ANTIOXIDANTS & REDOX SIGNALING
Volume 9, Number 12, 2007

DOI: 10.1089/ars.2007.1811

Forum Review

Protective Functions of Heme Oxygenase-1 and Carbon Monoxide in the Respiratory System

STEFAN W. RYTER,[1] HONG PYO KIM,[1] KIICHI NAKAHIRA,[1] BRIAN S. ZUCKERBRAUN,[2] DANIELLE MORSE,[1] and AUGUSTINE M. K. CHOI[1]

ABSTRACT

The respiratory system, including the lung and upper airways, succumbs to injury and disease through acute or chronic exposures to adverse environmental agents, in particular, those that promote increased oxidative or inflammatory processes. Cigarette smoke and other forms of particulate or gaseous air pollution, allergens, microorganisms infections, and changes in inspired oxygen may contribute to lung injury. Among the intrinsic defenses of the lung, the stress protein heme oxygenase-1 constitutes an inducible defense mechanism that can protect the lung and its constituent cells against such insults. Heme oxygenases degrade heme to biliverdin-IXα, carbon monoxide, and iron, each with candidate roles in cytoprotection. At low concentrations, carbon monoxide can confer similar cyto- and tissue-protective effects as endogenous heme oxygenase-1 expression, involving antioxidative, antiinflammatory, antiproliferative, and antiapoptotic effects. Lung protection by heme oxygenase-1 or its enzymatic reaction products has been demonstrated *in vitro* and *in vivo* in a number of pulmonary disease models, including acute lung injury, cigarette smoke–induced lung injury/chronic obstructive pulmonary disease, interstitial lung diseases, ischemia/reperfusion injury, and asthma/airway inflammation. This review summarizes recent findings on the functions of heme oxygenase-1 in the respiratory system, with an emphasis on possible roles in disease progression and therapies. *Antioxid. Redox Signal*. 9, 2157–2173.

INTRODUCTION

The lung, a complex organ of multiple cell types, functions primarily in gas exchange. This organ consists of bifurcating airways of decreasing diameter, which culminate in small sacs termed alveoli, and, together with the nasopharyngeal and tracheobronchial regions that form the pathway of inspired air, constitute the respiratory system. In the alveoli, oxygen diffuses from inspired air to the blood, resulting in the oxygenation of circulating hemoglobin, ultimately reaching the tissues for mitochondrial respiration. Through the same portal, the metabolic byproducts carbon dioxide (CO_2) and, to a lesser extent, carbon monoxide (CO), pass for elimination in the exhaled air. The principal cell types of the lung include epithelial cells of the airway, bronchi, and alveoli; interstitial fibroblasts, endothelial cells of the pulmonary vasculature; smooth muscle cells of the airway and pulmonary vasculature; and alveolar macrophages (62).

The lung and its individual cellular constituents represent an immediate target for injury arising from exposure to adverse environmental agents, through the inhalation of particulates, dusts, smokes, noxious gases, and fungal, viral, or bacterial pathogens (Fig. 1). Pulmonary damage may also arise from exposure to aberrant changes in O_2 concentration or pressure, as may occur during mechanical ventilation, high-altitude exposure, or deep-sea diving, as well as changes in perfusion dur-

[1]Department of Medicine, Division of Pulmonary, Allergy and Critical Care Medicine, and [2]Department of Surgery, The University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

