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Exogenous Myeloperoxidase Enhances Bacterial Phagocytosis and Intracellular Killing by Macrophages

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It is well documented that myeloperoxidase (MyPo) contributes to the bactericidal activities of neutrophils and monocytes. Since mature macrophages (Mϕ) are devoid of this enzyme, its participation in Mϕ-mediated phagocytosis and bacterial killing has not been completely defined. The present study demonstrates that exogenously added MyPo, at physiological levels, enhances both phagocytosis and killing of *Escherichia coli*. Murine peritoneal Mϕ were exposed to various concentrations of MyPo for different time intervals. Viable opsonized *E. coli* was added either prior to or after addition of MyPo. Thioglycolate-induced but not resident Mϕ exhibited an increase in the number of phagocytizing cells. Both resident and thioglycolate-induced Mϕ demonstrated increased bactericidal activity. Physiological levels of soluble MyPo also induced a significant increase in chemiluminescence. Since luminol-dependent chemiluminescence measures reactive oxygen intermediate production, studies were done to determine whether superoxide anion or H_2O_2 was involved in MyPo-induced Mϕ killing. Both superoxide dismutase and catalase ablated MyPo-induced bactericidal activity. The above data suggest that soluble MyPo, released from neutrophils at a site of infection or inflammation, can enhance both phagocytosis and killing of microorganisms.

Phagocytosis of foreign pathogens represents one of the most important host defenses against disease. Although various cells are capable of phagocytosis, only neutrophils and macrophages (Mϕ) are considered "professional" phagocytes (7). In addition to their ability to ingest invading pathogens, Mϕ scavenge effete cells (i.e., neutrophils) and cellular debris (15).

Phagocytosis by Mϕ is highly correlated with the respiratory burst. Following perturbation of the cell membrane during phagocytosis, the respiratory burst is initiated, and various reactive oxygen intermediates (ROI) are produced, including singlet oxygen, hydroxyl radicals, superoxide anion, and H_2O_2. In addition to the production of ROI during the respiratory burst, light is emitted. This production of light can be enhanced by using luminol. Light emitted during luminol-dependent chemiluminescence (CL) is directly proportional to the amount of ROI being secreted by the cell (4).

Neutrophils are frequently the first cells at the site of a tumor, infection, or inflammation (20). During phagocytosis of a pathogen, the neutrophil degranulates and releases myeloperoxidase (MyPo) into the extracellular environment (6). Myeloperoxidase plus halide plus H_2O_2 form a cytotoxic triad which is toxic to bacteria, fungi, and mammalian cells (9). Macrophages are usually devoid of MyPo and bind this enzyme via the Mϕ mannose receptor (MMR) (22). The MMR is a multivalent receptor capable of binding organisms which express mannose on their surface (i.e., *Pneumocystis carinii*) as well as other mannosylated proteins, including MyPo (24). Binding of the MMR results in alteration of Mϕ function (24).

Previous work by the present investigators suggests another role for MyPo, that of an immunoregulatory molecule. Studies done in this laboratory have shown that Mϕ exposed to MyPo exhibit enhanced secretion of immunomodulatory cytokines, such as tumor necrosis factor alpha, interleukin-1, and alpha/beta interferon (13, 14). Another study described the induction of tumor necrosis factor and interferon by intravenous injection of MyPo (17). The present study was undertaken to determine if MyPo could serve as a stimulus to increase both phagocytosis and intracellular killing of bacteria such as *Escherichia coli*. Enhancement of these Mϕ functions via exposure to MyPo would provide further evidence for the role of this enzyme as an immunomodulator.

MATERIALS AND METHODS

Animals. Age-matched C57BL/6 mice weighing between 18 and 22 g were obtained from Jackson Laboratories (Bar Harbor, Maine).

