Heme Induces the Expression of Adhesion Molecules ICAM-1, VCAM-1, and E Selectin in Vascular Endothelial Cells (44197)

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Abstract. **Heme is an important immunostimulating agent and oxidative factor contributing to endothelial cell activation. To investigate the mechanism of heme-induced endothelial cell activation, we analyzed the effect of heme and the inflammatory cytokine, tumor necrosis factor–α (TNF-α), on the expression of the heme-degrading stress protein, heme oxygenase (HO), and adhesion molecules in human umbilical vein endothelial cells (HUVEC). Indirect immunofluorescence double labeling studies demonstrated a simultaneous increase of ICAM-1 and HO-1 after exposure of cells to heme for 24 hr. Co-expression of HO-1 and ICAM-1 was also demonstrated in TNF-α–exposed cells. Dot blot immunoassay and quantitative analysis by ELISA demonstrated that heme treatment for 24 hr caused a 2-fold increase in ICAM-1 expression ($P < 0.002$) compared with quiescent cells, while in cells stimulated by TNF-α for 24 hr ICAM-1 gene expression increased by 5-fold. Moreover, heme exposure also resulted in a marked increase in VCAM-1 and E selectin expression (three and four times over control levels, respectively). On the other hand, TNF-α treatment showed similar expression levels for VCAM-1 and E selectin, compared with stimulation by heme (100 μM). The level of HO activity in endothelial cells exposed to heme or TNF-α was increased from 24.7 ± 5.7 pmol bilirubin/mg protein/min in control to 70.0 ± 9.5 and 36.7 ± 3.1 pmol bilirubin/mg protein/min in heme– and TNF-α–stimulated cells, respectively. These results suggest that upregulation of ICAM-1, VCAM-1, and E selectin expression is associated with oxidative stress induced by hemoglobin/heme and that HO-1 may play a modulating role *via* its ability to degrade heme to a substance with antioxidant properties.** [P.S.E.B.M. 1997, Vol 216]

Heme proteins play a vital role in diverse biological processes including cell respiration, energy generation, oxidative biotransformation, and growth/differentiation (1–3). The ubiquitous heme molecule has multiple immunoregulatory effects, which include the induction of cytokine production, such as tumor necrosis factor–α (TNF-α), interleukin-1 (IL-1), and IL-8 in mononuclear cells (4–7). However, heme also constitutes a major danger to vascular integrity by making the endothelium hypersensitive to oxidative damage (8–10). Oxidative damage includes lipid peroxidation resulting in loss of membrane integrity (11, 12) and structural alterations to DNA (13, 14). Free heme, which is highly hydrophobic, accumulates in the membranes of endothelial cells and acts in synergy with oxygen radicals to exacerbate oxidative damage (8). Iron released from the heme molecule is critical to this oxidative sensitization since ferritin synthesis is essential for protection against the cytotoxic effect of heme (15). Oxidative injury of the endothelium, due to a gross imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defenses of the cell, has been implicated in the pathogenesis of a number of pathological conditions, including hemorrhagic injury, ischemia/reperfusion injury, adult respiratory distress syndrome, and atherosclerosis (11, 16–19).

Heme oxygenase (HO) is the rate-limiting enzyme in heme degradation. Among the two isoforms of HO, HO-1 is

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highly responsive to stress and is induced by a variety of agents, such as H_2O_2, heavy metals, endotoxin, and heme, in several cell types, including endothelial cells (20, 21). Upregulation of HO-1 gene expression results in increase of the degradation of heme to equimolar amounts of carbon monoxide, iron, and the antioxidant biliverdin (22). Iron by itself is a potent oxidative stress agent, whereas the iron released by HO-1 activity further enhances ferritin synthesis and thereby its sequestering (23). In this regard, overexpression of HO-1 in endothelial cells has been shown to protect from heme- and hemoglobin-mediated toxicity (19), and is associated with increased tolerance of the cell to ischemia and trauma in a variety of tissues (24, 25).