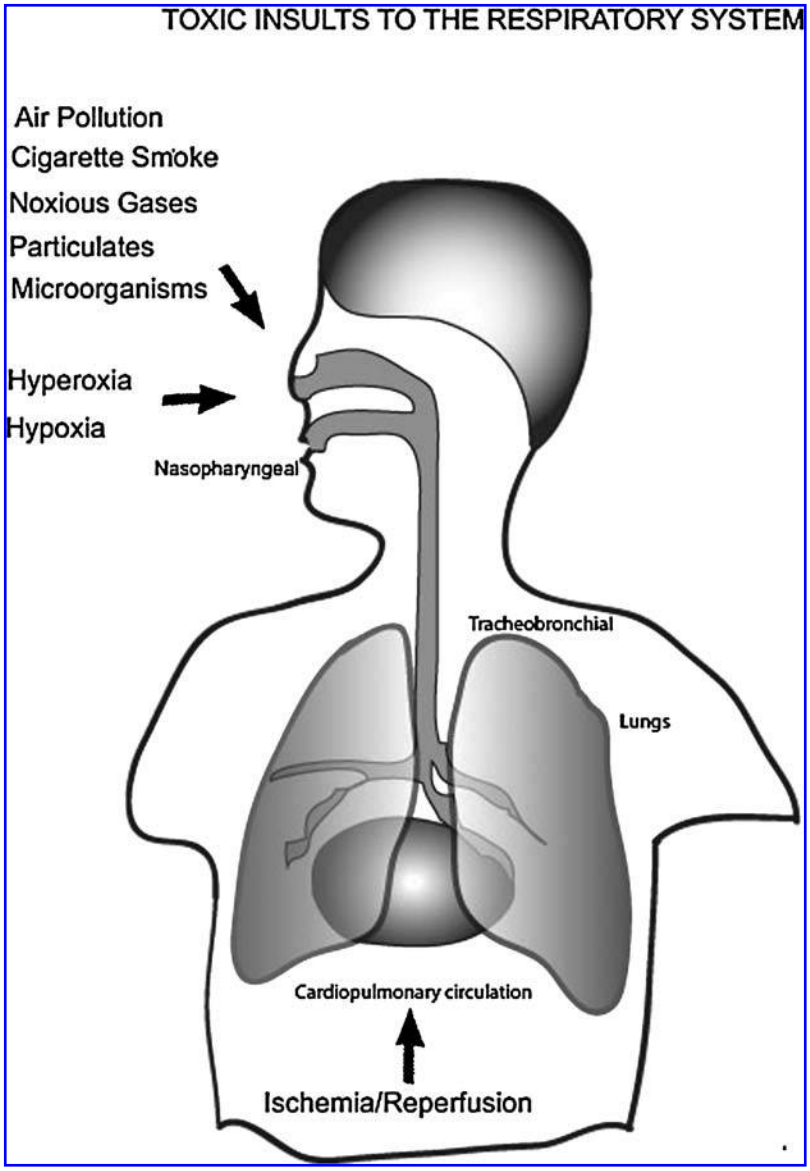

FIG. 1. Toxic insults to the respiratory system. The respiratory system with its three constituent parts (nasopharyngeal, tracheobronchial, and pulmonary regions) can sustain injury through the inhalation of toxic gases, particles, and microorganisms, or by fluxes in inspired oxygen. Additionally, the pulmonary vasculature can sustain injury through ischemia/reperfusion episodes.

ing disorders of the circulation such as ischemic episodes or transfusions (62).

As can be generalized to the other vital tissues, the lung possesses a number of innate defenses against the initiation and propagation of such injuries incurred by adverse environmental stimuli. In addition to systemic inflammatory and immune responses of the lung to invading microorganisms, the cellular components of the lung possess intrinsic defense mechanisms that include antioxidant systems and inducible stress protein responses. The antioxidants consist of water and lipid-soluble compounds (*e.g.*, glutathione, ascorbate, vitamin E, uric acid, and β-caroteine), which scavenge or detoxify reactive oxygen species (ROS) that arise through ordinary metabolism or as a consequence of toxicant exposure. Furthermore, lung tissue contains enzymatic antioxidant activities (*e.g.*, superoxide dismutases, catalases, and peroxidases) that catalyze the conversion of ROS to derivative forms (88). The inducible stress responses include several classes of proteins whose transcription and synthesis are increased by discrete classes of environmental stress, and that serve an endogenous protective function against damage elicited by agents that triggered their synthesis. These include the heat-shock proteins (which confer tolerance to hyperthermia), glucose-regulated proteins, and the low-molecular-weight stress response protein, heme oxygenase-1 (HO-1) (95). Although HO-1 is now known to confer tissue protection in multiple models of organ injury and disease, this review focuses on its relevance as an innate defense of the respiratory system against oxidative or inflammatory lung injury and disease. Particular disease processes that will be highlighted include chronic obstructive pulmonary disease (COPD) and its relation to cigarette smoking, idiopathic pulmonary fibrosis (IPF), asthma, acute lung injury, and ischemia/reperfusion (I/R) injury. Transplantation medicine and oncology are two important areas in which stress protein responses may influence the

outcome of lung pathogenesis, and these topics have been reviewed elsewhere (9, 109).

HEME OXYGENASE

Heme oxygenase (HO), a metabolic enzyme, provides the rate-determining step in the breakdown of heme. HO converts heme, in the presence of O_2 and an electron donor (NADPH/cytochrome p450 reductase), to the open-chain tetrapyrrole biliverdin-IXα, with the release of ferrous iron and carbon monoxide (CO) (115). Biliverdin-IXα is rapidly converted to bilirubin-IXα by NAD(P)H/biliverdin reductase. Elevated HO-1 levels occur in most cell types in response to harmful stimuli, and represent a general cellular response to oxidative stress (42). In addition to the natural HO substrate heme and many xenobiotics, inducing agents of particular relevance to lung physiology include nitric oxide (NO) (129), cigarette smoke, airborne particulate matter (49, 54, 105), bacterial endotoxins, and proinflammatory cytokines (14), as well as change in ambient O_2 tension above and below the normal physiologic range (26, 52, 94, 97). Cell-type and species-specific variations can occur in the inducible HO-1 response. For example, although hypoxic conditions appear to induce HO-1 in cultured animal endothelial cells, hypoxia appears to inhibit HO-1 expression in human endothelial cells (48, 73). In addition to the inducible form, HO-1, the HO system consists of a major constitutively expressed isozyme, heme oxygenase-2 (HO-2) (59).

