

Monocyte and Granulocyte-mediated Tumor Cell Destruction

A ROLE FOR THE HYDROGEN PEROXIDE-MYELOPEROXIDASE-CHLORIDE SYSTEM

STEPHEN J. WEISS and ADAM SLIVKA, *Simpson Memorial Institute, University of Michigan, Ann Arbor, Michigan 48109*

ABSTRACT Human monocytes stimulated with phorbol myristate acetate were able to destroy a T lymphoblast cell target (CEM). Stimulated human granulocytes were also capable of mediating CEM cytotoxicity to a comparable degree as the monocyte. CEM destruction was dependent on the pH and the effector cell number. Both monocyte or granulocyte mediated cytotoxicity were inhibited by the addition of catalase, whereas superoxide dismutase had no inhibitory effect. In addition, CEM were protected from cytolysis by the effector cells by the myeloperoxidase inhibitors, azide and cyanide, or by performing the experiment under halide-free conditions. Glucose oxidase, an enzyme system capable of generating hydrogen peroxide, did not mediate CEM cytotoxicity, while the addition of purified myeloperoxidase dramatically enhanced cytolysis. Hypochlorous acid scavengers prevented CEM destruction by the glucose oxidase-myeloperoxidase-chloride system but neither hydroxyl radical nor singlet oxygen scavengers had any protective effect. These hypochlorous acid scavengers were also successful in inhibiting monocyte or granulocyte-mediated CEM cytotoxicity. Based on these observations we propose that human monocytes or granulocytes can utilize the hydrogen peroxide-myeloperoxidase-chloride system to generate hypochlorous acid or a species of similar reactivity as a potential mediator of CEM destruction.

INTRODUCTION

Human monocytes and granulocytes play a key role in host defense and are capable of destroying a variety

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of targets including bacteria, fungi, viruses, and non-malignant or malignant cells (1-3). Although the mediators of granulocyte or monocyte mediated cytotoxicity are unclear, it appears that both oxygen dependent and independent mechanisms are important (3-6). The best characterized oxygen-dependent mechanism of cytotoxicity is the hydrogen peroxide (H_2O_2), myeloperoxidase (MPO),¹ halide system described by Klebanoff (3, 5, 7). Using a model system consisting of purified MPO, a source of H_2O_2 and various halides, Klebanoff and co-workers have demonstrated the ability of this system to destroy a wide spectrum of target cells (3, 5, 7).

Recent attention has focused on the ability of intact leukocytes to use the MPO system to destroy tumor cell targets. Clark and Klebanoff demonstrated that human granulocytes could destroy a murine tumor cell by a mechanism dependent on H_2O_2 , MPO and a halide (8-10). In addition, we recently demonstrated that human granulocytes could destroy a human T lymphoblast target (CEM) via the MPO system and suggested a potential role for hypochlorous acid (HOCl) as the lytic mediator (11).

At present, little work has dealt with the potential role of the MPO system in monocyte-mediated tumor cell cytotoxicity. Indeed, in separate reports Clark and Klebanoff reported that a mixture of human lymphocytes and monocytes were unable to destroy murine tumor cells (9, 10). In this study we have demonstrated the ability of purified suspensions of human monocytes or granulocytes to destroy CEM targets to a comparable degree via the H_2O_2 -MPO- Cl^- system. Analysis of the mechanism of CEM destruction by human

¹ Abbreviations used in this paper: CEM, T lymphoblast cell target; MPO, myeloperoxidase; PMA, phorbol myristate acetate; SOD, superoxide dismutase.

monocytes, granulocytes and a cell-free H_2O_2 -MPO- Cl^- system points to a common lytic mediator with characteristics similar, if not identical, with HOCl.