During the inflammatory response, the endothelium plays a critical role in the communication between the underlying site of inflammation and circulating leukocytes. Cytokines released from the inflamed tissue induce endothelial cells to express adhesion molecules on their cell surface, including intercellular adhesion molecule–1 (ICAM-1), vascular adhesion molecule–1 (VCAM-1), and endothelial leukocyte adhesion molecule (E-selectin) (26, 27). ICAM-1, VCAM-1, and E selectin on endothelial cells are able to interact specifically with ligands of passing leukocytes, promoting the recruitment, adherence, and extravasation of hematopoietic cells (28), which is thought to contribute to the pathogenesis of neurodegenerative diseases (29), sickle cell crisis (30), and vascular diseases like atherosclerosis (31). This is illustrated by the fact that sickle-cell reticulocytes express glycoprotein IV (GP-IV) and VLA-4, which may mediate binding to activated endothelial cells and initiate vascular occlusion in sickle-cell anemia (32). Cytokine-induced adhesion molecule expression and monocyte sequestration is also an important event in atherogenesis (33). In addition, activated neutrophils interacting with the activated microvascular endothelium, readily release ROS, which can contribute to free radical–mediated reperfusion injury, leading to endothelial cell injury (34, 35).

The mechanism by which heme and other factors induce HO-1 involves the activation of various promoter sites, including nuclear factor κB (NF-κB) and activator protein 2 (AP-2) responsive elements (1). NF-κB is involved in growth control and the rapid induction of various genes encoding defense and stress proteins (36). Cytokines, such as TNF-α and IL-1, activate NF-κB and modulate adhesion molecule expression on a variety of cells (36). ICAM -1, VCAM-1, and E selectin possess the regulatory sequences for the binding of NF-κB, and AP-2, and/or AP-1 in their promoter region (36–39). Since TNF-α and heme are major immunomodulators that share common features with respect to NF-κB activation, we considered that heme may have direct modulatory effects on adhesion molecules. In the present report, we examined the effects of heme and TNF-α on the coordinate expression of HO-1 and adhesion molecules ICAM-1, VCAM-1, and E selectin in human umbilical vein endothelial cells (HUVEC). Our results indicate that heme, in addition to its ability to induce HO-1 expression, is a potent inducer of ICAM-1, VCAM-1, and E selectin expression in endothelial cells, which may emphasize its importance in biological processes.

Materials and Methods

Reagents. Hemin (Sigma Chemical Co., St. Louis, MO) solution was prepared as previously described (3). Human recombinant TNF-α was obtained from Endogen (Cambridge, MA).

Antibodies. The following antibodies were used: a rabbit polyclonal HO-1 antibody directed against human HO-1 (Affinity Bioreagents, Golden, CO), a monoclonal mouse CD62E antibody against human E selectin (Pharmingen, San Diego, CA), a REK-1 monoclonal mouse antibody against human ICAM-1, a kind gift from Yvette van Kooyk (Academic University Hospital, Nijmegen, The Netherlands), and a mouse monoclonal 4B9 antibody against human VCAM-1, which was generously provided by Roy Lobb (Biogen Inc., Cambridge, MA). Horseradish peroxidase–conjugated sheep anti-mouse IgG (Boehringer Mannheim, Germany) was used as secondary antibody for the chemiluminscence studies. For fluorescence studies, we used as secondary antibodies goat anti-rabbit IgG coupled to the fluorochrome rhodamine (Pierce, Rockford, IL), and Cy-2 fluorochrome–conjugated to goat anti mouse IgG (Amersham/Life Sciences, Arlington Heights, IL). For enzyme-linked immunosorbent assay (ELISA), alkaline phosphatase (AM)–conjugated goat anti-mouse-IgG (H+L) (Gibco BRL, Grand Island, NY) was used as secondary antibody.