The molecular regulation of HO-1 by diverse agents occurs primarily at a transcriptional level. Two major upstream enhancers occurring at -4 and -10 kb relative to the transcriptional start site have been identified through comprehensive analysis of the murine *ho-1* gene 5′ regulatory region (2, 3). These enhancers consist of repeats of stress-response elements (StRE), which bear homology to the antioxidant responsive element (ARE) consensus sequence, and mediate the transcriptional response to many inducers of *ho-1*, such as lipopolysaccharide (LPS), heme, and heavy metals (1–3). Among the many transcription factors potentially involved with *ho-1* regulation, the NF-E2–related factor-2 (Nrf2) binds to StRE sequences as stable heterodimers with small Maf proteins, and mediates the induction response to heavy metals and polyphenolic compounds (4–6, 39, 54). A redox-sensitive inhibitor molecule (Keap-1), which anchors Nrf2 in the cytoplasm by direct binding and acts as an adaptor for ubiquitin ligases, promotes the proteolytic degradation of Nrf2 (40). Cellular stimulation with xenobiotics leads to the inhibition of Keap-1–dependent ubiquitination of Nrf2, the dissociation of the Keap-1/Nrf2 complex, and the nuclear translocation of Nrf2 (40, 50). A transcriptional repressor, Bach-1, antagonizes Nrf2-dependent transactivation of *ho-1* by heterodimerization and inactivation of Maf proteins (111). Bach-1 DNA binding and repressor function are relieved by heme binding, which leads to the nuclear exportation of Bach-1 (76). Certain stimuli, such as hypoxia, may increase the expression of Bach-1 (48). Other transcriptional regulators implicated in *ho-1* regulation include hypoxia-inducible factor (Hif-1), heat-shock factor-1 (HSF-1), activator protein-1 (AP-1), early growth-response factor-1 (Egr-1), ETS, and nuclear factor kappa-B (NF-κB) (1, 4, 95).

GENERAL MECHANISMS OF HO-INDUCED CYTOPROTECTION

The precise molecular mechanisms by which HO-1 confers tissue protection remain unclear. Among the possibilities is the accelerated removal of heme, to prevent exacerbation of oxidative stress by iron-dependent mechanisms. The end products of the heme metabolic pathway, iron, CO, and biliverdin, have all been implicated in cytoprotective mechanisms. This evidence is largely based on pharmacologic application of CO and biliverdin/bilirubin, with demonstrated protective effects in several models of cytotoxicity or organ injury (93, 95). Biliverdin and bilirubin can act as chain-breaking antioxidants in model systems (110). Although iron is considered a catalyst of prooxidant reactions, protective roles for HO-derived iron have been discussed, related to secondary increases in ferritin levels after HO-mediated iron release, as well as the direct coupling of HO-1 to active iron-transport mechanisms (reviewed in 95).

The overall importance of HO-1 in systemic homeostasis is emphasized by the only documented case of HO-1 deficiency in humans, the subject of which displayed extensive endothelial cell damage, anemia, and aberrant tissue iron deposition (125). Furthermore, hepatic and renal iron deposition, anemia, and increased susceptibility to oxidative stress were observed in knockout mice bearing the *ho-1*$^{-/-}$ genotype (86, 87).

CARBON MONOXIDE

The occurrence of CO within the respiratory system can originate from both exogenous (intake of contaminated air) and endogenous (heme degradation, lipid and xenobiotic metabolism) sources (Fig. 2) (96). In the absence of environmental exposure, the majority of systemic CO arises from hemoglobin turnover, which will circulate with existing hemoglobin, for eventual diffusion through the lung and elimination in exhaled air (18). The lethal properties of environmental CO have been described elsewhere, and arise primarily from hypoxemia caused by hemoglobin binding and competitive displacement of O_2, leading to tissue hypoxia and asphyxiation (31). Although NO presents a broader spectrum of biologic reactivity because of its free radical character, the reactivity of CO appears to be restricted to the liganding of heme iron centers. Both NO and CO can act as ligands for soluble guanylate cyclase, which stimulates the synthesis of guanosine 3′,5′ monophosphate (cGMP), although experimental evidence indicates that NO activates this enzyme system *in vitro* and corresponding vasodilatory action *in vivo*, with considerably more potency (30). cGMP flux has been implicated in both the vasoactive and neuromodulatory effects of both gases (reviewed in 24, 95). Exogenous or endogenous (HO-derived) CO can modify cellular processes by acting on intracellular signaling pathways, resulting in apparent antiinflammatory, antiapoptotic, and antiproliferative effects (reviewed in 43, 95).