METHODS

Cell preparations. Granulocytes and monocytes were obtained from the venous blood of normal healthy volunteers. Granulocyte preparations were isolated by Ficoll-Hypaque density centrifugation followed by dextran sedimentation (12). Purified preparations of human monocytes were isolated as previously described (13). Briefly, mononuclear cells obtained by Ficoll-Hypaque separation were washed twice in Selegman's balanced salt solution (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) with 13% autologous serum. Approximately 50×10^6 mononuclear cells were added to Falcon 3003 tissue culture dishes (Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif.) and incubated for 90 min at $37^\circ C$ in a humidified 95% air/5% CO_2 atmosphere. The dishes were vigorously washed five times with RPMI (Gibco Laboratories) supplemented with 10% fetal calf serum to remove nonadherent cells. The adherent cells were then incubated with Selegman's balanced salt solution containing 0.2% bovine albumin (Sigma Chemical Co., St. Louis, Mo.) and 0.1% EDTA for 1 min at which time the adherent cells were gently removed from the surface with a rubber policeman. These cells were washed and resuspended in Dulbecco's phosphate-buffered saline (Gibco Laboratories; pH 7.4 with 1 mg/ml glucose).

Cell preparations routinely contained >92% monocytes as assessed by Wright stain morphology, esterase staining, or latex phagocytosis (14). Contaminating cells consisted of no more than 5–6% lymphocytes and 2–3% granulocytes. Viability was always >95% by trypan blue exclusion.

CEM targets were radioactively labeled by incubating 10^7 cells with 100 μCi of $Na_2^{51}CrO_4$ (New England Nuclear, Boston, Mass.) for 90 min at $37^\circ C$ (14). The cells were then washed and resuspended in Dulbecco's phosphate-buffered saline with 1.5% fetal calf serum.

Cytotoxicity assay. The cytotoxicity assay was performed as previously described (11). Briefly, various numbers of neutrophils or monocytes were incubated with 2×10^4 ^{51}Cr -labeled CEM targets in Dulbecco's phosphate-buffered saline (pH 6.5, 7.4, or 8.0) with a final serum concentration of 0.25%. In some experiments Dulbecco's phosphate-buffered saline was replaced with a halide-free Na_2SO_4 -phosphate buffer at pH 7.4 as described by Clark and Klebanoff (15). Assays were done in triplicate and the final cell volume of each reaction mixture was 0.3 ml. Leukocytes were stimulated to generate oxygen metabolites and secrete lysosomal enzymes by the addition of phorbol myristate acetate (PMA) (Consolidated Midland Corp., Forrester, N. Y.) (11, 16).

In additional experiments, granulocytes or monocytes were replaced by varying concentrations of glucose oxidase (Type V, Sigma Chemical Co.) in the presence or absence of purified canine myeloperoxidase (17; kindly provided by Dr. J. Schultz, Papanicolaou Cancer Research Inst., Miami, Fla.). MPO was assayed by the *o*-dianisidine method and activity expressed in International Units (18). Immediately after the addition of PMA or glucose oxidase, the samples were centrifuged at 50 *g* for 3 min. The samples were then placed in a humidified atmosphere of 95% air/5% CO_2 at $37^\circ C$. After 4 h of incubation the cells were pelleted (100 *g* for 3 min) and 0.1-ml aliquots of the supernatant removed to determine the amount of ^{51}Cr released. Cytotoxicity was

expressed as the percentage of ^{51}Cr released calculated by the formula:

$$\% \text{ cytotoxicity} = \left[\frac{(A - B)}{(C - B)} \right] \times 100,$$

where A is the mean counts per minute in the supernatant of samples containing PMA-treated granulocytes, monocytes or glucose oxidase in the presence or absence of MPO and CEM, B is the mean counts per minute in the supernatant of samples containing CEM alone, and C is the mean counts per minute of target cells added to each sample.