Cell Culture and Incubations. HUVEC (second passage), kindly provided by Eric Jaffe (New York Hospital, New York, NY), were passaged two to four times and used in the experiments described below. Cells were cultured in 199 medium containing 16% fetal calf serum, 4% human serum, 25 m*M* HEPES, antibiotics-antimycotics and endothelial cell growth factor, at 37°C, at 5% CO_2.

Indirect Immunofluorescence Detection of ICAM-1 and HO-1 Expression. HUVEC were grown on fibronectin-coated cover glasses to 70% confluency, and subsequently treated with heme (100 μ*M*), TNF-α (20 ng/ml), or a combination of both, for 24 hr. The cells were carefully washed with phosphate-buffered saline (PBS)(0.1 g/l $CaCl_2/MgCl_2$), and fixed with 80% acetone for 5 min at room temperature. After being washed twice with PBS/0.05% Tween 20 (PBST), the cells were incubated with 5% bovine serum albumin (BSA) in PBST for 1 hr at room temperature. Protein expression was evaluated by indirect fluorescence using primary antibodies against ICAM-1 and/or HO-1. After incubation for 1 hr at room temperature, the cells were washed three times with PBST, followed by incubation for 1 hr with fluorescent-labeled secondary antibodies and washing for three times with PBST. The cover glasses were mounted with one drop of aqua polymount

(Poly sciences, Warrington, PA), and analyzed using fluorescence microscopy.

Chemiluminescent Detection of Cellular Adhesion Molecule Expression. Control and treated HUVEC were washed twice using cold PBS (0.1 g/l $CaCl_2$/$MgCl_2$), incubated with ice-cold Giordano buffer (50 m*M* Tris/HCl [pH = 7.4], 0.25 *M* NaCl, 0.1 % Triton X-100, 5 m*M* EDTA, 50 m*M* NaF, 0.1 m*M* Na Orthovanadate, 0.5 m*M* phenyl-methylsulfonyl fluoride), and gently scraped. The cell preparation was incubated for 30 min on ice, and vortexed. After centrifugation, the protein content of the supernatant was determined by the method of Lowry (40). One hundred micrograms of total protein was then transferred to nitrocellulose membranes using the minifold II slotblotter (Schleicher & Schuell, Keene, NH), crosslinked by UV light, and incubated for 1 hr with 1% blocking buffer at room temperature. Membranes were then incubated with appropriate dilutions of the primary antibodies against ICAM-1, VCAM-1, and E selectin in 0.5% blocking buffer for 1 hr at room temperature, washed twice with Tris-buffered saline containing 0.05% Tween 20 (pH = 8.0)(TBST), and incubated with horseradish peroxidase–conjugated secondary antibodies for 1 hr at room temperature. After membranes were washed with TBST, proteins were detected by chemiluminescence and the bands representing the expressed adhesion molecules were quantified by scanning densitometry. The results are presented as arbitrary units of absorbance at 560 nm (AU) (Beckman spectrophotometer DU 7400).

Endothelial Cell ELISA for Surface Expression of ICAM-1, VCAM-1, and E Selectin. Aliquots of HUVEC were grown in 24-well plates to near confluence (95%), followed by exposure to heme, TNF-α, or a combination thereof for 24 hr. The cells were washed with PBS (0.1 g/l $CaCl_2$/$MgCl_2$) and fixated with 500 μl/well 2.5% paraformaldehyde for 10 min at room temperature. Cells were washed with PBS and incubated for 2 hr with antibodies against ICAM-1, VCAM-1, and E selection (1:1000 dilution in PBS) at room temperature. Cells were washed three times and incubated for another hour with AP-conjugated goat anti-mouse IgG (H+L)(1:000 dilution in PBS) at room temperature and washed three times with PBS. Binding of the secondary antibody was determined by the addition of 350 μl of *p*-nitrophenyl phosphate substrate (Sigma) (1 mg/ml in 0.2 *M* Tris [pH 9.8], containing 5 m*M* $MgCl_2$). The 24-well plate was incubated in the dark for 30 min, and the reaction was terminated by the addition of 150 μl of 3 *M* NaOH. The solution was transferred into 96-well plates and absorbance was read at 405 nm by an ELISA microplate reader. The surface expression of ICAM-1, VCAM-1, and E selectin is shown as mean ± SD of the optical density (OD) at 405 nm. OD in the absence of primary antibody serves as the blank value. The values are the mean of four experiments using triplicate wells.

