Exposure of Macrophages to an Enzymatically Inactive Macrophage Mannose Receptor Ligand Augments Killing of *Candida albicans* (44208)

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Abstract. **Macrophages (Mϕ) are involved in host defenses against opportunistic pathogens. Previous studies by the present investigators indicate that Mϕ exposed to enzymatically active myeloperoxidase (MPO), exhibited both increased phagocytosis and killing of *Candida albicans.* The purpose of this study was to determine if enzymatically inactive Mϕ-mannose receptor (MMR) ligands could function similarly. Resident murine peritoneal Mϕ were exposed to the MMR ligands, mannosylated bovine serum albumin (mBSA), and enzymatically inactive myeloperoxidase (iMPO), followed by exposure to opsonized *C. albicans.* Both mBSA and iMPO induced a slight increase in the number of phagocytizing cells; however, candidacidal activity was significantly higher in treated cultures compared to controls ($P \leq 0.001$). The production of reactive oxygen intermediates (ROI) was detected using chemiluminescence. After employment of ROI scavengers, a decrease in candidacidal activity was observed. The data suggest that MMR-ligand interaction alone is sufficient to significantly enhance the candidacidal activity of Mϕ *via* ROI, and that iMPO which is released at a site of inflammation induces Mϕ-mediated killing of microorganisms. These findings indicate a previously unrecognized role of iMPO.** [P.S.E.B.M. 1998, Vol 217]

Opportunistic fungal infections are commonly observed in immunocompromised individuals (1, 2). Because of AIDS, transplantation, and immunosuppressive therapies, the incidence of infections caused by *Candida albicans* has increased (3, 4). This resurgence of *C. albicans*-related illnesses has resulted in a renewed interest in the control of this organism.

Phagocytosis is one of the early lines of defense against foreign pathogens. The neutrophil and the macrophage (Mϕ) are the most important cells involved in phagocytosis (5, 6). During phagocytosis, the neutrophil degranulates, and myeloperoxidase (MPO) is released into the extracellular environment (7, 8). It is estimated that approximately 40% of the enzyme is inactivated within the microenvironment (9).

Peroxidases belong to a group of heme-containing enzymes that use hydrogen peroxide (H_2O_2) as a substrate. Myeloperoxidase is one of the most abundant peroxidases in the human body. About 4% of MPO is composed of carbohydrates in the form of high-mannose asparagine-linked oligosaccharides that form a ligand with the Mϕ mannose receptor (MMR) (10, 11).

Macrophages obtain and utilize MPO *via* the MMR, a receptor for mannosylated or fucosylated proteins (11, 12). In addition to binding MPO, the MMR is a receptor that readily binds organisms such as *C. albicans,* which also express mannose residues on their surfaces (13). Previous studies by the present investigators have demonstrated that Mϕ exposed to enzymatically active MPO and other mannosylated proteins exhibited enhanced secretion of cytokines including tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interferon α/β (IFN α/β) (14–16). In ad-

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dition, recombinant human myeloperoxidase (rec-MPO), also an enzymatically active MMR ligand, induced an increase in Mϕ-mediated phagocytosis and intracellular killing of *C. albicans* (17). Therefore, MPO appears to be a potent immunomodulator of the immune response.

As stated above, both MPO and enzymatically inactive myeloperoxidase (iMPO) are present at the site of infection (9). Bradley *et al.,* have determined that there are 20–45 U/ml of MPO at a site of infection whereas other investigators have estimated that there are 150 nM of MPO in an infected microenvironment (9, 18). In support of the presence of iMPO in the microenvironment, King *et al.,* recently reported that a major portion of released MPO is inactivated upon secretion from the neutrophil (44% secreted with only 3% active enzyme) (19). In addition, Edward's *et al.,* have reported that 16–29 μg/ml of iMPO were present in the joints of rheumatoid arthritis (RA) patients previously considered to be MPO negative (20). Investigators have also reported that the loss of enzymatic activity of MPO was not due to proteolytic destruction (19). The iMPO used in the present study represents an inactivated intact molecule (21). Therefore, the iMPO used in this study was a relevant *in vitro* correlate to MPO inactivated *in vivo.* The present study was undertaken to determine whether receptor-ligand interaction alone was sufficient to induce the enhancement of certain Mϕ functions such as ROI production, phagocytosis, and intracellular killing of *C. albicans.* In the present study, two high-affinity MMR ligands, mannosylated bovine serum albumin (mBSA) and iMPO, were employed. One low affinity ligand, galactosylated BSA (gBSA), was also used. The data presented herein indicate that Mϕ exposed to the above mentioned high-affinity MMR ligands exhibited a modest increase in phagocytosis with a significant increase in candidacidal activity. These data also support the hypothesis that iMPO is a previously unrecognized immunoregulatory substance which could play a role in immune function.