Other additions to the cytotoxicity assay included bovine superoxide dismutase (SOD; 3,000 U/mg; Sigma Chemical Co.), bovine catalase (120,000 U/mg; Worthington Biochemical, Co., Freehold, N. J.), glycine, L-alanine, L-serine, L-valine, L-isoleucine (Sigma Chemical Co.), sodium azide, sodium cyanide (Fisher Scientific Co., Fairlawn, N. J.), 2,5-dimethylfuran (Aldrich Chemical Co., Milwaukee, Wis.) or ethanol. Catalase was washed over an XM-100A ultrafiltration membrane (Amicon Corp., Lexington, Mass.) before use and 2,5-dimethylfuran was purified by passage over a column of W-200 basic aluminum oxide (International Chemical and Nuclear Corp., Irvine, Calif.).

Superoxide and H_2O_2 production. Superoxide anion generation was measured by SOD inhibitable reduction of ferricytochrome *c* (horse heart, grade III, Sigma Chemical Co.) according to Babior et al. (19). H_2O_2 generation by the glucose oxidase system, granulocytes or monocytes was determined in the presence of 1 mM azide by the method of Thurman et al. (20).

RESULTS

Monocyte and neutrophil-mediated CEM destruction. At an effector to target cell ratio of 10:1 PMA-stimulated monocytes were capable of destroying almost half of the CEM targets (Table I). Neither monocytes nor PMA alone mediated significant toxicity. Under identical conditions granulocyte-mediated cytotoxicity was not significantly different from that observed with the monocytes (Table I). Maximal cytolysis was obtained with a PMA dose of 5 ng/ml and a 4-h incubation period with both effector cells (data not shown). Fig. 1 illustrates the effect of pH on monocytes and granulocyte-mediated CEM destruction. Mono-

TABLE I
Monocyte and Granulocyte-mediated Cytotoxicity of CEM

Additive	% Cytotoxicity*	
	Monocyte system	Granulocyte system
PMA† alone	2.4±2.5	2.2±1.9
Effector cell‡	4.8±3.1	5.7±4.5
Effector cell + PMA	49.9±7.8	51.1±4.9

* Expressed as mean percent cytotoxicity±1 SD of 2×10^4 CEM tumor cells (*n* = 22) in 4 h. The spontaneous release of ^{51}Cr at 4 h was 8.2±1.9%. The final serum concentration was 0.25%.

† Phorbol myristate acetate 5 ng/ml.

‡ Effector cell number was 2×10^5 .

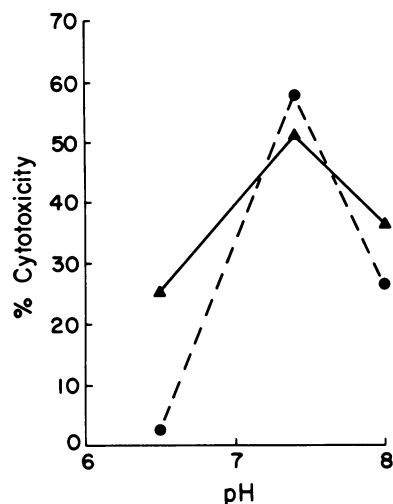


FIGURE 1 Effect of pH on the destruction of 2×10^4 CEM by 2×10^5 monocytes (●---●) or granulocytes (▲—▲) at a PMA concentration of 5 ng/ml over a 4-h period of incubation. Results are expressed as the mean percentage of cytotoxicity of two experiments.

cytes and granulocytes destroyed CEM targets maximally at a pH of ~ 7.4 . Fig. 2 illustrates CEM cytotoxicity as a function of the effector cell number. Monocytes and granulocytes mediated comparable cytotoxicity over the entire range studied.

The role of H_2O_2 , MPO, and Cl^- in CEM-mediated cytotoxicity. We have previously demonstrated that PMA-stimulated granulocytes can destroy a variety of target cells by generating superoxide anion (O_2^-), H_2O_2 and releasing MPO (11, 21–23). If the cytotoxic system employed by monocytes is dependent on the H_2O_2 -MPO- Cl^- system then the addition of catalase should inhibit cytolysis, whereas superoxide dismutase should have no inhibitory effect. Indeed, catalase signifi-

