ANTIOXIDANTS & REDOX SIGNALING
Volume 4, Number 2, 2002

Forum Review

Modulation of Endothelial Cell Apoptosis by Heme Oxygenase-1-Derived Carbon Monoxide

MIGUEL P. SOARES,[1,3] ANNY USHEVA,[2] SOPHIE BROUARD,[1] PASCAL O. BERBERAT,[1] LUKAS GUNTHER,[1] EDDA TOBIASCH,[1] and FRITZ H. BACH[1]

ABSTRACT

It is well established that expression of heme oxygenase-1 (HO-1) acts in a cytoprotective manner in a variety of cell types, including in endothelial cells (EC). We have recently shown that HO-1 expression protects EC from undergoing apoptosis. We have also shown that the antiapoptotic effect of HO-1 is mediated through heme catabolism into the gas carbon monoxide (CO). In this review, we discuss the possible molecular mechanisms by which HO-1-derived CO suppresses EC apoptosis. We will review data suggesting that the antiapoptotic effect of CO acts through the activation of the p38 mitogen-activated protein kinase signal transduction pathway and requires the activation of the transcription factor nuclear factor-κB (NF-κB), as well as the expression of a subset of NF-κB-dependent antiapoptotic genes. Antioxid. Redox Signal. 4, 321–329.

INTRODUCTION

ACUTE INFLAMMATORY REACTIONS, as they occur most often during microbial infections, are essential to initiate the immune responses that lead to microbial clearance and restore normal tissue and organ function. To avoid tissue injury and organ damage, inflammatory reactions must be tightly regulated in a manner that they are terminated as soon as microbial infections have been cleared. When this does not occur, chronic inflammation develops, leading to tissue injury, organ failure, and disease. Due to their localization and function, endothelial cells (EC) play a pivotal role in regulating inflammatory reactions in a manner that prevents disease associated with the development of chronic inflammation.

ENDOTHELIAL CELLS

In their normally quiescent state, EC perform a number of critical functions, including maintenance of anticoagulation and prevention of platelet aggregation, as well as trafficking of cellular and soluble components within blood and neighboring tissues. To achieve these functions, quiescent EC must promote some level of vasorelaxation and inhibit leukocyte adhesion and coagulation (for review, see 15). Generation of basal levels of the gaseous molecules nitric oxide (NO), by endothelial nitric oxide synthase (NOS-1), and carbon monoxide (CO), by heme oxygenase (HO-2), contributes in a critical manner to these functions.

When exposed to proinflammatory stimuli, EC become "activated" and their phenotype changes to one that promotes vasoconstriction, leukocyte adhesion, as well as thrombosis (for reviews, see 15, 43). These changes are the direct result of the induced expression of a series of early responsive proinflammatory genes that encode cytokines/chemokines, adhesion, and costimulatory as well as procoagulant molecules (for reviews, see 15, 43). Expression of these proinflammatory genes is regulated primarily at the level of transcription through a mechanism requiring the activation of the transcription factor nuclear factor-κB (NF-κB) (2, 16, 69, 80).

The NF-κB family of transcription factors consists of several homo- or heterodimeric complexes formed by Rel family members, *e.g.,* p65/RelA, p50, and c-Rel (for review, see 31). In quiescent EC, NF-κB dimers are bound to cytoplasmic

[1]Immunobiology Research Center, Department of Surgery, and [2]Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, 02115 Boston, MA, U.S.A.
[3]Instituto Gulbenkian de Ciência, Apartado 14, 2781–901 Oeiras, Portugal.

molecules of the inhibitor nuclear factor-κB (IκB) family, *i.e.,* IκBα, IκBβ, and IκBε, which mask the nuclear localization domain of NF-κB, thereby preventing its nuclear translocation and transcription activity (7, 31). Once EC are stimulated by proinflammatory stimuli, IκB molecules are rapidly degraded through the 26S proteasome pathway and NF-κB dimers translocate into the nucleus, where they bind to specific decameric recognition motifs in the promoter region of NF-κB-dependent genes (7, 31). This results in up-regulation of the transcription of NF-κB-dependent genes, including proinflammatory genes associated with EC activation. Expression of these genes is transitory, suggesting that EC activation is regulated in a manner that limits the extent of NF-κB activation and thus the expression NF-κB-dependent proinflammatory genes (3, 31). This is a critical feature of EC activation given that prolonged expression of proinflammatory genes would exacerbate inflammatory reactions resulting in EC overstimulation and apoptosis.

One of the mechanisms by which EC are protected from undergoing apoptosis relies on their ability to respond to a large spectrum of inflammatory stimuli by up-regulating the expression of antiapoptotic genes, also referred to as "protective genes" (5). Work by our colleague Christiane Ferran suggests that these genes have a dual function in that they not only protect EC from undergoing apoptosis, but also block NF-κB activation, therefore limiting the extent of expression of NF-κB-dependent proinflammatory genes associated with EC activation (5). According to this "functional definition," protective genes include several genes of the bcl family, *e.g.,* bcl-2, bcl-x_L, and A1, the zinc finger protein A20, the antioxidant manganese superoxide dismutase (MnSOD), and the inducible form of nitric oxide synthase (5).

There are, however, additional antiapoptotic/protective genes that are expressed during EC activation and that can limit the expression of proinflammatory genes without interfering with the NF-κB signal transduction pathway. These include the heat shock protein (hsp)-70 and hsp-32, the latter being also referred to as HO-1. We will review in this article recent data emerging from several laboratories, including our own, suggesting that hsp-32/HO-1 protects EC from undergoing apoptosis and thus contributes in a critical manner to regulate inflammatory reactions.

THE HEME OXYGENASE SYSTEM

Heme oxygenases are the rate-limiting enzymes in the catabolism of heme to yield equimolar amounts of biliverdin, free iron, and CO, with biliverdin being subsequently catabolized into bilirubin by the enzyme biliverdin reductase (34; for reviews, see 14, 42). In their quiescent state, EC express only the noninducible HO-2 isoform. However, when exposed to proinflammatory stimuli, EC up-regulate HO-1 expression, which is critical to maintain EC integrity during inflammatory reactions. The reason for this relates to the fact that reactive oxygen species, generated during inflammatory reactions, denature heme proteins, *e.g.,* hemoglobin and myoglobin, thereby releasing free prooxidant heme (see Fig. 1). As it accumulates, free heme intercalates into EC membranes and becomes internalized, acting as a cytotoxic prooxidant (8–10). The ability of EC to up-regulate the expression of HO-1 under these circumstances is the only known mechanism by which free heme can be rapidly eliminated. In the process of degrading heme, HO-1 generates the gas CO, which others and we have shown to act as a cytoprotective molecule that limits the deleterious effects of inflammatory reactions (see Fig. 1).

CARBON MONOXIDE

CO is a signaling molecule that exerts a large spectrum of biological functions in neurons (27, 74), smooth muscle cells (17, 40), platelets (12, 75), monocyte/macrophages (55), and EC (11). CO can modulate the activation of several signal transduction pathways, including guanylyl cyclase/cyclic GMP (cGMP) (46, 74), and p38 mitogen-activated protein kinase (MAPK) (11, 55) and regulate the expression of vasoconstrictor, proinflammatory, and procoagulant molecules in these cells (21, 55). Presumably this broad action accounts for the ability of CO to promote vasodilation (17, 64), to inhibit inflammation (55), and to suppress apoptosis (11, 57), cell-cycle progression (17, 37), as well as thrombosis (12, 21, 75).

The only molecular target of CO identified so far is iron such as it exists in heme and iron-sulfur clusters contained in a variety of proteins, *e.g.,* guanylyl cyclase, cytochromes, peroxidases, catalase, and nitric oxide synthases. The biological functions attributed to CO are thought to result directly or indirectly from binding of CO to iron in these proteins. Presumably, when this occurs, the conformational structure of these proteins is modified in a manner that modulates their biological activity and thus mediates the different biological functions of CO (see Fig. 1). The paradigm for CO-mediated signal transduction is provided by the well described interaction of CO with the heme moiety of guanylyl cyclase (32). This results in conformational changes in guanylyl cyclase, increasing by two- to fourfold its enzymatic activity and leading to the generation of cGMP (32). Generation of cGMP activates the cGMP-dependent protein kinases (cGKI and II) that act in smooth muscle cells to mediate vasodilation and in platelets to prevent activation/aggregation (17, 58, 64). Presumably, this accounts for the vasodilatory and antithrombotic effects of CO, which are thought to contribute in a critical manner to the overall antiinflammatory effect of CO. However, there are additional biological functions of CO that may contribute to its antiinflammatory effect as well. These include the ability of CO to modulate monocyte/macrophage activation (55) (see accompanying article by Otterbein *et al.*) and to suppress EC apoptosis (11, 57). We will focus in this review on the possible mechanisms by which CO protects EC from undergoing apoptosis and how this may contribute to the overall cytoprotective effect of CO.

