Chemotactic Factor Inactivation by the Myeloperoxidase-Hydrogen Peroxide-Halide System

AN INFLAMMATORY CONTROL MECHANISM

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ABSTRACT Polymorphonuclear leukocytes may modulate the acute inflammatory response by the secretion of enzymes capable of inactivating mediators of inflammation. The ability of the myeloperoxidase-H_2O_2-halide system of the neutrophil to inactivate chemoattractants was examined using both a radioassay and a morphologic assay of chemotaxis. Incubation of either a complement-derived agent, C5a, or a synthetic formyl-methionyl peptide chemoattractant with the myeloperoxidase system for 15 min at 37°C resulted in essentially complete loss of chemotactic activity. Inactivation was dependent on enzymatically active myeloperoxidase, H_2O_2 or a peroxide-generating enzyme system, and a halide cofactor. It was blocked by agents which inhibit peroxidase (azide) or degrade H_2O_2 (catalase). Inactivation of chemoattractants was time-dependent, reaching maximal levels within 1–5 min, and temperature-dependent with no significant inactivation occurring at 0°C. H_2O_2 alone had no significant inactivating ability at concentrations as high as 10 mM, whereas in the presence of myeloperoxidase and a halide, 0.1 μM H_2O_2 showed significant activity and 10 μM H_2O_2 caused complete inactivation. On a molar basis, the order of effectiveness of the halide cofactors was $Br^- > I^- > Cl^-$, although only chloride was fully active at physiologic concentrations. Neutrophils stimulated by phagocytosis or by membrane-perturbing agents secrete enzymatic constituents, including myeloperoxidase, and metabolic products such as H_2O_2. Thus, it is suggested that the myeloperoxidase system acting at an extracellular site serves as an inflammatory control mechanism by virtue of its ability to inactivate neutrophil chemoattractants.

INTRODUCTION

The hallmark of the acute inflammatory response is an influx of polymorphonuclear leukocytes (PMN).[1] This occurs by the directed migration, or chemotaxis, of PMN from the vascular space into the tissues and is mediated by a number of humoral agents of both host and microbial origin (1, 2). The control of the inflammatory response has been a subject of intense study, the progress of which has been facilitated by the availability in recent years of quantitative in vitro assays of PMN chemotaxis. In particular, it has been established that PMN themselves produce agents capable of either augmenting or limiting the chemotactic response. Thus, PMN contain factors which either have direct chemotactic activity (3–8) or can form chemotactic agents by interacting with the serum complement system (5, 8–16), factors which immobilize leukocytes, thus preventing the exodus of cells from the inflammatory site (17, 18), and factors which inactivate chemotactic agents (8, 9, 14–16, 19). The focus of the current work is on a specific mechanism by which PMN may modulate inflammation through the myeloperoxidase-catalyzed inactivation of leukotactic factors.

Myeloperoxidase, together with hydrogen peroxide and an oxidizable halide cofactor, forms an important microbicidal system of the PMN (2, 20, 21). Although the primary role of this system is the destruction of microorganisms within the phagocytic vacuole, it has become apparent that it may also participate in extra-

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[1] *Abbreviations used in this paper*: f-met-leu-phe, *N*-formyl-methionyl-leucyl-phenylalanine; MPO, myeloperoxidase; PMN, polymorphonuclear leukocyte.

cellular reactions. When the PMN is activated by contact with phagocytic particles or immune reactant-coated surfaces or by exposure to certain soluble stimuli, including chemoattractants, exocytosis of cytoplasmic granules results in the secretion of granule enzymes (2, 22, 23). Among the secretory products of this degranulation process is the azurophil granule constituent, myeloperoxidase (MPO) (24–27). The stimulated PMN also undergoes a burst of oxidative metabolism (2, 20, 21) which results in the extracellular release of various activated oxygen species, including H_2O_2 (28–30). These components of the MPO system might be expected to react with extracellular halides to exert effects on host tissues present in the vicinity of the inflammatory response because the cell-free peroxidase system has been shown to affect a number of mammalian cells, including spermatozoa (31), erythrocytes (32), leukocytes (33), platelets (34), and tumor cells (35, 36). Furthermore, the effects of activated PMN on adjacent tumor cells (27, 37) and platelets (38) have been shown to be mediated in part by the MPO-H_2O_2-halide system.