Materials. Two enzymatically active recombinant MyPo preparations were generously supplied by Nicole Moguilevsky and Alex Bollen of Université Libre de Bruxelles, Brussels, Belgium. These contained 350 μg of protein per ml (4.2 μM) as determined by the Lowry method and approximately 75 U/ml as determined by the *O*-dianisidine assay (10, 18). One unit of MyPo activity is defined as the amount degrading 1 μmol of peroxide per min at 25°C. *E. coli* K-12 was supplied by Randall Jeter, Texas Tech University, Lubbock. Dulbecco's modified Eagle's medium (DMEM), RPMI 1640, and guinea pig complement were purchased from GIBCO. Auto-POW medium (Flow Laboratories Inc., McLean, Va.), fetal bovine serum (Intergen, Purchase, N.Y.), crystal violet (color index no. 42555; dye content, 95%; Fisher, Pittsburgh, Pa.), and tryptic soy broth (TSB; Difco, Detroit, Mich.) were purchased as noted. Phosphate-buffered saline (PBS, pH 7.2) was made as needed. The following reagents were purchased from Sigma (St. Louis, Mo.): superoxide dismutase, zymosan, gentamicin sulfate, HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), bovine serum albumin (essentially globulin free), and acridine orange (color index no. 46005; dye content, 90%). MyPo preparations were tested for endotoxin contamination with the *Limulus* amoebocyte lysate test (Associates of Cape Cod, Woods Hole, Mass.). Working dilutions of MyPo contained a maximum of ≤0.2 ng of endotoxin per ml. Stock preparations of MyPo with levels of endotoxin higher than 1 ng/ml were adsorbed with Endex beads (Associates of Cape Cod) and then retested.

Mϕ collection. Resident and thioglycolate-induced Mϕ (TG-Mϕ) were collected from the peritoneal cavity as previously described (10). Briefly, mice were killed by cervical dislocation, and Mϕ were collected by peritoneal lavage with DMEM. The cell suspension was removed and centrifuged at 250 × *g* for 10 min

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at 4°C. The supernatants were decanted, and the Mϕ were resuspended in DMEM containing 25 mM HEPES without gentamicin. Erythrocytes were lysed with 0.83% NH_4Cl.

Measurement of reactive oxygen and reactive nitrogen intermediates. The chemiluminescence assay was essentially that reported previously (12). Briefly, 100 μl of peritoneal cells containing 10^5 Mϕ in medium without phenol red (Auto-POW) was added to a tube (6 by 50 mm; Evergreen Scientific, Los Angeles, Calif.). The medium was supplemented with 0.6 g of HEPES, 0.2 g of sodium bicarbonate (Sigma), and 1.0 g of bovine serum albumin per dl. After 30 min of incubation at 37°C in 5% CO_2, the cells were washed three times with PBS to remove nonadherent cells. Following another 30 min of incubation, the cultures were washed, and the following were added to each tube: 100 μl of luminol, 100 μl of zymosan (opsonized with guinea pig complement), and 100 μl of medium alone or containing MyPo. The tubes were placed in a luminometer (model 20e; Turner, Mountain View, Calif.), and five 2-min counts were recorded. The results were plotted as time versus counts. The mean of triplicate treatments ± the standard error of the mean (SEM) was determined. Each experiment was repeated at least twice. In other experiments, readings were recorded for 1 h. Reactive nitrogen intermediates were measured using the method described by Ding et al. (5).

Bacterial preparation. One loop of *E. coli* was added to 10.5 ml of TSB in a 250-ml Erlenmeyer flask. With an orbital shaker, the suspension was grown to an optical density (OD) of 1.0 at a wavelength of 450 nm. This OD corresponded to approximately 10^9 CFU/ml. To determine the precise number of bacteria, a plate count was performed. The culture was centrifuged at 10,000 rpm for 15 min at 4°C. Subsequently, the culture was washed with 10 ml of PBS and stored at 4°C for 10 h in TSB. Prior to use, *E. coli* cells were washed in PBS and opsonized twice with 400 μl of guinea pig complement in an orbital shaker at 150 rpm, 24°C, for 30 min. The bacterial cell suspension was subsequently washed twice in 10 ml of PBS.

Phagocytosis assay. The procedure used to assay phagocytosis was similar to that described by Lian et al. (16). Resident Mϕ and TG-Mϕ were collected and suspended in DMEM without gentamicin. The Mϕ number was adjusted to 1×10^6/ml for resident Mϕ and 4×10^5/ml for TG-Mϕ. A total of 100 μl of the cell suspension was added to each well of a 16-well tissue culture chamber slide (Nunc Inc., Naperville, Ill.). The cells were allowed to attach for 2 h at 37°C under 5% CO_2. Following attachment, cultures were washed twice with 200 μl of warm medium to remove nonadherent cells. Subsequently, cultures were treated in one of two ways: (i) Mϕ were exposed to MyPo for 10 min and washed vigorously, and *E. coli* cells suspended in DMEM with 10% fetal bovine serum were added at a ratio of 10:1; or (ii) Mϕ were exposed to *E. coli* for 30 min, washed to remove uningested bacteria, and then exposed to MyPo. After incubation for 30 min, the cells were stained for 45 s with 0.1 mg of acridine orange per ml (16). The slides were counterstained for 1 min with 1 mg of crystal violet per ml to quench the fluorescence of extracellular bacteria. Viable bacterial cells, having intact cell membranes, do not absorb acridine orange. However, cells devoid of intact membranes allow acridine orange to intercalate within the DNA (14). Bacteria which fluoresced green were scored as live, and those which fluoresced red-orange were scored as dead.

