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The Endothelium and Cytokine Secretion: The Role of Peroxidases as Immunoregulators

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The endothelium is frequently exposed to many proinflammatory mediators. The present study was done to determine the effects of human recombinant myeloperoxidase (MPO) and porcine eosinophil peroxidase (EPO) on certain endothelial cell (HUVEC) functions. The following areas were evaluated: (1) production of reactive oxygen intermediates (ROI), (2) cytokine secretion, and (3) regulation of mRNA cytokine transcripts. Both MPO and EPO induced the production of ROI, but an enzymatically inactive form of MPO (iMPO) was the most effective. Enzymatically inactive MPO, but not MPO, induced the secretion of interleukins 6 and 8 and granulocyte–monocyte colony-stimulating factor. A ribonuclease protection assay indicated that both iMPO and MPO upregulated mRNA cytokine transcripts; however, the former was markedly more effective. The simultaneous addition of EPO and iMPO resulted in a decrease in cytokine-specific mRNA. These data indicate a major role for peroxidases in the regulation of inflammation.

INTRODUCTION

Inflammation is the body's reaction to invasion by an infectious agent, antigen challenge, or physical damage. It represents the consequence of capillary dilation with edema and the emigration of leukocytes (1). Once the initiating event has occurred, the persistence of the inflammatory process has been attributed to a variety of events, including: (a) superantigen expression (2), (b) antigen–antibody complexes (3), (c) activated T cells (4–6), and (d) abnormal cytokine expression (5–8). This persistent immune activation can result in various pathological consequences, such as rheumatoid arthritis, Crohn's disease, chronic asthma, and various allergic diseases (e.g., allergic rhinitis) (9–11).

Over time it has become increasingly clear that the endothelium is not a passive participant in the inflammatory process. Endothelial cells (EC) provide an interface between the blood and extravascular space, monitoring the flow of various macromolecules into the tissues. In addition, other investigators have reported that EC are a source of reactive oxygen intermediates (ROI) as well as numerous cytokines implicated in cell trafficking and activation. These cells are also involved in inducing inflammation by secreting "proinflammatory" mediators (12). Specifically, in response to TNF-α, EC secrete interleukin-1 (IL-1), interleukin-8 (IL-8), and granulocyte–macrophage colony-stimulating factor (GM-CSF). All of these would further enhance the inflammatory response (13).

At the site of inflammation, both neutrophils (PMN) and macrophages (Mϕ) predominate. If the inflammatory process becomes chronic, lymphocytes also become participants (14). After an initiating event, however, PMNs are among the first cells to arrive at the site of inflammation (15). Activation of PMNs results in the release of myeloperoxidase (MPO) into the extracellular matrix, where it can bind to various cells, including EC (14).

Myeloperoxidase, which is present in the azurophilic granules of neutrophils, makes up 5% of their dry weight (16). Bradley *et al.* reported that neutrophils found in the synovial fluid of rheumatoid arthritis (RA) patients degranulated and released approximately 50% of their MPO into the microenvironment. One of the functions of MPO involves the infiltration of cells into the microenvironment. It has been reported that binding of monocytes and neutrophils to the endothelium is enhanced by MPO (17). However, in the microenvironment of an inflammatory response, approximately 40% of the MPO is rapidly inactivated via oxidation (18). Studies by Edwards *et al.* have revealed the presence of 16–29 μg/ml of enzymatically inactive MPO (iMPO) in an arthritic joint (19). Therefore, at the site of inflammation both MPO and iMPO would be present.

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In general, in addition to neutrophils and macrophages, eosinophils are frequently present at a site of inflammation (20). Moreover, eosinophils have been found to be a striking feature of 40% of certain severely afflicted RA patients (21). Among the granular contents of eosinophils, eosinophil peroxidase (EPO) is one of the most abundant substances present (22). At the site of inflammation there may be approximately 100–200 ng/ml of EPO (23). It is not known how much enzymatically inactivated EPO would be present at an inflammatory site. However, it has been reported that upon exposure to certain stimuli, eosinophils degranulate and release their contents into the microenvironment (22, 23), and as such EC would be exposed to the products of eosinophil degranulation, including EPO.