ANTIAPOPTOTIC ACTION OF HO-1-DERIVED CO

EC express "death receptors" that can be triggered to initiate signaling transduction pathways leading to apoptosis. These include the tumor necrosis factor-α (TNF-α) receptor 1

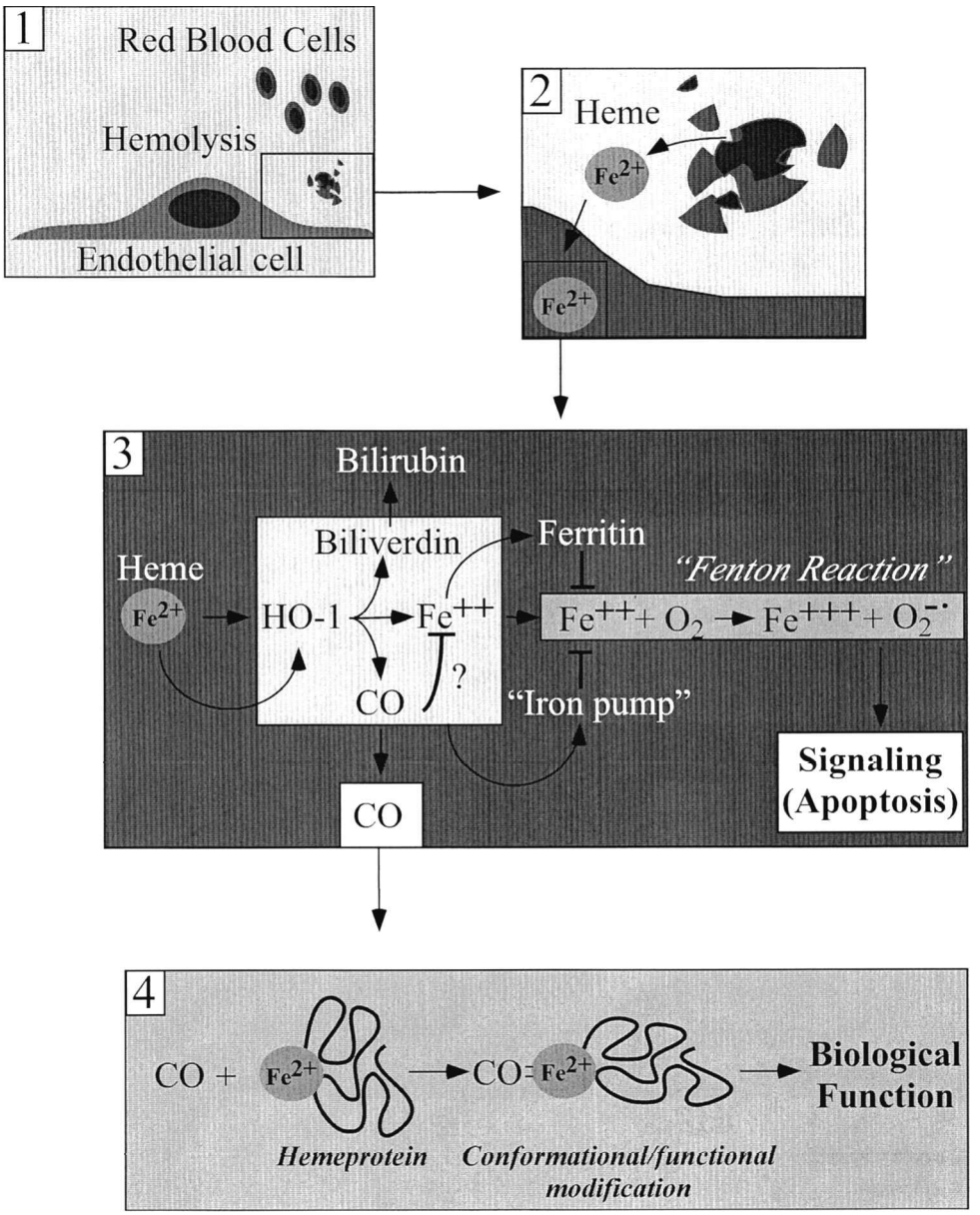

FIG. 1. HO-1-derived CO acts as a signaling molecule in EC. Acute inflammatory reactions are most often associated with tissue necrosis and hemolysis and with release of heme proteins, *e.g.,* myoglobin and/or hemoglobin from red blood cells and myocytes, respectively (see panel **1**). Once exposed to free radicals, these extracellular heme proteins become oxidized, generating prooxidant free heme (see panel **2**). Free heme can intercalate into EC membranes to become incorporated in the intracellular pool of free heme (see panel **2**). The ability of EC to up-regulate HO-1 expression when exposed to extracellular heme allows the levels of intracytoplasmic prooxidant heme to decrease rapidly (see panel **3**). Biliverdin generated through this enzymatic reaction is subsequently converted into bilirubin, which acts as an antioxidant (see panel **3**). Free Fe^{2+}, also released through the action of HO-1 on heme, can promote the generation of free radicals through the Fenton reaction and therefore act as a potent prooxidant (see panel **3**). However, the prooxidant action of free Fe^{2+} may be "neutralized" by the ability of HO-1 to induce the up-regulation of the iron chelator ferritin, as well as the activation/up-regulation of iron pumps that release Fe^{2+} into the extracellular space. CO, the other end product of heme catabolism by HO-1, may also contribute directly to neutralize the availability of Fe^{2+} to participate in the Fenton reaction (see text). In addition, CO can interact with heme groups and iron sulfur clusters in several proteins to induce conformational changes in these proteins and modulate their biological functions (see panel **4**). Presumably, this is the molecular basis underlining the different biological functions of CO, including its ability to suppress EC apoptosis.

(TNFR1/CD120a), which is particularly relevant for EC given the high levels of TNF-α generated by activated monocyte/macrophages at sites of inflammation (59). The intracytoplasmic region of TNFR1 contains one or several sequence homology domains, referred to as "death domains" (for review, see 4). Signaling via these death domains is constitutively repressed through binding of the antiapoptotic protein SODD (silencer of death domains) (30). Once TNFR1 is cross-linked,

SODD is released and the death domains of TNFR1 are exposed (30). The exposed domains recruit other signaling molecules containing additional death domains, *e.g.,* FADD (Fas-associated death domain), TRADD (TNF receptor-associated death domain), and RIP (receptor-interacting protein) (47; for review, see 4). This process results in the generation of a catalytic protein complex referred to as DISC (death-inducing signaling complex) (56), which activates cysteine proteases referred to as caspases (for review, see 73). Interaction between the DISC and the caspase signal transduction pathway occurs via FADD-dependent proteolytic cleavage of the inactive zymogen of caspase 8 and/or caspase 10 and subsequent dimerization of the "active" fragments of these caspases (47). Once activated, these initiator caspases act as cysteine proteases to activate additional caspases, *e.g.,* caspases 2, 3, 6, and 7, that cleave a series of cellular substrates (for review, see 73). In addition to the caspase signal transduction pathway, TNFR1 also activates a signal transduction pathway that results in depolymerization of the mitochondria membrane (81) and release of the proapoptotic proteins, *e.g.,* cytochrome *c* (33), apaf-1 (83), caspase 2 and 9 (70), apoptosis-inducing factor (AIF) (71), and the direct inhibitor of apoptosis (IAP) binding protein with low pI (DIABLO) from the mitochondria into the cytosol (13). These proapoptotic proteins amplify the signal transduction pathway leading to the activation of effector caspases, *e.g.,* caspase 3, thus promoting apoptosis (for review, see 73). Whether or not release of these proapoptotic molecules by the mitochondria is essential for TNF-α-mediated EC apoptosis is not clear. However, the observation that bcl family members that act at the level of the mitochondria, *e.g.,* bcl-2, bcl-x_L, or A1, can suppress TNF-α-mediated EC apoptosis (6) suggests that this is the case.