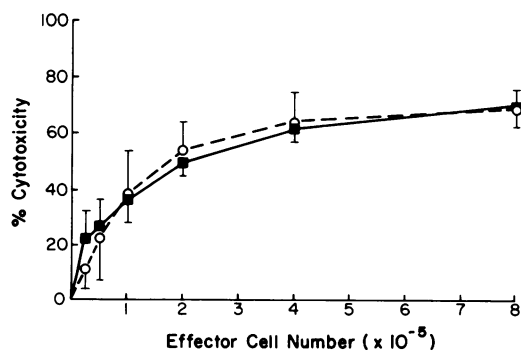


FIGURE 2 Effect of monocyte (○---○) or granulocyte (■—■) number on the cytolysis of 2×10^4 CEM at a PMA concentration of 5 ng/ml over a 4-h period of incubation. Results are expressed as the mean percentage of cytotoxicity ± 1 SD of eight experiments.

cantly inhibited both monocyte and granulocyte mediated cytolysis while SOD had no inhibitory effect (Fig. 3). Heat-inactivated catalase did not inhibit cell-mediated cytotoxicity. If catalase was added to the monocyte or granulocyte system 60 min after the start of the incubation, the suspension gently mixed and repelleted, there was no inhibition of cytotoxicity. Thus, the lethal hit occurred within the first hour of incubation. A role for MPO in CEM destruction was underlined by the ability of two heme-enzyme inhibitors, azide and cyanide, to significantly inhibit cytotoxicity in both effector cell systems (Fig. 3). In order to demonstrate a requirement for Cl^- in the cytotoxic event, paired experiments were performed in Cl^- -containing and Cl^- -free buffers. When 2×10^5 PMA-stimulated monocytes or granulocytes were incubated with 2×10^4 CEM targets in a Cl^- -free buffer cytotoxicity was inhibited 95 and 75%, respectively ($n = 2$). Thus, it appears that both monocytes and granulocytes are capable of destroying CEM targets to a comparable degree by a mechanism dependent on H_2O_2 , MPO, and Cl^- .

Glucose oxidase-MPO- Cl^- -mediated CEM destruction. The cytotoxic potential of H_2O_2 , MPO, and Cl^- alone against CEM targets was reinforced by using a model system consisting of purified MPO, glucose oxidase as a source of H_2O_2 , and Cl^- in a glucose-containing buffer. Exposure of the CEM targets to H_2O_2 alone, generated by increasing doses of glucose oxidase, failed to mediate cytotoxicity (Table II, line 1). However, the addition of purified MPO (8 mU) to the

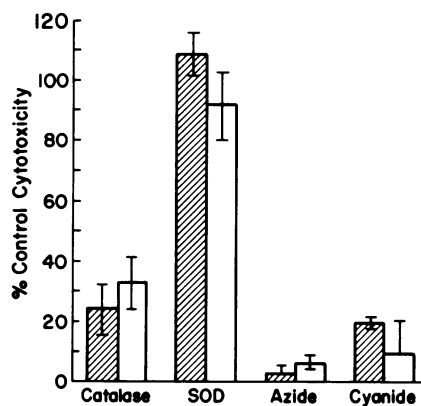


FIGURE 3 Effect of superoxide dismutase (SOD, 10 μ g/ml), catalase (10 μ g/ml), azide (1 mM), and cyanide (1 mM) on the cytolysis of 2×10^4 CEM by 2×10^5 monocytes (▨) or granulocytes (□) stimulated with 5 ng/ml of PMA over a 4-h incubation. All of the enzymes and inhibitors were incubated with the effector and target cells for 5 min before the addition of PMA. None of the agents alone stimulated the spontaneous release of ^{51}Cr above background. Results are expressed as the mean percentage of control cytotoxicity ± 1 SD of five experiments.

