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MACROPHAGE-MEDIATED CANDIDACIDAL ACTIVITY IS AUGMENTED BY EXPOSURE TO EOSINOPHIL PEROXIDASE: A Paradigm for Eosinophil-Macrophage Interaction

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Abstract—Various disease states are associated with eosinophilia and the release of eosinophil peroxidase (EPO) into the microenvironment. The present study targets the effects of low levels of EPO on macrophage (MØ) phagocytosis and intracellular killing of *Candida albicans* as well as MØ oxidative activity measured as the luminescence product of luminol dioxygenation. Resident murine peritoneal MØ were exposed to various concentrations of EPO. Chemiluminescence data indicate that nanomolar concentrations of EPO markedly enhanced the dioxygenation activity (respiratory burst) of MØ. In other studies, the exposure of MØ to 0.17 μM EPO for 10 min. enhanced MØ-mediated candidacidal activity 10 fold. The above data indicate that EPO enhances certain MØ functions. Also the results illustrate a previously un-recognized interaction between eosinophils and MØ and implicate yet another possible role for EPO in host defenses against disease.

INTRODUCTION

The incidence of *Candida albicans* infections has been increasing primarily because of the AIDS epidemic and, to a lesser extent, because of the use of immunosuppressive therapies for transplantation (1, 2). The host's initial response to *Candida* involves neutrophil and monocyte/macrophage-mediated

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microbicidal action. The role of the neutrophil in scavenging and killing of this organism has been studied extensively (3–5). Additionally neutrophil myeloperoxidase (MPO), a halide : peroxide oxidoreductase, has been shown to be critically important to this action (3–5). Although it is known that macrophages (MØ) also participate in the host's clearance of *Candida*, the role of this cell with respect to candidacidal activity has not been fully elucidated.

Candida tends to colonize anatomic spaces such as the oropharyngeal space and the upper respiratory tract. Although the neutrophil and MØ have been associated with clearance of *Candida* from a host, the eosinophil is generally not thought to be involved in this process. However, eosinophils are often found in close physical proximity to MØ , and as such, eosinophil-MØ interactions are possible. It has been reported that, upon exposure to various stimuli, eosinophils degranulate and release their granular contents into the milieu (6) and as such MØ would be exposed to the products of eosinophil degranulation including eosinophil peroxidase (EPO).

Eosinophil peroxidase, a highly cationic enzyme, is the most abundant substance present in the crystalloid-containing granules of eosinophils (15 $\mu g/10^6$ cells) (6) and at the site of an inflammatory reaction there may be approximately 100–200 μg/ml of EPO (7). As with the neutrophil, eosinophils are capable of secreting both a peroxidase, EPO, and hydrogen peroxide (H_2O_2) which participate in a "cytotoxic triad" (8–11). Eosinophil peroxidase is capable of oxidizing halide to a hypohalous acid which can further react with peroxide to generate singlet oxygen. Additional reactive oxygen radicals (ROI), such as superoxide anion (O_2^-), H_2O_2, and hydroxyl radical (OH·), can be produced during the respiratory burst (RB) due to the rapid increase in phagocyte oxygen consumption (12). These agents are toxic to various microorganisms including *Candida* (8, 10, 11). Even without the presence of ROI, EPO can damage various microorganisms as well as the respiratory epithelium of the host (13, 14). Damaged tissue would offer access for colonization by *C. albicans*.

Released EPO is also reported to trigger the host's inflammatory response via induction of mast cell degranulation and activation of platelets (15, 16). In addition, it has been reported that eosinophils secrete the pro-inflammatory cytokines, tumor necrosis factor alpha (TNF), interleukins (IL) 1α, 6, as well as granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-8 which also augment inflammation (17, 18).

Previous studies by the present investigators have shown that enzymatically active and enzymatically inactive recombinant myeloperoxidase (MPO and iMPO respectively) can function to modulate various immune functions including: (1) oxidative activity such as the RB, (2) phagocytosis of *C. albicans* (3) intracellular killing of *C. albicans* or *Escherichia coli*, (4) secretion of TNF α, IL's 1, 6, 10, transforming growth factor β (TGF-β) and GM-CSF. As stated above, it is well established that eosinophils release EPO into the microenviron-

ment during degranulation (6, 19) and this enzyme would be readily available to interact with MØ at the site of inflammation. Therefore, the present study was undertaken to determine if MØ exposed to purified EPO would exhibit (1) an enhanced RB and (2) enhanced phagocytosis and intracellular killing of *Candida*. Finally, and possibly more important, the results of this study could indicate possible cellular interactions between eosinophils and MØ at a site of inflammation which have not been previously recognized.