Because the MPO system generates labile intermediates capable of reacting with a variety of substances, it was of interest to us to determine whether it might affect humoral mediators of the inflammatory process. In this report, we document the ability of MPO in combination with H_2O_2 and a halide to destroy the biological activity of two different leukocyte chemotactic factors, one of complement system origin and one a synthetic oligopeptide. The studies provide the basis for an inflammatory control mechanism involving the MPO system.

METHODS

Chemotactic factors. The chemotactic fragment of the fifth component of complement was partially purified by a minor modification of a previously described technique (39). Blood from normal adult volunteers was allowed to clot in glass tubes at room temperature for 1 h and the serum was harvested by centrifugation at 550 *g* for 10 min at 4°C. A 10-ml portion of serum was incubated with 5 mg of zymosan (ICN Nutritional Biochemicals, Cleveland, Ohio) for 1 h at 37°C and residual complement was then inactivated by heating at 56°C for 30 min. Zymosan was removed by centrifugation at 550 *g* for 20 min at 4°C and the activated serum was then subjected to molecular seive chromatography on a 2.5 × 100-cm column of Sephadex G-75. Fractions were eluted with 0.05 M sodium phosphate buffer, pH 7.0, and tested for chemotactic activity. Essentially all of the activity was contained in fractions eluting just before cytochrome *c*, indicating an apparent mol wt of 13,000–15,000. The chemotactic activity in these pooled fractions was nearly completely inhibited by antibody to human C5, but not by antibody to human C3 (Meloy Laboratories Inc., Springfield, Va.). Currently available evidence indicates that the active material should be designated $C5a_{des\ arg}$ (40), but for convenience will be referred to here as C5a.

The synthetic chemotactic peptide, *N*-formyl-methionyl-leucyl-phenylalanine (f-met-leu-phe), was kindly supplied by Dr. Elliot Schiffmann of the National Institute of Dental Research (Bethesda, Md.). It was dissolved at 10 mM in dimethyl sulfoxide and subsequent dilutions were made in sterile distilled water.

Chemotaxis assays. Chemotactic activity was determined by two previously described methods, the ^{51}Cr assay (41) (C5a and some f-met-leu-phe experiments) and the leading front assay (6) (f-met-leu-phe experiments). Blood was obtained from normal adult human volunteers and the PMN harvested by dextran sedimentation and hypotonic lysis of erythrocytes. For the ^{51}Cr assay, PMN were labeled with $Na_2{}^{51}CrO_4$ (New England Nuclear, Boston, Mass., 200–500 μCi/μg Cr), washed, and suspended in Gey's medium (Microbiological Associates, Walkersville, Md.) at 2.3×10^6 granulocytes/ml (purity 80–90% granulocytes). For the leading front assay, the labeling step was omitted, but washing and suspension in Gey's medium was the same. In the ^{51}Cr assay, chambers were incubated for 3 h and chemotaxis was determined as the mean counts per minute of ^{51}Cr in the lower of two micropore filters (a 3-μm mean pore diameter, Sartorius Membranfilter, Göttingen, West Germany). In the leading front assay, chambers were incubated for 1 h and chemotaxis was determined as the mean depth of penetration in micrometers into the filter (3-μm pore, Sartorius) of the leading two cells. In both assays, each sample was tested in four replicate chambers.

Exposure of chemotactic factors to the MPO system. The chemotactic factors, C5a and f-met-leu-phe, were incubated with the components of the MPO system as detailed in the legends to the figures and tables. Each sample contained either 0.8 ml of C5a in a total volume of 1.0 ml of 40 mM sodium phosphate buffer, pH 7.0, or 50 pmol of f-met-leu-phe in a total volume of 0.5 ml of 20 mM sodium phosphate buffer, pH 7.0. Incubations were performed in 12 × 75-mm glass tubes in a 37°C water bath for 15 min, then placed in an ice bath and diluted to a total volume of 4 ml with iced gelatin-Veronal-buffered saline containing 70 μM Ca^{2+} and 0.5 mM Mg^{2+}. 0.8-ml aliquots were placed in the lower compartments of each of four chemotaxis chambers for the determination of chemotactic activity. Controls in each experiment included samples without the chemotactic factor (background) and samples with the chemotactic factor, but none of the components of the MPO system (positive control). After the chemotactic activity in each sample was determined as either mean counts per minute (^{51}Cr assay) or mean micrometers migration (leading front assay), the data were expressed as percent inactivation of chemotactic activity according to the following formula: Inactivation (percent) = (positive control-experimental sample)/(positive control-background) × 100. The mean background activity was 159±26 cpm (^{51}Cr assay) or 62.8±3.4 μm (leading front assay); the mean positive control for C5a was 1,281±131 cpm and for f-met-leu-phe was 627±66 cpm or 136.1±2.9 μm.