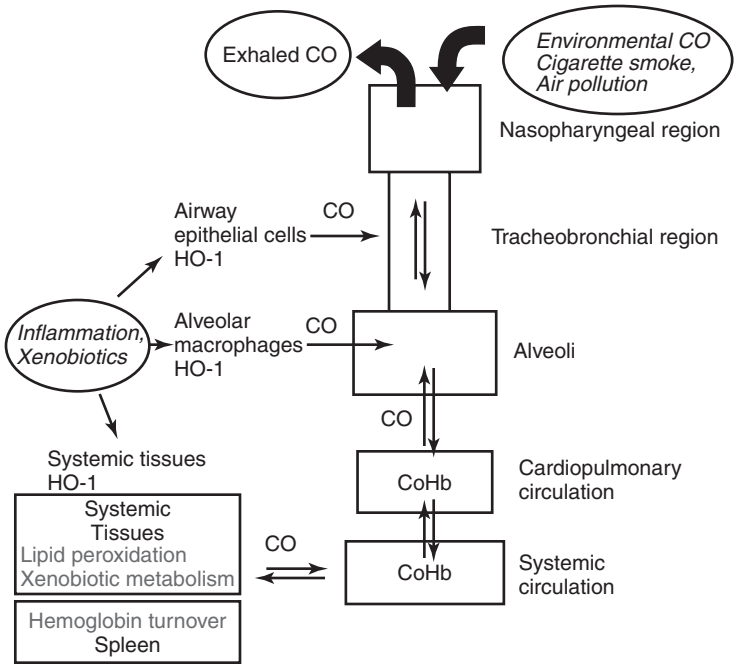

FIG. 2. Carbon monoxide cycle. Systemic CO arises in the body by two mechanisms: the inhalation of contaminated air, or through endogenous heme oxygenase (HO) activity. Inspired CO will enter the circulation through diffusion through the lung and form complexes with circulating hemoglobin (CO-Hb). CO-Hb can dissociate and exchange CO to the exhaled air, also through the alveoli. Inhaled toxicants can potentially induce HO-1 in the tracheobronchial and alveolar epithelium, as well as in alveolar macrophages. Thus, HO activity may provide a major source of CO in the respiratory system. Additionally, HO activity in the systemic tissues, whether induced by inflammation or engaged in hemoglobin turnover (as in the spleen and liver), can contribute to the CO appearing in the systemic and pulmonary circulation as CO-Hb. CO can also arise in the systemic circulation as the byproduct of lipid peroxidation or metabolism of certain xenobiotics.

Antiinflammatory effects of carbon monoxide

The antiinflammatory effects of CO were described in cultured RAW264.7 murine macrophages (Fig. 3). Exogenous CO application or HO-1 expression prevented the lipopolysaccharide (LPS)-induced production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) interleukin-1β (IL-1β) and macrophage inflammatory protein-1β (MIP-1β) in cultured macrophages, whereas it increased the production of the antiinflammatory cytokine interleukin-10 (IL-10) during LPS challenge. The original characterization of these effects excluded cGMP signaling and implicated differential activation of p38 mitogen-activated protein kinase (p38 MAPK) in CO-treated cells (78).

Recent studies in this laboratory have identified a novel mechanism by which CO may exert antiinflammatory effects involving the downregulation of Toll-like receptor (TLR) trafficking (Fig. 4) (71). The effects of CO on cytokine production were investigated in RAW 264.7 cells induced with various TLR ligands. CO inhibited TLR4 ligand (*e.g.*, LPS) and TLR (-2, -5, and -9) ligands induced TNF-α production, but did not affect TLR3 ligand (*e.g.*, poly[I:C])-induced signaling or TNF-α production. The trafficking of TLRs to plasma membrane lipid-raft domains represents an early upstream event in the activation of TLR-dependent signaling pathways associated with proinflammatory stimuli. Trafficking of TLR4 to lipid rafts in response to LPS was ROS-dependent, because it was diminished by chemical inhibitors of NADPH oxidase, and in macrophages deficient in the *gp91^{phox}* component of the oxidase. CO selectively inhibited LPS-induced recruitment of TLR4 to lipid rafts, which was associated with the inhibition of NADPH oxidase activity and LPS-inducible ROS production in macrophages. CO also inhibited the translocation of TLR adaptors to the lipid raft, including MyD88 and TRIF, as well as their association with TLR4. In summary, CO differentially regulated TLR signaling pathways by inhibiting translocation of TLR4 to lipid rafts through suppression of NADPH oxidase–dependent ROS generation (71). Suppression of NADPH oxidase activity in RAW 264.7 macrophages by HO-1 expression has also been demonstrated through a mechanism involving reduced heme bioavailability for cytochrome b_{558} synthesis (114).