Other investigators have reported that radicals induce cytokine secretion (24, 25) as well as induce tissue damage associated with inflammation. Studies completed in this laboratory have shown that exposure of murine Mϕ to various peroxidases resulted in an increase in the respiratory burst (RB), with a concomitant increase in H_2O_2 and other ROI (26, 27). It is known that ROI cause tissue destruction associated with inflammation, and the secretion of ROI by EC would be another means by which these cells could contribute to the inflammatory process.

Previous work by the present investigators has focused on the effect of MPO, iMPO, or EPO on Mϕ. Since it is established that both forms of MPO as well as EPO could be present at a site of inflammation, the present study was undertaken to elucidate the effects of these peroxidases on EC. The specific areas of investigation included: (a) production of ROI, (b) cytokine secretion, and (c) regulation of cytokine gene transcription.

MATERIALS AND METHODS

Reagents

Enzymatically active human recombinant and enzymatically inactive human recombinant myeloperoxidases were generously supplied by Dr. Nicole Moguilevsky and Dr. Alex Bollen from the Universite Libre de Bruxelles, Belgium. All experiments in these studies were done with human recombinant MPO, and throughout the remainder of this paper, recombinant human MPO will be designated as either MPO or iMPO. Protein determination of either MPO or iMPO was performed by the Lowry method and stock solutions were as follows: MPO (preparation 1) was 1180 μg/ml (177 U/ml), and iMPO was 1400 μg/ml ($\leq$1 U/ml) of enzymatic activity as determined by *o*-dianisidine assay. Other preparations of MPO (2 and 3) were approximately the same concentration. Characteristics of the different myeloperoxidase preparations have been published previously (28–31). Dr. R. C. Allen, ExOxiMis Corp. (Little Rock, AR), supplied porcine EPO. Protein determination of EPO was carried out utilizing the Lowery method, and the EPO contained 1184 μg/ml. This enzyme exhibited 1830 U/ml of enzymatic activity. Fetal bovine serum (FBS) was purchased from Intergen (Purchase, NY). Guinea pig complement (serum) and Auto-POW MEM were purchased from Gibco (Long Island, NY). The following reagents were obtained from Sigma (St. Louis, MO): gentamycin sulfate, heparin, Hepes, bovine serum albumin (BSA), dimethyl sulfoxide (DMSO), and endothelial cell growth supplement from bovine neural tissue. A 20 mM stock solution of luminol (Eastman Kodak, Rochester, NY) 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, MA). Preparations of the stock solutions of MPO, iMPO, and EPO contained $\leq$8.0, $\leq$4.0, and $\leq$0.02 ng/ml of endotoxin, respectively. Although macrophages respond to subnanogram levels of LPS, EC did not response to less than 1 μg/ml of LPS (data not shown).

Cell Culture

Human umbilical vein endothelial cells (HUVEC) were obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained in Kaighn's nutrient mixture F-12K (Gibco BRL Products, Grand Island, NY) supplemented with 20% FBS, 50 mg/ml gentamycin sulfate, 100 μg/ml heparin, and 30 μg/ml endothelial cell growth supplement.

Chemiluminescence Assay

Methods used were modified from the chemiluminescence assay described by Lefkowitz *et al.* (32). Briefly, HUVEC were suspended at a concentration of 4×10^5 cells/ml in Kaighn's nutrient mixture F-12K supplemented with 2% FBS. Each well of a 96-well microtiter plate was seeded with 100 μl of the HUVEC suspension. Following a 2-h incubation at 37°C, under 5% CO_2, the monolayers were washed three times with medium without phenol red (Auto-POW), which was supplemented with 0.6 g/dl Hepes, 0.2 g/dl sodium bicarbonate, and 1.0 g/dl BSA. This mixture was termed CL medium. One hundred microliters was added to each of the wells, and culture fluids were allowed to equilibrate for an additional 20 min at 37°C under 5% CO_2. Subsequently, medium was removed, and 100 μl of one of the following was added to each well: medium alone or medium containing MPO, iMPO, or EPO. In addition, 50 μl of zymosan opsonized with guinea pig complement and 50 μl of luminol (80 mM working concentration) were added to all of the wells. The plate was placed in a Dynatech ML 3000 plate luminometer, and the mean luminescence was recorded using a 2-min interval. The results were plot-

ted as time vs relative light units (RLU). The mean of triplicate treatments ± SEM was determined. Each experiment was repeated at least twice.