Special materials. MPO was prepared from canine PMN by the method of Agner (42) through the end of step 6. Peroxidase activity was determined before each experiment by the *o*-dianisidine method (43); 1 U of activity is that causing the utilization of 1 μmol of substrate/min at 25°C (32). H_2O_2 (30% solution) was obtained from Fisher Scientific Co., Pittsburgh, Pa. Catalase (beef liver, 6.7 mg/ml, 62,900 U/mg) was from Worthington Biochemical Corp., Freehold, N. J.; 1 U of catalase catalyzes the oxidation of 1 μmol of H_2O_2/min at 25°C, pH 7.0. Glucose oxidase (type V, 1,460 U/ml, 200 U/mg) was obtained from Sigma Chemical Co., St. Louis, Mo.; 1 U of glucose oxidase catalyzes the oxidation of 1 μmol of glucose/min at 35°C, pH 5.1.

Statistics. Standard error was used throughout as an estimate of variance and means were compared by Student's *t* test (two-tailed).

RESULTS

Exposure of chemoattractants to the complete MPO system (MPO, H_2O_2, Cl^-) for 15 min resulted in essentially complete loss of their biologic activity (Table I). This inactivation was comparable for both C5a and f-met-leu-phe and was documented with two different assays of PMN chemotaxis, the ^{51}Cr method and the leading front technique. The presence of MPO was essential for inactivation and this requirement was related to its enzymatic activity because heated MPO was ineffective and inactivation was blocked by azide ($P < 0.001$), an inhibitor of peroxidase activity. The amount of MPO employed in the experiments summarized in Table I was in excess of that required; under the same conditions, 1/10th as much MPO (1.6 mU/ml) plus H_2O_2 and chloride resulted in complete inactivation of f-met-leu-phe (104.9±5.5%, $n = 8$). H_2O_2 was also an essential component of the system and could be supplied by either reagent H_2O_2 or a peroxide-generating enzyme system, glucose plus glucose oxidase. Furthermore, inactivation of the chemotactic agents was blocked by the peroxide-degrading enzyme, catalase ($P < 0.001$), but not by heat-inactivated catalase. Loss of activity on omission of chloride indicated a role for a halide and this requirement could be fulfilled by iodide or bromide, as well as by chloride.

Investigation of the time-course of inactivation of the chemoattractants demonstrated a rapid reaction (Fig. 1). Using the standard concentrations of MPO, H_2O_2, and chloride, complete inactivation of f-met-leu-phe occurred within the 1st min of incubation. When lower concentrations of reactants were used, a period of 5–7.5 min was required for complete loss of activity. Inactivation was temperature-dependent because little or no loss of chemotactic activity was observed when f-met-leu-phe was incubated in a melting ice bath (0°C) for 15 min with the MPO (4 mU/ml)-H_2O_2 (10 μM)-Cl^- (10 mM) system; inactivation at 0°C was 11.4±14.4% compared with 92.0±5.9% in control samples incubated at 37°C ($P < 0.001$).

H_2O_2 alone is a reactive form of reduced oxygen,

TABLE I
*Chemotactic Factor Inactivation by the MPO System**

Supplements	Inactivation, %		
	C5a	f-met-leu-phe	
	^{51}Cr assay	^{51}Cr assay	Leading front assay
MPO + H_2O_2 + Cl^-	94.4±1.8 (17)‡§	94.8±1.8 (14)§	98.3±4.4 (15)§
MPO omitted	8.6±5.4 (5)	5.5±18.8 (6)	4.1±8.5 (9)
MPO heated	2.9±14.6 (6)	0.1±10.2 (6)	13.8±7.0 (8)
H_2O_2 omitted	6.4±10.7 (6)	10.6±6.6 (9)	4.8±5.5 (13)
Cl^- omitted	2.1±11.9 (6)	3.2±8.3 (7)	3.2±6.2 (8)
Azide added	23.9±14.7 (6)	15.4±15.1 (5)	13.4±7.4 (7)
Catalase added	17.6±23.2 (5)	0.6±6.1 (4)	6.7±6.5 (8)
Heated catalase added	89.8±5.0 (5)§	96.1±1.1 (4)§	112.5±2.9 (5)§
MPO + glucose + glucose oxidase + Cl^-	87.6±11.2 (5)‖	—	73.6±3.0 (2)¶
MPO omitted	23.0±5.4 (2)	—	—
MPO + H_2O_2 + I^-	54.2±15.5 (5)¶	80.9±5.1 (7)§	—
MPO + H_2O_2 + Br^-	—	89.6±10.4 (3)¶	87.8±6.1 (4)§