A total of 300 cells were counted for each well with a fluorescence microscope at ×1,000 magnification. Each experiment was repeated at least three times. The results of representative experiments are given in the text and figures.

Statistical determinations. With the exception of the CL data, all other data represent the means of replicate counts ± SEM. A one-way analysis of variance and Student-Keuls-Newmann tests were used to determine significance levels.

RESULTS

Experiments were done to determine if exogenously supplied MyPo would enhance phagocytosis of bacteria. From previous studies involving cytokine induction, a MyPo concentration of 840 nM (71 μg of protein per ml) was chosen. At this concentration, the effect of MyPo on the phagocytic function of resident and TG-Mϕ was examined. When resident Mϕ were exposed to 840 nM MyPo for various time intervals, there were no significant differences in the number of phagocytizing cells between treated and control cultures (Table 1). During these studies, the mean number of intracellular bacteria per resident Mϕ was not markedly different between control and treated resident Mϕ cultures. TG-Mϕ exposed to 840 nM MyPo exhibited a time-dependent increase in the number of phagocytizing cells at each time point studied (Fig. 1). As with the resident Mϕ population, there were no significant differences between the mean number of phagocytized bacteria per TG-Mϕ in either control or treated cultures (data not shown).

Experiments were done to determine the effect of different concentrations of MyPo on bacterial killing by resident Mϕ. It can be seen that there was a direct relationship between the concentration of MyPo and the extent of bactericidal activity. At the lowest dose, the percentage of dead bacteria was 23%, compared with 54% at the highest dose (Fig. 2). An intermediate concentration of 840 nM, which caused 38% bacterial death, was chosen for further studies involving bacterial cell killing.

When bacteria were exposed to MyPo directly, 90% killing was observed. Because of this, all assays were done with a protocol which did not involve the simultaneous presence of soluble MyPo and *E. coli*. In most of the studies, Mϕ were exposed to MyPo, washed extensively, and then exposed to bacteria. A second design was implemented to address whether extracellular killing was caused by membrane-bound MyPo participating in the cytotoxic triad. In these studies, resident Mϕ were exposed to bacteria for 30 min, washed to remove uningested bacteria, and subsequently exposed to either medium with MyPo or medium alone. With the second design, the percent killing was not significantly different from that in experiments in which the cells were exposed to MyPo prior to bacterial exposure (Fig. 3).

TABLE 1. Kinetics of bacterial phagocytosis by resident Mϕ exposed to recombinant MyPo[a]

Time (min)	Mean no. of phagocytizing Mϕ ± SEM		Mean no. of bacteria/cell ± SEM	
	Control	MyPo	Control	MyPo
15	23.50 ± 1.55	24.33 ± 0.67	2.72 ± 0.23	2.67 ± 0.03
30	29.67 ± 1.45	33.00 ± 0.58	2.90 ± 0.06	3.13 ± 0.24
60	32.75 ± 1.11	35.67 ± 0.88	3.43 ± 0.25	3.66 ± 0.15

[a] Mϕ were cultured on 16-well tissue culture chamber slides. After attachment, monolayers were exposed to 840 nM MyPo for 10 min. Subsequently, monolayers were washed, and *E. coli* was added at a ratio of 10 *E. coli* cells per Mϕ. At various times, the cells were stained with acridine orange, and the number of cells with ingested bacteria were counted. All values are expressed as the mean of three 100-cell counts ± SEM. Each experiment was repeated at least three times. There were no significant differences between control and MyPo-treated cultures.