Measurement of HO Activity. HO activity was assayed by preparing microsomes from HUVEC treated with heme, TNF-α, or a combination of both. Microsomes were incubated with 50 μ*M* heme, 2 mg/ml rat liver cytosol (source of biliverdin reductase), 1 m*M* $MgCl_2$, 3 units glucose-6-phosphate dehydrogenase, 1 m*M* glucose-6-phosphate, and 2 m*M* $NADP^+$ in 0.5 ml 0.1 *M* potassium phosphate buffer [pH = 7.4] for 30 min at 37°C. The reaction was stopped by placing the tubes on dry ice, and bilirubin was extracted with chloroform as previously described (41). The amount of bilirubin generated was determined by using a scanning spectrophotometer (Lambda 17 UV/VIS; Perkin-Elmer, Norwalk, CT) and defined as the difference between 464 and 530 nm (41). Results are expressed as picomoles of bilirubin per milligram of protein per minute.

Results

Visualization of HO-1 and ICAM-1 Proteins Using Indirect Immunofluorescence. We employed indirect immunofluorescence double labeling to simultaneously visualize the expression of ICAM-1 and HO-1 after exposure of the cells to heme, and/or TNF-α. We observed that 24-hr incubation of HUVEC with 100 U/ml TNF-α resulted in the simultaneous expression of HO-1 and ICAM-1 (Fig. 1A). TNFα–treated cells showed a distinctive expression pattern of ICAM-1 on the cell surface, while HO-1 seemed to be widely expressed within the cell. Furthermore, TNF-α caused an apparent morphological change of the HUVEC from a spherical appearance to a more elongated shape.

Incubation of HUVEC for 24 hr with 100 μ*M* heme resulted in an enhanced co-expression of HO-1 and of ICAM-1 (Fig. 1B). The cell surface expression pattern of ICAM-1 differs from the cells incubated with TNF-α in its dispersed distribution of ICAM-1 over the cell membrane, and the higher level of HO-1 expression. No significant morphology changes were apparent after treatment with heme.

Indirect immunofluorescence single labeling showed that HO-1 was also highly induced when HUVEC were incubated with 100 μ*M* heme and 100 U/ml TNF-α for 24 hr (Fig. 1C).

The Effect of Heme and TNF-α on Adhesion Molecule Expression in Endothelial Cells as Measured by Chemiluminescent Dot Blot Immunoassay. To confirm the immunofluorescence results, we further determined the effect of heme and TNF-α on the cellular adhesion molecule content by dot blot immunoassay techniques. HUVEC were incubated with heme (50 μ*M*) or TNF-α (20 ng/ml) for 24 hr, and cell lysates were then analyzed for the expression of ICAM-1, VCAM-1, and E selectin. Densitometry analysis performed after chemiluminescence, showed that untreated HUVEC expressed ICAM-1 (Fig. 2) and VCAM-1 (data not shown) constitutively, while E selectin (data not shown) could not be detected. After treatment for 24 hr with a single dose of heme (50 μ*M*) or TNF-α (20 ng/ml), the expression level of the