* The complete reaction mixture contained either 0.8 ml of C5a in 40 mM sodium phosphate buffer pH 7.0 (total volume 1.0 ml) or 50 pmol f-met-leu-phe in 20 mM sodium phosphate buffer pH 7.0 (total volume 0.5 ml). The supplements listed were as follows: MPO, 16 mU/ml; H_2O_2, 10 μM; sodium salts of Cl^-, 0.1 M, I^-, 1 mM, and Br^-, 0.1 mM; sodium azide, 0.1 mM (C5a and f-met-leu-phe with leading front assay) or 1 mM (f-met-leu-phe with ^{51}Cr assay); catalase, 4,200 U/ml; glucose, 10 mM; glucose oxidase, 3 mU/ml (C5a experiments) or 30 mU/ml (f-met-leu-phe experiments). MPO or catalase was heated at 100°C for 15 min where indicated. After incubation at 37°C for 15 min, all samples were diluted in gelatin-Veronal-buffered saline to a total volume of 4.0 ml and tested for chemotactic activity.

‡ Mean±SE, number of experiments in parentheses.

§ $P < 0.001$ vs. control.

‖ $P < 0.01$ vs. control.

¶ $P < 0.05$ vs. control.

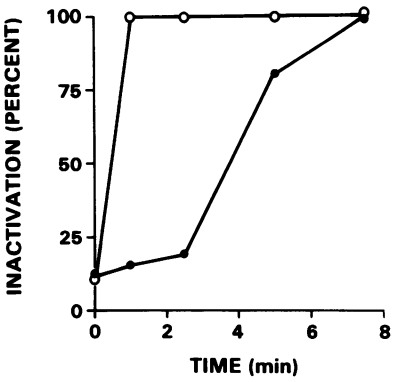

FIGURE 1 Time-course of inactivation of f-met-leu-phe by the MPO system. The reaction mixture and supplements were the same as described in Table I for the MPO-H_2O_2-Cl^- system (○) or the MPO and Cl^- concentrations were reduced to 4 mU/ml and 10 mM, respectively (●). The samples were incubated at 37°C for varying times as indicated and the reaction stopped by rapid cooling to 0°C and dilution in iced buffer. The zero time samples were kept at 0°C and iced buffer was added simultaneously with the MPO. The leading front assay of chemotaxis was used. The data shown are means of quadruplicate determinations in a single experiment; similar results were obtained in three additional experiments.

although its activity in microbicidal and cytotoxic systems is markedly enhanced by the addition of MPO and a halide (20, 32–34). Thus, we analyzed the dose-response characteristics of chemotactic factor inactivation by H_2O_2 alone and as a component of the complete MPO system (Fig. 2). H_2O_2 alone failed to inactivate C5a under the conditions employed. At the highest concentration tested, 10 mM, there was a suggestion of partial inactivation, although this did not prove to be significant. In contrast, when MPO and Cl^- were present, H_2O_2 had a significant effect at concentrations as low as 0.1 μM and inactivation was essentially complete at 10 μM H_2O_2.

The bactericidal activity of MPO requires a halide, with chloride being the least effective on a molar basis, iodide the most effective, and bromide intermediate in activity (44). The rank order observed for chemoattractant inactivation was similar with respect to chloride, although iodide and bromide were reversed (Fig. 3). The concentrations of chloride, iodide, and bromide required for 50% inactivation were 0.5 mM, 50 μM, and 15 μM, respectively.