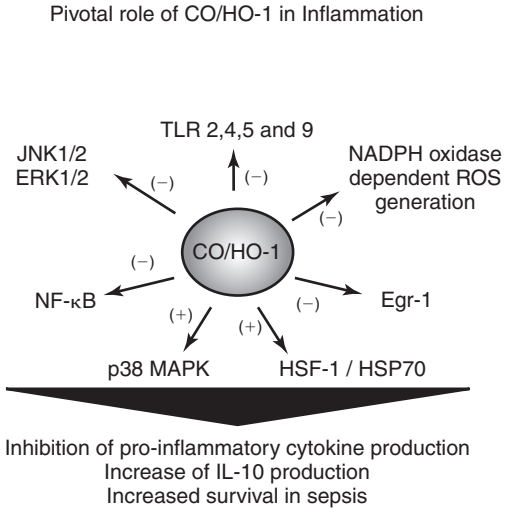

FIG. 3. Pivotal role of HO-1/CO in inflammation. HO-1/CO can downregulate inflammation by several possible mechanisms. CO inhibits LPS-inducible proinflammatory cytokine production in macrophages by modulation of p38 MAPK, and also through inhibition of ERK1/2 and JNK-dependent pathways. Recent studies indicate that CO can downregulate NADPH:oxidase-dependent ROS production, resulting in the inhibition of Toll-like–receptor trafficking (TLR -4, -6, -9), and early events in LPS-initiated signaling (71). Furthermore CO may inhibit nuclear transcription factor activities during inflammatory signaling such as NF-κB (71) or EGR-1 (64). Upregulation of the heat-shock protein-70 (hsp70) through heat-shock factor-1 (HSF-1) has also been implicated in CO-mediated antiinflammatory tissue protection (45).

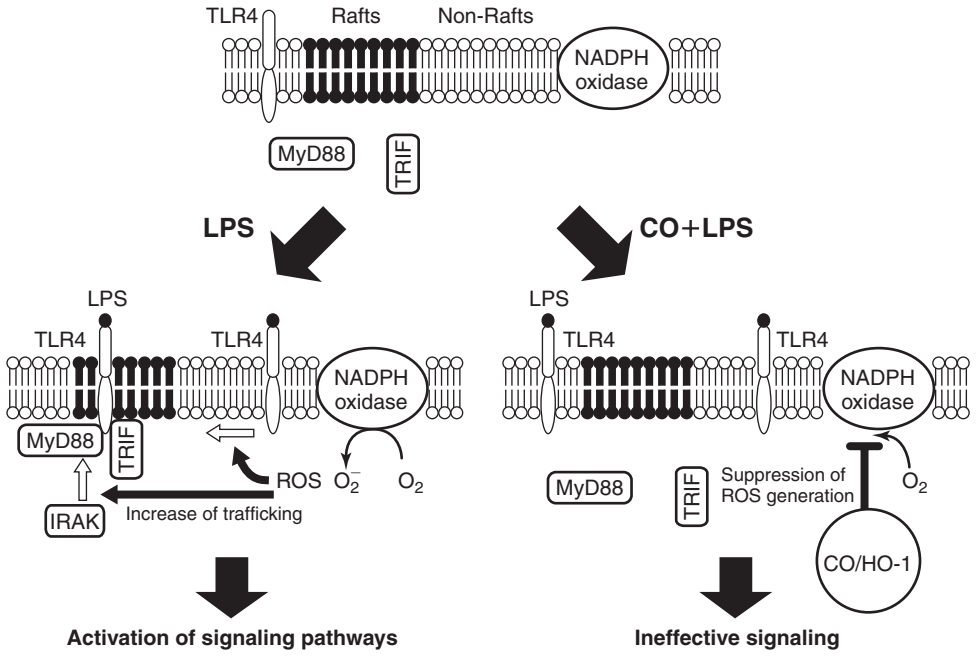

FIG. 4. CO inhibits Toll-like–receptor trafficking. The trafficking of Toll-like receptors (TLRs) to specialized membrane lipid raft domains represents an early upstream event in the activation of TLR-dependent signaling pathways associated with proinflammatory stimuli (71). The activated receptors associate with multiple adaptor molecules (*i.e.*, MyD88, TRIF). Proinflammatory signaling culminates in the activation of proinflammatory cytokine genes (*e.g.*, TNF-α). TLR4 trafficking to lipid rafts in response to LPS stimulation depended on the endogenous production of ROS. CO, which has known antiinflammatory effects with respect to macrophage production of cytokines, specifically inhibited TLR4 trafficking to the lipid raft during LPS stimulation, through a mechanism involving the downregulation of NADPH:oxidase-dependent ROS production (71).

Antiapoptotic effects of carbon monoxide

Apoptosis is a form of programmed cell death that requires the regulated activation of proteases (*i.e.*, caspases) and nucleases. Apoptosis serves a critical function in the maintenance of tissue homeostasis under physiologic conditions, as a component of developmental programs, but may also contribute to disease pathogenesis. Two apoptotic pathways have been identified by which cells can initiate and execute the cell-death process: an intrinsic (mitochondria-dependent) pathway and an extrinsic (receptor-dependent) pathway (122).