Materials and Methods

Animals. C57BL/6 mice, age matched, of either sex, and weighing between 18–22 g were purchased from Jackson Laboratories (Bar Harbor, ME). All animals were maintained in facilities that abided by federal guidelines for animal care.

Materials. *Candida albicans,* strain 3153 A, was generously supplied by Dr. L. Chaffin, Texas Tech University Health Sciences Center, Lubbock, Texas. Enzymatically inactive recombinant MPO, containing 487 μg/ml protein as determined by the Lowry method, and 0 U/ml activity as determined by O-dianisidine assay (10, 22), was supplied by Drs. Nicole Moguilevsky and Alex Bollen of Universite Libre de Bruxelles, Nivelles, Belgium. The iMPO represents a mutated form of the active enzyme that is minus the histidine residue from the heme moiety (21). Dulbecco's modified Eagle's medium (DMEM) and guinea pig complement were purchased from GIBCO (Long Island, NY). Phosphate buffered saline pH 7.2 (PBS) was prepared as described previously (23). The following reagents were purchased from Sigma (St. Louis, MO): gentamicin sulfate, superoxide dismutase (SOD) from bovine erythrocytes, HEPES, bovine serum albumin (essentially globulin-free) (BSA), acridine orange (color index #46005, dye content 90%), catalase from bovine liver, phorbol myristate acetate (PMA), and D-mannitol. Other reagents used, which were commercially purchased, included: crystal violet (color index #42555, dye content 95%), Fisher (Pittsburgh, PA); fetal bovine serum (FBS) Intergen (Purchase, NY); mBSA and gBSA, EY Labs (San Mateo, CA). Luminol was obtained from Eastman Kodak (Rochester, NY). All reagents were tested for endotoxin contamination using the *Limulus* amebocyte lysate test (LAL) (Associates of Cape Cod, Woods Hole, MA). Preparations of MPO with a level of endotoxin higher than 1 ng/ml were adsorbed with END-X beads (Associates of Cape Cod) and then retested for endotoxin. The iMPO dilution employed in all studies contained $\leq$ 0.2 ng/ml of endotoxin.

Macrophage Collection. Resident peritoneal Mϕ were collected as previously described (23). Briefly, mice were sacrificed by cervical dislocation, and Mϕ were collected by peritoneal lavage using cold PBS. The cells were washed and resuspended in DMEM with 25 mM HEPES. The DMEM did not contain either gentamicin or FBS. The Mϕ cell number was adjusted to 1×10^6 Mϕ/ml. One hundred μl of the Mϕ-suspension were added to each well of a 16-well tissue culture chamber slide (Nunc Inc., Naperville, IL) and incubated for 24 hr at 37°C under 5% CO_2.

Candida Albicans. *C. albicans* was cultured in 10 ml of yeast extract peptone-dextrose (YPD) broth at 30°C with slight agitation. This medium was composed of glucose (20 g/l), yeast extract (10 g/l), and peptone (20 g/l) (Difco Laboratories, Detroit, MI). After 14 hr, a stationary growth phase was reached, and the density of the culture was approximately 5×10^8 cfu/ml. By microscopic examination a pure yeast form without budding or hyphae was obtained. The precise number and viability of the yeast were determined by plate count. Stock cultures of *C. albicans* were maintained on YPD agar plates at 4°C and were transferred every 4–6 weeks. After the stationary growth phase was reached, the cell suspension was centrifuged at 15,000 rpm for 15 min (at 4°C) and washed one time in PBS. Using slight agitation, cells were opsonized twice for 30 min at 30°C with 400 μl guinea pig complement or homologous murine sera. Following opsonization, cells were washed twice and diluted in PBS to a concentration of 5×10^6 yeast/ml.