TABLE II
Effect of Scavengers on Glucose Oxidase-MPO-Cl⁻-mediated Cytotoxicity of CEM

Additive*	% Cytotoxicity†			
	Glucose oxidase concentration (mU)			
	26	2.6	1.3	0.26
Glucose oxidase	1.0±1.3	0.2±0.1	0.1±0.0	0.1±0.0
Glucose oxidase + MPO§	61.8±1.4	55.1±3.4	51.6±6.6	6.9±5.6
+ Alanine	38.2±12.8	10.2±7.7	1.7±1.8	0.7±0.5
+ Glycine	53.1±3.3	42.1±15.5	19.4±12.2	0.3±0.6
+ Serine	34.9±4.7	2.8±2.8	1.7±0.3	0.8±0.8
+ Valine	26.0±4.7	5.9±4.7	1.0±1.6	0.8±0.8
+ Isoleucine	26.3±6.8	6.2±4.4	2.6±1.0	1.4±0.3
+ 2,5-dimethylfuran	55.7±2.8	54.1±5.4	53.4±3.6	12.4±5.1
+ Ethanol	62.4±0.3	55.0±4.0	52.0±1.9	6.0±5.9

* Each reaction mixture was preincubated 5 min with L-amino acids (20 mM), 2,5-dimethylfuran (1 mM), and ethanol (20 mM). None of the scavengers stimulated spontaneous release from the CEM targets. The final serum concentration was 0.25%.

† Expressed as the mean percent cytotoxicity±1 SD of 2×10^4 CEM tumor cells ($n = 4$) after a 4-h incubation.

§ 8 mU MPO were added to each assay.

glucose-oxidase system dramatically enhanced CEM destruction (Table II, line 2). Increasing the glucose oxidase concentration to 80 mU or the MPO dose to 16 mU did not increase CEM destruction.

The role of HOCl in CEM cytotoxicity. The H₂O₂-MPO-Cl⁻ system is capable of peroxidizing Cl⁻ to HOCl (24). This species has a strong cytotoxic potential and we have previously demonstrated that CEM targets are extremely sensitive to small amounts of commercial HOCl (11). If the model glucose oxidase-MPO-Cl⁻ system is mediating cytotoxicity by generating HOCl then the addition of compounds known to scavenge this species should inhibit cytolysis. Table II illustrates the effect of five amino acids, compounds known to react rapidly with HOCl (25), on CEM destruction mediated by the model MPO system. At the highest concentration of glucose oxidase used (26 mU), MPO-mediated cytotoxicity was modestly inhibited by the HOCl scavengers. As the glucose oxidase concentration was lowered to 2.6 or 1.3 mU, cytotoxicity remained constant but the ability of the scavengers to inhibit cytotoxicity was dramatically increased (Table II). None of the amino acids tested impaired H₂O₂ generation by the glucose oxidase system or the rate of *o*-dianisidine oxidation by MPO. The hydroxyl radical (OH[•]) scavenger ethanol and the singlet oxygen (¹O₂) scavenger 2,5-dimethylfuran (12) did not inhibit cytotoxicity at any of the glucose oxidase concentrations studied. Thus, in the model glucose oxidase-MPO-Cl⁻ system a variety of compounds known to scavenge HOCl dramatically inhibited CEM destruction.

In order to demonstrate a role for the HOCl in

monocyte- and granulocyte-mediated cytotoxicity, the effect of the amino acids was studied in the cell system. Monocyte-mediated cytolysis was significantly impaired by all of the amino acids tested (Fig. 4). As previously demonstrated (11) the scavengers were also effective in inhibiting granulocyte-mediated CEM destruction (Fig. 4). Although glycine consistently impaired monocyte-mediated cytolysis, the effect on the granulocyte system was variable. In four of nine experiments performed, glycine inhibited cytotoxicity 83±6%, while in the remainder of the studies killing was decreased by only 39±12%. Interestingly, this same amino acid was the least effective scavenger stud-