MATERIALS AND METHODS

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

Materials. *Candida albicans*, strain 3153A, was generously donated by Dr. L. Chaffin, Texas Tech University Health Sciences Center, Lubbock, Texas. Purified porcine EPO (pEPO) was generously supplied by ExOxEmis Corp., Little Rock, Arkansas, and human EPO (hEPO) was a generous gift of Dr. Gerald Gleich, Mayo Clinic, Rochester, Minnesota. Dulbecco's modified Eagle's medium (DMEM) as well as guinea pig complement (serum) were purchased from GIBCO (Long Island, New York). Phosphate buffered saline pH 7.2 (PBS) was prepared as needed. The following reagents were obtained from Sigma (St. Louis, Missouri): enzymatically inactive horseradish peroxidase (dHRP), gentamicin sulfate, superoxide dismutase from bovine erythrocytes (SOD), catalase from bovine liver, D-mannitol, HEPES, bovine serum albumin (essentially globulin-free) (BSA), acridine orange (color index #46005, dye content 90%), and dimethyl sulfoxide (DMSO). Other commercially purchased reagents were: crystal violet (color index #42555, dye content 95%), Fisher (Pittsburgh, Pennsylvania) and fetal bovine serum (FBS) Intergen (Purchase, New York). A 20 mM stock solution of luminol (Eastman Kodak, Rochester, New York) dissolved in DMSO was made and stored at 4°C until needed. All reagents were tested for endotoxin contamination using the Limulus amoebocyte lysate test (LAL) (Associates of Cape Cod, Woods Hole, Massachusetts). Preparations of pEPO employed in all studies contained ≤0.02 ng/ml of endotoxin.

Macrophage Collection. Murine resident peritoneal MØ were collected as described previously (20). Mice were sacrificed by cervical dislocation and MØ collected by peritoneal lavage using PBS at 4°C. Cells were washed and resuspended in DMEM without gentamicin or FBS. The media were supplemented with 25 mM HEPES. Macrophage cell number was adjusted to 1×10^6 MØ/ml and 100 μl of the cell suspension were added to each well of a 16 well tissue culture chamber slide (Nunc Inc., Naperville, Illinois). Following incubation for 2 hrs at 37°C under 5% CO_2, cell monolayers were washed to remove non-adherent cells. The MØ population was ≥99% as determined by microscopy.

Candida albicans. *C. albicans* were cultured in 10 ml of yeast extract peptone-dextrose (YPD) broth at 30°C with slight agitation. The composition of the medium purchased from Difco Laboratories, Detroit, Michigan, was: glucose (20 g/l), yeast extract (10 g/l) and peptone (20 g/l). After 14 hrs, a stationary growth phase was reached and the density of the culture was determined to be approximately 5×10^8 cfu/ml by spread plate counts. Subsequently, the cell suspension was centrifuged at 20,000 × g 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. After 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 described by Lian et al. and others (21–24). Briefly, following a 2 hr attachment period and subsequent

washing, MØ monolayers were treated according to one of the following protocols: (A) MØ were first exposed for 10 min. to pEPO and monolayers washed vigorously. Subsequently, *C. albicans*, suspended in DMEM supplemented with 10% FBS, were added at a ratio of 5 yeast/MØ for 60 min. or (B) MØ were first exposed to *C. albicans* at a ratio of 5 yeast/MØ for 60 min., washed extensively to remove uningested yeast, and then exposed to pEPO for 10 min. It should be noted that the term "extracellular" denotes those *Candida* which are not in contact with the MØ membrane and would therefore be removed by vigorous washes. Both protocols were followed by staining with acridine orange (0.1 mg/ml) for 90 sec., and counterstained for 40 sec. with crystal violet (1 mg/ml). Crystal violet was employed in order to quench the fluorescence of extracellular yeast (21, 25). Macrophage-mediated phagocytosis and intracellular killing of *C. albicans* was determined using fluorescence microscopy. Yeast which fluoresced green were scored as live and those which fluoresced orange were scored as dead. Values represent the mean ± *SEM* of three 100 cell counts.

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 standard microbiological plate count assay (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 to ensure proper staining had occurred 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 described by Lefkowitz, et al. (26). Briefly, resident peritoneal MØ were obtained as described above and suspended at 1×10^6 cells/ml in media without phenol red (Auto-POW, Flow Labs., McLean, Virginia) but supplemented with 0.6 g/dl HEPES, 0.2 g/dl sodium bicarbonate, and 1.0 g/dl BSA. This mixture was termed CL media. Each well of a 96 well microtiter plate was seeded with 100 μl of the MØ cell suspension. Following a 30 min. incubation at 37°C, 5% CO_2, the monolayers were washed 3× with CL media to remove non-adherent cells. By differential staining, cultures were estimated to be ≥99% MØ. Culture fluids were allowed to equilibrate at 37°C, 5% CO_2 for an additional 30 min. Subsequently, media were removed and the following added to each well: 50 μl of luminol (40 μM working concentration), 50 μl of zymosan opsonized with guinea pig complement (10 zymosan: MØ), and 100 μl of media alone or media containing pEPO or hEPO. The plate was placed in a Dynatech ML 3000 plate luminometer and luminescence recorded for 2 sec. over a 2 min. interval. The results were plotted as time vs. 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-Neuwman-Keuls multiple comparison tests were performed to determine significance levels among the different treatment groups and controls. Non-transformed means are illustrated in the appropriate figures.

