule out possible contributing effects of catalase or pseudoperoxidase activities, although murine PMN have been reported to contain little or no catalase activity. To do this, the disappearance of exogenously added hydrogen peroxide was measured in the presence of cell lysates from both HD- and ID-PMN fractions. No changes were observed in the concentration of hydrogen peroxide, indicating the absence of competing hydrogen peroxide utilization under our assay conditions. Moreover, the peroxidase activity in lysates of PEC harvested from 5 to 11 hr after elicitation was quantitatively precipitated using rabbit antiserum to purified murine MPO. This demonstrated that the enzyme activity being measured in these experiments was due to MPO and not to some other peroxidase, such as eosinophilic peroxidase.

**Relationship of Differences in Cell-Associated MPO Activity to Degranulation**

Granule exocytosis of PMN can be induced under appropriate conditions by chemotactic factors, as well as by leukocyte pyrogen, immune complexes, or phagocytosis of particles. Since, under our conditions, the inflammatory response in the peritoneum was initiated by particulate-eliciting agents, degranulation of PMN might be expected to accompany the response. Consistent with this idea was our repeated observation that when cells were stained using rhodamine-labeled antilactoferrin, there appeared to be a general decrease in fluorescence intensity associated with ID-PMN as compared to HD-PMN. However, particle phagocytosis is known to cause degranulation of both specific and azurophilic granules. Since we used a particulate-eliciting agent in our experiments, it was possible that partial degranulation of both major granule populations was occurring in vivo. Indeed, measurement of lysozyme, an enzyme found in both azurophilic and specific granules, indicated that HD-PMN contained more enzyme than ID-PMN: 3.2 μg and 2.2 μg/10⁷ cells, respectively. In addition, MPO activity was found to be 5%–10% less in ID-PMN than in HD-PMN. Taken together, these observations suggested that a partial degranulation of PMN during in vivo elicitation might be related to the differences in MPO activity between the HD- and ID-PMN subpopulations. In order to test directly whether degranulation was related to observed changes in MPO activity between HD- and ID-PMN, the following in vitro experiments were carried out.

Elicited PEC were fractionated by density gradient centrifugation to yield a population of HD-PMN. These cells were then induced to degranulate by first preincubating in the presence of cytochalasin-B, followed by treatment with endotoxin-activated serum, a procedure that has been shown to cause partial degran-
ulation of both specific and azurophilic granules. Such treatment resulted in the secretion of 44% of the cellular lysozyme and 25% of the total cellular β-glucuronidase as compared to control cells (HD-PMN incubated with and without endotoxin-activated serum). Furthermore, secretion was specific, since the cytoplasmic marker enzyme, lactate dehydrogenase, was not significantly released (<5% of total). It was not possible to measure directly the amount of MPO secreted because of the presence of numerous components with pseudoperoxidase or oxidase activity in the serum. However, it was possible to measure the proportion of cell-associated MPO remaining after induction of secretion and to determine the sensitivity of this enzymatic activity to inhibition by AT. The results of a typical experiment are presented in Fig. 3A. It can be seen that the MPO remaining in cells that had undergone extensive degranulation was considerably more sensitive to AT inhibition than the MPO in control cells that had been treated under nondegranulating conditions.

In order to test the generality of our observations with elicited murine PMN, we extended our in vitro investigation to a more controlled system in which degranulation of purified human peripheral blood PMN was induced with the synthetic chemotactic peptide FMLP. This approach allowed quantitation of enzymatic activity released into the extracellular medium because of the absence of serum, and also provided larger numbers of cells necessary for further biochemical analysis. Initially, total MPO activity was detergent-solubilized from granule preparations after PMN had been extensively degranulated, and AT sensitivity was compared to the solubilized activity in granule preparations obtained from control (nondegranulated) PMN. Degranulation under the conditions employed resulted in a marked loss of 40%-50% of the total solubilized granule MPO activity. Moreover, the lost MPO activity could be accounted for by exocytosed enzyme activity in the extracellular fluid. Furthermore, as seen in Fig. 3B, MPO activity from granule preparations recovered after extensive exocytosis was more sensitive to inhibition by AT than the activity isolated from control granules. These data confirmed similar observations described above with whole cell lysates of murine PMN and additionally demonstrated that changes in MPO activity were associated specifically with a subcellular granule fraction.