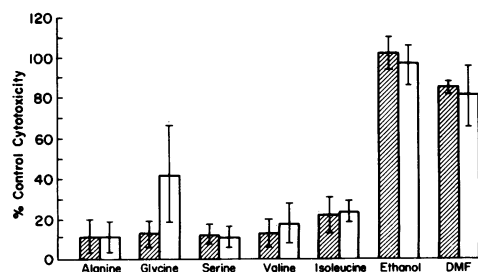


FIGURE 4 Effect of L-alanine (20 mM), glycine (20 mM), L-serine (20 mM), L-valine (20 mM), L-isoleucine (20 mM), ethanol (20 mM), and 2,5-dimethylfuran (DMF, 1 mM) on the cytolysis of 2×10^4 CEM by 2×10^5 monocytes (▨) or granulocytes (□) with a PMA dose of 5 ng/ml for 4 h of incubation. Scavengers were added to the effector and target cells 5 min before the addition of PMA. Addition of the scavengers alone did not stimulate ⁵¹Cr release above background. Results are expressed as the mean percentage of control cytotoxicity ±1 SD of five experiments except with the glycine-granulocyte incubations ($n = 9$).

TABLE III
Effect of Glycine on Granulocyte-mediated CEM Destruction

Granulocyte number $\times 10^{-5}$	% Cytotoxicity*		
	- Glycine	+ Glycine (20 mM)	% Inhibition
2.0	57.6 \pm 0.5	36.1 \pm 1.1	37.3
1.0	47.1 \pm 1.2	3.8 \pm 0.7	91.9
0.5	30.4 \pm 2.2	1.0 \pm 0.2	96.7
0.25	20.8 \pm 1.1	0.1 \pm 0.2	99.5

* Expressed as mean percent cytotoxicity \pm 1 SD of triplicate samples in a single representative experiment of four performed after a 4-h incubation. The phorbol myristate acetate and serum concentration was 5 ng/ml and 0.25%, respectively.

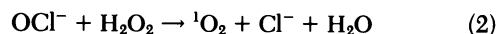
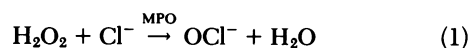
ied in the model system (Table II). Because lowering the concentration of glucose oxidase in the model MPO system increased the effectiveness of the HOCl scavengers (Table II), we studied the effect of glycine on granulocyte-mediated cytotoxicity as the effector cell concentration was decreased. As predicted, the ability of glycine to inhibit CEM destruction increased as the granulocyte concentration was lowered (Table III). None of the amino acids studied inhibited O_2^- or H_2O_2 generation by the effector cells.² In addition, neither ethanol nor 2,5-dimethylfuran had any effect on cell-mediated cytotoxicity (Fig. 4). Thus, it appears that monocytes, granulocytes, and the model glucose oxidase-MPO- Cl^- system all use HOCl or a species of similar reactivity as the common lytic mediator of CEM destruction.

DISCUSSION

The ability of human granulocytes and monocytes to destroy tumor cell targets supports a potential role for these effector cells in host defense against neoplasia (8–11, 14, 27). Human granulocytes incubated with opsonized zymosan, concanavalin A, or PMA are capable of destroying either murine lymphoma cells (LSTRA) (8–10), or CEM (11). In all of these systems granulocyte-mediated cytotoxicity is dependent on H_2O_2 , MPO, and a halide. In contrast, a mixture of human lymphocytes and monocytes stimulated with concanavalin A or PMA have been reported unable to mediate tumor cell cytotoxicity (9, 10). In this study we have demonstrated that purified preparations of human monocytes can destroy CEM targets compar-

ably to granulocytes. Although monocyte suspensions contained up to 3% granulocytes, this concentration did not appear to enhance cytotoxicity values. In several experiments monocyte-mediated cytotoxicity was not decreased when suspensions containing <1% granulocytes were obtained. Monocyte or granulocyte-mediated cytotoxicity was optimal at a pH of \sim 7.4 and could be inhibited by the addition of catalase, MPO inhibitors, or Cl^- -free conditions. The MPO system has been reported to have an acid pH optimum (3) but the activity of the enzyme is actually dependent on pH, H_2O_2 , and Cl^- concentrations (28). Sbarra et al. (29) demonstrated that at low H_2O_2 concentrations MPO exerted the strongest bactericidal effect at an alkaline pH. In addition, as the pH of the system becomes more alkaline the magnitude of the respiratory burst is enhanced (30). In this study it appears that both effector cells can effectively utilize the H_2O_2 -MPO- Cl^- system to destroy CEM targets under the conditions employed.