The antiapoptotic effects of CO were originally described *in vitro* (13, 85) and involve several potential mechanisms (Fig. 5). Exogenous CO inhibited tumor necrosis factor-α (TNF-α)–initiated apoptosis in mouse fibroblasts (85) and endothelial cells (13). In fibroblasts, an antiapoptotic effect also was observed with HO-1 overexpression (85). In the endothelial cell model, the inhibitory effect of CO on TNF-α–induced apoptosis could be abolished with the selective p38α/β MAPK inhibitor, SB203580, or a p38 MAPK dominant negative mutant, implying a critical role for the p38 MAPK pathway (13). Furthermore, HO-1 or CO cooperated with NF-κB–dependent antiapoptotic genes (c-IAP2 and A1) to protect against TNF-α–mediated endothelial cell apoptosis (12). In mouse lung endothelial cells, protection against TNFα/actinomycin-D–induced cell death likewise involved p38β MAPK activation, in this instance leading to upregulation of HSF-1 and heat-shock protein-70 (hsp70) expression (45). The cytoprotective effects of CO were diminished in heat-shock factor-1 knockout ($hsf1^{-/-}$) fibroblasts, indicating a potential role of the heat-shock response in CO-mediated cytoprotection (45). The requirement for p38 MAPKs and the participation of signal transducer and activator of transcription (STAT) proteins in the antiapoptotic effects of CO were recently described in several O_2-dependent injury models (*i.e.*, hyperoxia, and anoxia/reoxygenation), as discussed in the subsequent sections (133, 134, 136, 137).

In contrast to the aforementioned studies, Thom *et al.* (116) reported proapoptotic effects of CO in endothelial cells at low concentrations (10–100 ppm) in the absence of other proapoptotic stimuli.

Antiproliferative effects of carbon monoxide

CO also exerts antiproliferative effects on various cell types, including fibroblasts, and vascular or airway smooth muscle cells, and thus has potential application in preventing tissue remodeling (Fig. 6) (67, 82, 106, 113). CO inhibits smooth muscle proliferation by activation of soluble guanylate cyclase and p38β MAPK, and increased expression of the cyclin-dependent kinase inhibitor $p21^{Waf1/Cip1}$ (44, 67, 82). In human airway smooth muscle cells, the antiproliferative effects of CO involved the increased production of mitochondrial ROS or the downregulation of cytosolic ROS as a consequence of NAD(P)H:oxidase inhibition, or both, leading to ERK1/2 downregulation and decreased cyclin D expression (113).

Additional mechanisms for antiproliferative effects of CO include the downregulation of endothelium-derived smooth muscle mitogenic factors (66). Caveolin-1, a candidate tumor-sup-

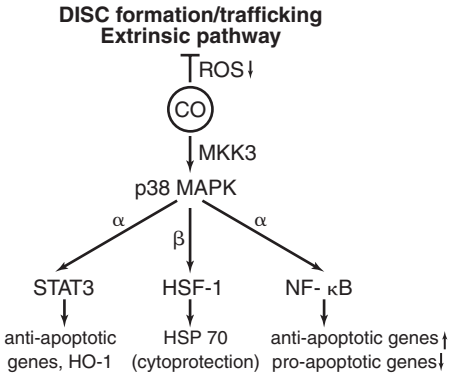

FIG. 5. Antiapoptotic mechanisms of HO-1/CO. Antiapoptotic effects of HO-derived CO (in endothelial cells) were originally assigned to modulation of p38 MAPK, and the upregulation of NF-κB–dependent antiapoptotic genes (12, 13). The p38 MAPK-dependent modulation of the STAT-3 pathway was also implicated in antiapoptotic effects of CO (in the lung I/R model) (134). Downstream activation of HSF-1, also p38 MAPK dependent, may contribute to antiapoptotic cytoprotection (in fibroblasts) (45). Recent studies using the hyperoxia model demonstrate that CO, through downregulation of ERK1/2-dependent ROS production, can inhibit the initiation of the extrinsic apoptotic pathway in endothelial cells (122).

pressor protein, and the major structural component of plasma membrane caveolae, serves an intermediate role in the antiproliferative effects of CO (44) (Fig. 7), and potentially in the antiinflammatory effects of this gas as well (unpublished observations).

HO-1/CO IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND CIGARETTE SMOKE–RELATED LUNG INJURY/CELL DEATH

Chronic obstructive pulmonary disease (COPD) is defined as "a preventable and treatable disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and is associated with an abnormal inflammatory response of the lungs to noxious particles or gases, primarily caused by cigarette smoking" (15). Restricted breathing, leading to reduced efficiency of blood oxygenation, may also precipitate cardiovascular disease. In the developed nations, tobacco smoking remains a primary cause of COPD, which develops in 15–20% of smokers. Environmental and occupational exposures to particulate air pollution, chronic microbiologic infections, and asthma may contribute to the disease process. Genetic deficiencies in α_1-antitrypsin, although rare, also produce COPD (10).

Airway inflammation is a major component of COPD, characterized by the presence of macrophages, neutrophils, and inflammatory mediators. Cigarette smoke will react primarily on alveolar macrophages and epithelial cells, which react to the oxidative stress by producing proinflammatory cytokines and chemokines, and releasing growth factors (92). The etiology of COPD involves two hypotheses, both of which relate to smoke exposure: dysbalances in redox homeostasis and protease/antiprotease equilibria (11, 89, 118). Proteases with tissue-degrading capacity (*e.g.*, elastases and matrix metalloproteinases), if insufficiently counterregulated by antiproteases, can induce tissue damage, leading to emphysema. Oxidant stress, in addition to causing direct tissue damage, can inactivate antiproteases and interfere with the repair of the extracellular matrix (89). Apoptosis, a process not only triggered but also amplified by oxidative stress, also potentially contributes to the pathogenesis of COPD/emphysema (84, 119).