Figure 1. ICAM-1 and HO-1 expression of HUVEC, visualized by indirect immunofluorescence. The expression of HO-1 is represented by the red appearance of rhodamine. The expression of ICAM-1 is represented by the green fluorochrome Cy-2. The yellow appearance represents a combination of both green and red fluorescent labels. (Magnification: ×100.) (A) ICAM-1 and HO-1 expression of HUVEC treated with 100 U/ml TNF-α for 24 hr, visualized by indirect immunofluorescence double labeling. (B) ICAM-1 and HO-1 expression of HUVEC treated with 100 μ*M* heme for 24 hr, visualized by indirect immunofluorescence double labeling. (C) HO-1 expression of HUVEC treated with 100 μ*M* heme and 100 U/ml TNF-α for 24 hr, visualized by indirect immunofluorescence single labeling.

adhesion molecules were analyzed. ICAM-1 expression after heme stimulation was 4.4-fold over the control values ($P < 0.03$), while in TNF-α–treated cells ICAM-1 expression was 6-fold higher ($P = 0.051$) (Fig. 2). Similarly, VCAM-1 and E selectin expression showed to be increased after exposure to heme or TNF-α, as compared to untreated cells (data not shown).

Endothelial Cell ELISA for Surface Expression of ICAM-1, VCAM-1, and E Selectin. To quantify further the inductive effect of heme on the expression of adhesion molecules, we employed ELISA for the cell surface expression of ICAM-1, VCAM-1, and E selectin in HUVEC. Treatment of HUVEC with different concentrations of heme for 24 hr resulted in a significant concentra-

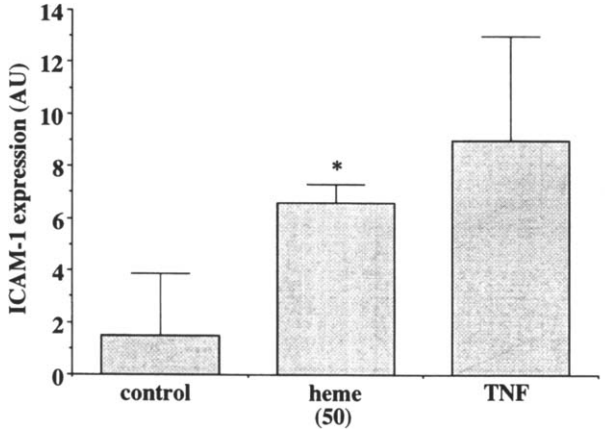

Figure 2. The expression of ICAM-1 was examined by immunochemiluminescent dot blot assay of isolated total proteins after exposure of HUVEC monolayers to heme (50 μM), or TNF-α (20 ng/ml) for 24 hr. The expression was quantitated by densitometry and represented as arbitrary units of absorbance at 560 nm (AU). Results show the mean ± SD of three separate experiments. Significance was defined by Student's *t* tests. *P* values exceeding 0.05 were considered not to be significant. (*Significantly different from control value; $P < 0.03$.)

tion-related increase in the surface expression of ICAM-1 (Fig. 3A). Heme at 100 μM caused a 2-fold increase in ICAM-1 expression compared to control values ($P < 0.002$). TNF-α (20 ng/ml) caused an increase in cell surface expression of ICAM-1 of about 5-fold compared with nontreated cells (Fig. 3B). VCAM-1 surface expression in HUVEC was markedly increased by 3-fold compared with control levels after exposure to heme (100 μM) for 24 hr ($P < 0.02$) (Fig. 3C) TNF-α showed a 2.5-fold increase in VCAM-1 expression (Fig. 3D). In addition, heme (100 μM) increased the expression of E selectin by 4-fold in comparison with untreated cells (Fig. 3E). TNF-α mediated ICAM-1 expression was about 2.5-fold higher compared with cells exposed to heme (100 μM) for 24 hr, but TNF-α–stimulated VCAM-1 and E selectin expression was similar to that stimulated by heme (100 μM) (Figs. 3, D and F). Although the addition of heme tended to increase TNF-α–mediated ICAM-1 and E selectin expression, this was not significant ($P > 0.05$) (Figs. 3, B and F). However, TNF-α–mediated VCAM-1 expression was significantly enhanced, when HUVEC were co-exposed to heme (100 μM)($P < 0.03$)(Fig. 3D).