Despite the well-known cytotoxic potential of the MPO system in the intact leukocyte the final mediator of this effect has been debated. Early studies postulated that the H_2O_2 -MPO- Cl^- system could generate HOCl (31–33) but this has only recently been confirmed (24). Although HOCl is a potentially cytotoxic oxidant capable of reacting with a variety of substrates (25, 34, 35) attention has focused on a potential role for 1O_2 in MPO-mediated reactions (5). Allen et al. (36) reported the ability of the H_2O_2 -MPO-halide system to chemiluminesce and concluded that OCl^- (HOCl at an alkaline pH) reacted with H_2O_2 to form 1O_2 .



This hypothesis was reinforced by the observations of Rosen and Klebanoff who demonstrated the MPO-dependent conversion of 2,5-diphenylfuran to cis-dibenzoyl ethylene, a reaction thought to be specific for 1O_2 (37). The formation of this product could be inhibited by a number of 1O_2 scavengers and quenchers (37). Recently, it has been appreciated that most 1O_2 traps are nonspecific and can react with a variety of oxidants including HOCl (38–40). The conversion of cholesterol to the 5- α -hydroperoxide is presently considered the most specific detection technique for 1O_2 but, attempts to demonstrate this product in cholesterol droplets internalized by neutrophils have been unsuccessful (40). Thus, strong evidence is not available for the generation of 1O_2 by intact leukocytes.

The ability of HOCl to oxidize a variety of substrates and its known bactericidal activity (31, 41–43) led us

² In an earlier report we used L-methionine as a scavenger in granulocyte-mediated cytotoxicity since it did not interfere with O_2 consumption (11). In this study, methionine decreased H_2O_2 generation by the glucose oxidase system or PMA-stimulated effector cells probably by a direct reaction between methionine and H_2O_2 (26).

to examine the ability of HOCl alone to destroy CEM targets. In a preliminary report we demonstrated that CEM were rapidly destroyed by small aliquots of a HOCl solution (11). HOCl-mediated cytotoxicity was inhibited by amino acids, compounds known to react rapidly with HOCl, but not by OH⁻ or ¹O₂ scavengers (11). In this study we demonstrated the ability of five amino acids to inhibit glucose oxidase-MPO-Cl⁻-mediated cytotoxicity. As the concentration of glucose oxidase was decreased from 26 to 1.3 mU, cytotoxicity remained constant but the ability of the amino acids to inhibit CEM destruction increased. Apparently, at the lower doses of glucose oxidase (2.6 or 1.3 mU) the rate or absolute quantity of HOCl generated was decreased sufficiently such that the scavengers could effectively compete with the CEM targets for the oxidant generated. Thus, the protective ability of the amino acids may be a more sensitive index for HOCl generation than cytotoxicity alone.

Subsequently, we studied the effect of these amino acids on monocyte- and granulocyte-mediated CEM destruction. Alanine, serine, valine, and isoleucine were all capable of impairing cytotoxicity, whereas neither the OH⁻ nor ¹O₂ scavenger had any inhibitory effect. However, the addition of glycine had contrasting effects in the monocyte and granulocyte systems. While glycine consistently inhibited monocyte-mediated cytotoxicity, its protective ability in the granulocyte system was decreased in half of the experiments performed. Apparently, glycine (the most inefficient scavenger studied in the model MPO system) could not completely scavenge the HOCl generated by 2×10^5 granulocytes. Thus, as the granulocyte concentration was decreased, the protective ability of glycine increased. It appears that the granulocyte may generate HOCl at a faster rate or in greater quantities than the monocyte despite the ability of both effector cells to mediate a similar degree of CEM cytotoxicity.