Phagocytosis Assay. The procedure employed in the present study was similar to the one originally described by Lian *et al.* and others (24–26). This procedure was further modified by Lefkowitz *et al.* (17). Resident Mϕ were incubated for 24 hr, then washed twice with warm media to

remove nonadherent cells. Subsequently, the monolayers were treated according to one of the following protocols: (A) Mϕ were exposed for 10 min to either a MMR-ligand or control media. After incubation, the Mϕ were washed vigorously, and *C. albicans,* suspended in DMEM supplemented with 10% FBS, was added at a ratio of five yeast/Mϕ for 60 min. (B) Mϕ were exposed to *C. albicans* for 60 min, washed extensively to remove uningested yeast, and then exposed to a MMR ligand for 10 min. Both protocols were followed by staining with acridine orange (0.1 mg/ml) for 90 sec, and counterstaining for 40 sec with crystal violet (1 mg/ml). Crystal violet was used to quench the fluorescence of extracellular yeast (27). A total of 400 cells were counted for each treatment using a fluorescence microscope at 1000× magnification using oil immersion. Fungal cells that fluoresced green were scored as live and those that fluoresced orange were scored as dead.

In order to insure the veracity of the assay procedure, the following was done: (1) results of phagocytosis and intracellular killing were initially verified using a plate count assay (results not shown); (2) viability of *C. albicans* was determined by plate count at the beginning of each experiment; (3) yeast were boiled for 30 min prior to each experiment and added to wells as positive controls, all of which were orange; and (4) readings of the primary examiner were checked "blind" by a second party periodically to insure consistency. Each experiment was repeated at least three times. Representative experiments are shown in the text.

Chemiluminescence Assay. Methods used were modified from a chemiluminescence assay as described by Lefkowitz *et al.* (23). Briefly, 100 μl of murine peritoneal cells, containing 10^6 Mϕ/ml, in media without phenol red (Auto-POW, Flow Lab., McLean, VA), were added to 6 × 50-mm tubes (Evergreen Sci., Los Angeles, CA). The media were supplemented with 0.6 g/dl HEPES, sodium bicarbonate 0.2 g/dl, and 1.0 g/dl BSA (Sigma). After 30 min incubation at 37°C under 5% CO_2 the cells were washed three times with media without phenol red to remove nonadherent cells. Although no neutrophils were detected, occasionally a lymphocyte was observed. After another 30 min incubation, the cultures were washed and the following added to each tube: 30 μl of luminol, 100 μl of phorbol myristate acetate (PMA) (600 nM) (Sigma), and 200 μl of media alone or media containing mBSA or iMPO. The tubes were placed in a Turner luminometer model 20e (Mountainview, CA) and five 2-min counts were recorded. The results were plotted as time versus relative light units. The mean of triplicate treatments ± SEM was determined. Each experiment was repeated at least twice.

Statistical Analysis of Data. One-way Analysis of Variance (ANOVA) and Student-Newman-Keuls multiple comparison tests were performed to determine significance levels among the different treatment groups and controls. Nontransformed means are illustrated in the appropriate figures.

Results

The present study was done to determine if MMR-ligand interaction was sufficient to enhance the phagocytosis and killing of *C. albicans* by murine peritoneal Mϕ. In order to insure a more homogeneous cell population, resident peritoneal Mϕ were cultured for a minimum of 24 hr prior to their use in experiments. After incubation and washing, cultures were >99% Mϕ as determined by differential staining.

To ensure that intracellular killing alone was measured and not the ingestion of *C. albicans* killed externally, two experimental designs were employed as described in materials and methods. In the first design, exposure to treatments was done prior to contact with *C. albicans* whereas in the second design, exposure to *C. albicans* was followed by exposure to treatments. For most experiments, the former was employed. The results using both protocols are depicted in figure 1. The percent of dead cells after Mϕ were exposed to 840 nM mBSA was approximately 64% for protocol A, as compared to approximately 60% for protocol B. Therefore, there were no significant differences noted between the results obtained with either protocol.