The Effect of Heme and TNF-α on HO Activity.

In parallel experiments, we examined the effects of heme and TNF-α on HO activity (Fig. 4). This was of particular interest since heme and TNF-α were found to be significant inducers of adhesion molecules in HUVEC (Figs. 1–3), as well as HO-1 expression (Fig. 1). As seen in Figure 4, heme (50 μM) was a powerful inducer of HO activity in HUVEC,

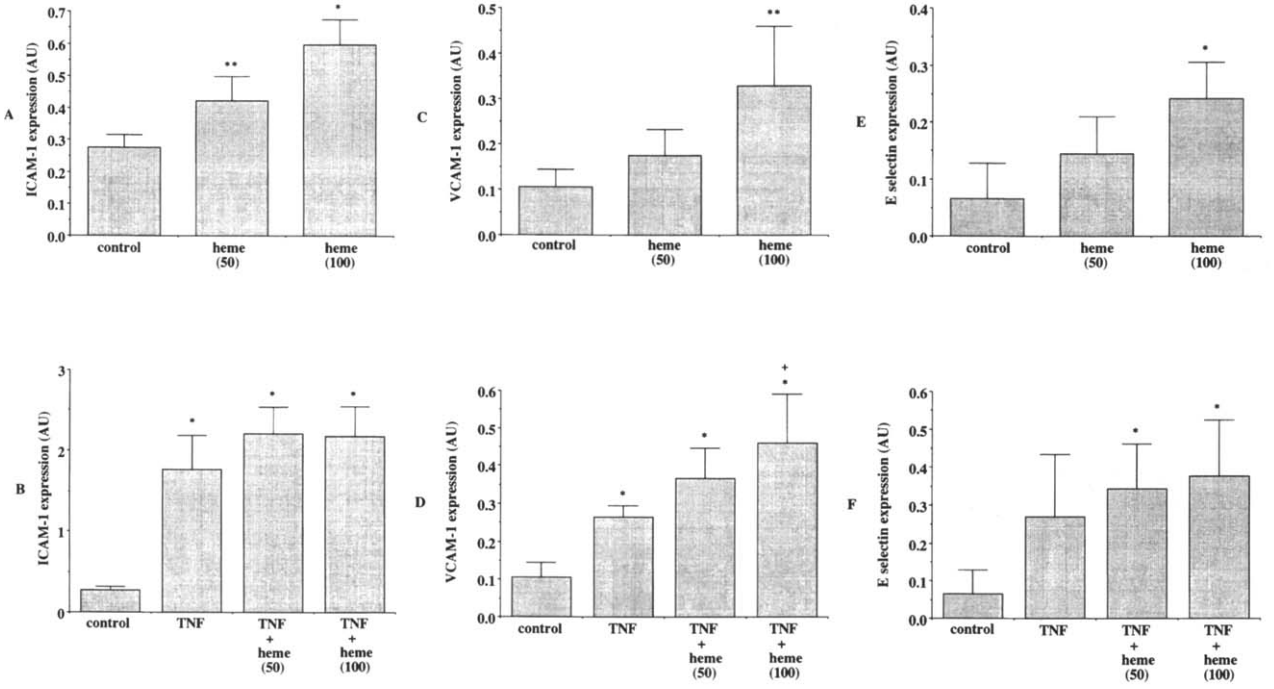

Figure 3. The effect of heme on endothelial surface expression of ICAM-1, VCAM-1, and E selectin in cultured endothelial cells was analyzed using ELISA. HUVEC subconfluent monolayers in 24-well plates were exposed for 24 hr to heme (50 μM; 100 μM), TNF-α (20 ng/ml), or combinations thereof. ICAM-1, VCAM-1, and E selectin expression was assayed by ELISA by measuring absorbance at 405 nm. Results show the mean ± SD of four separate experiments (triplicate). The data are presented as arbitrary units of absorbance at 405 nm (AU). (A) Expression of ICAM-1 after stimulation with heme (50 μM; 100 μM) for 24 hr. (B) Expression of ICAM-1 after exposure to TNF-α (20 ng/ml), with or without heme (50 μM; 100 μM) for 24 hr. (C) Expression of VCAM-1 after stimulation with heme (50 μM; 100 μM) for 24 hr. (D) Expression of VCAM-1 after exposure to TNF-α (20 ng/ml), with or without heme (50 μM; 100 μM) for 24 hr. (E) Expression of E selectin after stimulation with heme (50 μM; 100 μM) for 24 hr. (F) Expression of E selectin after exposure to TNF-α (20 ng/ml), with or without heme (50 μM; 100 μM) for 24 hr. Significance was defined by Student's *t* tests. *P* values exceeding 0.05 were considered not to be significant. Significantly different from control: $^{*}P < 0.01$; $^{**}P < 0.03$. Significantly different from TNF-α: $^{+}P < 0.03$