We have analyzed whether CO modulates the TNF-α-derived signal transduction pathways leading to EC apoptosis. Cultured EC undergo apoptosis when exposed to either high levels of TNF-α or low levels of TNF-α in the presence of the transcription inhibitor actinomycin D (67, 69). When exposed to extracellular heme, EC up-regulate the expression of HO-1 and develop resistance to TNF-α-mediated apoptosis (11). That HO-1 is directly responsible for the protective effect of heme is supported by the observation that overexpression of HO-1 protects EC from TNF-α-mediated apoptosis (11, 68). The protective effect of HO-1 requires its enzymatic activity, indicating that its antiapoptotic effect requires the generation of one or several end products of heme catabolism by HO-1, *i.e.,* Fe^{2+}, biliverdin, and/or CO (11). Because heme-derived Fe^{2+} up-regulates the expression of the iron chelator ferritin (18), we tested whether Fe^{2+} chelation *per se* could account for the antiapoptotic effect of HO-1 in EC. Our data (11), as well as those of others (20), indicate that this is the case: the antiapoptotic effect of HO-1 can be mimicked by the exogenously administered iron chelator deferoxamine (11, 20) and/or by overexpressing the heavy chain of ferritin (P. Berberat *et al.,* unpublished observations), which supports the iron chelation activity of ferritin (25). There is also evidence to suggest that HO-1 up-regulates the expression/activity of an "iron pump" that decreases the levels of intracytoplasmic Fe^{2+} (20). The exact nature of this iron pump remains to be fully characterized. However, these data indicate that the combined effect of ferritin expression and expression/activation of this iron pump decreases the intracytoplasmic pool of free Fe^{2+} available to promote the generation of free radicals through the Fenton reaction (20). Given that antioxidants such as *N*-acetylcysteine and pyrrolidine dithiocarbamate can suppress TNF-α-mediated EC apoptosis (C. Ferran, unpublished observations), it would be reasonable to suggest that one of the main mechanisms by which HO-1 exerts its antiapoptotic function relates to its antioxidant properties and, in particular, to its ability to limit the levels of free Fe^{2+} available to generate reactive oxygen species through the Fenton reaction. This hypothesis, however, remains to be tested. Based on this assumption, others have suggested that reduction of the levels of free intracytoplasmic Fe^{2+}, associated with HO-1 expression, is the main mechanism accounting for the antiapoptotic effect of HO-1 (20). Our recent data, however, provide a more complex picture in that we found that HO-1-derived CO may be equally important for the antiapoptotic effect of HO-1 in EC (11). This is illustrated by the observation that when the action of HO-1-derived CO is inhibited by hemoglobin, HO-1 is no longer able to prevent TNF-α-mediated EC apoptosis (11). Furthermore, exposure of EC to exogenous CO also suppresses TNF-α-mediated EC apoptosis (11). Similar results have been observed in fibroblasts (57), as well as in islet β-cells (21a) and hepatocytes (L. Otterbein *et al.,* unpublished observations). Based on these data, we have concluded that HO-1-derived CO is a cytoprotective molecule that can act in several cell types, including EC, to suppress apoptosis. Others, however, have suggested that levels of CO similar to the ones we have used in our studies can induce EC apoptosis (72). The reason for this discrepancy is not clear at this point. Most likely, this is due to differences in methodology used to evaluate EC apoptosis.

The mechanistic details underlining the antiapoptotic effect of CO in EC are presently unclear. It is possible that this effect of CO may be related to its potential antioxidant properties and, in particular, to the ability of CO to limit the generation of reactive oxygen species (see accompanying article by Otterbein *et al.*). It is well established that CO is a strong π-acceptor and as such might participate and contribute to the formation of cellular *d*-metal–protein clusters. There is such a precedent in cells. Cytochrome P-450 forms mixed (CO/peptide) ligand–Fe^{2+} clusters. This phenomenon is easily identified as it is responsible for the absorption at 450 nm, which gives the protein family its name (44). Further, for example, by changing the $Fe^{2+/3+}$ or $Cu^{+/2+}$ coordination equilibrium in reactions of free metal ions with CO and cellular protein ligands, CO might lower the presence of free metal ions. Therefore, redox reactions would be less likely to complicate the free metal ion behavior, and there will be little risk of generating free radicals that might contribute to TNF-α-mediated EC apoptosis. The idea that CO might participate in the formation of intracellular mixed ligand–metal clusters is echoed in organometallic chemistry, where it has been demonstrated that metal clusters with mixed ligands including CO exist (35).

Another property of CO that may contribute to suppress EC apoptosis relates to its ability to down-regulate the activity of the mitochondrial electron transport chain through direct binding to heme or iron-sulfur clusters in proteins (complexes) of the electron transport chain. This could have at least two important implications. First, CO might inhibit the

generation of free radicals, generated through basal activity of the electron transport chain. Second, CO has been shown in cells other than EC to suppress ATP synthesis, an event required for apoptosis to occur (38). Whether CO acts in such a manner in EC remains to be established.

INTERACTION OF CO WITH ANTIAPOPTOTIC SIGNAL TRANSDUCTION PATHWAYS

The antiapoptotic action of CO in EC seems to be strictly dependent on the activation of the p38 MAPK signal transduction pathway (11). This notion is supported by the observation that expression of HO-1 in EC or exposure of EC to exogenous CO enhances the activation of p38 MAPK and that specific inhibition of p38 MAPK activation abrogates the antiapoptotic effect of HO-1 and/or CO (11). How HO-1 derived CO acts to modulate the activation of p38 MAPK and how the activation of this specific signal transduction pathway acts to suppress EC apoptosis remain to be elucidated.

The family of p38 MAPK groups comprises several kinases, *i.e.,* p38α (CSBP-1 and CSBP-2; 38 kDa) (36), p38β (p38-2/P38β1 and p38β2; 39 kDa) (19, 28), p38γ (ERK6/SAPK3; 43 kDa) (29, 39), and p38δ (SAPK4; 40 kDa) (26, 76) (for review, see 49). These p38 MAPK share sequence homology ranging from 74% (p38α versus p38β) (28) to 98% (p38β versus p38β2) (19) and have a canonical dual phosphorylation site (Thr-Gly-Tyr) (for review, see 49). Activation of p38 MAPK is associated with the phosphorylation of Thr and Tyr residues in the Thr-Gly-Tyr canonical site (61; for review, see 49). The signal transduction pathways leading to the phosphorylation of Thr and/or Tyr residues in these kinases are diverse, but are mostly associated with cell stress (61). In addition, most of the proinflammatory stimuli that lead to EC activation also activate p38 MAPK, *e.g.,* bacterial lipopolysaccharides, interleukin-1α, and TNF-α (11, 79). The signal transduction pathways initiated by these stimuli converge into the activation of one or several MAPK kinases (MAPKK) that phosphorylate/activate p38 MAPK directly (for review, see 49). These MAPKK include MKK3 (19, 62), MKK4 (49), MKK6 (45, 62), and probably MKK7 (26), but MKK3 and MKK6 are thought to play a predominant role in activating p38 MAPK (62, 79). MKK3 can activate all p38 isoforms, whereas MKK6 activates p38α, γ, and δ preferentially (19).

Given that the antiapoptotic effect of CO in EC is dependent on the activation of p38 MAPK, it becomes important to understand how CO modulates the activation of this specific signal transduction pathway. One hypothesis is that CO interacts directly with one or several upstream kinases and/or phosphatases involved in the activation of p38 MAPK. Considering this, one would expect that these kinases and/or phosphatases contain iron-sulfur clusters and/or heme groups with which CO could interact to modulate their activity. Another possibility would be that CO modulates this signal transduction pathway indirectly, such as by interfering with the generation of reactive oxygen species that can modulate the expression/activation of kinases and/or phosphatases involved in p38 MAPK activation.