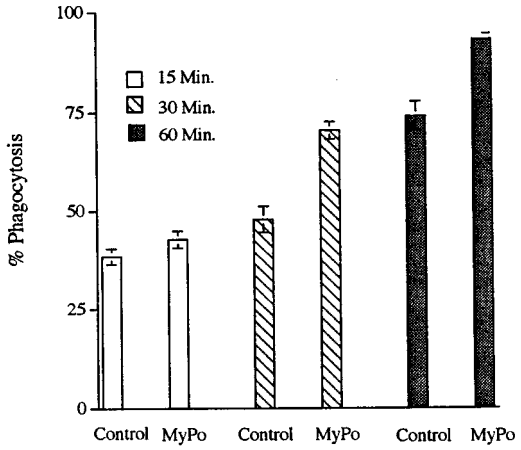

FIG. 1. Kinetics of phagocytosis by TG-Mϕ. TG-Mϕ were cultured on 16-well chamber slides. After attachment, the monolayers were exposed to recombinant MyPo (840 nM) for 10 min. Subsequently, the monolayers were washed vigorously, and *E. coli* cells were added at a ratio of 10 *E. coli* cells per Mϕ. Following incubation, the cells were stained with acridine orange, and the number of cells with ingested bacteria was counted. Each value represents the mean ± SEM of three 100-cell counts. $P \leq 0.005$ for controls versus corresponding MyPo-treated cultures for 30 and 60 min. $P \geq 0.05$ for control versus MyPo-treated culture for 15 min.

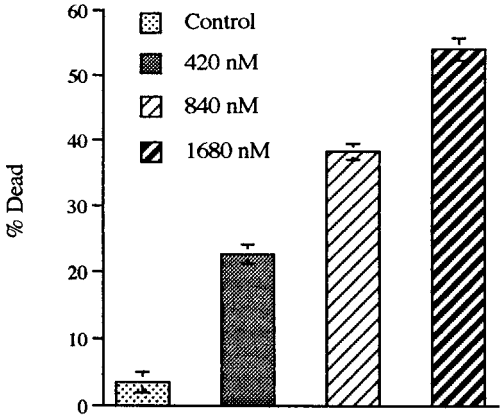

FIG. 2. Effect of various concentrations of MyPo on intracellular killing of bacteria. Resident Mϕ were cultured on 16-well chamber slides. After attachment, the monolayers were exposed to various concentrations of recombinant MyPo for 10 min. Subsequently, the monolayers were washed vigorously, and *E. coli* cells were added at a ratio of 10 *E. coli* cells per Mϕ. Following 30 min of incubation, the cells were stained with acridine orange and scored as live or dead according to the color observed by fluorescence microscopy. $P \leq 0.001$ for control versus MyPo-treated cultures.

Once it was established that MyPo-treated Mϕ exhibited enhanced bactericidal activity, the kinetics of bacterial killing were determined. Studies with resident Mϕ indicated that there was a time-dependent increase in bacterial killing (Fig. 4a), with greater killing by MyPo-exposed resident Mϕ at all time points examined. Significantly greater bacterial killing was observed when TG-Mϕ were used (Fig. 4a and b). It can be seen that at 15 min, there was approximately 34% killing after exposure to MyPo, compared with approximately 12% in the controls. By 60 min, virtually all bacteria were dead in both treated and control TG-Mϕ cultures (Fig. 4b).

Phagocytosis is highly correlated with the respiratory burst, and the ROI generated during the respiratory burst are involved in Mϕ-mediated killing. In the present study, the respiratory burst was measured by CL. The ability of various concentrations of MyPo to induce a CL response was determined, and a dose-dependent response was obtained. Concentrations of MyPo as low as 17 nM induced a threefold increase in CL over that of the control (Fig. 5a). Since phagocytosis and intracellular killing were measured over 30 to 60 min, CL was observed for 60 min. A comparison was made between resident Mϕ and TG-Mϕ following exposure to MyPo (Fig. 5b). It can be seen that the respiratory burst of resident Mϕ exposed to 35 nM MyPo rose for approximately 40 min and then declined slowly. In control cultures, peak CL occurred earlier. Compared with resident Mϕ, TG-Mϕ exhibited greater activity in both control and treated cultures. However, at all time points measured, the CL of cultures exposed to MyPo was significantly higher than that of those exposed to medium alone (Fig. 5a and b). Levels of reactive nitrogen intermediates were not significantly different from that of controls (data not shown).

Since MyPo induced an enhanced respiratory burst concomitant with increased levels of ROI production, studies were undertaken to determine if the ROI induced by MyPo were necessary for bacterial killing. Resident peritoneal Mϕ cultures were exposed to either medium alone or medium containing MyPo. After incubation, cultures were exposed to *E. coli* in the presence of medium alone, medium plus catalase, or medium plus superoxide dismutase. The presence of catalase decreased intracellular killing in a dose-dependent manner, with 0.2 μM catalase completely ablating the effects of MyPo (Fig. 6). Superoxide dismutase was more effective than catalase at inhibiting the cytocidal effects of Mϕ exposed to MyPo.