Once it was established that exposure of Mϕ to mBSA induced intracellular killing, Mϕ were exposed to various concentrations of mBSA. Concentrations of 420 nM of

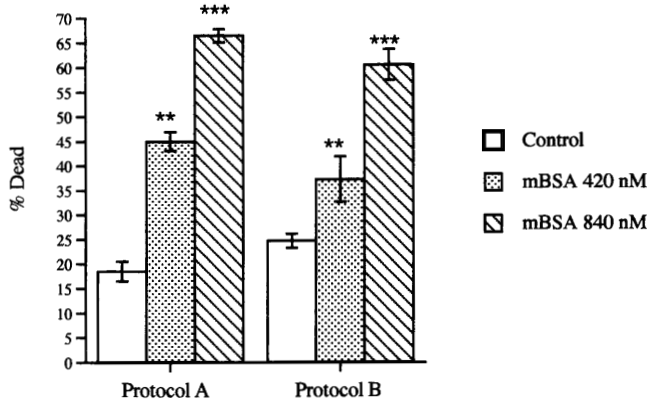

Figure 1. Candidacidal activity of Mϕ exposed to mannosylated bovine serum albumin (mBSA) prior to and after exposure to *Candida albicans.* Protocol A: Resident Mϕ were cultured on 16-well chamber slides, exposed to either 420 nM or 840 nM mBSA for 10 min, then washed. *C. albicans* was added at a ratio of five years/Mϕ. After 60 min incubation, the cells were stained with acridine orange. Protocol B: Resident Mϕ cultured on 16-well chamber slides were exposed to *C. albicans* first at a ratio of five yeast/Mϕ for 60 min. Subsequently, the monolayers were washed to remove uningested yeast. Next, Mϕ monolayers with ingested yeast were exposed to either media alone or mBSA for 10 min, followed by staining with acridine orange. Intracellular yeast which fluoresced orange were scored as dead whereas yeast fluorescing green were scored as live. Each experiment was repeated at least three times, and each value represented the mean ± SEM of four 100-cell counts. Expressed significance represents treated compared to respective controls. $P \leq 0.01$**, $P \leq 0.001$***

mBSA and greater induced significant ($P \leq 0.01$) candidacidal activity compared to controls (Fig. 1). However, no significant difference existed between the percent of candidacidal activity induced by 840 nM of mBSA and that induced by 1680 nM of mBSA (data not shown). If 5 mg/ml of mannans were present simultaneously with the mBSA, the percent killing was less than that of the controls (data not shown).

Other experiments were done to compare the effects of mBSA (a high-affinity ligand of the MMR) and gBSA (a low-affinity ligand of the MMR) on Mϕ-mediated *Candida* killing. As depicted in Figure 2, the low dose of mBSA (420 nM) induced approximately 45% killing, whereas the higher dose of mBSA (840 nM) induced 57% killing compared to controls (20%). Only the higher concentration (840 nM) of gBSA induced marked killing (35%) of *Candida* as compared to the respective controls (23%).

The effects of iMPO on Mϕ-mediated phagocytosis and killing of *Candida* are depicted in Figures 3a and 3b. There was a dose-dependent increase in phagocytosis. If 210 nM of iMPO were employed, the percentage of phagocytosis was equivalent to the controls. Approximately 25% phagocytosis occurred compared to 16% in the control when 420 nM of iMPO was used. If 840 nM iMPO were employed, treated cultures exhibited 42% phagocytosis compared to controls. If 210 nM of iMPO were employed, the percent killing was essentially the same as controls. The percent killing increased from 5% in the controls to approximately 40% in cultures treated with 420 nM of iMPO, to 50% in cultures treated with 840 nM of iMPO.

Phagocytosis is highly correlated with the respiratory burst (RB). Since chemiluminescence (CL) is one of the end products of the RB, light emission was employed to measure the RB. The production of ROI by resident Mϕ exposed to mBSA was approximately two-fold that of controls, and approximately three-fold that of the controls after exposure to iMPO (Fig. 4). Since both mBSA and iMPO induced an enhanced RB concomitant with increased levels of ROI, experiments were done to determine if scavengers of ROI had any effect on candidacidal activity. As depicted in Fig. 5, the presence of ROI scavengers decreased intracellular killing (lower dose not shown). All of the ROI scavengers (catalase at 132 μg/ml: mannitol at 200 mM; SOD 100 μg/ml) ablated the candidacidal activity so that the values of the mBSA-treated cultures in combination with one of the scavengers were lower than controls. Upon decreasing the concentration of the ROI scavengers (50%), values observed were equal to control values (data not shown). The presence of scavengers alone had no direct effect on either Mϕ or yeast viability.