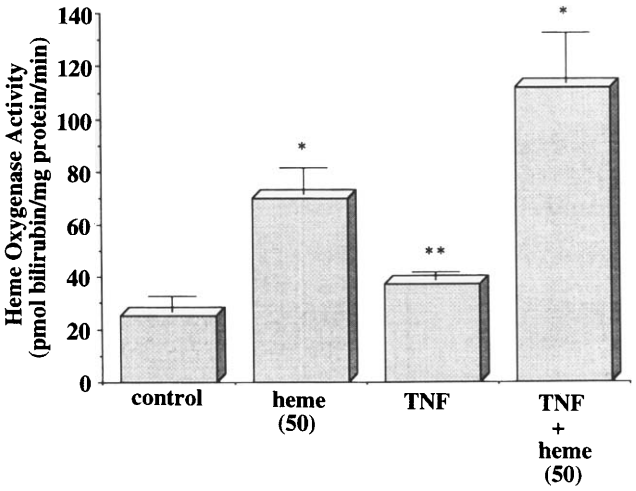

Figure 4. HO activity of HUVEC treated with combinations of heme (50 μ*M*) and TNF-α (20 ng/ml) for 24 hr. Activity is expressed as picomoles of bilirubin per milligram protein per minute. Significance was defined by Student's *t* tests. *P* values exceeding 0.05 were considered not to be significant. Significantly different from control: $^{*}P < 0.003$; $^{**}P < 0.035$.

increasing the basal level of HO activity from 24. 7 ± 5.7 mmol bilirubin/mg of protein/min to 70.0 ± 9.5 pmol bilirubin/mg protein/min after 24 hr of addition of heme ($P < 0.003$). On the other hand, exposure of HUVEC to TNF-α (20 ng/ml) for 24 hr showed a smaller increase in HO activity (36.7 ± 3.1 pmol bilirubin/mg protein/min) compared with the untreated cells ($P < 0.035$), whereas the combined addition of heme and TNF-α produced an enhanced response that was higher than that observed with heme alone (112.3 ± 18.6 pmol bilirubin/mg protein/min; $P < 0.003$).

Discussion

The experiments reported in this communication assessed the expression of HO-1 and adhesion molecules ICAM-1, VCAM-1, and E selectin in endothelial cells in response to oxidative stress generated by heme. The level of expression of ICAM-1, VCAM-1, and E selectin proteins was consistently enhanced by heme in comparison to basal levels (Figs. 1–3). Simultaneously, HO-1 expression and HO activity were increased in heme-stimulated HUVEC (Figs. 1 and 4). TNF-α, a potent inducer of these adhesion molecules, also induced HO-1 expression and HO activity (Figs. 1–4).