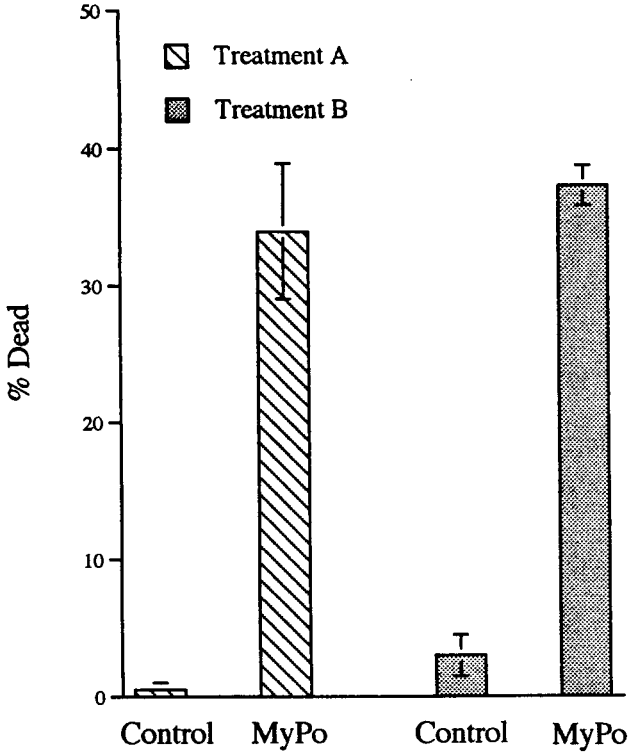

FIG. 3. Comparison of resident Mϕ-mediated intracellular bacterial killing pre- and postexposure to MyPo. (Treatment A) Resident Mϕ were cultured on 16-well chamber slides. After incubation, Mϕ were exposed to 840 nM MyPo for 10 min. After incubation, the monolayers were washed vigorously, and *E. coli* was added at a ratio of 10 cells per Mϕ. Following 30 min of incubation, the cells were stained with acridine orange and scored as live or dead according to color. $P \leq 0.001$. (Treatment B) Resident Mϕ were cultured on 16-well chamber slides. After incubation, Mϕ were exposed to *E. coli* (ratio, 10:1) for 30 min. Subsequently, monolayers were washed to remove uningested bacteria. Mϕ with ingested bacteria were exposed to 840 nM MyPo for 10 min and stained with acridine orange. $P \leq 0.001$ for controls versus corresponding MyPo treatments..

DISCUSSION

The present studies were done with murine resident Mϕ and TG-Mϕ and human MyPo. The use of a homologous system would have been preferable; however, it is established that the murine neutrophil expresses very low levels of MyPo (23), making a homologous study impractical if not impossible.

MyPo is released into the microenvironment by neutrophils during phagocytosis and can be taken up by Mϕ via the MMR (22). Therefore, neutrophils at a site of infection or inflammation could serve as a source of this enzyme for Mϕ. Previous work by the present investigators demonstrated that Mϕ exposed to MyPo exhibited enhanced cytokine production (13, 14, 17). The present studies were undertaken to determine if soluble MyPo, which is normally found in the microenvironment, could activate Mϕ to increase phagocytosis of bacteria, intracellular killing of bacteria, or both.

Adams and Marino (1) reported that Mϕ activation is a multistep process, with resident Mϕ being less capable of various functions and capacities than inflammation-induced Mϕ. The results of the present study indicate that MyPo did not alter the number of cells phagocytosed by resident Mϕ but did increase the number of cells phagocytosed by TG-Mϕ. Because