Chromatographic Differences Between Exocytosed and Cell-Associated MPO

We have recently shown that under conditions in which there is minimal proteolysis of endogenous PMN protein, normal human PMN contain three chromatographically distinct forms of MPO designated I, II, and III. Form I is more sensitive to inhibition by AT than either forms II or III. Thus, it seemed possible that preferential exocytosis of individual forms of MPO might be related to the observed cell-associated changes in MPO activity described above. This possibility was investigated by comparing the chromatographic profile of granule-associated MPO activity from control cells with that of residual MPO activity from PMN that had been degranulated. Figure 4A shows the carboxymethylcellulose elution profile of MPO activity solubilized from granules obtained from control PMN. Three distinct chromatographic peaks were resolved (I, II, III), as previously described. When enzyme from residual granules isolated from PMN that had undergone exocytosis was subjected to chromatography, a typical elution profile, such as depicted in Fig. 4B, was obtained. Form I showed no quantitative change relative to control profiles (Fig. 4A). In contrast, forms II and III decreased relative to control profiles, presumably as a

Fig. 3. (A) AT inhibition of cell-associated MPO activity solubilized from murine HD-PMN following in vitro induced degranulation. HD-PMN were obtained by Percoll density gradient centrifugation of PEC harvested 2-3 hr after i.p. injection (0.4% caseinate) of C57BL/FnLn male and female mice. Equivalent amounts of MPO (11 units) were tested for AT inhibition from cells exposed 30 min to control serum (O-O), endotoxin-activated serum (A-A), and endotoxin-activated serum after preincubation (10 min) of PMN with cytochalasin-B (e-e). Values represent means from 2-3 experiments. (B) Sensitivity to AT inhibition of human MPO activity from control PMN granules (e-e) or from residual granules (O-O) after FMLP-induced exocytosis. The same amount of activity (equivalent to 33 mouse MPO units) was compared from each granule preparation.
result of preferential exocytosis. This was confirmed by chromatography of activity recovered in the cell suspension medium after induced degranulation (Fig. 4C). Forms II and III were present in this profile, but form I was absent. These results clearly indicated that under these conditions of degranulation, forms II and III were exocytosed from the cell, while form I remained cell-associated. The enhanced AT sensitivity of total MPO activity solubilized from residual granules after PMN exocytosis could therefore be related to the increased presence of form I relative to forms II and III. These data also suggested that forms II and III were compartmentalized differently than form I, since they appeared to be under different secretory control.

**Peroxidase Activity of Murine Macrophages**

Murine macrophages were purified from peritoneal exudates using Percoll density gradient centrifugation and were identified and characterized as described in the accompanying article. Macrophages, banding at a density of about 1.04 g/ml, were isolated from both resident (nonelicited) and elicited peritoneal exudate cells (PEC). Resident macrophages contained no bio-
chemically detectable peroxidase activity and were histochemically negative at the level of the light microscope. In contrast, elicited macrophages contained variable histochemical and biochemical peroxidase activity, the amount being dependent on the time of harvest following stimulation. For example, in the macrophage fraction isolated from exudates harvested at 15 hr, 30%-40% of the cells contained histochemically detectable peroxidase corresponding to 80 units/5 x 10^6 cells of biochemical activity (about 20% of the amount found in the same number of PMN). Although this particular fraction contained greater than 90% monocytes/macrophages, the possibility that some of the peroxidase activity was of PMN origin was suggested by the frequent observation of globular lactoferrin immunocytofluorescence in a significant number of macrophages.

**DISCUSSION**

We have recently shown that Percoll density gradient centrifugation may be utilized to fractionate "activated" from "resting" PMN based on differences in buoyant density.12 Wright and Gallin have reported that PMN undergo preferential exocytosis of specific granules during in vivo elicitation using sterile skin blister and skin chamber techniques.35 On the other hand, a variety of stimuli, including immune complexes,30 chemotactic agents,28 33 and phagocytic particles,31 32 are known to activate PMN and to cause degranulation of both specific and azurophilic granules under appropriate conditions. Since our in vivo experiments were based on the use of a particulate-eliciting agent, the observation that ID-PMN appeared to have undergone degranulation relative to HD-PMN was consistent with the recognized effects of such agents on PMN. This observation, in turn, led to the question of whether differences in cell-associated MPO activity might perhaps be related to degranulation. Support for such an idea came from two findings: (1) there was a linear increase in the sensitivity to AT inhibition of MPO activity derived from elicited PMN of decreasing density across the Percoll density gradient, and (2) the demonstration of in vitro induction of changes in MPO activity following degranulation of HD-PMN.