We have previously shown that transfection of the human HO-1 gene into rabbit coronary endothelial cells, using adenovirus or lipofectin as a means of gene delivery, protected endothelial cells against heme and hemoglobin toxicity (19). Nath *et al.* (42) demonstrated that *in vivo* induction of HO-1 prevents renal failure and drastically reduces mortality after glycerol-induced rhabdomyolysis in the rat, a pathological condition that is characterized by an increased release of myoglobin and hemoglobin into the extracellular renal space. HO may play an important role in moderating tissue damage associated with many pathologic processes in humans, such as trauma, hemorrhage, thrombosis, and reperfusion injury. The iron release resulting from HO activity is believed to cause increased expression of ferritin synthesis, which serves to sequester the metal, thus rendering this potential cellular oxidant inactive (42). Bilirubin and biliverdin both act as antioxidants *in vitro* and *in vivo* (24), and their increased local concentrations after HO-1 induction may be beneficial in the protection of endothelial cells from injury.

Overexpression of HO-1 may not only offer a means of cellular protection against heme/hemoglobin-derived oxidative injury, but possibly also against heme-mediated adhesion molecule expression, *via* its degradation and elimination of heme-dependent transcriptional activation of these cell surface molecules. Previous studies in our laboratory, using electrophoretic mobility-shift assays (EMSA) of nuclear protein extracts from heme-treated and control cells with specific oligonucleotide probes containing binding sites for known transcription factors, showed that heme, like IL-1 and TNF-α, is a powerful and rapid activator of transcription factors NF-κB, AP-2, and, to a lesser extent, AP-1 (1). NF-κB is an early mediator of immune and inflammatory responses, which, after activation, is translocated to the nucleus to activate responsive genes encoding defense and signaling proteins (43). Each of the examined adhesion molecules, ICAM-1, VCAM-1, and E selectin, possesses the regulatory sequences for the binding of NF-κB and AP-2, and/or AP-1 in their promoter regions, just as the acute-phase protein HO-1 (1, 38, 39). It is therefore possible that the oxidative agent heme mediates the activation of these genes *via* this pathway. Preliminary immunofluorescent results (data not shown) demonstrated that in cells pretreated with tin mesoporphyrin (SnMP), an inhibitor of HO activity, co-exposure to heme led to induction of ICAM-1. It is therefore likely that heme, and not one of its breakdown products, is responsible for the induction of adhesion molecules.

The fact that the stress protein HO-1 is simultaneously expressed with the adhesion molecules ICAM-1, VCAM-1, and E selectin after exposure to heme suggests a form of feedback system. It is tempting to consider this as a general defense against heme-mediated adhesion molecule expression, since, after inflammation, acute-phase proteins HO-1, haptoglobin, and hemopexin are rapidly expressed. Hemopexin is a heme scavenger protein, and haptoglobin scavenges hemoglobin. Insufficient amounts of HO-1, hemopexin, and haptoglobin may result in cells unable to defend themselves against large amounts of free heme, leading to oxidative injury and adhesion molecule expression. This may explain the various complications found in sickle-cell disease, and the formation of lesions after hemorrhagic injuries (29–31, 44–46).

TNF-α–induced adhesion molecule expression in endothelial cells has been shown to enhance the adherence of hematopoietic cells (47, 48). We are currently investigating the effect of heme, heme scavengers, and heme oxygenase

on the binding of hematopoietic cells to the vascular wall in relation to inflammatory disorders.

In summary, our data demonstrate that heme, in addition to catalyzing the formation of ROS (8–10), may have a regulatory effect on the expression of ICAM-1, VCAM-1, and E selectin in endothelial cells. The inducing effect of heme on the expression of adhesion molecules may explain part of the mechanism by which heme and HO-1 modulate cellular response to inflammatory stimuli. Free heme serves, therefore, as a potential threat for the development of diseases in which inflammatory lesions and oxidative stress play a role. In order to be able to correlate heme-mediated adhesion molecule expression to a clinical situation, we will further investigate the functional importance of the heme-mediated expression of ICAM-1, VCAM-1, and E selectin by studying the adherence of hematopoietic cells to the heme-activated vascular wall, and the possible regulatory role of HO-1 in this process.

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