Enzyme-Linked Immunosorbent Assay

After HUVEC were adjusted to 2.0×10^5 cells/ml, 100 μl of the suspension was added to a 96-well Falcon Primaria plate (Becton Dickinson, Lincoln Park, NJ). Following incubation overnight, the cells were washed with F-12K/0% FBS without supplement. Different concentrations of either MPO or iMPO were added to the cells and cultures were incubated for various time intervals. Supernatants were collected and stored at −70°C until assayed. Sandwich ELISA minikits for the detection of various cytokines were purchased from the following sources and utilized per the manufacturers' instructions: IL-8 OptEIA kit (PharMingen, San Diego, CA); IL-6 OptEIA kit (PharMingen); hGM-CSF (Endogen, Woburn, MA); and mouse TNF-α OptEIA kit (PharMingen). Briefly, Maxi-sorb 96-well microtiter plates (Nunc, Inc., Naperville, IL) were coated with monoclonal antibody specific for the cytokine of interest and incubated overnight. The wells were exposed to a blocking buffer, and subsequently samples were added and incubated for 1 h. Wells were washed, and a secondary polyclonal antibody for the cytokine of interest along with an avidin–horseradish peroxidase (HRP) conjugate was added. Following incubation with the conjugate, the amount of HRP-labeled antibody was detected with tetramethylbenzidine. Absorbances were read at a wavelength of 450 nm on a microtiter plate reader (Dynatech Laboratories, Chantilly, VA).

Ribonuclease Protection Assay

HUVEC were cultured in 6-well plates with MPO, iMPO, or both iMPO and EPO for 3 h. Cells were lysed using TriZol (Gibco, Long Island) and total cell RNA was collected by phenol:chloroform extraction and ethanol precipitation. A RNase protection assay (RPA) was then performed on equal amounts of total cell RNA from each treatment group using the RiboQuant multiprobe RNase protection assay kit (PharMingen) per the manufacturer's instructions. Briefly, ^{32}P-labeled antisense RNA probes (specific to MIP-1β, GM-CSF, IL-1β, MCP-1, IL-8, and the two housekeeping genes L32 and GAPDH) were hybridized to sample RNA, a ribonuclease specific for single-stranded RNA was added to digest nonhybridized RNA, and the final products were resolved on an 8 M urea–polyacrylamide gel. The bands on the gel were visualized by autoradiography. To determine differences between treatments, the amount of mRNA loaded in the lanes was normalized based on GAPDH or L32 band intensities using densitometry.

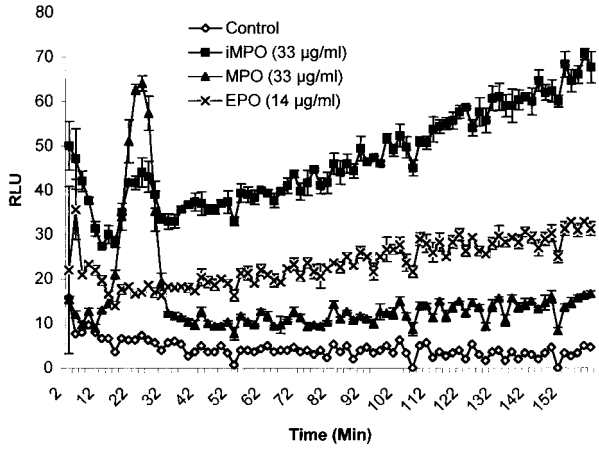

FIG. 1. Effects of peroxidases on respiratory burst in HUVEC. HUVEC were added to 96-well luminometry plates and cultured for 2 h. Media were removed and the cells were treated with EPO, MPO, or iMPO. Opsonized zymosan and luminol were added and the relative light units (RLU) were analyzed on a luminometer. Values represent the means ± SEM of duplicate wells. All experiments were repeated at least twice.

Statistical Analysis of Data

A Student *t* test was used to determine the significance between two treatments. A one-way analysis of variance and Tukey posttest were used to determine significance among multiple groups. All values are reported as means ± SEM. Differences were considered significant at $P \leq 0.05$. In general, significance is reported between control and experimental values unless otherwise noted. All experiments were repeated at least two times in order to ensure reproducibility.