An analysis of this phenomenon at the biochemical and cellular levels was made possible by taking advantage of the degranulating effect of the synthetic chemotactic peptide FMLP on purified human peripheral blood PMN pretreated with cytochalasin-B.36 37 These results confirmed the data obtained with the in vivo elicited mouse PMN and also established that the changes in MPO activity were associated with changes in the granule fraction of the cell.

We have previously reported that MPO can be resolved by ion-exchange chromatography into several components having differences in sensitivity to inhibition by AT and ability to oxidize halides; MPO from elicited murine PEC and human PMN (single donor) were resolved into two (A, B) and three components (I, II, III), respectively.6 31 These data suggested that changes in cell-associated MPO activity in different density subpopulations of PMN might be due to a selective exocytosis of these different forms of the enzyme. Indeed, the chromatographic studies reported on in the present study showed that MPO forms II and III were selectively secreted by cytochalasin-B-treated cells in response to FMLP. Moreover, MPO form I, which was not secreted under these in vitro conditions, was more sensitive to inhibition by AT than either forms II or III6 and was also less active in the oxidation of iodide.38 Furthermore, recent evidence has indicated that form I also differs from forms II and III in its compartmentalization, molecular weight, and amino acid composition.38 Taken together, these data strongly support the notion that differences in MPO activity between elicited and unelicited PMN, as well as among PMN of differing density, are attributable to the differential specific exocytosis of distinct forms of the enzyme with different biochemical and enzymatic properties.

It is known that the intra- and extracellular recognition and transfer of lysosomal enzymes is dependent on the presence or absence of specific carbohydrate markers involving a mannose-6-phosphate moiety.39 40 Whether or not a similar structural element is involved in the selective exocytosis of the different forms of MPO is an intriguing but presently unanswered question. In this regard, it is interesting to note that MPO is a glycoprotein.41 42 Moreover, differential elution from Con-A-Sepharose of several forms of MPO from a human leukemic cell line leaves open the possibility that a carbohydrate moiety may be involved.

Our results indicate a physical heterogeneity of PMN that appears to be associated with a molecular heterogeneity of MPO. In addition, the selective exocytosis of distinct forms of MPO suggests that the molecular heterogeneity of the enzyme may be associated with functional specificity of the PMN as well. Functional specificity may be related to differences in compartmentalization of the individual forms.38 Multiple molecular forms of kidney cortex lysosomal acid phosphatase have been shown to be compartmentalized differently; distinct forms are distributed between glomerulus and tubules within the cortex.43 Dopamine β-hydroxylase of adrenal chromaffin granules has been shown to be localized both in the soluble contents of the granule and in the granule membrane.44 In this regard, MPO is known to be associated with at least two
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subtypes of peroxidase-positive azurophilic granules that have been reported to contain different enzymes distributed among individual granule subtypes. Therefore, it is possible that individual forms of MPO may also be distributed differently, perhaps associated with distinct subtypes of azurophilic granules that undergo independent exocytosis in response to various activating stimuli.

Our identification of different forms of MPO having somewhat different enzymatic properties provides an explanation for the reported differences between neutrophil MPO and a putative cell-line-specific monocyte/macrophage peroxidase. Nevertheless, there is still uncertainty as to the presence or absence of biochemically measurable peroxidase/MPO activity in macrophages from various sources. Based on the present studies with elicited PMN from murine PEC, it appeared that macrophages purified using Percoll fractionation of PEC did not contain endogenous peroxidase activity. Biochemical activity was not found in the macrophage fraction except under conditions where the simultaneous presence of PMN-specific lactoferrin was also found by immunocytofluorescence. These results suggested that the observed peroxidase activity could have been entirely derived from PMN, either by phagocytosis of PMN themselves or by endocytosis of PMN debris. The latter process has been documented in vitro, and the former process was observed in this study. Thus, the apparent lack of endogenous biochemically detectable peroxidase in peritoneal macrophages would be consistent with the absence of the enzyme reported by others in this cell type and the disappearance of the enzyme as monocytes differentiate to macrophages.

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