The evidence presented in this study suggests that the MPO system mediates cytotoxicity by generating HOCl but the proposal is partly based on the action of nonspecific scavengers. The amino acids employed did not react with O₂⁻ or H₂O₂ but they can react with OH⁻ (44). Amino acids can also trap ¹O₂ but only tryptophan, methionine, and histidine are known to react rapidly with this oxygen metabolite (45, 46). Based on the inability of either ethanol or 2,5-dimethylfuran to inhibit cytotoxicity, it does not appear that these species play an important role in CEM destruction. However, we would expect that at higher concentrations either ethanol (47) or 2,5-dimethylfuran (38, 39) could inhibit cytotoxicity by reacting with HOCl.

HOCl reacts with amino acids at rapid rates to form the resultant chloramine (25). The chloramine deriv-

atives of amino acids retain a strong oxidizing potential and could potentially exert a destructive effect (31, 41, 42). The ability of the amino acids to impair cytotoxicity in our systems is probably related to the instability of their respective chloramines (33). These species spontaneously undergo oxidative decarboxylation and deamination to NH₃, CO₂, Cl⁻, and an aldehyde (33). Therefore, the oxidizing potential of the chloramine would be rapidly dissipated. Alternatively, chloramines may be unable to penetrate cell membranes to exert an intracellular cytotoxic effect (42). Thus, HOCl appears capable of mediating CEM destruction directly but caution must be exercised in ascribing all lytic effects to this species since it may be in equilibrium with other strong oxidizing agents including chlorine, chlorine monoxide, or H₂OCl₂ (48, 49). Although we cannot rule out the possibility that the amino acids prevented cytotoxicity by scavenging HOCl and thus inhibiting ¹O₂ generation more efficiently than 2,5-dimethylfuran (Eq. 2), several points should be reviewed. First, direct evidence exists that the H₂O₂-MPO-Cl⁻ system is capable of generating free HOCl (24). Second, HOCl is an extremely powerful oxidant capable of degrading a host of biologically relevant compounds (25, 34, 35). Third, HOCl is a potent cytotoxic agent with demonstrated bactericidal and tumoricidal activity (11, 41-43). Finally, amino acids, compounds known to rapidly react with HOCl, protected CEM from the H₂O₂-MPO-Cl⁻ system both in a model system or by intact monocytes and granulocytes. Taken together, increasing evidence points to HOCl as the common lytic mediator generated by the H₂O₂-MPO-Cl⁻ system.

Do granulocytes and monocytes utilize HOCl to destroy all biological targets? Although this agent is cytotoxic, it is important to note that PMA-stimulated, human leukocytes can destroy other target cells by MPO-independent mechanisms. In earlier reports we examined the ability of granulocytes to destroy either erythrocytes or vascular endothelial cells. Erythrocyte targets were destroyed by a mechanism dependent on an interaction between O₂⁻ and oxyhemoglobin (22) while endothelial cells were sensitive to H₂O₂ alone (23). Indeed, MPO-deficient granulocytes successfully destroyed both erythrocyte³ and endothelial cell targets (23). Recent reports have also demonstrated that human monocytes and neutrophils can destroy target cells in antibody dependent (14, 50-52) or serum independent systems (53). These studies have clearly shown that these effector cells can mediate cytotoxicity by both oxygen-dependent and independent mechanisms. Apparently, intact leukocytes can generate or release a host of potentially destructive species whose

³ Weiss, S. J. Unpublished observation.

role in cytotoxicity is in part dictated by the stimulating agent and the biochemical characteristics of the target cell. In this study, we have demonstrated that both human monocytes and granulocytes can destroy CEM targets by a mechanism dependent on H_2O_2 , MPO, and chloride. It appears that these effector cells and a model MPO system mediate CEM destruction by generating a species with characteristics similar, if not identical, with HOCl. This powerful oxidant should prove to be a potent addition to the arsenal of human monocytes and granulocytes.

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