RESULTS

The respiratory burst (RB) with concomitant production of ROI resulted after exposure of HUVEC to certain peroxidase preparations. Cells exposed to iMPO resulted in more RLU than observed with other treatments. The amount of RLU induced by iMPO continued to increase for the duration of the experiment. Exposure of these cells to EPO resulted in a similar but reduced level of activity. Active MPO caused a rapid response, which peaked in approximately 25 min but returned to baseline by 35 min (Fig. 1). If cells were not present during the assay, chemiluminescence was not observed.

Interleukin-8 is a potent chemotactant for neutrophils that could serve as a source of MPO and iMPO. Cells were incubated with various preparations of either MPO or iMPO for 6 h. Following incubation, supernatants were collected and analyzed for IL-8 by ELISA. None of the 3 different MPO preparations induced IL-8 secretion above control titers (Fig. 2). However, both preparations of iMPO employed consistently

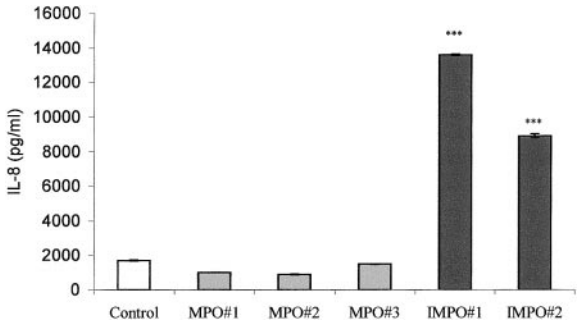

FIG. 2. Comparison of active and inactive MPO induction of IL-8 secretion. HUVEC were added to the wells of a microtiter plate and incubated overnight. Monolayers were washed and the cells treated with media alone, media containing 13 U/ml of different preparations of MPO (1, 2, or 3), or equivalent amounts of iMPO (preparations 1 and 2). Following a 6-h incubation, supernatants were removed and assayed for IL-8 by an ELISA. Values represent the means ± SEM of duplicate wells. All experiments were repeated at least twice. Values without indicators demonstrate no significant differences relative to controls. $***P \leq 0.001$.

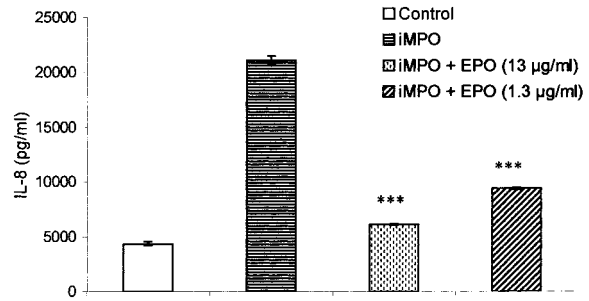

FIG. 4. IL-8 secretion by HUVEC in response to EPO and iMPO stimulation. HUVEC were added to a microtiter plate. After overnight incubation, monolayers were washed and treated with either 100 μg/ml iMPO alone or 100 μg/ml of iMPO with various concentrations of EPO. After 6 h incubation, supernatants were collected and assayed for IL-8 by ELISA. Values represent the means ± SEM of duplicate wells. All experiments were repeated at least twice. $***P \leq 0.001$.

enhanced IL-8 secretion. When HUVEC were incubated with different concentrations of iMPO for 6–24 h, dose- and time-dependent increases in IL-8 secretion were observed (Fig. 3). At 6 h, 100 μg/ml of iMPO induced a 3.5-fold increase over the control, whereas at 24 h, an 18-fold increase was observed. Because of these studies, iMPO was used for the majority of subsequent studies.

Initially, studies were carried out to determine the effect of EPO on HUVEC IL-8 secretion. HUVEC exposed to EPO exhibited a minimal reduction in cytokine secretion; i.e., HUVEC incubated with 1.3–13 mg/ml of EPO for 6 h exhibited a slight, but not significant, decrease in IL-8 compared to that in medium controls (data not shown). The next set of experiments investigated whether EPO would inhibit iMPO-induced cytokine secretion. HUVEC were exposed to iMPO and EPO simultaneously, and after incubation, IL-8 titers were determined. In these studies, EPO markedly decreased iMPO-induced IL-8 secretion (Fig. 4).