RESULTS

The present study was performed to determine if MØ exposed to pEPO exhibited enhanced phagocytosis and killing of *C. albicans*. Because contact with pEPO by itself sometimes causes death of microorganisms, all experimental protocols were designed to exclude the simultaneous exposure of pEPO to *Candida*. Initially MØ cultures were exposed to various concentrations of pEPO washed and both phagocytosis as well as candidacidal activity were measured. As depicted in Figure 1a, 0.09 μM, 0.17 μM, and 0.34 μM of pEPO significantly

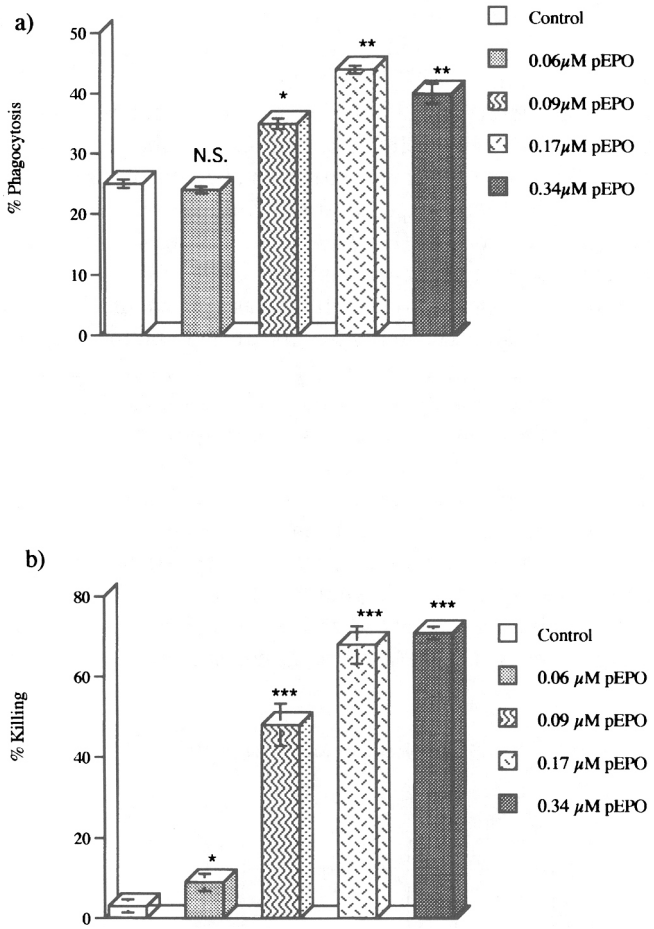


Fig. 1. Resident MØ were cultured on 16-well chamber slides for 2 hr. MØ monolayers were subsequently treated for 10 min. with either media containing pEPO at various concentrations or media alone. MØ monolayers were washed vigorously and *C. albicans* added at a ratio of 5/MØ. Cells were incubated for 60 min., subsequently stained with acridine orange, and the percentage of MØ having ingested *Candida* determined (Fig. 1a). Ingested yeast were scored as live or dead using fluorescence microscopy (Fig. 1b). Values represent the mean ± *SEM* for three 100 cell counts. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

enhanced phagocytosis ($P \leq 0.05$). No differences were noted, however, between 0.17 and 0.34 μM concentrations. Also, the mean number of *Candida* per MØ was not different between treatment groups and controls (data not shown). However, with respect to candidacidal activity, MØ exposed to pEPO for 10 min. demonstrated a dose-dependent increase in candidacidal activity with approx-

imately 70% of the yeast being killed by MØ exposed to 0.17 μM of pEPO (Figure 1b). The % killing in controls exposed to media alone was <5%. Subsequent experiments utilized 0.17 μM pEPO.

To ensure that observed MØ-mediated candidacidal activity was due to ingestion and subsequent intracellular killing and not the ingestion of *C. albicans* killed externally, two different experimental protocols were employed. In the first design (A), MØ were exposed to pEPO prior to challenge with *C. albicans* while in the second design (B), MØ were challenged with *C. albicans* and subsequently exposed to pEPO. Macrophages exposed to 0.17 μM pEPO, as described in protocol A, demonstrated an increase in candidacidal activity from 5% in the controls to approximately 45% for treated cultures (Figure 2). Cells treated with an equimolar concentration of pEPO and treated as described in protocol B, demonstrated essentially the same results. As no significant differences were noted between the results obtained using either protocol, future experiments employed protocol A.