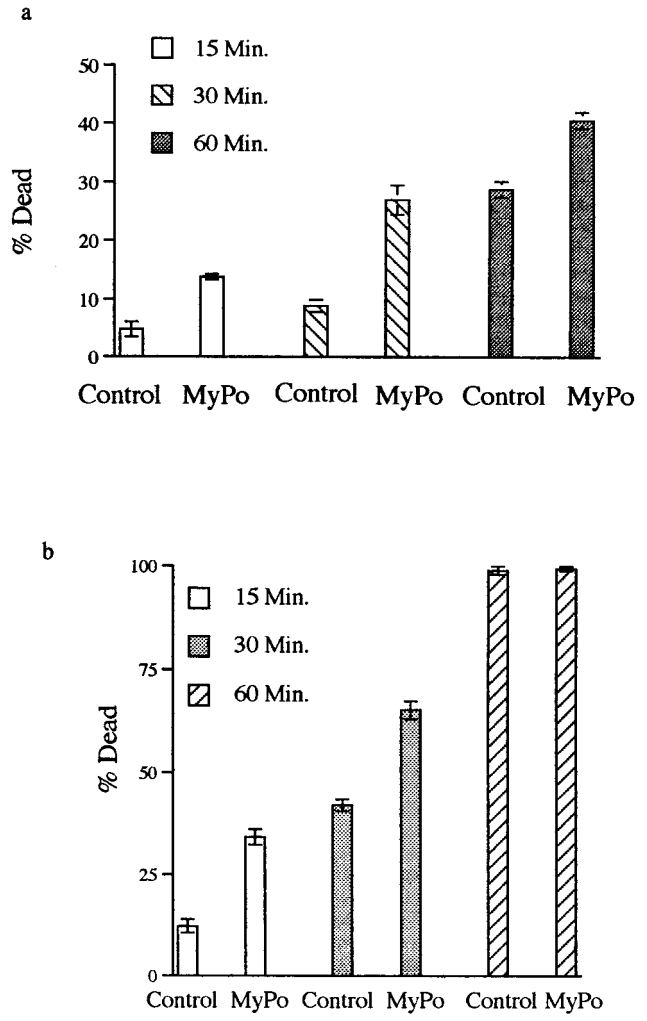

FIG. 4. Kinetics of bacterial killing by resident Mϕ and TG-Mϕ exposed to MyPo. (a) Resident Mϕ were cultured on 16-well chamber slides. After attachment, the monolayers were exposed to MyPo (840 nM) for 10 min. Subsequently, the monolayers were washed vigorously, and *E. coli* cells were added at a ratio of 10 *E. coli* cells per Mϕ. $P \leq 0.005$ for controls versus corresponding MyPo-treated cultures. (b) TG-Mϕ were cultured on 16-well chamber slides. After attachment, the monolayers were exposed to recombinant MyPo (840 nM) for 10 min. Subsequently, the monolayers were washed vigorously, and *E. coli* cells were added at a ratio of 10 *E. coli* cells per Mϕ. After incubation, the cells were stained with acridine orange and scored as live or dead according to the color observed by fluorescence microscopy. $P \leq 0.001$ for controls versus corresponding MyPo-treated cultures for 15 and 30 min; $P \geq 0.05$ for controls versus corresponding MyPo-treated cultures for 60 min.