Interleukin-6 is a cytokine that has the capacity to enhance the inflammatory cascade. To determine whether either MPO or iMPO was capable of stimulating IL-6, HUVEC were incubated with either MPO or iMPO for 6–24 h. Supernatants were collected and analyzed for IL-6 by ELISA. As was seen in assays for IL-8, neither concentration of MPO induced IL-6 secretion (Fig. 5). However, 11 and 33 μg/ml iMPO induced 2-fold and 4-fold increases in IL-6 secretion at 6 h,

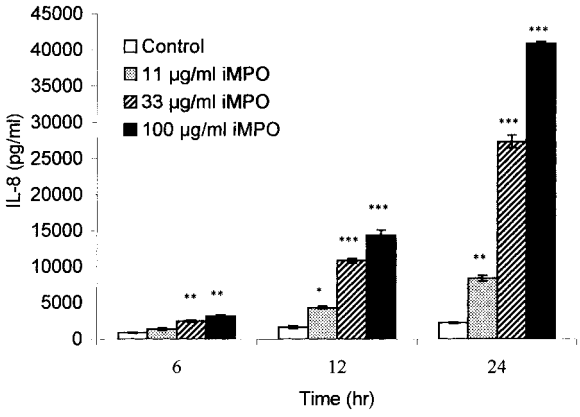

FIG. 3. IL-8 secretion by HUVEC in response to iMPO stimulation. HUVEC were added to a microtiter plate. After overnight incubation, monolayers were washed and cells treated with either media alone or media containing various concentrations of iMPO. Supernatants were removed after various time periods and assayed for IL-8 by ELISA. Values represent the means ± SEM of duplicate wells. All experiments were repeated at least twice. Values without indicators demonstrate no significant differences relative to control. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$.

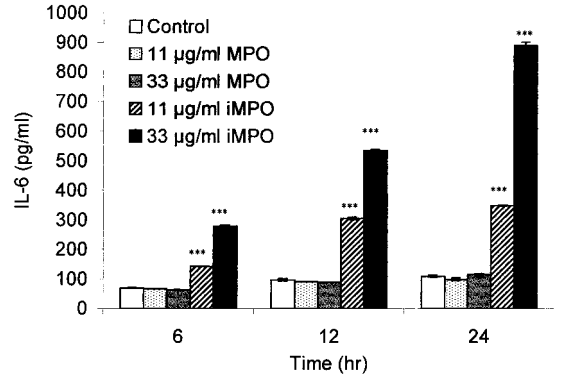

FIG. 5. IL-6 secretion by HUVEC in response to stimulation by either MPO or iMPO. HUVEC were added to a microtiter plate. After overnight incubation, monolayers were washed and treated with media alone, media containing various concentrations of MPO, or media containing various concentrations of iMPO. Supernatants were removed at various time intervals and assayed for IL-8 by ELISA. Values represent the means ± SEM of duplicate wells. All experiments were repeated at least twice. $***P \leq 0.001$.

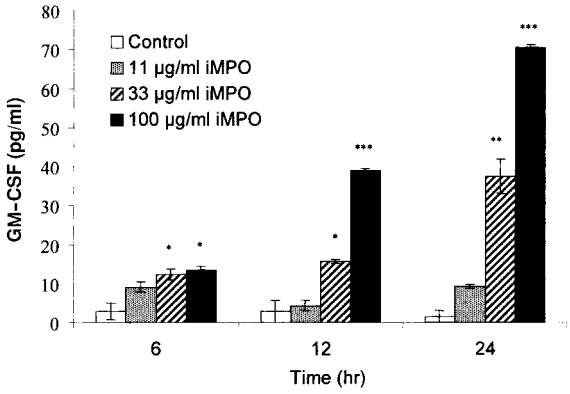

FIG. 6. GM-CSF secretion by HUVEC in response to iMPO stimulation. HUVEC were added to wells of a microtiter plate. After overnight incubation, monolayers were washed and treated with either media alone or media containing various concentrations of iMPO. Supernatants were removed at various time periods and assayed for GM-CSF by ELISA. Values represent the means ± SEM of duplicate wells. All experiments were repeated at least twice. Values without indicators demonstrate no significant differences relative to controls. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$.