Discussion

Previous studies by the present investigators have shown that enzymatically active MPO enhances the RB of Mϕ and increases Mϕ-mediated phagocytosis and intracellular killing of both bacteria and *C. albicans* (28). Since approximately 40% of MPO released into the microenvironment during phagocytosis by neutrophils is rapidly inactivated (26), the present *in vitro* study was undertaken to determine if receptor-ligand interaction alone was sufficient to enhance Mϕ-mediated RB, phagocytosis, and intracellular killing of *C. albicans.*

It would have been preferred to use a homologous system (i.e., murine Mϕ and murine iMPO). However, it is documented that murine neutrophils do not express high levels of MPO (29). Also, the number of animals required to obtain enough MPO for this study would have made this work impractical. The present studies employed human recombinant MPO because of its purity and availability (21).

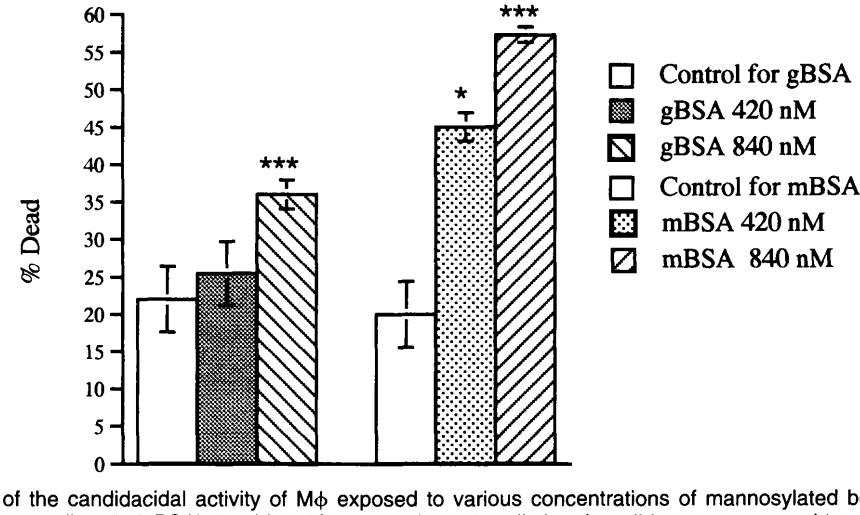

Figure 2. Comparison of the candidacidal activity of Mϕ exposed to various concentrations of mannosylated bovine albumin (mBSA) or galactosylated bovine serum albumin (gBSA). Resident Mϕ cultured on 16-well chamber slides were exposed to mBSA or gBSA for 10 min. Subsequently, the monolayers were washed, and *C. albicans* was added at a ratio of five yeast/Mϕ. After 60 min incubation, the cells were stained with acridine orange. Intracellular *C. albicans,* which fluoresced orange, were scored as dead whereas yeast fluorescing green were scored as live. Each experiment was repeated at least three times, and each value represented the mean ± SEM of four 100-cell counts. Expressed significance represents treated compared to control. $P \leq 0.05$*, $P \leq 0.001$***