Enhancement of phagocytosis as well as candidacidal activity of MØ exposed to pEPO increased in a time-dependent manner. Significant differences in phagocytosis were observed only after 60 min. (approximately 32% in controls vs 47% in the treated ($P \leq 0.01$) (Figure 3a). However, MØ exposed to pEPO demonstrated a significantly enhanced both early and late candidacidal activity. As depicted in Figure 3b, the pEPO treated group exhibited a significant increase

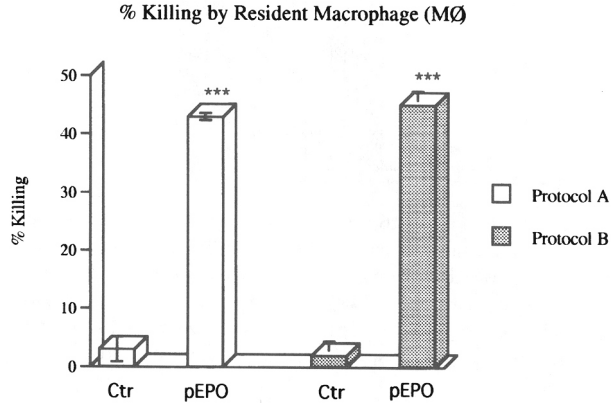


Fig. 2. Protocol A: Resident MØ were exposed to 0.17 μM pEPO for 10 min. Following incubation, monolayers were washed and *C. albicans* added at a rato of 5/MØ. After 60 min. incubation, the cells were stained with acridine orange and scored as live or dead according to color. Protocol B: Resident MØ were exposed to *C. albicans*, at a ratio of 5/MØ, for 60 min. Following incubation, monolayers were washed to remove uningested yeast. Subsequently, MØ monolayers were exposed to 0.17 μM pEPO for 20 min., stained with acridine orange, and scored as described above. Values represent the mean $\pm$ *SEM* for three 100 cell counts. $^{***}P \leq 0.001$.

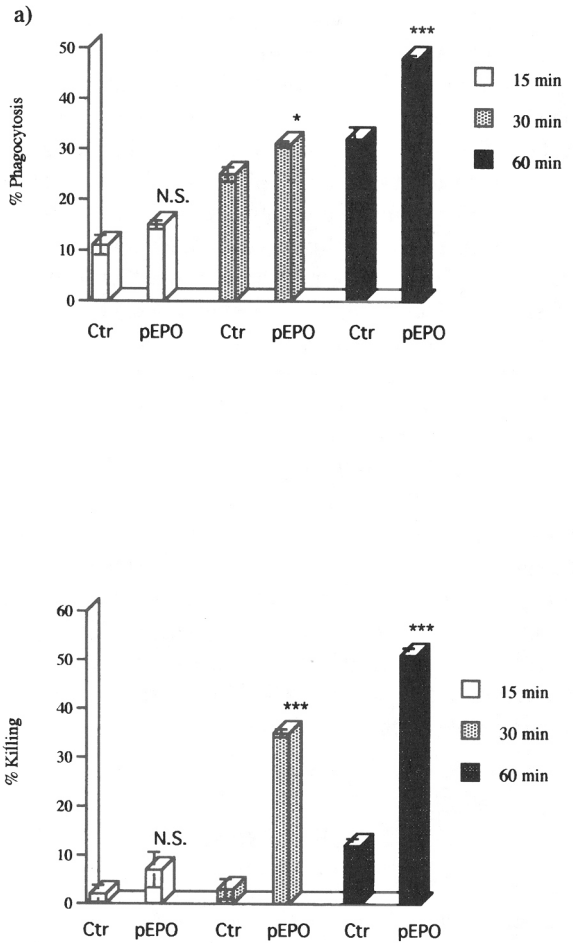


Fig. 3. Resident MØ were cultured on 16-well chamber slides for 2 hr. MØ monolayers were subsequently treated for 10 min. with either media containing 0.17 μM pEPO or media alone. MØ monolayers were washed vigorously and *C. albicans* added at a ratio of 5/MØ. Cells were incubated for the indicated time periods, subsequently stained with acridine orange, and the percentage of MØ having ingested *Candida* determined. Ingested yeast were scored as live or dead using fluorescence microscopy. Values represent the mean $\pm$ *SEM* for three 100 cell counts. $^{*}P \leq 0.05$, $^{***}P \leq 0.001$.

in candidacidal activity over controls after 30 min. incubation (approximately 3% in the controls vs approximately 37% in the treated) ($P \leq 0.001$).

With regard to the phagocyte RB, glucose metabolism is required for effective microbicidal action (27–29). Moreover, since it is known that the RB is highly correlated with phagocytosis (30), studies were undertaken to determine

if MØ exposed to pEPO or hEPO would exhibit an enhanced RB. Chemiluminescence was employed to determine the effect of various doses of pEPO or hEPO or the MØ RB. Macrophages exposed to pEPO demonstrated a dose-dependent and time-dependent increase in RB. As shown in Figure 4a, MØ exposed to 0.006 μM pEPO demonstrated a 2 fold increase in relative light units (RLU) by 24 min., whereas, exposure to 0.17 μM pEPO resulted in a 50 fold increase in RLU by 24 min. Similar results were obtained with hEPO (data not shown).

Eosinophil peroxidase is a highly mannosylated, cationic molecule. A competitive binding study was performed to test whether pEPO binds MØ via its mannose residues. Macrophages were exposed to 10 μM dHRP, an enzymatically inactive but antigenically equivalent form of horseradish peroxidase which is a high affinity ligand for the MØ mannose receptor (MMR) (31). Cells were subsequently challenged with opsonized zymosan and assayed for CL. Macrophages exposed simultaneously to pEPO and dHRP demonstrated approximately a 30–40% reduction in CL (Figure 4b).