The most likely explanation of the findings described above is that the MPO system exerts its inhibitory effect on chemotaxis by the inactivation of the chemoattractant during the initial preincubation. An alternative possibility is that the peroxidase system components carried over into the chemotaxis chamber have an inhibitory effect on the assay of PMN migration, for example, by damaging the responding cells (33). This possibility seemed unlikely because of the dilution of the MPO system components which occurred before the chemotaxis assay (Methods) and because the high concentration of protein present in the chemotaxis chamber might be expected to limit the toxicity of the MPO system (2). Furthermore, the lack of inactivation when the preincubation was short (Fig. 1) or was performed at 0°C rather than 37°C (see above) suggests that the loss of chemotactic activity occurred during the preincubation step. Two additional approaches to this question are summarized in Table II. In the first series of experiments, the chemotactic factor was added

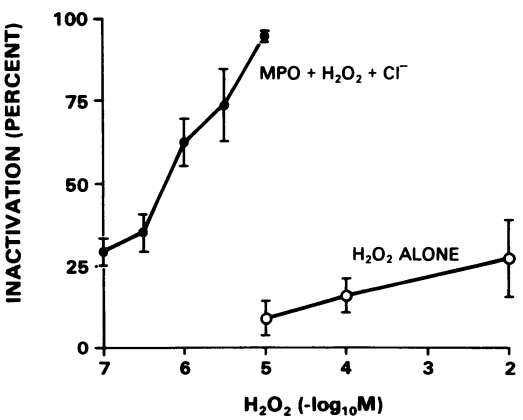

FIGURE 2 Inactivation of C5a by the MPO-H_2O_2-Cl^- system and by H_2O_2 alone. The reaction mixture and supplements were the same as described in Table I for C5a and the ^{51}Cr assay. The effect of the MPO-H_2O_2-Cl^- system with varying H_2O_2 concentrations (●) is compared with that of H_2O_2 alone (○). The data shown are means ±SE of five experiments. The activity of the complete MPO system is significant from 0.1 to 10 μM ($P < 0.01$ vs. control), whereas H_2O_2 had no significant activity ($P > 0.05$ vs. control).

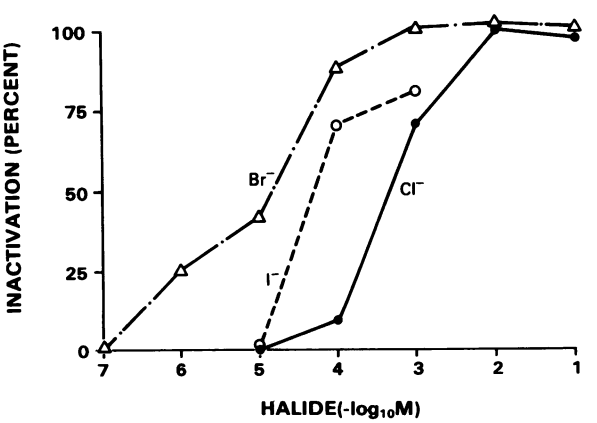

FIGURE 3 Inactivation of f-met-leu-phe by the MPO system with various halide cofactors. The reaction mixture and supplements were the same as described in Table I for the f-met-leu-phe experiments except that the concentrations of chloride (●), iodide (○), or bromide (△) were varied as indicated. Data from the ^{51}Cr assay and leading front assay were pooled. Values shown represent the means of four or more experiments.

TABLE II

Inactivation of the Chemotactic Factor Occurs during Its Preincubation with the MPO System

Conditions	Inactivation
	%
*Chemotactic factor added:	
Before preincubation	94.4±1.8 (17)§
After preincubation	10.0±10.1 (4)
‡Catalase added:	
Before preincubation	0.6±6.1 (4)
After preincubation:	
MPO system present	92.9±2.9 (3)
MPO system absent	10.0±11.4 (3)

* The reaction mixture and supplements were the same as described in Table I for C5a by the ^{51}Cr assay using the MPO-H_2O_2-Cl^- system except that C5a was added either before or after the 15-min preincubation.

‡ The reaction mixture and supplements were the same as described in Table I for f-met-leu-phe by the leading front assay using the MPO-H_2O_2-Cl^- system except that catalase (4,200 U/ml) was added either before or after the 15-min preincubation as indicated. In the latter case, the components of the MPO system were either included or omitted as indicated.