Accumulating clinical, genetic, and *in vitro* evidence suggests that HO-1 may play important roles in the development of COPD. The protective potential of HO-1 against the development of COPD is underscored by a recent report demonstrating that *ho-1* gene transfer protects against the development of experimental elastase-induced emphysema (103). However, apparent differences exist in endogenous HO-1 expression in the lung in the context of chronic smoke exposure implicated in COPD pathogenesis, *versus* the end state of COPD. Elevated levels of HO-1 were reported in the alveolar spaces of chronic smokers with and without COPD relative to nonsmokers (57). However, a subsequent study found decreased HO-1 expression in alveolar macrophages of severe COPD patients relative to those of smokers without lung-function impairment (58). Finally, ex-smoking COPD patients were reported to have reduced HO-1 expression in alveolar macrophages relative to healthy ex-smokers (104).

Systemic CO production and its evolution on the exhaled breath have been explored as potential biomarkers of diseases involving inflammation, and this has been studied in the contexts of asthma, smoking, and COPD (35, 36). The source of increased exhaled or systemic CO or both during inflammatory diseases of the lung and other tissues is not entirely clear and may represent a net increase in heme metabolic activity in the airways, lung, and systemic tissues contributed by inducible HO-1 activity. Alveolar macrophages and airway epithelial

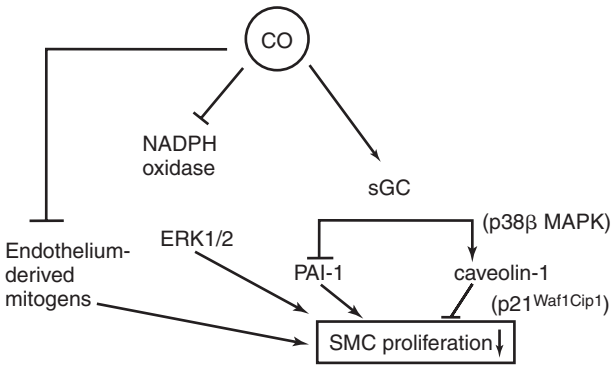

FIG. 6. General antiproliferative mechanisms of HO-1/CO. CO inhibits smooth muscle cell proliferation in a cGMP-dependent manner. This has been associated with the upregulation of caveolin-1, $p21^{Waf1/Cip1}$, and the downregulation of plasminogen activator inhibitor-1 (PAI-1) (28, 44, 67, 82). Additional mechanisms may involve the downregulation of endothelial cell–derived mitogenic factors, and the downregulation of the NADPH oxidase-dependent ERK1/2 pathway (66, 113).

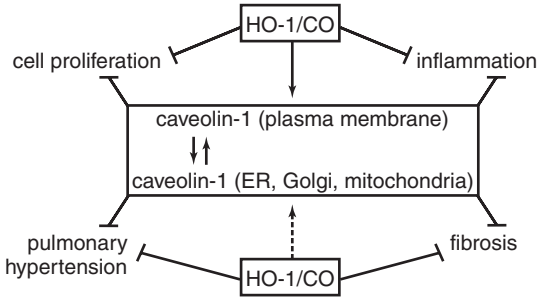

FIG. 7. Possible involvement of caveolin-1 in HO-1/CO–mediated protection. Caveolin-1, a tumor-suppressor protein and major structural component of plasma membrane caveolae, has recently been implicated as an intermediate in cellular signaling initiated by CO. CO treatment upregulated caveolin-1 in vascular smooth muscle, and this was required for antiproliferative effects of CO in this cell type (44). Work in progress implicates caveolin-1 in the antiinflammatory effects of CO (unpublished studies). Although recent studies have implicated caveolin-1 in preventing pulmonary hypertension and fibrosis (60, 123), further studies are needed to examine the possible relations with HO-1/CO.

cells, which can produce a strong HO-1 response to proinflammatory stimuli, may represent a major source of endogenous CO production in the airways (22, 35, 36). These relations are complicated by smoking, which may induce HO-1 in the airways (57) but also introduces systemic CO by inhalation (20). Thus, higher levels of exhaled CO appear on the breath of smokers than nonsmokers in healthy subjects in the absence of lung pathology and correlate with the degree of tobacco consumption (20).

COPD patients that were smoking displayed higher exhaled CO values than ex-smoking COPD patients, the values of whom were also higher than those of healthy nonsmoking controls (65). A recent study examined arterial carboxyhemoglobin (Hb-CO) levels in 119 subjects consisting of COPD patients or healthy normal patients. Patients with COPD displayed higher Hb-CO levels than healthy controls, which furthermore correlated with disease severity and increased during COPD exacerbations (128). Thus, although an apparent correlation between exhaled CO and increased HO-1 production in the airways was reported in asthmatics (35), the relations between exhaled CO and HO-1 expression in severe COPD, in which lung HO-1 expression may be reduced, remains unclear, and further studies that involve smoking status are needed to clarify these relations (104).