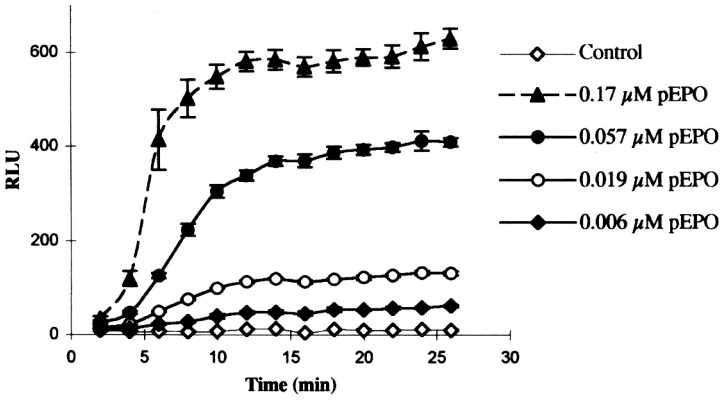
Once it had been established that pEPO or hEPO enhanced the MØ-mediated RB, various scavengers of ROI were tested for their effect on the observed MØ -mediated intracellular killing of yeast. The presence of superoxide dismutase (SOD) and mannitol completely ablated intracellular killing of *Candida*. Catalase was only slightly less effective and reduced killing to control levels (Figure 5). There was no significant decrease in intracellular killing of MØ exposed to pEPO and either boiled SOD or catalase thus showing the necessity for enzymatic activity (data not shown).

DISCUSSION

In the present studies, pEPO, guinea pig complement (serum), and murine MØ were employed. It would have been ideal if the source of EPO, complement, and cells were homologous. However, it was not feasible to obtain the quantities of purified murine EPO necessary to carry out the present studies. In addition, if murine serum was employed as the opsonin, results were essentially the same as those obtained with guinea pig complement. For these reasons, guinea pig complement was employed for the balance of the study.

The results of the present study indicate that EPO enhances both phagocytosis and killing of *Candida* by MØ. The fact that EPO enhanced cytocidal activity more than phagocytosis could possibly be explained by: (1) the assay is not as sensitive for the measurement of phagocytosis as it is for killing, (2) phagocytosis is a more complex function than killing and therefore requires more time and/or higher doses of EPO, (3) that ROI generated by engulfment of the yeast and/or exposure to EPO oxidized receptors utilized in phagocytizing the

a)



b)

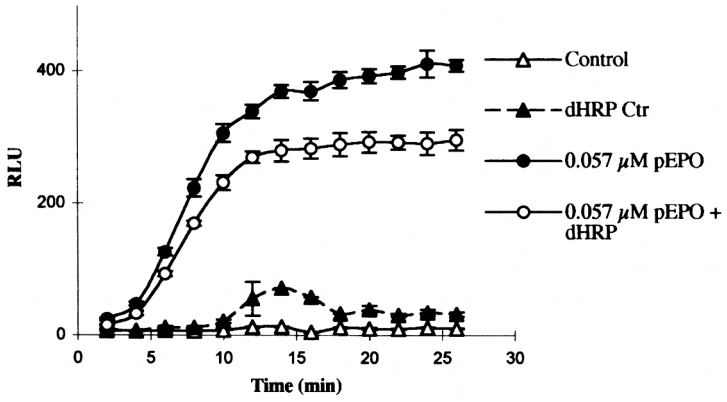


Fig. 4. (a) Resident MØ were simultaneously exposed to either media alone or media containing various concentrations of pEPO, luminol, and zymosan and then incubated at 37°C. The amount of light released for a 24 min. period was measured using a luminometer. Values represent the mean of triplicate counts. (b) Resident MØ were simultaneously exposed to media alone or media containing 0.057 μM pEPO or 0.057 μM pEPO and dHRP, luminol, and zymosan and then incubated at 37°C. The amount of light released for a 24 min. period was measured using a luminometer. Values represent the mean of triplicate counts.

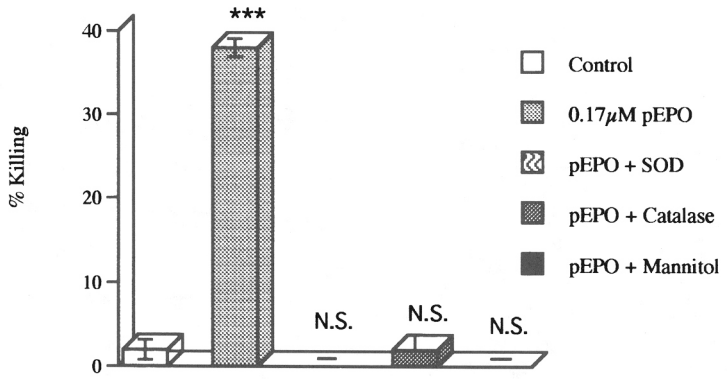


Fig. 5. Resident MØ were cultured on 16-well chamber slides for 2 hr. MØ monolayers were subsequently treated for 10 min. with either media containing 0.17 μM pEPO or media alone. MØ monolayers were washed vigorously and *C. albicans*, at a ratio of 5/MØ, added with either 50 μg/ml SOD, 0.2 μM catalase, or 100 mM mannitol. Cells were incubated for 60 min., subsequently stained with acridine orange, and scored as live or dead using fluorescence microscopy. Values represent the mean ± *SEM* for three 100 cell counts. $^{***}P \leq 0.001$.