§ Mean±SE, number of experiments in parentheses.

to the complete MPO system either before or after the 15-min preincubation at 37°C. As shown previously (Table I), the former approach resulted in complete inactivation. In contrast, no significant inactivation was observed when the addition of the chemoattractant was withheld until the reaction mixture was placed on ice and diluted after the preincubation (Table II, chemoattractant added before vs. after preincubation—$P < 0.001$), indicating that the peroxidase system was not acting at a subsequent step (i.e., in the chemotaxis chamber). The second approach involved the termination of the MPO-H_2O_2-halide system activity by the addition of catalase either before or after the preincubation step. As previously demonstrated (Table I), when catalase was added before the preincubation, inactivation of chemotaxis was prevented. However, when catalase was added to the MPO system-chemoattractant mixture after preincubation, no effect was observed; i.e., there was essentially complete inactivation of chemotaxis by the MPO system (Table II, catalase added before vs. after preincubation—$P < 0.001$), again indicating that the MPO system was exerting its effect during the preincubation and not during the subsequent chemotaxis assay. The incubation of catalase with the chemoattractant in the absence of the MPO system did not result in significant inhibition of chemotaxis (Table II).

We also considered the possibility that exposure of a chemotactic peptide to the MPO system might modify the peptide to generate an inhibitor of chemotaxis. A small quantity of such an inhibitor could conceivably mask the chemotactic activity of residual intact peptide. This possibility was assessed by preincubating 50 pmol of f-met-leu-phe with the MPO-H_2O_2-Cl^- system as in Table I and then adding an additional 50 pmol of f-met-leu-phe before testing for chemotactic activity. Although complete loss of activity of the f-met-leu-phe present during the preincubation was observed as expected, the peptide added after the preincubation expressed full activity in the leading front chemotaxis assay (148.7±3.3 μm compared with 143.1±4.6 μm for control f-met-leu-phe assayed after preincubation in buffer alone without the MPO system). Thus, the MPO-treated peptide did not inhibit the activity of the added f-met-leu-phe, indicating that loss of activity on exposure to the MPO system was due to true inactivation rather than to the generation of an inhibitor.

DISCUSSION

The foregoing data indicate that the MPO-H_2O_2-halide system of the PMN is capable of destroying the biological activity of inflammatory mediators. Under the conditions employed, there was virtually complete inactivation of both a complement-derived chemotactic factor, C5a, and a synthetic peptide chemoattractant, f-met-leu-phe. Inactivation was demonstrated by two different assays of PMN chemotaxis, the ^{51}Cr radiolabel method and the leading front technique. In each instance, loss of chemotactic activity was dependent on each component of the system-MPO, H_2O_2, and a halide. Chemoattractant inactivation by the MPO system was rapid, being essentially complete within 1–5 min depending on the concentrations of reactants. The reaction was temperature-dependent because no significant inactivation occurred when the chemoattractant was incubated with the MPO system at 0°C rather than 37°C. The requirement for MPO was related to its enzymatic activity since heated peroxidase was ineffective. The H_2O_2 requirement was satisfied by either reagent peroxide or a peroxide-generating enzyme, glucose oxidase. Inactivation of the chemotactic factors was blocked by agents which inhibit MPO (azide) or degrade H_2O_2 (catalase). In the presence of MPO and a halide, H_2O_2 was effective at concentrations as low as 0.1 μM, although 10 μM was required for complete inactivation. H_2O_2 alone had little or no activity at levels as high as 10 mM. The order of effectiveness of halides as cofactors was $Br^- > I^- > Cl^-$, although only Cl^- supported complete inactivation at physiologic concentrations.

A number of potential mechanisms for controlling the inflammatory response have been described and several of these involve the inactivation of chemoattractants. Inactivating agents may be found in serum

or associated with leukocytes. Normal human serum contains chemotactic factor inactivators which degrade both complement-derived and bacterial chemoattractants (45, 46), apparently functioning as aminopeptidases (47). Serum carboxypeptidase B converts C5a to its desarginyl derivative with loss of anaphylatoxic, but not chemotactic activity (40). PMN granule lysates contain proteases capable of inactivating chemotactic factors (8, 9, 15, 19). The inactivation of C5a (15) and hydrolysis of the synthetic chemoattractant, formyl-norleucyl-leucyl-phenylalanine (19), was attributed to azurophil granule constituents and, in the case of C5a inactivation, two different agents with mol wt of 65,000 and 30,000 were detected (15). Two purified human PMN granule neutral proteases, chymotrypsin-like cationic protein (cathepsin G) (14, 16) and elastase (16), have been shown to inactivate C5a.