The mechanism by which CO-derived p38 MAPK activation suppresses EC apoptosis is also not clear. Activation of p38 MAPK can either promote or suppress apoptosis in several cell types, including L929 fibroblasts (63), myocytes (41, 48), HeLa kidney epithelial embryonic cells, or Jurkat T cells (48, 63), as well as other cell types. These opposing effects may be explained by the fact that distinct p38 isoforms seem to have opposite effects in terms of regulating apoptosis. For example, activation of p38α induces apoptosis of cardiac myocytes, HeLa epithelial cells, and Jurkat T cells, whereas activation of p38β suppresses apoptosis in the same cell types (77, 82). The fact that different cell types express different profiles of p38 isoforms (23) and that different signal transduction pathways can lead to specific activation of different p38 isoforms (for review, see 49) may explain the apparent discrepancy of p38 MAPK in controlling apoptosis. One possibility to explain the ability of CO to suppress EC apoptosis would be that somehow CO promotes the activation of p38β, but not that of the p38α isoform. This hypothesis however remains to be tested. It should be noticed that at least in EC, activation of the p38 MAPK γ and δ isoforms does not seem to be required for the protective effect of CO. This notion is supported by the observation that the pyridinyl imidazol SB203580, which blocks the activation of the p38 MAPK α and β isoforms, but not that of the γ and δ isoforms, inhibits the antiapoptotic effect of CO in EC (11).

The mechanism(s) underlining the antiapoptotic effect of CO are probably cell-specific. This notion is supported by the observation that the antiapoptotic effect of CO in fibroblasts is not dependent on the activation of the p38 MAPK signal transduction pathway but instead requires the activation of a signal transduction pathway, that involves the activation of guanylyl cyclase and the generation of cGMP (57). Activation of this cGMP-dependent signal transduction pathway is not required for the antiapoptotic effect of CO in EC (11).

Besides the p38 MAPK signal transduction pathway, HO-1-derived CO also interacts with signal transduction pathways initiated through the activation of the transcription factor NF-κB and that suppress EC apoptosis (11a). This is supported by the observation that inhibition of NF-κB activation by overexpression of the natural inhibitor of NF-κB, *i.e.,* IκBα, suppresses the antiapoptotic effect of CO (11a). Although CO does not activate NF-κB directly, it requires basal NF-κB activity to suppress EC apoptosis. Coexpression of IκBα with basal levels of the NF-κB family members p65/RelA or p65/RelA and c-Rel, but not with p65/RelA and p50, at levels that *per se* do not suppress apoptosis, restores the antiapoptotic effect of CO. Coexpression of IκBα with the NF-κB-dependent antiapoptotic genes A1 or c-IAP2, at levels that *per se* do not suppress apoptosis, also restores the antiapoptotic effect of CO (11a). These data indicate that HO-1-derived CO interacts with a subset of NF-κB-dependent antiapoptotic genes, *i.e.,* A1 and c-IAP-2, to suppress EC apoptosis. The ability of CO to interact with NF-κB-dependent antiapoptotic genes is highly selective in that it does not occur with the NF-κB-dependent antiapoptotic genes A20 or MnSOD. This excludes the possibility that CO would interact randomly with any given set of antiapoptotic genes to promote EC survival and points to a rather selective type of interaction between CO and a restricted subset of antiapoptotic

genes. The antiapoptotic genes A1 and c-IAP2 belong to this group, which does not exclude that other antiapoptotic genes may have similar effects as well.

The physiological significance of the "cross-talk" between CO and NF-κB-dependent antiapoptotic genes in preventing EC apoptosis is not clear. One possibility is that this would act as a mechanism of controlling the antiapoptotic action of CO. In the light of this hypothesis, one should consider that expression of HO-1 and subsequent generation of CO are the immediate reaction of most cell types exposed to oxidative stress (for reviews, see 14, 42). If there were to be no additional mechanisms to control the antiapoptotic action of CO, one would expect that all cells that are exposed to oxidative stress would become resistant to apoptosis, based on their ability to express HO-1 and generate CO. As this is clearly not the case, there must be strict mechanisms by which the antiapoptotic action of CO is controlled. Our hypothesis is that one of such mechanisms relies on the ability to activate additional signal transduction pathways leading to NF-κB activation. In EC, these additional stimuli can be provided through a multitude of sources, including integrin-mediated signaling (66) and/or proinflammatory stimuli such as TNF-α, interleukin-1, and/or lipopolysaccharide (for review, see 43). When this occurs, EC express a subset of NF-κB-dependent antiapoptotic genes, *e.g.,* A1 and c-IAP2, that interact with CO to suppress apoptosis.

CONCLUDING REMARKS

Expression of HO-1 suppresses inflammatory responses associated with endotoxic shock (50, 51, 60), hyperoxia (53, 54), acute pleurisy (78), ischemia/reperfusion injury (1), and rejection of transplanted organs (24, 68). The current data emerging from several laboratories would suggest that these effects of HO-1 are largely mediated through the generation of CO. In several instances, CO alone (in the absence of HO-1 function) will mediate the same effects as HO-1: inhaled CO prevents inflammatory reactions associated with hyperoxia (52), ischemia/reperfusion injury (21), and graft rejection (65) in a manner that mimics that of HO-1. The ability of HO-1-derived CO to prevent EC apoptosis may account in large measure for its cytoprotective function because EC apoptosis is a proinflammatory event that contributes in a critical manner to the pathogenesis of these inflammatory reactions (22). We believe that the data reviewed here, which reveal the potent antiapoptotic effect of CO, will contribute to the development of new approaches to overcome pathologic conditions associated with acute and/or chronic inflammation, including septic shock, atherosclerosis, and/or the rejection of immediately vascularized transplanted organs, as well as cell transplants.

ACKNOWLEDGMENTS

Miguel P. Soares was a recipient of the Phyllis and Paul Fireman 2000 Fellowship from Harvard Medical School. Fritz H. Bach is the Lewis Thomas professor at Harvard Medical School. The work from our laboratories was supported by a grant from the Roche Organ Transplantation Research Foundation (ROTRF; 998521355) awarded to M.P.S.; NIH grants awarded to M.P.S. (HL67040), F.H.B. (HL58688), and A.U. (HL62458); American Heart Association grant awarded to A.U.; and the Edward Mallinckrodt, Jr., Foundation grant awarded to A.U. Sophie Brouard was supported by a fellowship grant from Association pour la Recherche sur le Cancer (ARC) and from Institut National de la Santé et de la Recherche Medicale (INSERM), France. Pascal O. Berberat was supported by a fellowship grant from the Swiss National Science Foundation. Lukas Gunther was supported by a fellowship grant from the Deutsche Forschungsgemeinschaft (DFG), Germany.

ABBREVIATIONS

cGMP, cyclic GMP; CO, carbon monoxide; DISC, death-inducing signaling complex; EC, endothelial cell; FADD, Fas-associated death domain; HO, heme oxygenase; hsp, heat shock protein; IAP, inhibitor of apoptosis; IκB, inhibitor nuclear factor-κB; MAPK, Mitogen-activated Protein Kinases; MAPKK, MAPK kinase; MnSOD, manganese superoxide dismutase; NF-κB, nuclear factor-κB; SODD, silencer of death domains; TNF-α, tumor necrosis factor-α; TNFR1, TNF-α receptor 1.