fungi and thereby limited engulfment (32, 33). Although it would be expected that the complement receptor would be the primary receptor involved in phagocytosing the opsonized *Candida*, the role of other receptors such as the MMR and Fc receptors cannot be ruled out.

Eosinophil peroxidase is highly cationic and is reported to cause peroxide-dependent and independent damage (6, 34). For this reason, all experiments were conducted in a manner so that *Candida* and EPO were not simultaneously present. In addition, there were no differences in observed MØ-mediated candidacidal activity using either of the two different protocols (A and B) explained previously. If "A" and "B" were measuring intracellular vs extracellular killing, differences in data from the two protocols would be expected. If the *Candida* were killed extracellularly, then using protocol "B" should have resulted in less killing. Since no differences were detected, either killing occurred at the intercellular junction or intracellularly. In fact, when MØ cultures were stained with Giemsa after 5 vigorous washes, there were 3–5 *Candida* adhered to the surface of most MØ in both control and treated cultures. When trypan blue, the non-vital stain, was employed, a small percentage (≤10%) of the *Candida* adhering to the MØ membrane were dead. Other investigators have reported that ROI production is concentrated at the phagocyte-microbe junction (35). Therefore, there is the possibility that some *Candida* were killed at the intercellular junction and then phagocytized.

The mean number of *Candida* per MØ were essentially the same for treated and control cultures. These data would, therefore, suggest that the observed

enhancement in phagocytosis and killing were the result of a greater number of MØ within the treated population engulfing *Candida* rather than more yeast per individual MØ being engulfed.

Luminol-dependent CL does not allow dissection of the radicals being measured. Therefore, it should be noted that the CL data presented does not provide information as to whether a particular radical is involved in the actual killing process of EPO stimulated MØ. Rather the data indicate that upon challenge with zymosan, EPO initiates a rapid MØ-derived RB as demonstrated by the results of the luminol-dependent CL studies. Various scavengers were employed to determine which radical species was pivotal in the killing *C. albicans*. The presence of SOD, catalase, or mannitol reduced killing to levels either the same as, or below those observed with media alone. Therefore, it appears that all three of the radicals investigated participate to a greater or lesser extent in the candidacidal activity by EPO stimulated MØ. However, it should be noted that the concentration of mannitol was such that it could have affected the osmolarity of the cell, and the decrease in candidacidal activity was independent of any antioxidant function.

A competitive binding study was performed to test whether pEPO binds MØ via its mannose residues. The fact that 10 μM dHRP did not completely inhibit EPO induced CL may be explained by the fact that the MMR is only one of several scavenger receptors which can bind mannosylated substances (36). The affinity of dHRP for these other mannose binding receptors is not known. Macrophages exposed simultaneously to pEPO and dHRP demonstrated approximately 30–40% reduction in CL. In lieu of the above, complete inhibition of CL by dHRP would seem improbable.

As stated previously the effect of EPO treatment on *Candida* phagocytosis is relatively minor in comparison to the major increases in candidacidal activity. As such, the increased candidacidal activity can not be explained on the basis of increased phagocytosis alone. Eosinophil peroxidase is a highly cationic enzyme which would adhere to the surface of the cell (6, 28). Therefore, the additional killing observed above phagocytosis could be the result of the cytotoxic triad functioning at the intercellular junction. Eosinophil peroxidase readily catalyzes the H_2O_2-dependent oxidation of bromide to hypobromous acid (HOBr). Hypobromous acid can react with a second molecule of H_2O_2 to yield singlet molecular oxygen and can serve as a direct microbicidal agents. As such, EPO provides a mechanism for realizing the microbicidal potential of H_2O_2, the product of the MØ RB metabolism.

The increased candidacidal activity is probably consequent to EPO-dependent improvement in MØ-oxidant generation. This conclusion is supported by the finding that MØ treated with EPO showed proportional increases in oxidative activity as measured using CL.

As stated previously, it has been reported that there is approximately 15

μg or 0.220 μM of EPO/10^6 cells (6). At the site of inflammation there is frequently 90–200 μg/ml or 1.3 μM to 2.86 μM of EPO (7; Dr. Gerald Gleich, personal communication). Since the concentrations utilized in the present study are markedly less than that found in various human pathological conditions, the results of this study could be extremely relevant to in vivo situations.