respectively. A time-dependent effect was also observed with both concentrations of iMPO, and by 24 h there was a 9-fold increase in the concentration of iMPO compared to controls (Fig. 5). Another cytokine assayed, GM-CSF, has been shown to have numerous functions, including the induction of neutrophil degranulation. HUVEC were incubated with control medium or 11, 33, or 100 μg/ml of the iMPO for 6–24 h. At each time period, a dose-dependent increase in the titer of GM-CSF was observed (Fig. 6).

To determine if the increase or decrease in cytokine secretion observed with (a) iMPO, (b) iMPO, or (c) EPO stimulation was the result of an effect on mRNA transcription, an RPA was done. Initially, HUVEC were incubated with 33 μg/ml of either MPO or iMPO for 3 h. Figure 7 illustrates that there was an increase in the transcripts for IL-8, MCP-1, and GM-CSF from cells incubated with iMPO, but only a slight increase in mRNA was observed in the cells exposed to MPO. Densitometry indicated that there was a 16-fold increase in IL-8 mRNA and a 10-fold increase in GM-CSF mRNA from iMPO-treated HUVEC over the amounts of each from control cells. In addition, iMPO-treated cells exhibited a marked increase in mRNA for MCP-1. No changes were observed in the levels of the constitutively expressed transcripts for GAPDH and L32. Also, to verify results obtained using ELISAs, an RPA assay was done after HUVEC were exposed to both iMPO and EPO for 3 h. The combination of iMPO and EPO resulted in a *reduction* of cytokine mRNA. As can be seen in Fig. 8, there was a marked *reduction* of IL-8, MCP-1, and GM-CSF.

DISCUSSION

The physiological process by which vascularized tissues respond to trauma is termed inflammation. When this process is not properly controlled, massive tissue damage frequently associated with disease states can result. The inflammatory process is characterized by an initial neutrophil influx, which involves extravasation of cells through the endothelium. No longer considered a passive barrier, the endothelium secretes various substances involved in the inflammatory processes. Among these substances are cytokines, which are chemotactants for neutrophils and other cells (33). Neutrophil influx would ensure an abundance of MPO in a particular area. With chronic inflammation such as that associated with RA, in addition to the neutrophil influx, there may be an influx of eosinophils concomitant with the release of EPO into the microenvironment (21). The present study was undertaken to determine the effects of these peroxidases on EC functions.

Since neutrophils are the hallmark cells of inflammation and they secrete MPO, there has been some

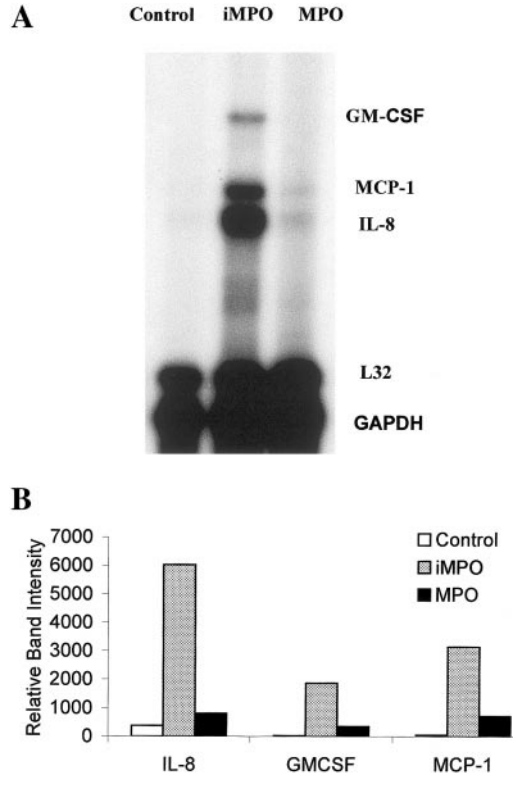

FIG. 7. Upregulation of HUVEC mRNA levels by iMPO. HUVEC were added to 6-well cluster plates and incubated overnight. Media were removed and cells incubated with control media, 33 μg/ml iMPO, or 33 μg/ml MPO for 3 h. Total cell RNA was then collected and an RPA was performed as described under Materials and Methods. (A) The autoradiogram is a representative experiment. Densitometry (B) was performed to determine relative differences in mRNA levels between treatments.