REFERENCES

1. Amersi F, Buelow R, Kato H, Ke B, Coito A, Shen X, Zhao D, Zaky J, Melinek J, Lassman C, Kolls J, Alam J, Ritter T, Volk H, Farmer D, Ghobrial R, Busuttil R, and Kupiec-Weglinski J. Upregulation of heme oxygenase-1 protects genetically fat Zucker rat livers from ischemia/reperfusion injury. *J Clin Invest* 104: 1631–1639, 1999.
2. Anrather J, Csizmadia V, Brostjan C, Soares MP, Bach FH, and Winkler H. Inhibition of bovine endothelial cell activation in vitro by regulated expression of a transdominant inhibitor of NF-κB. *J Clin Invest* 99: 763–772, 1997.
3. Anrather J, Csizmadia V, Soares MP, and Winkler H. Regulation of NF-κB RelA phosphorylation and transcriptional activity by p21(ras) and protein kinase Cζ in primary endothelial cells. *J Biol Chem* 274: 13594–13603, 1999.
4. Ashkenazi A and Dixit VM. Death receptors—signaling and modulation. *Science* 281: 1305–1308, 1998.
5. Bach FH, Hancock WW, and Ferran C. Protective genes expressed in endothelial cells—a regulatory response to injury. *Immunol Today* 18: 483–486, 1997.
6. Badrichani AZ, Stroka DM, Bilbao G, Curiel DT, Bach FH, and Ferran C. Bcl-2 and Bcl-XL serve an anti-inflammatory function in endothelial cells through inhibition of NF-κB. *J Clin Invest* 103: 543–553, 1999.
7. Baeuerle PA and Baltimore D. IκB: a specific inhibitor of the NF-κB transcription factor. *Science* 242: 540–546, 1988.
8. Balla G, Jacob HS, Balla J, Rosenberg M, Nath K, Apple F, Eaton JW, and Vercellotti GM. Ferritin: a cytoprotective antioxidant strategem of endothelium. *J Biol Chem* 267: 18148–18153, 1992.
9. Balla J, Jacob HS, Balla G, Nath K, Eaton JW, and Vercellotti GM. Endothelial-cell heme uptake from heme pro-

teins: induction of sensitization and desensitization to oxidant damage. *Proc Natl Acad Sci U S A* 90: 9285–9289, 1993.

10. Balla J, Nath KA, Balla G, Juckett MB, Jacob HS, and Vercellotti GM. Endothelial cell heme oxygenase and ferritin induction in rat lung by hemoglobin in vivo. *Am J Physiol* 268: l321–l327, 1995.
11. Brouard S, Otterbein LE, Anrather J, Tobiasch E, Bach FH, Choi AMK, and Soares M. Carbon monoxide generated by heme oxygenase-1 suppresses endothelial cell apoptosis. *J Exp Med* 192: 1015–1025, 2000.

11a. Brouard S, Berberat OP, Seldon PM, Tobiasch E, Bach FH, and Soares MP. Heme oxygenase-1 derived carbon monoxide requires the activation of the transcription factor NF-κB to protect endothelial cells from TNF-α mediated apoptosis. *J Biol Chem* 2002 (in press).

12. Brune B and Ullrich V. Inhibition of platelet aggregation by carbon monoxide is mediated by activation of guanylate cyclase. *Mol Pharmacol* 32: 497–504, 1987.
13. Chai J, Du C, Wu JW, Kyin S, Wang X, and Shi Y. Structural and biochemical basis of apoptotic activation by Smac/DIABLO. *Nature* 406: 855–862, 2000.
14. Choi AM and Alam J. Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am J Respir Cell Mol Biol* 15: 9–19, 1996.
15. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, Pober JS, Wick TM, Konkle BA, Schwartz BS, Barnathan ES, McCrae KR, Hug BA, Schmidt AM, and Stern DM. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* 91: 3527–3561, 1998.
16. Collins T, Read MA, Neish AS, Whitley MZ, Thanos D, and Maniatis T. Transcriptional regulation of endothelial cell adhesion molecules: NF-κB and cytokine-inducible enhancers. *FASEB J* 9: 899–909, 1995.
17. Duckers HJ, Boehm M, True AL, Yet S, San H, Park JLR, Webb C, Lee M, Nabel GJ, and Nabel EG. Heme oxygenase-1 protects against vascular constriction and proliferation. *Nat Med* 7: 693–698, 2001.
18. Eisenstein RS, Garcia MD, Pettingell W, and Munro HN. Regulation of ferritin and heme oxygenase synthesis in rat fibroblasts by different forms of iron. *Proc Natl Acad Sci U S A* 88: 688–692, 1991.
19. Enslen H, Raingeaud J, and Davis RJ. Selective activation of p38 mitogen-activated protein (MAP) kinase isoforms by the MAP kinase kinases MKK3 and MKK6. *J Biol Chem* 273: 1741–1748, 1998.
20. Ferris C, Jaffrey S, Sawa A, Takahashi M, Brady S, Barrow R, Tysoc S, Wolosker H, Baranano D, Dore S, Poss K, and Snyder SH. Haem oxygenase-1 prevents cell death by regulating cellular iron. *Nat Cell Biol* 1: 152–157, 1999.
21. Fujita T, Toda K, Karimova A, Yan SF, Naka Y, Yet SF, and Pinsky DJ. Paradoxical rescue from ischemic lung injury by inhaled carbon monoxide driven by derepression of fibrinolysis. *Nat Med* 7: 598–604, 2001.

21a. Gunther L, Berberat PO, Haga M, Brouard S, Smith RN, Soares MP, Bach FH, and Tobiasch E. Carbon monoxide protects pancreatic β-cells from apoptosis and improves islet function/survival after transplantation. *Diabetes* 51: 994–999, 2002.

22. Haimovitz FA, Cordon-Cardo C, Bayoumy S, Garzotto M, McLoughlin M, Gallily M, Edwards C III, Schuchman EH, Fuks Z, and Kolesnick R. Lipopolysaccharide induces disseminated endothelial cell apoptosis requiring ceramide. *J Exp Med* 186: 1831–1841, 1997.
23. Hale KK, Trollinger D, Rihanek M, and Manthey CL. Differential expression and activation of p38 mitogen-activated protein kinase alpha, beta, gamma, and delta in inflammatory cell lineages. *J Immunol* 162: 4246–4252, 1999.
24. Hancock WW, Buelow R, Sayegh MH, and Turka LA. Antibody-induced transplant arteriosclerosis is prevented by graft expression of anti-oxidant and anti-apoptotic genes. *Nat Med* 4: 1392–1396, 1998.
25. Harrison PM and Arosio P. The ferritins: molecular properties, iron storage function and cellular regulation. *Biochim Biophys Acta* 1275: 161–203, 1996.
26. Hu MC, Wang YP, Mikhail A, Qiu WR, and Tan TH. Murine p38-delta mitogen-activated protein kinase, a developmentally regulated protein kinase that is activated by stress and proinflammatory cytokines. *J Biol Chem* 274: 7095–7102, 1999.
27. Ingi T, Cheng J, and Ronnett GV. Carbon monoxide: an endogenous modulator of the nitric oxide–cyclic GMP signaling system. *Neuron* 16: 835–842, 1996.
28. Jiang Y, Chen C, Li Z, Guo W, Gegner JA, Lin S, and Han J. Characterization of the structure and function of a new mitogen-activated protein kinase (p38β). *J Biol Chem* 271: 17920–17926, 1996.
29. Jiang Y, Gram H, Zhao M, New L, Gu J, Feng L, Di Padova F, Ulevitch RJ, and Han J. Characterization of the structure and function of the fourth member of p38 group mitogen-activated protein kinases, p38δ. *J Biol Chem* 272: 30122–30128, 1997.
30. Jiang Y, Woronicz JD, Liu W, and Goeddel DV. Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. *Science* 283: 543–546, 1999.
31. Karin M and Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-κB activity. *Annu Rev Immunol* 18: 621–663, 2000.
32. Kharitonov VG, Sharma VS, Pilz RB, Magde D, and Koesling D. Basis of guanylate cyclase activation by carbon monoxide. *Proc Natl Acad Sci U S A* 92: 2568–2571, 1995.
33. Kluck RM, Bossy WE, Green DR, and Newmeyer DD. The release of cytochrome *c* from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* 275: 1132–1136, 1997.
34. Kutty RK, Daniel RF, Ryan DE, Levin W, and Maines MD. Rat liver cytochrome P-450b, P-420b, and P-420c are degraded to biliverdin by heme oxygenase. *Arch Biochem Biophys* 260: 638–644, 1988.
35. Lavigne G. Cluster-assisted ligand transformations. In: *The Chemistry of Metal Cluster Complexes,* edited by Shriver DF, Kaesz HD, and Adams RD, Weinheim, Germany: VCH, 1990, pp. 320–415.
36. Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D, McNulty D, Blumenthal MJ, Heys JR, Landvatter SW, *et al.* A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 372: 739–746, 1994.