The above data taken in their entirety, as well as that of others, indicate that there exists an unrecognized interaction between eosinophils and MØ (37). The binding of EPO by MØ can lead to the enhancement of the following MØ functions: (1) RB (2) phagocytosis and (3) intracellular killing of *C. albicans*. From this study as well as others, it is apparent that there may be other, yet previously unrecognized, functions of EPO.

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REFERENCES

1. AMPEL, N. M. 1996. Emerging Disease Issues and Fungal Pathogens Associated with HIV Infection. *Emerging Infect. Dis.* **2:**109–116.
2. HIBBERD, P. L. and R. H. RUBIN. 1994. Clinical Aspects of Fungal Infection in Organ Transplant Recipients. *Clin. Infect. Dis.* **19:**S33–S40.
3. FABIAN, I., K. YEHUDITH, S. MOR, C. GELLER-BERNSTEIN, M. BEN-YAAKOV, B. VOLOVITZ, and D. W. GOLDE. 1992. Activation of human eosinophil and neutrophil functions by haematopoietic growth factors: comparisons of IL-1, IL-3, IL-5 and GM-CSF. *Brit. J. Haematology.* **80:**137–143.
4. DOMER, J. E. and R. I. LEHRER. 1993. Introduction to *Candida:* Systemic Candidiasis. *In:* Fungal Infections and Immune Responses, J. W. Murphy, H. Friedman, and M. Bendinelli (ed.). Plenum Press, New York. pp. 54–66.
5. CALDERONE, R. and J. STURTEVANT. 1994. Macrophage Interactions with *Candida. In:* Macrophage-Pathogen Interactions, B. S. Zwilling and T. K. Eisenstein (ed.). Marcel Dekker, Inc., New York. pp. 505–515.
6. VENGE, P. 1993. Human Eosinophil Granule Proteins: Structure, Function, and Release. *In:* The Handbook of Immunopharmacology, H. Smith and R. M. Cook (ed.). Academic Press, San Diego, California. pp. 43–55.
7. SEDGWICK, J. B., W. J. CALHOUN, G. J. GLEICH, H. KITA, J. S. ABRAMS, L. B. SCHWARTZ, B. VOLOVITZ, M. BEN-YAAKOV, and W. W. BUSSE. 1991. Immediate and Late Airway Response of Allergic Rhinitis Patients to Segmental Antigen Challenge. Characterization of Eosinophil and Mast Cell Mediators. *Am. Rev. Resp. Dis.* **144:**1274–1281.
8. CARR, A. C., C. C. WINTERBOURN, and J. M. VAN DEN BERG. 1996. Peroxidase-Mediated Bromination of Unsaturated Fatty Acids to Form Bromohydrins. *Arch. Biochem. Biophysics.* **327:**227–233.
9. MAYENO, A. N., A. J. CURRAN, R. L. ROBERTS, and C. S. FOOTE. 1989. Eosinophils preferentially use bromide to generate halogenating agents. *J. of Biol. Chem.* **264:**5660–5668.
10. JONG, E. C., W. R. HENDERSON, and S. J. KLEBANOFF. 1980. Bactericidal activity of eosinophil peroxidase. *J. Immunol.* **124:**1378–1382.