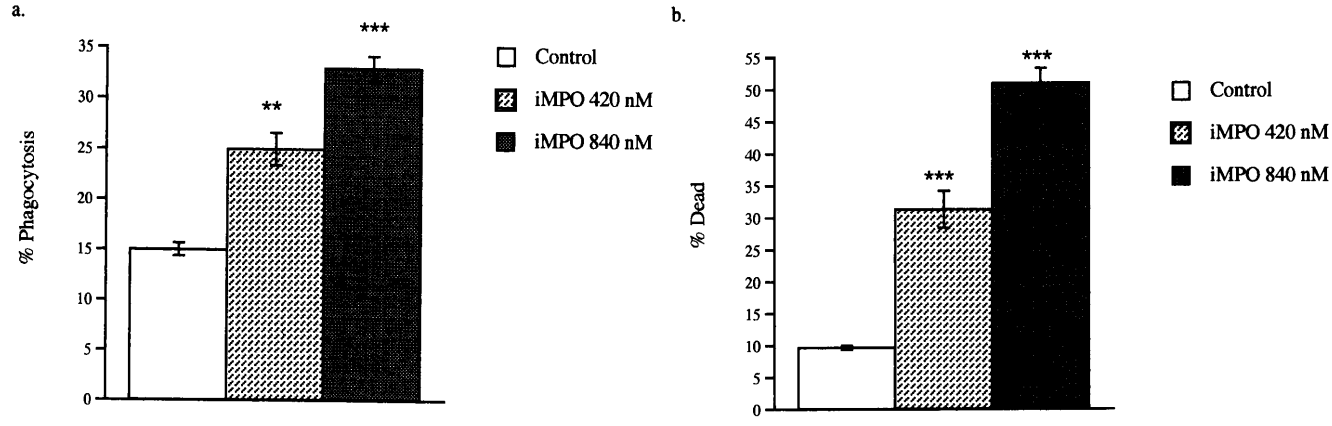

Figure 3. Effect of inactivated myeloperoxidase (iMPO) on (A) phagocytosis and (B) candidacidal activity. Resident Mϕ cultured on 16-well chamber slides were exposed to iMPO for 10 min. Subsequently, the monolayers were washed, and *C. albicans* was added at a ratio of five yeast/Mϕ. After 60 min incubation, the cells were stained with acridine orange. (a) Phagocytosis of *C. albicans* was assessed by percent Mϕ with ingested yeast. Each experiment was repeated at least three times, and each value represented the mean ± SEM of four 100-cell counts. Expressed significance represents treated compared to controls. $P \leq 0.001$*** (b) Intracellular *C. albicans,* which fluoresced orange, were scored as dead whereas yeast fluorescing green were scored as live. Each experiment was repeated at least three times, and each value represented the mean ± SEM of four 100 cell-counts. Expressed significance represented treated compared to controls. $P \leq 0.001$***

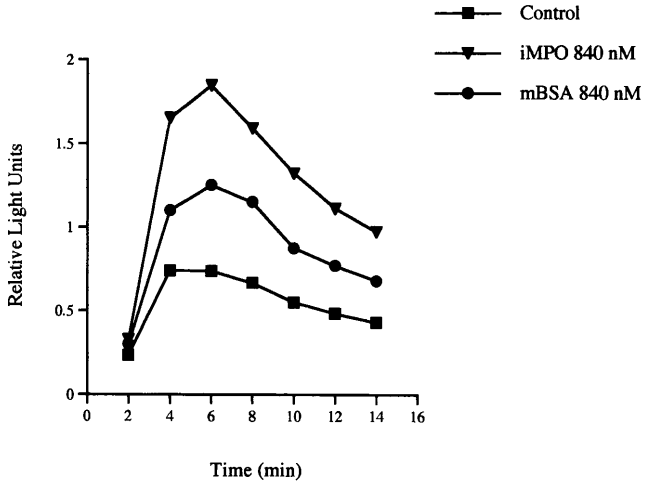

Figure 4. Effect of mannosylated bovine serum albumin (mBSA) and enzymatically inactive myeloperoxidase (iMPO) on the respiratory burst. One hundred ml media containing 10^5 resident Mϕ were cultured for 30 min. After incubation, the cultures were washed extensively, and the following reagents were added: 100 ml PMA, 100 ml mBSA or iMPO, 100 ml media, and 30 ml of luminol. The tube was agitated and placed in a luminometer. Chemiluminescence was measured at 2-min intervals for 10 min. The results were plotted as relative light units (RLU) versus time.

Since our supply of iMPO was limited, most of the experiments in this study were done using mBSA as a model. Confirmatory experiments were done using iMPO.

It is known that the optimal pH for MPO to function as a catalyst is approximately pH 5 (30). The acidity of the phagolysosome affords this type of environment. Studies by the present investigators have shown that if bacteria were incubated with MPO alone, 90% death occurred (28). Incubation of *Candida* with mBSA, an enzymatically inactive MMR ligand, did not alter viability.