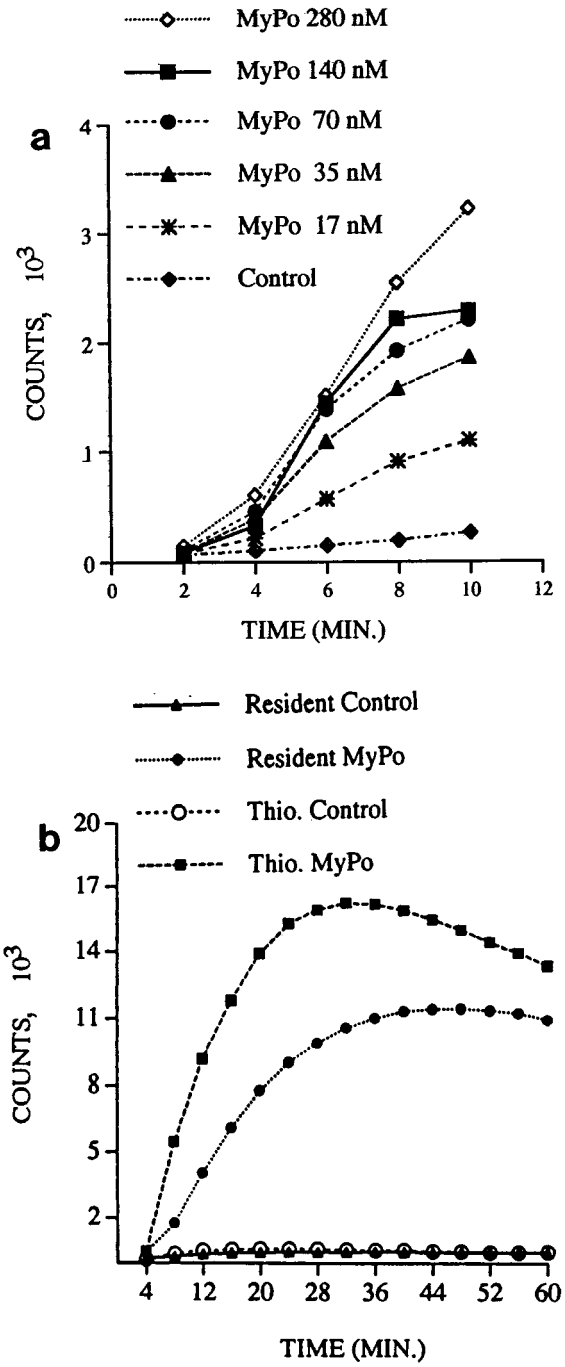

FIG. 5. Enhancement of respiratory burst by MyPo. Macrophages exposed to MyPo, luminol, and opsonized zymosan were incubated at 37°C, and the amount of light released was measured with a luminometer. (a) Various dilutions of MyPo were incubated with resident Mϕ, and CL was measured over a 10-min interval. (b) Resident Mϕ and TG-Mϕ (Thio.) were incubated without (control) or in the presence of 35 nM MyPo. CL was determined over a 1-h interval.

of their low level of activation, resident Mϕ, unlike TG-Mϕ, may require another signal in addition to MyPo in order to exhibit increased phagocytosis. T cells activated by the presence of a pathogen may secrete cytokines which could serve as a second signal. TG-Mϕ, on the other hand, are considered the prototype of inflammation-induced Mϕ and are generally more active than resident Mϕ (1).

Addition of MyPo directly to *E. coli* killed approximately 90% of the bacteria in 30 min. Aerobically cultured bacteria produce H_2O_2 during normal cell respiration (21). Hydrogen peroxide produced by bacteria can react with MyPo plus halide, forming a cytotoxic triad capable of killing bacteria (9). Because of this, experimental designs used in this study did not combine the bacteria and MyPo simultaneously; either the MyPo was washed off prior to the addition of bacteria, or the MyPo was added to the cultures after the Mϕ were exposed to the bacteria. The fact that there was no significant difference in bacterial killing with resident Mϕ exposed to MyPo either before or after exposure to bacteria suggests that the bactericidal activity of the resident Mϕ observed was intracellular. If membrane-bound MyPo was present after the cultures were

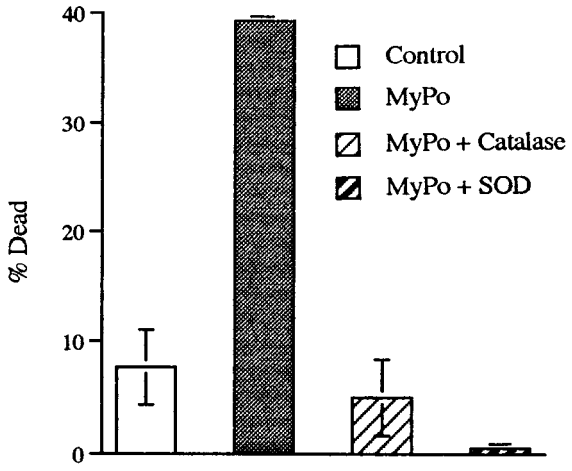

FIG. 6. Effect of radical scavengers on bacterial killing induced by MyPo. Resident Mϕ were cultured on 16-well chamber slides. After attachment, the monolayers were exposed to 850 nM MyPo for 10 min. Subsequently, the monolayers were washed vigorously, and one of the following was added: *E. coli* alone, superoxide dismutase (SOD, 1 μg/ml) plus *E. coli*, or 0.2 μM catalase plus *E. coli*. Bacteria were added to Mϕ at a ratio of 10:1. Following a 30-min incubation, the cells were stained with acridine orange and scored as live or dead according to the color observed by fluorescence microscopy. $P \leq 0.002$.

washed, it was not present at levels sufficient to alter the results.