11. KLEBANOFF, S. J., J. M. AGOSTI, A. JORG, and A. M. WALTERSDORPH. 1989. Comparative toxicity of the horse eosinophil peroxidase-H_2O_2-halide system and granule basic proteins. *J. Immunol.* **143:**239–244.
12. BABIOR, B. M., J. T. CURNUTTE, and N. OKAMURA. 1988. The Respiratory Burst Oxidase of the Human Neutrophil. *In*: B. Halliwell (ed.), Oxygen Radicals and Tissue Injury, Federation of American Societies for Experimental Biology, M.R. pp. 43–48.
13. GLEICH, G. J. 1990. The eosinophil and bronchial asthma: Current understanding. *J. Allergy and Clin. Imm.* **85:**422–436.
14. HISAMATSU, K., T. GANBO, T. NAKAZAWA, Y. MURAKAMI, G. J. GLEICH, K. MAKIYAMA, and H. KOYAMA. 1990. Cytotoxicity of human eosinophil granule major basic protein to human nasal sinus mucosa in vitro. *J. of Allergy and Clin. Immun.* **86:**52–63.
15. HENDERSON, W. R., E. Y. CHI, and S. J. KLEBANOFF. 1980. Eosinophil peroxidase-induced mast cell secretion. *J. Exp. Med.* **152:**265–279.
16. ROHRBACH, M. S., C. L. WHEATLEY, N. R. SLIFMAN, and G. J. GLEICH. 1990. Activation of platelets by eosinophil granule proteins. *J. Exp. Med.* **172:**1271–1274.
17. HANSEL, T. T. and C. WALKER. 1993. Experimental Techniques with Human Eosinophils. In: The Handbook of Immunopharmacology, H. Smith and R. M. Cook (ed.). Academic Press, San Diego, California. pp. 217–235.
18. SIMON, H. U., S. YOUSEFI, M. WEBER, D. SIMON, C. HÖLZER, K. HARTUNG, and K. BLASER. 1995. Human Peripheral Blood Eosinophils Express and Release Interleukin-8. *Int. Arch. Allergy Immunol.* **107:**124–126.
19. MATSUNAGA, Y., H. KIDO, K. KAWAJI, K. KAMOSHITA, N. KATUNUMA, and T. OGURA. 1994. Inhibitors of Chymotrypsin-like Proteases Inhibit Eosinophil Peroxidase Release from Activated Human Eosinophils. *Arch. Biochem. and Biophysics.* **312:**67–74.
20. LEFKOWITZ, D. L., S. S. LEFKOWITZ, R. Q. WEI, and J. EVERSE. 1986. Activation of macrophages with oxidative enzymes. In: *Methods in Enzymology*, G. DiSabato and J. Everse (ed.). Academic Press Inc., Orlando, FL. pp. 537–548.
21. LIAN, C. J., W. S. HWANG, and C. H. PAI. 1987. Plasmid-mediated resistance to phagocytosis in Yersinia enterolitica. *Infect. Immun.* **55:**1176–1183.
22. PETERSON, K. P., E. GAZIANO, H. J. SUH, M. DEVALON, L. PERTERSON, and W. F. KEANE. 1985. Antimicrobial Activities of Dialysate-elicited and Resident Human Peritoneal Macrophages. *Infect. Immun.* **49:**212–218.
23. SHUIT, K. E. 1979. Phagocytosis and Intracellular Killing of Pathogenic Yeasts by Human Monocytes and Neutrophils. *Infect. Immun.* **24:**932–938.
24. LEFKOWITZ, S. S., M. P. GELDERMAN, D. L. LEFKOWITZ, N. MOGUILEVSKY, and A. BOLLEN. 1996. Phagocytosis and Intracellular Killing of *Candida albicans* by Macrophages Exposed to Myeloperoxidase. *J. Infect. Dis.* **173:**1202–1207.
25. SASADA, M. and R. B. JOHNSTON. 1980. Macrophage microbicidal activity. *J. Exp. Med.* **152:**85–98.
26. LEFKOWITZ, D. L., S. S. LEFKOWITZ, J. MONE, and J. EVERSE. 1988. Peroxidase-induced enhancement of chemiluminescence by murine peritoneal macrophages. *Life Scs.* **43:**739–745.
27. KLEBANOFF, S. J. and R. C. CLARK. 1978. The Neutrophil: Function and Clinical Disorders. Amsterdam: North-Holland. pp. 283–316.
28. ALLEN, R. C., S. J. YEVICH, R. W. ORTH, R. H. STEELE. 1974. The Superoxide Anion and Singlet Molecular Oxygen: Their Role in the Microbicidal Activity of the Polymorphonuclear Leukocyte. *Biochem. Biophys. Res. Commun.* **60:**909–917.
29. ALLEN, R. C. 1994. Role of oxygen in phagocyte microbicidal action. *Environ. Health Perspect.* **102(Suppl 10):**210–208.
30. GREENBERG, S. and S. C. SILVERSTEIN. 1993. Phagocytosis. *In:* W. E. Paul (ed.), Fundamental Immunology, Raven Press, New York. pp. 941–964.

31. GORDON, S., P. M. STARKEY, D. HUME, R. A. B. EZEKOWITZ, S. HIRSCH, and J. AUSTYN. 1986. Plasma membrane markers to study differentiation, activation and localization of murine macrophages. Ag F4/80, and the mannosyl, fucosyl receptor. *In:* D. M. Weir ed. Handbook of experimental immunology v 2. Blackwell Pub. Oxford. **43:**1–15.
32. SHEPHERD, V. L., R. ABDOLRASULNIA, J. STEPHENSON, and C. CRENSHAW. 1990. Modulation of mannose receptor activity by proteolysis. *Biochem. J.* **270:**771–776.
33. BOSEMAN, P. M., J. R. HOIDAL, and V. L. SHEPHERD. 1988. Oxidant-mediated Inhibition of Ligand Uptake by the Macrophage Mannose Receptor. *J. Biol. Chem.* **263:**1240–1247.
34. BUTTERWORTH, A. E. and K. J. I. THORNE. 1993. Experimental Techniques with Human Eosinophils. *In:* The Handbook of Immunopharmacology, H. Smith and R. M. Cook (ed.) Academic Press, San Diego, CA. pp. 217–235.
35. HIRAI, K., K. MORIGUCHI, and G. Y. WANG. 1991. Human Neutrophils Produce Free Radicals from the Cell-Zymosan Interface during Phagocytosis and from the Whole Plasma Membrane When Stimulated with Calcium Ionophore A23187. *Exp. Cell Res.* **194:**19–27.
36. PEARSON, A. M. 1996. Scavenger receptors in innate immunity. *Cur. Opin. Immunol.* **8:**20–28.
37. RAMSEY, P. G., T. MARTIN, E. CHI, and S. J. KLEBANOFF. 1982. Arming of mononuclear phagocytis by eosinophil peroxidase bound to *Slaphylococcus aureus. J. Immunol.* **128:**415.