37. Lee PJ, Alam J, Wiegand GW, and Choi AM. Overexpression of heme oxygenase-1 in human pulmonary epithelial cells results in cell growth arrest and increased resistance to hyperoxia. *Proc Natl Acad Sci U S A* 93: 10393–10398, 1996.
38. Leist M, Single B, Castoldi AF, Kuhnle S, and Nicotera P. Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J Exp Med* 185: 1481–1486, 1997.
39. Li Z, Jiang Y, Ulevitch RJ, and Han J. The primary structure of p38 gamma: a new member of p38 group of MAP kinases. *Biochem Biophys Res Commun* 228: 334–340, 1996.
40. Liu Y, Christou H, Morita T, Laughner E, Semenza GL, and Kourembanas S. Carbon monoxide and nitric oxide suppress the hypoxic induction of vascular endothelial growth factor gene via the 5′ enhancer. *J Biol Chem* 273: 15257–15262, 1998.
41. Mackay K and Mochly-Rosen D. An inhibitor of p38 mitogen-activated protein kinase protects neonatal cardiac myocytes from ischemia. *J Biol Chem* 274: 6272–6279, 1999.
42. Maines MD. The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* 37: 517–554, 1997.
43. Mantovani A, Bussolino F, and Introna M. Cytokine regulation of endothelial cell function—from molecular level to the bedside. *Immunol Today* 18: 231–240, 1997.
44. Moore GR and Pettigrew G. Cytochrome *c*: structural and physicochemical aspects. Berlin: Springer Verlag, 1990.
45. Moriguchi T, Toyoshima F, Gotoh Y, Iwamatsu A, Irie K, Mori E, Kuroyanagi N, Hagiwara M, Matsumoto K, and Nishida E. Purification and identification of a major activator for p38 from osmotically shocked cells. Activation of mitogen-activated protein kinase kinase 6 by osmotic shock, tumor necrosis factor-alpha, and H_2O_2. *J Biol Chem* 271: 26981–26988, 1996.
46. Morita T, Perrella MA, Lee ME, and Kourembanas S. Smooth muscle cell-derived carbon monoxide is a regulator of vascular cGMP. *Proc Natl Acad Sci U S A* 92: 1475–1479, 1995.
47. Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, Scaffidi C, Bretz JD, Zhang M, Gentz R, Mann M, Krammer PH, Peter ME, and Dixit VM. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. *Cell* 85: 817–827, 1996.
48. Nemoto S, Xiang J, Huang S, and Lin A. Induction of apoptosis by SB202190 through inhibition of p38beta mitogen-activated protein kinase. *J Biol Chem* 273: 16415–16420, 1998.
49. Ono K and Han J. The p38 signal transduction pathway: activation and function. *Cell Signal* 12: 1–13, 2000.
50. Otterbein L, Sylvester SL, and Choi AM. Hemoglobin provides protection against lethal endotoxemia in rats: the role of heme oxygenase-1. *Am J Respir Cell Mol Biol* 13: 595–601, 1995.
51. Otterbein L, Chin BY, Otterbein SL, Lowe VC, Fessler HE, and Choi AM. Mechanism of hemoglobin-induced protection against endotoxemia in rats: a ferritin-independent pathway. *Am J Physiol* 272: L268–L275, 1997.
52. Otterbein LE, Mantell LL, and Choi AM. Carbon monoxide provides protection against hyperoxic lung injury. *Am J Physiol* 276: L688–L694, 1999.
53. Otterbein LE, Kolls JK, Mantell LL, Cook JL, Alam J, and Choi AM. Exogenous administration of heme oxygenase-1 by gene transfer provides protection against hyperoxia-induced lung injury. *J Clin Invest* 103: 1047–1054, 1999.
54. Otterbein LE, Lee PJ, Chin BY, Petrache I, Camhi SL, Alam J, and Choi AM. Protective effects of heme oxygenase-1 in acute lung injury. *Chest* 116: 61S–63S, 1999.
55. Otterbein LE, Bach FH, Alam J, Soares MP, Tao HL, Wysk M, Davis R, Flavell R, and Choi AMK. Carbon monoxide mediates anti-inflammatory effects via the mitogen activated protein kinase pathway. *Nat Med* 6: 422–428, 2000.
56. Peter ME and Krammer PH. Mechanisms of CD95 (APO-1/Fas)-mediated apoptosis. *Curr Opin Immunol* 10: 545–551, 1998.
57. Petrache I, Otterbein LE, Alam J, Wiegand GW, and Choi AM. Heme oxygenase-1 inhibits TNF-α-induced apoptosis in cultured fibroblast. *Am J Physiol Lung Cell Mol Physiol* 278: 312–319, 2000.
58. Pfeifer A, Klatt P, Massberg S, Ny L, Sausbier M, Hirneiss C, Wang GX, Korth M, Aszodi A, Andersson KE, Krombach F, Mayerhofer A, Ruth P, Fassler R, and Hofmann F. Defective smooth muscle regulation in cGMP kinase I-deficient mice. *EMBO J* 17: 3045–3051, 1998.
59. Pober JS, Bevilacqua MP, Mendrick DL, Lapierre LA, Fiers W, and Gimbrone MA Jr. Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. *J Immunol* 136: 1680–1687, 1986.
60. Poss KD and Tonegawa S. Reduced stress defense in heme oxygenase 1-deficient cells. *Proc Natl Acad Sci U S A* 94: 10925–10930, 1997.
61. Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch RJ, and Davis RJ. Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *J Biol Chem* 270: 7420–7426, 1995.
62. Raingeaud J, Whitmarsh AJ, Barrett T, Derijard B, and Davis RJ. MKK3- and MKK6-regulated gene expression is mediated by the p38 mitogen-activated protein kinase signal transduction pathway. *Mol Cell Biol* 16: 1247– 1255, 1996.
63. Roulston A, Reinhard C, Amiri P, and Williams LT. Early activation of c-Jun N-terminal kinase and p38 kinase regulate cell survival in response to tumor necrosis factor alpha. *J Biol Chem* 273: 10232–10239, 1998.
64. Sammut IA, Foresti R, Clark JE, Exon DJ, Vesely MJ, Sarathchandra P, Green CJ, and Motterlini R. Carbon monoxide is a major contributor to the regulation of vascular tone in aortas expressing high levels of haeme oxygenase-1. *Br J Pharmacol* 125: 1437–1444, 1998.
65. Sato K, Balla J, Otterbein L, Smith NR, Brouard S, Lin Y, Csizmadia E, Sevigny J, Robson SC, Vercellotti G, Choi AMK, Bach FH, and Soares MP. Carbon monoxide generated by heme oxygenase-1 suppresses the rejection of mouse-to-rat cardiac transplants. *J Immunol* 166: 4185–4194, 2001.