It is established that MPO is released into the extracellular microenvironment prior to closure of the phagolysosome during phagocytosis (8). Because of the pH of the microenvironment (pH 7.2), as well as other factors present, much of the MPO is either inactive or functions suboptimally (8). If indeed receptor-ligand interaction was sufficient to induce the above Mϕ functions, the implications are that iMPO at a site of infection could have immunoregulatory functions *in vivo.*

It has been reported that MPO binds to Mϕ *via* the MMR (11). This same receptor is reported to bind unopsonized yeast. Since *in vivo* conditions favor opsonization, all experiments were done using opsonized *C. albicans.* It should be noted that most of the studies employed guinea pig complement as the opsonin. Results obtained from experiments using homologous mouse serum were essentially the same (data not shown). Since the yeast were opsonized, one would expect the F_c and complement receptors to be preferentially involved in the ingestion of yeast. In the present study, both high- and low-affinity ligands of the MMR were employed (12, 13). It has been shown by other investigators that mannans inhibit MMR binding (11, 31, 32). In order to show specificity of the ligands for the MMR, mannans were added to block receptor-ligand interactions. The presence of 5 mg/ml mannans with 1680 nM mBSA ablated Mϕ-mediated killing (results not shown) but not phagocytosis. Mannans, employed by themselves at a concentration of 5 mg/ml, did not enhance Mϕ functions described in this study.

The similarity of MPO to iMPO was determined by gel electrophoresis and ELISA using both monoclonal and polyclonal antibodies. Furthermore, no differences existed between the carbohydrate moieties of the two forms of the enzyme (21, 30, 33). Because of the above, the ability of both forms of MPO should be similar with respect to bind-

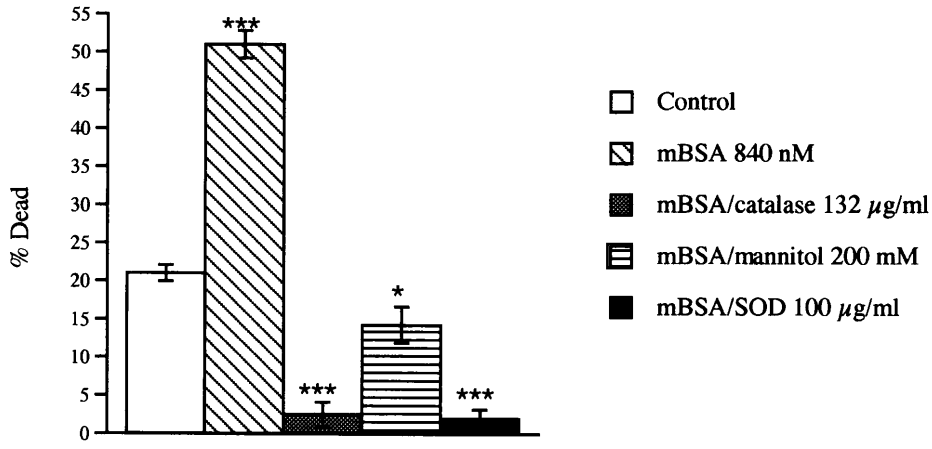

Figure 5. Role of reactive oxygen intermediates (ROI) in killing of *Candida albicans*. Resident Mϕ cultured on 16-well chamber slides were exposed to 840 nM mBSA for 10 min. Subsequently, the monolayers were washed, and one of the following treatments was added: *C. albicans* alone, catalase plus *C. albicans,* superoxide dismutase (SOD) plus *C. albicans,* mannitol plus *C. albicans. C. albicans* were added at a ratio of five yeast/Mϕ. After 60 min incubation, the cells were stained with acridine orange. Intracellular *C. albicans,* which fluoresced orange, was scored as dead whereas yeast fluorescing green was scored as live. Each experiment was repeated at least three times, and each value represented the mean ± SEM of four 100-cell counts. Expressed significance represents treated compared to control. $P \leq 0.001$***

ing to the MMR. Although the MMR is probably not the only means by which MPO can bind to a cell (i.e., *via* cationic charge), blocking of this receptor by mannans negated Mϕ function as measured in the present study.

The MMR receptor has eight binding domains and does not require cross-linking for Mϕ activation (34, 35). Rather, it has been reported by Taylor *et al.,* that certain domains must be bound for activation to occur (34, 35). Thus, the configuration of a molecule would determine if the domains necessary for activation of Mϕ had been bound. This could explain why iMPO and mBSA activate Mϕ and why gBSA was less effective.