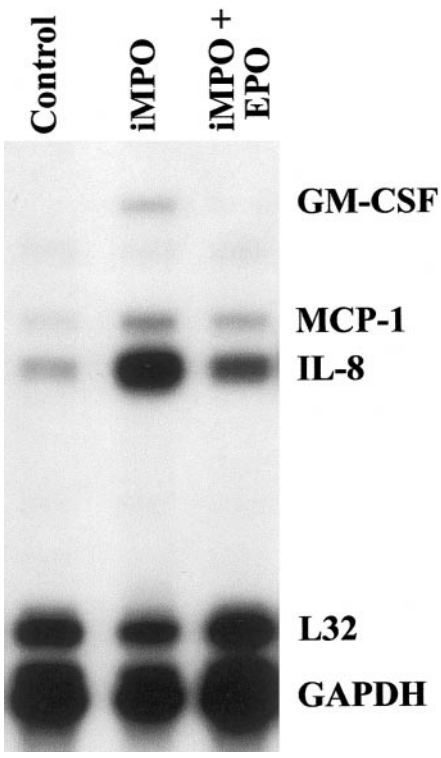

FIG. 8. Downregulation of HUVEC mRNA levels by EPO in response to stimulation with iMPO. HUVEC were added to 6-well cluster plates and incubated overnight. Media were removed and cells incubated with control media, 33 μg/ml iMPO, or 33 μg/ml MPO with 14 μg/ml EPO for 3 h. Total cell mRNA was collected and an RPA was performed as described under Materials and Methods. A representative experiment is presented.

debate over the amount of both MPO and iMPO at a site of inflammation (18, 19). Some investigators have reported the quantity of active enzyme, while others have reported the amount of inactive enzyme present. King *et al.* reported that most of the MPO that is secreted is inactivated (more than 80%) within 10 min (34). Others have reported that there is approximately 16–29 μg/ml of iMPO alone in an inflamed RA joint. Likewise, Flugge *et al.* have reported high concentrations of MPO in RA synovial fluid (35). Therefore, at least two of the concentrations (11 and 33 μg/ml) employed in this study were within "physiological range." With respect to EPO, it is known that there is 100–200 ng/ml of EPO secreted into the milieu of the microenvironment (24). Also, both of these enzymes can participate in a cytotoxic triad as well as induce a strong RB in Mϕ (14, 17, 27). Products of the RB, ROI, can create enlarged foci of tissue damage, which augment the inflammatory process disproportionately (12).

In the present study, it is interesting to note that EPO, MPO, and iMPO induced an EC RB. It has been reported by others that peroxidase-induced chemiluminescence measures the relative secretion of H_2O_2 formed during the RB (36). The products of the RB could function as follows: ROI (a) would induce tissue damage, which would recruit inflammatory cells; (b) would enhance neutrophil adherence to the endothelium (37); (c) could oxidize receptors and/or cell surface proteins, resulting in either cell death or cell inactivation (35); and (d) act as second messengers for cytokines (38). The fact that MPO induced a rapid increase in the RB followed by a return to control values indicates that after the initial RB, the cells were no longer responding to the stimulant (Fig. 1). This lack of responsiveness could be the result of oxidized receptors (39). When iMPO was employed, the data indicate that iMPO initiated a slow, steady increase in ROI (Fig. 1). Also, the iMPO would not have the enzymatic activity to cause oxidation of receptors; it appears that receptor–ligand interaction was sufficient to induce a prolonged RB. Even though MPO, iMPO, and EPO are highly mannosylated and cationic, it appears that neither charge nor interaction with a mannose-specific receptor could explain the differences obtained in the data. Other investigators have reported that neutrophils avidly bind EPO, but not MPO, via a mannose receptor (10). Perhaps the same mechanism of differentiation between EPO and MPO may be employed by the EC and could account for differences in RLU released. Further studies are needed to elucidate this point.