Phagocytosis is highly correlated with the respiratory burst, and the respiratory burst in turn is correlated with bactericidal activity (7). It has been demonstrated that the enhanced light emission in luminol-dependent CL was correlated with the level of peroxidase (MyPo) used. The enhanced CL is proportional to the amounts of O_2^- and H_2O_2 species present (4). In the presence of MyPo, an increase in the respiratory burst was observed for both resident Mϕ and TG-Mϕ (Fig. 5a and b). Resident Mϕ exposed to MyPo exhibited an enhanced respiratory burst compared with control cultures; however, the respiratory burst was significantly less than that of TG-Mϕ under similar conditions (Fig. 5b). These facts correlate with the bactericidal data obtained from resident Mϕ and TG-Mϕ exposed to MyPo. Bactericidal activity was directly proportional to both the time and level of exposure to the products of the respiratory burst (Fig. 4a and b). For all time points examined, the level of CL with TG-Mϕ was significantly higher than that seen in resident Mϕ cultures. This difference in CL, through increased ROI production, may account for the enhanced ability of the TG-Mϕ to kill bacteria during the incubation period.

Since the CL studies indicated that MyPo enhanced the production of ROI, studies were undertaken to determine the role of H_2O_2 and superoxide anion in Mϕ-mediated bactericidal activity. As noted in Fig. 6, the presence of catalase and superoxide dismutase significantly reduced the intracellular killing of *E. coli* by Mϕ exposed to MyPo. A cytochrome *c* reduction assay was done to confirm the induction of superoxide anion by MyPo. In addition, boiling of catalase or superoxide dismutase ablated their effects, confirming the enzymes' specificities. These results suggest that both H_2O_2 and superoxide anion are required for bacterial killing. It is not known whether ROI are directly responsible for the killing observed by MyPo-stimulated Mϕ. Exogenous MyPo may be internalized by the Mϕ, with a concomitant production of ROI. MyPo, the ROI, and chloride present in the medium could form the cytotoxic triad, as observed in neutrophils (9). This cytotoxic triad (MyPo plus halide plus H_2O_2) would then form hypochlorous acid, which would cause bacterial death. If, indeed, the radicals themselves are not responsible for killing, another possible explanation for why superoxide dismutase ablates bactericidal activity is that (i) H_2O_2 crosses membranes easily (3) and (ii) high levels of H_2O_2, which could be produced in the presence of superoxide dismutase, can inactivate MyPo (8). Therefore, the enzyme would not be available to participate in the above reaction.

When neutrophils ingest pathogens at the site of inflammation, soluble MyPo is released into the microenvironment. Within 5 s of perturbation of the membrane of neutrophils by bacteria, MyPo is observed on the surface of these cells (19). It has been reported that, at the site of inflammation, as much as 150 nM MyPo could be present (26). Routinely, 840 nM MyPo was used for both the phagocytosis and intracellular killing studies. Various concentrations of MyPo from 17 to 140 nM were able to induce a CL response. Since 17 nM MyPo was adequate to induce a significant increase in CL, these data imply that there is sufficient MyPo present in the microenvironment of an inflammatory site to initiate the respiratory burst. Higher concentrations of MyPo were needed in this study to induce enhancement of phagocytosis compared with CL. Since phagocytosis is a more complex function than the respiratory burst, other factors which are present in the microenvironment (cytokines, growth factors, etc.) may be necessary for the Mϕ to respond to the concentrations of MyPo found at a site of inflammation. Also, the higher concentrations of MyPo needed for enhancement of phagocytosis may simply reflect differences in the sensitivity of the assays. Finally, it must be taken into account that it is extremely difficult to duplicate in vivo conditions using in vitro models.

The lower levels of MyPo used in these studies are comparable to the amounts present under natural conditions. It has been reported that there is 20 to 45 U of MyPo per ml in the extracellular environment in an inflamed area (2). With the same assay used for determining a unit of activity, the units of activity used in the present study (0.5 to 35 U/ml) were within physiological levels for both the CL and phagocytosis studies.

In order to ascertain if the above Mϕ responses were specific for prokaryotic cells, experiments were done with the eukaryotic cell *Candida albicans*. The results obtained with *C. albicans* were similar to those obtained with *E. coli*, indicating that neither phagocytosis nor killing is specific for *E. coli* (unpublished data).

Considering the above collectively, the data suggest another role for MyPo, i.e., that of an immunomodulator. At the site of infection, the neutrophil is frequently the first cell to arrive and carry out phagocytosis. During phagocytosis, MyPo is released into the microenvironment. This enzyme binds to the MMR of Mϕ and enhances the ability of resident Mϕ to kill bacteria. Inflammation-induced Mϕ at the site would bind MyPo and exhibit enhanced phagocytosis and bactericidal activity. After the infection was cleared, the supply of MyPo would diminish, and the system would return to the baseline state.

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