Unlike active MPO, neither mBSA nor iMPO were directly toxic to the *Candida* alone. However, protocols were still employed in which the stimulant and the *Candida* would not be present simultaneously. Once the question of direct toxicity was addressed, studies were undertaken to determine if these same ligands of the MMR, as well as gBSA (a low-affinity ligand), could enhance Mϕ-mediated candidacidal activity. The two different protocols described previously for these studies were done to ensure that the yeast were killed intracellularly rather than externally. If yeast were being killed externally, then a higher percentage of killed *Candida* should have been obtained using protocol "A." Since similar results were obtained with both protocols (Fig. 1), it was reasoned that intracellular killing or killing at the intercellular junction was being measured. When Mϕ and yeast cultures were stained with Giemsa after five vigorous washes, 3–5 *Candida* were observed adhering to the surface of Mϕ. If trypan blue was employed, $\geq$ 10% of the adhering cells were dead according to staining properties. Hiral *et al.* have reported that ROI production is concentrated at the phagocyte-microbe junction (36). Therefore, one cannot rule out the possibility that a small percentage of *Candida* were killed at the above junction and then phagocytized.

Figure 2 depicts the results of experiments employing different doses of mBSA and gBSA. The low affinity of gBSA for the MMR would explain the reduced killing compared to mBSA. When iMPO was employed as a stimulant, there was a significant increase in Mϕ-mediated phagocytosis (Fig. 3a). However, the mean number of yeast ingested per Mϕ was not significantly different between control and treated cultures. This implies that within a given population of Mϕ, an increase in phagocytosis is a reflection of an increased number of cells ingesting yeast rather than increased activity of individual Mϕ. With respect to candidacidal activity, iMPO induced a significant increase in Mϕ-mediated *Candida* killing compared to controls (Fig. 3b). These results confirm that enzymatic activity was not necessary for activation of Mϕ to the candidacidal state and that iMPO present at the site of infection could be immunoregulatory.

Chemiluminescence studies were undertaken to determine if ROI were involved in the intracellular killing of *C. albicans.* Upon perturbation of the Mϕ membrane, ROI are released. The results of the CL studies confirmed the presence of ROI. Additionally, the presence of ROI scavengers, catalase, mannitol, and superoxide dismutase (SOD) reduced Mϕ-mediated candidacidal activity below the controls (Fig. 5). However, of the three scavengers, mannitol was the least effective. This could be interpreted to mean that H_2O_2 or superoxide anion plays a more pivotal role in *Candida* killing than hydroxyl ions. Another interpretation is that mannitol is not as efficient a scavenger as SOD or catalase. Boiling of the enzymes, SOD and catalase, had no effect on activity, thereby showing the necessity for enzymatic activity (26).

It has been reported that one of the first cells to arrive at a site of infection is the neutrophil (7). Once at the site, this cell actively engages in phagocytosis and intracellular killing of pathogens such as *C. albicans.* The role of the Mϕ

in the elimination of *C. albicans* is not as well defined. It is our contention that there is a dynamic interaction between neutrophils and Mϕ at the site of infection. This interaction allows MPO released from the neutrophil during phagocytosis as well as iMPO formed by rapid oxidation of the enzyme to bind to Mϕ *via* the MMR (9). The results of the present study indicate that MMR-ligand interaction is sufficient to induce an increase in: (1) the RB; (2) phagocytosis; and (3) intracellular killing of *C. albicans* by Mϕ. The mechanism of killing appears to be similar to that observed with the enzymatically active enzyme (i.e., generation of ROI).

Comparison of *in vitro* and *in vivo* levels of an agent required to cause a cellular response is difficult. Numerous factors *in vivo* could enhance or depress a response. However, as stated previously, 16–29 μg/ml of iMPO was found in the synovial fluid of arthritic joints. Also, since the concentration of MPO has usually been determined by measuring peroxidase activity, some investigators feel that reported amounts of MPO are "grossly underestimated" (37). The concentrations employed in the present study (33–66 μg/ml) were biologically active *in vitro* and approached the reported concentrations *in vivo.*

These studies, taken in their entirety, suggest that binding of iMPO to the MMR or other Mϕ scavenger receptors is sufficient to enhance an array of Mϕ functions including microbial killing. Therefore, iMPO, which is present at a site of an infection, has a previously unrecognized immunoregulatory property.

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