As alluded to above, the migration of neutrophils into an area is an early sign of inflammation, and these cells are considered pivotal to inflammation (12). Once at a site, the cell degranulates and most of the MPO is inactivated in the microenvironment (19, 35). Considering the role of the neutrophil in inflammation and the fact that IL-8 is a potent chemotactant for this cell (40), initial studies involved the effects of peroxidases on IL-8 secretion by EC. Although IL-8 was the first cytokine assayed, a pattern emerged that held true for all of the cytokines investigated; i.e., MPO did not induce cytokine secretion, whereas iMPO did (Figs. 2 and 4). Like MPO, EPO did not induce cytokine secretion above that of controls. Since a substantial amount of the MPO would be in the form of iMPO in the microenvironment *in vivo*, EC would be stimulated to secrete IL-8. This cytokine, in particular, would recruit more neutrophils, stimulate their transendothelial migration, and induce their degranulation (concomitant with release of MPO, which would be rapidly inactivated). Therefore, cyclic events would follow, which could perpetuate inflammation.

As with IL-8, iMPO, but not MPO, induced significantly higher IL-6 and GM-CSF titers than control cultures ($P \leq 0.005$) (Figs. 5 and 6). IL-6 affects the acute phase response and participates in the pathology of certain chronic inflammatory diseases (40). In addition to IL-6, iMPO induced a dose- and time-dependent increase in GM-CSF (Fig. 6). Along with IL-8, GM-CSF functions as a chemotactant for neutrophils (41). Gran-

ulocyte–macrophage CSF also enhances development of neutrophils (41), adhesion to the endothelium (42), transmigration of neutrophils across the vascular endothelium (42), degranulation of neutrophils (43), activation of eosinophils (44), and release of histamine from basophils. This cytokine also induces migration and proliferation of EC, i.e., neovascularization (44). With various diseases such as RA, neovascularization is pivotal to the pathogenic process (45).

Because cytokine secretion was upregulated in response to iMPO stimulation, an RPA was carried out to determine whether this effect was due to an increase in mRNA or to an increase in secretion of IL-8 and GM-CSF. In addition, the mRNA levels of another cytokine, MCP-1, were assessed. The RPA corroborated the results of the ELISAs; that is, iMPO was more stimulatory than MPO. In addition, the results of the RPA indicated that the peroxidase was not enhancing cytokine secretion, but was in fact causing upregulation of gene transcripts.

Upregulation of MCP-1 mRNA transcript was obvious from the radiogram (Fig. 7). With all the other cytokines, upregulation of transcript was concomitant with enhanced secretion measured by ELISA. It would be logical to speculate the same was true for MCP-1. If, indeed, there is an increase in secreted MCP-1, this cytokine promotes Th_2 responses, is a chemotactant for monocytes, and causes basophil release of histamine. Obviously, all of the above would have implications for inflammatory-based diseases.

With respect to eosinophils, it should be noted that IL-8 recruits these cells, albeit to a lesser extent than it recruits neutrophils (30). Also, it has been reported that EPO bound to neutrophils increases neutrophil adhesion to EC. Therefore, IL-8 would cause an influx of cells, which would release either MPO or EPO as well as induce a RB. The result of this would be increased tissue damage and augmentation of the inflammatory response. However, there seems to be a duality of function for EPO. That is, EPO can function in either a proinflammatory or an anti-inflammatory manner within the body. While an enhanced RB would augment inflammation, negating the induction of IL-8 by iMPO would be anti-inflammatory. These anti-inflammatory observations were corroborated by the results of the RPA, which indicated a downregulation of mRNA gene transcripts after EC were exposed to iMPO and EPO simultaneously (Fig. 8). This type of response is not unique, as inhibition of proinflammatory cytokine secretion and mRNA transcription has been observed previously when enzymatically inactive EPO and Mϕ have been employed (46).

This study also describes a dichotomy of function for certain peroxidases. That is, both enzymatically active MPO and EPO are known to be participants in the "cytotoxic triad," i.e., killing potential (27, 33), whereas this study suggests that the *enzymatically inactive* form of MPO serves as an immunoregulator. Taken in its entirety, these results imply that different peroxidases can regulate each other with respect to cell activation and inflammation.

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