66. Scatena M, Almeida M, Chaisson ML, Fausto N, Nicosia RF, and Giachelli CM. NF-kappaB mediates alphavbeta3 integrin-induced endothelial cell survival. *J Cell Biol* 141: 1083–1093, 1998.
67. Slowik MR, Min W, Ardito T, Karsan A, Kashgarian M, and Pober JS. Evidence that tumor necrosis factor triggers apoptosis in human endothelial cells by interleukin-1-converting enzyme-like protease-dependent and -independent pathways. *Lab Invest* 77: 257–267, 1997.
68. Soares MP, Lin Y, Anrather J, Csizmadia E, Takigami K, Sato K, Grey ST, Colvin RB, Choi AM, Poss KD, and Bach FH. Expression of heme oxygenase-1 (HO-1) can determine cardiac xenograft survival. *Nat Med* 4: 1073–1077, 1998.
69. Soares MP, Muniappan A, Kaczmarek E, Koziak K, Wrighton CJ, Steinhauslin F, Ferran C, Winkler H, Bach FH, and Anrather J. Adenovirus mediated expression of a dominant negative mutant of p65/RelA inhibits proinflammatory gene expression in endothelial cells without sensitizing to apoptosis. *J Immunol* 161: 4572–4582, 1998.
70. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Brenner C, Larochette N, Prevost MC, Alzari PM, and Kroemer G. Mitochondrial release of caspase-2 and -9 during the apoptotic process. *J Exp Med* 189: 381–394, 1999.
71. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, and Kroemer G. Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 397: 441–446, 1999.
72. Thom SR, Fisher D, Xu YA, Notarfrancesco K, and Ischiropoulos H. Adaptive responses and apoptosis in endothelial cells exposed to carbon monoxide. *Proc Natl Acad Sci U S A* 97: 1305–1310, 2000.
73. Thornberry N and Lazebnik Y. Caspases: enemies within. *Science* 281: 1312–1316, 1998.
74. Verma A, Hirsch DJ, Glatt CE, Ronnett GV, and Snyder SH. Carbon monoxide: a putative neural messenger. *Science* 259: 381–384, 1993.
75. Wagner CT, Durante W, Christodoulides N, Hellums JD, and Schafer AI. Hemodynamic forces induce the expression of heme oxygenase in cultured vascular smooth muscle cells. *J Clin Invest* 100: 589–596, 1997.
76. Wang XS, Diener K, Manthey CL, Wang S, Rosenzweig B, Bray J, Delaney J, Cole CN, Chan-Hui PY, Mantlo N, Lichenstein HS, Zukowski M, and Yao Z. Molecular cloning and characterization of a novel p38 mitogen-activated protein kinase. *J Biol Chem* 272: 23668–23674, 1997.
77. Wang Y, Huang S, Sah VP, Ross J Jr, Brown JH, Han J, and Chien KR. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. *J Biol Chem* 273: 2161–2168, 1998.
78. Willis D, Moore AR, Frederick R, and Willoughby DA. Heme oxygenase: a novel target for the modulation of the inflammatory response. *Nat Med* 2: 87–90, 1996.
79. Wysk M, Yang DD, Lu HT, Flavell RA, and Davis RJ. Requirement of mitogen-activated protein kinase kinase 3 (MKK3) for tumor necrosis factor-induced cytokine expression. *Proc Natl Acad Sci U S A* 96: 3763–3768, 1999.
80. Wrighton CJ, Hofer-Warbinek R, Moll T, Eytner R, Bach FH, and de Martin R. Inhibition of endothelial cell activation by adenovirus-mediated expression of IκBα, an inhibitor of the transcription factor NF-κB. *J Exp Med* 183: 1013–1022, 1996.
81. Zamzami N, Marchetti P, Castedo M, Decaudin D, Macho A, Hirsch T, Susin SA, Petit PX, Mignotte B, and Kroemer G. Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death. *J Exp Med* 182: 367–377, 1995.
82. Zechner D, Craig R, Hanford DS, McDonough PM, Sabbadini RA, and Glembotski CC. MKK6 activates myocardial cell NF-κB and inhibits apoptosis in a p38 mitogen-activated protein kinase-dependent manner. *J Biol Chem* 273: 8232–8239, 1998.
83. Zou H, Henzel WJ, Liu X, Lutschg A, and Wang X. Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome *c*-dependent activation of caspase-3 [see comments]. *Cell* 90: 405–413, 1997.

Address reprint requests to:
Miguel P. Soares, Ph.D.
Immunobiology Research Center
Beth Israel Deaconess Medical Center
Harvard Medical School
99 Brookline Avenue
Boston, MA 02215

E-mail: msoares@caregroup.harvard.edu

Received for publication June 15, 2001; accepted August 15, 2001.

This article has been cited by:

1. Nishani T. Hettiarachchi , John P. Boyle , Claudia C. Bauer , Mark L. Dallas , Hugh A. Pearson , Shuichi Hara , Nikita Gamper , Chris Peers . 2012. Peroxynitrite Mediates Disruption of Ca2+ Homeostasis by Carbon Monoxide via Ca2+ ATPase Degradation. *Antioxidants & Redox Signaling* **17**:5, 744-755. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
2. Julie Devallière, Béatrice Charreau. 2011. The adaptor Lnk (SH2B3): An emerging regulator in vascular cells and a link between immune and inflammatory signaling. *Biochemical Pharmacology* **82**:10, 1391-1402. [CrossRef]
3. Mathias Chatelais, Julie Devallière, Cesare Galli, Béatrice Charreau. 2011. Gene transfer of the adaptor Lnk (SH2B3) prevents porcine endothelial cell activation and apoptosis: implication for xenograft's cytoprotection. *Xenotransplantation* **18**:2, 108-120. [CrossRef]
4. Urte Hinkelmann, Nina Grosser, Kati Erdmann, Henning Schröder, Stephan Immenschuh. 2010. Simvastatin-dependent up-regulation of heme oxygenase-1 via mRNA stabilization in human endothelial cells#. *European Journal of Pharmaceutical Sciences* **41**:1, 118-124. [CrossRef]
5. Ruey-Horng Shih , Shin-Ei Cheng , Wei-Hsuan Tung , Chuen-Mao Yang . 2010. Up-Regulation of Heme Oxygenase-1 Protects Against Cold Injury-Induced Brain Damage: A Laboratory-Based Study. *Journal of Neurotrauma* **27**:8, 1477-1487. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. Raffaella Gozzelino, Viktoria Jeney, Miguel P. Soares. 2010. Mechanisms of Cell Protection by Heme Oxygenase-1. *Annual Review of Pharmacology and Toxicology* **50**:1, 323-354. [CrossRef]
7. Atsushi Ikeda, Shinya Ueki, Atsunori Nakao, Koji Tomiyama, Mark A. Ross, Donna B. Stolz, David A. Geller, Noriko Murase. 2009. Liver graft exposure to carbon monoxide during cold storage protects sinusoidal endothelial cells and ameliorates reperfusion injury in rats. *Liver Transplantation* **15**:11, 1458-1468. [CrossRef]
8. Barbara Wegiel, Leo Otterbein. 2009. Heme oxygenase 1. *AfCS-Nature Molecule Pages* . [CrossRef]
9. Amy Bishop, Renea Gooch, Asuka Eguchi, Stephanie Jeffrey, Lorraine Smallwood, James Anderson, Alvaro G. Estevez. 2009. Mitigation of peroxynitrite-mediated nitric oxide (NO) toxicity as a mechanism of induced adaptive NO resistance in the CNS. *Journal of Neurochemistry* **109**:1, 74-84. [CrossRef]
10. Zhongqiu Wang, Junwen Zhang, Hao Liu, Hairong Huang, Changtian Wang, Yi Shen, Demin Li, Hua Jing. 2009. Melatonin, a potent regulator of hemeoxygenase-1, reduces cardiopulmonary bypass-induced renal damage in rats. *Journal of Pineal Research* **46**:3, 248-254. [CrossRef]
11. Hyun Ju Song, Chang Yell Shin, Tae Young Oh, Young Sil Min, Eon Sub Park, Uy Dong Sohn. 2009. Eupatilin with Heme Oxygenase-1-Inducing Ability Protects Cultured Feline Esophageal Epithelial Cells from Cell Damage Caused by Indomethacin. *Biological & Pharmaceutical Bulletin* **32**:4, 589-596. [CrossRef]
12. Daya R. Varma, Shree Mulay, Sylvain ChemtobCarbon Monoxide: From Public Health Risk to Painless Killer 271-292. [CrossRef]
13. Agnieszka Loboda , Agnieszka Jazwa , Anna Grochot-Przeczek , Andrzej J. Rutkowski , Jaroslaw Cisowski , Anupam Agarwal , Alicja Jozkowicz , Jozef Dulak . 2008. Heme Oxygenase-1 and the Vascular Bed: From Molecular Mechanisms to Therapeutic Opportunities. *Antioxidants & Redox Signaling* **10**:10, 1767-1812. [Abstract] [Full Text PDF] [Full Text PDF with Links]
14. Masato Tachibana, Kenta Watanabe, Yuki Yamasaki, Hiroshi Suzuki, Masahisa Watarai. 2008. Expression of heme oxygenase-1 is associated with abortion caused by Brucella abortus infection in pregnant mice. *Microbial Pathogenesis* **45**:2, 105-109. [CrossRef]
15. Hyun Ju Song, Chang Yell Shin, Tae Young Oh, Uy Dong Sohn. 2008. The protective effect of eupatilin on indomethacin-induced cell damage in cultured feline ileal smooth muscle cells: Involvement of HO-1 and ERK. *Journal of Ethnopharmacology* **118**:1, 94-101. [CrossRef]