Other studies have indicated that PMN secrete agents into the extracellular fluid during phagocytosis or on exposure to soluble PMN activators which can inactivate C5a, synthetic chemotactic peptides, and bacterial chemotactic factors (8, 11, 15, 16, 19). The identity of these secreted inactivators has not been securely established in most studies, although blocking of C5a inactivation by specific inhibitors of elastase (16) suggests that this neutral protease may be partially responsible. In other studies, the evidence suggested the participation of two different azurophil granule constituents (15). It should be noted that proteases in PMN granule lysates and in extracellular fluid of activated PMN may generate as well as degrade C5a (8, 9, 12, 14–16). In general, it appears that limited proteolytic cleavage of the C5 parent molecule results in the formation of biologically active C5a, but that more extensive proteolysis leads to cleavage of the C5a to an inactive product (9, 14, 16).

Apart from the potential control of inflammation by PMN proteases through the formation and inactivation of chemoattractants, it has been demonstrated that such enzymes are involved in the chemotactic response of the PMN; the potency of various f-met peptides as chemoattractants is related to the rate at which the peptides are hydrolyzed by the PMN (48). Furthermore, chloromethyl ketone protease inhibitors block chemotaxis (48). It was suggested that binding of the chemotactic peptide to a cell receptor results in peptide hydrolysis and that this process allows the sensing of the chemical gradient of the attractant. It is not known whether this peptidase is of granule origin or is associated with the cell membrane. Thus, its relationship to the chemoattractant-cleaving proteases detected in lysosomal lysates is unclear.

The mode of chemotactic factor inactivation observed in our studies is clearly different from the previously described proteolytic mechanisms. Unlike proteases, the MPO system requires H_2O_2 and a halide, indicating a peroxidative reaction. Although MPO is an azurophil granule constituent, its large molecular weight (49, 50) distinguishes it from the C5a-inactivating agents isolated from azurophil granules by Wright and Gallin (15). Furthermore, the activities which these investigators observed did not depend on H_2O_2 or other products of oxygen metabolism. It is not surprising that MPO-mediated inactivation of chemoattractants has not been previously detected in studies using granule lysates or supernates of activated PMN. These preparations should contain MPO, but it is likely that any H_2O_2 initially present would have been dissipated and thus fresh H_2O_2 would have to be supplied. It remains to be seen whether stimulated PMN can inactivate chemoattractants by secretion of MPO and H_2O_2. However, such a sequence is quite plausible since activated PMNs are known to release these constituents of the MPO system into the extracellular fluid (24–30). Among the stimuli inducing release of granule enzymes and metabolic products are chemotactic factors (2, 22, 51–54). These observations raise the interesting possibility that chemoattractants could initiate their own inactivation by triggering the release of either MPO and H_2O_2 or neutral proteases. The MPO system may, in addition, manifest other biological activities, including the lysis of mammalian cells (27, 31–38) and the inactivation of other humoral mediators such as the slow-reacting substance of anaphylaxis (55) and serum antiproteases (56).

The molecular mechanism of MPO-catalyzed inactivation of chemoattractants has not been addressed in the experiments reported here. Two general mechanisms of MPO-mediated toxicity to microorganisms have been demonstrated, halogenation and oxidation (2, 20). Halogenation involves the covalent binding of the halide cofactor to the target cell or molecule. Among the possible products formed with protein acceptors and iodide as the cofactor are iodotyrosines, iodohistidines, and sulfenyl iodides (2, 57). The presence of these chemical configurations near the active site of the chemoattractant could account for the loss of biological activity. Oxidation reactions may be mediated by such peroxidase system intermediates as halogens, hypohalous acids, chloramines, aldehydes, and singlet molecular oxygen (2, 20). A wide variety of oxidizable groups may be attacked. Of these, the thioether group of methionine may be especially pertinent because methionine is present both in the synthetic peptide attractant used in this study and in highly purified C5a (40). The sulfone and sulfoxide derivatives of the f-met peptides are known to be inactive as chemoattractants (58). Thus, methionine oxidation is a potential mechanism of action in our system. We are currently examining the role of both halogenation and oxidation reactions in the inactivation of chemoattractants by the MPO-H_2O_2-halide system.

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