16. Ulrich Goebel, Matthias Siepe, Anne Mecklenburg, Phillip Stein, Martin Roesslein, Christian I. Schwer, Rene Schmidt, Torsten Doenst, Klaus K. Geiger, Heike L. Pahl, Christian Schlensak, Torsten Loop. 2008. Carbon Monoxide Inhalation Reduces Pulmonary Inflammatory Response during Cardiopulmonary Bypass in Pigs. *Anesthesiology* **108**:6, 1025-1036. [CrossRef]
17. Stefan Mustafa, Ansgar Weltermann, Robert Fritsche, Claudia Marsik, Oswald Wagner, Paul A. Kyrle, Sabine Eichinger. 2008. Genetic variation in heme oxygenase 1 (HMOX1) and the risk of recurrent venous thromboembolism. *Journal of Vascular Surgery* **47**:3, 566-570. [CrossRef]
18. Thomas Laumonier, Sheng Yang, Stephane Konig, Christine Chauveau, Ignacio Anegon, Pierre Hoffmeyer, Jacques Menetrey. 2008. Lentivirus Mediated HO-1 Gene Transfer Enhances Myogenic Precursor Cell Survival After Autologous Transplantation in Pig. *Molecular Therapy* **16**:2, 404-410. [CrossRef]
19. Jozef Dulak . 2007. Changing Faces of Heme Oxygenases. *Antioxidants & Redox Signaling* **9**:12, 2043-2048. [Citation] [Full Text PDF] [Full Text PDF with Links]
20. A I Goodman, R Olszanecki, L M Yang, S Quan, M Li, S Omura, D E Stec, N G Abraham. 2007. Heme oxygenase-1 protects against radiocontrast-induced acute kidney injury by regulating anti-apoptotic proteins. *Kidney International* **72**:8, 945-953. [CrossRef]
21. Faikah Gueler, Joon-Keun Park, Song Rong, Torsten Kirsch, Carsten Lindschau, Wen Zheng, Marlies Elger, Anette Fiebeler, Danilo Fliser, Friedrich C. Luft, Hermann Haller. 2007. Statins Attenuate Ischemia-Reperfusion Injury by Inducing Heme Oxygenase-1 in Infiltrating Macrophages. *The American Journal of Pathology* **170**:4, 1192-1199. [CrossRef]
22. D SACERDOTI, C COLOMBRITA, M DIPASCOLI, M SCHWARTZMAN, M BOLOGNESI, J FALCK, A GATTA, N ABRAHAM. 2007. 11,12-Epoxyeicosatrienoic acid stimulates heme-oxygenase-1 in endothelial cells#. *Prostaglandins & Other Lipid Mediators* **82**:1-4, 155-161. [CrossRef]
23. Libor Vítek, Harvey A. SchwertnerThe Heme Catabolic Pathway and its Protective Effects on Oxidative Stress#Mediated Diseases **43**, 1-57. [CrossRef]
24. T UCHIYAMA, H ATSUTA, T UTSUGI, Y OHYAMA, T NAKAMURA, A NAKAI, M NAKATA, I MARUYAMA, H TOMURA, F OKAJIMA. 2006. Simvastatin induces heat shock factor 1 in vascular endothelial cells. *Atherosclerosis* **188**:2, 265-273. [CrossRef]
25. Paulo N. A. Martins, Anil Chandraker, Stefan G. Tullius. 2006. Modifying graft immunogenicity and immune response prior to transplantation: potential clinical applications of donor and graft treatment. *Transplant International* **19**:5, 351-359. [CrossRef]
26. Y HUANG, J LI, Q CAO, S YU, X LV, Y JIN, L ZHANG, Y ZOU, J GE. 2006. Anti-oxidative effect of triterpene acids of Eriobotrya japonica (Thunb.) Lindl. leaf in chronic bronchitis rats. *Life Sciences* **78**:23, 2749-2757. [CrossRef]
27. Alexandra Kadl , Norbert Leitinger . 2005. The Role of Endothelial Cells in the Resolution of Acute Inflammation. *Antioxidants & Redox Signaling* **7**:11-12, 1744-1754. [Abstract] [Full Text PDF] [Full Text PDF with Links]
28. Beata Kie#-Wilk, Anna Polus, Joanna Grzybowska, Magdalena Miko#ajczyk, Jadwiga Hartwich, Juliusz Pryjma, Joanna Skrzeczy#ska, Aldona Dembi#ska-Kie#. 2005. #-Carotene stimulates chemotaxis of human endothelial progenitor cells. *Clinical Chemistry and Laboratory Medicine* **43**:5, 488-498. [CrossRef]
29. Pascal O Berberat, Yousif I A-Rahim, Kenichiro Yamashita, Michel M Warny, Eva Csizmadia, Simon C Robson, Fritz H Bach. 2005. Heme oxygenase-1-generated biliverdin ameliorates experimental murine colitis. *Inflammatory Bowel Diseases* **11**:4, 350-359. [CrossRef]
30. C HOLWEG, A BALK, A UITTERLINDEN, H NIESTERS, L MAAT, W WEIMAR, C BAAN. 2005. Functional heme oxygenase-1 promoter polymorphism in relation to heart failure and cardiac transplantation. *The Journal of Heart and Lung Transplantation* **24**:4, 493-497. [CrossRef]

31. Ping Zhou, Nagesh Kalakonda, Raymond L. Comenzo. 2005. Changes in gene expression profiles of multiple myeloma cells induced by arsenic trioxide (ATO): possible mechanisms to explain ATO resistance in vivo. *British Journal of Haematology* **128**:5, 636-644. [CrossRef]
32. Niels Olsen Saraiva Camara, Miguel Parreira Soares. 2005. Heme oxygenase-1 (HO-1), a protective gene that prevents chronic graft dysfunction. *Free Radical Biology and Medicine* **38**:4, 426-435. [CrossRef]
33. A BISHOP, S YET, M LEE, M PERRELLA, B DEMPLE. 2004. A key role for heme oxygenase-1 in nitric oxide resistance in murine motor neurons and glia. *Biochemical and Biophysical Research Communications* **325**:1, 3-9. [CrossRef]
34. St??phanie Le Bas-Bernardet, St??phanie Coupel, Annabelle Chauveau, Jean-Paul Soulillou, B??atrice Charreau. 2004. Vascular Endothelial Cells Evade Apoptosis Triggered by Human Leukocyte Antigen-DR Ligation Mediated by Allospecific Antibodies. *Transplantation* **78**:12, 1729-1739. [CrossRef]
35. Shuo Quan, Liming Yang, Sylvia Shnouda, Michal L. Schwartzman, Alberto Nasjletti, Alvin I. Goodman, Nader G. Abraham. 2004. Expression of human heme oxygenase-1 in the thick ascending limb attenuates angiotensin II-mediated increase in oxidative injury1. *Kidney International* **65**:5, 1628-1639. [CrossRef]
36. D METHY. 2004. Differential MnSOD and HO-1 expression in cerebral endothelial cells in response to sublethal oxidative stress. *Brain Research* **1003**:1-2, 151-158. [CrossRef]
37. N.G. Abraham, G. Scapagnini, A. Kappas. 2003. Human heme oxygenase: Cell cycle-dependent expression and DNA microarray identification of multiple gene responses after transduction of endothelial cells. *Journal of Cellular Biochemistry* **90**:6, 1098-1111. [CrossRef]
38. 2003. Trend of Most Cited Papers (2001-2002) in ARS. *Antioxidants & Redox Signaling* **5**:6, 813-815. [Citation] [Full Text PDF] [Full Text PDF with Links]
39. C Colombrita, G Lombardo, G Scapagnini, N.G Abraham. 2003. Heme oxygenase-1 expression levels are cell cycle dependent. *Biochemical and Biophysical Research Communications* **308**:4, 1001-1008. [CrossRef]
40. Nándor Marczin, Ruth E. Bundy, Ginette S. Hoare, Magdi Yacoub. 2003. Redox regulation following cardiac ischemia and reperfusion. *Coronary Artery Disease* **14**:2, 123-133. [CrossRef]
41. Augustine M.K. Choi , Leo E. Otterbein . 2002. Emerging Role of Carbon Monoxide in Physiologic and Pathophysiologic States. *Antioxidants & Redox Signaling* **4**:2, 227-228. [Abstract] [Full Text PDF] [Full Text PDF with Links]