Recent studies have explored the genetic basis of COPD. A microsatellite $(GT)_n$ dinucleotide-length polymorphism can occur in the promoter region of the *ho-1* gene, resulting in a lower production of HO-1 in individuals that carry the long (L) allele [$(GT)_n \geq 30$] of this polymorphism (126). This polymorphism was linked with the development of COPD in a cohort of Japanese men (126), and more recently, in a Chinese population (27). In a retrospective study of French smokers, the L allele [(GT)n $\geq$33] was associated with decreased lung-function parameters relative to noncarriers. The greatest decline in lung function was observed in heavy smokers that carried the L allele (32). These observations suggest that a genetically dependent downregulation of HO-1 expression may arise in subpopulations, possibly linked to increased susceptibility to smoke-induced oxidative stress (126).

Expression-profiling experiments have demonstrated that many of the antioxidant and redox-related molecules (*e.g.*, catalase, thioredoxin, glutaredoxin, heat-shock protein-70, and HO-1) were downregulated in total lung tissue from COPD individuals when compared with control smokers (75). Although these antioxidant and stress-responsive genes generally appear upregulated in cellular and tissue-injury models in response to specific stresses (*e.g.*, cigarette smoke), the decreased expression of these stress-response molecules in human COPD supports a hypothesis of compromised oxidant/antioxidant balance in COPD (75).

Of related importance, Nrf2, which regulates *ho-1*, as well as a number of other genes encoding enzymes involved in xenobiotic detoxification (*e.g.*, NADPH:quinone oxidoreductase and glutathione-*S*-transferase) (39), appears to regulate tissue defenses against emphysema (90). Genetic deletion of Nrf2, as in *nrf2*$^{-/-}$ mice, rendered the mice susceptible to experimental emphysema induced by chronic smoke inhalation (90). In parallel with the compromised expression of >50 genes, *nrf2*$^{-/-}$ mice displayed increased epithelial and endothelial cell apoptosis, increased markers of oxidative stress and pulmonary inflammation, relative to wild-type mice, after chronic smoke exposure (90). These experiments further support the hypothesis of compromised cellular defense in COPD.

HO-1 in cytoprotection against cigarette smoke–induced cell death

Experimentally, cigarette smoke (CS) represents a complex model of toxicant exposure, as it contains >4,500 distinct chemical species. Because CS contains ROS, NO, and other free radicals, electrophilic substances, and heavy metals, and furthermore can trigger the intracellular production of ROS and deplete natural antioxidants, CS is generally regarded to be an oxidative cellular stress (88). Whereas inhalation studies with animals are typically exposed to mainstream or sidestream CS, cell-culture studies may also use aqueous cigarette smoke extract (CSE) or CS condensate (91). Although some reports have suggested that CSE causes primarily necrotic cell death in bronchial epithelial cells (56), apoptotic phenotypes have also been observed, depending on cell model and experimental conditions (37, 105). Lower dose and shorter kinetics of CSE exposure generally favor apoptosis (105).

Several *in vivo* and *in vitro* studies have studied the regulation of HO-1 by smoke exposure (105). We recently demonstrated induction of *ho-1* mRNA in total lung after prolonged CS exposure of AKR/J mice. HO-1 gene expression was increased at 2 to 12 weeks' exposure and declined to control values at 24 weeks (Fig. 8) (105). The induction of HO-1 by cigarette smoke or CSE was observed in a number of pulmonary and nonpulmonary cell types, including fibroblasts, alveolar macrophages, and epithelial cells (29, 49, 83, 105).

In our recent studies, human bronchial epithelial cells (Beas-2b) subjected to CSE responded with a time- and dose-dependent upregulation of HO-1 protein and enzymatic activity. HO-1 protected against CSE-induced cell death and preserved cellular ATP levels, which were depleted by the smoke treat-

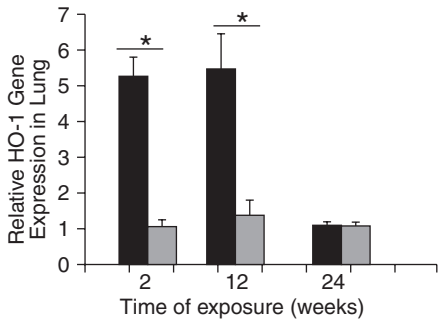

FIG. 8. Induction of *HO-1* by prolonged smoke exposure in rat lung. AKR/J mice were exposed to 24 weeks of cigarette smoke exposure (*black bars*) or air (*grey bars*). At 2, 12, and 24 weeks' exposure, lungs were excised. Relative heme oxygenase-1 gene expression in lung tissue was determined at each indicated exposure time, by real-time PCR, in three to eight animals per time point per exposure condition. $*p < 0.01$. Figure reprinted from ref. 105, with permission from the American Thoracic Society.

ment. Curiously, a portion of the HO-1 induced by the CSE treatment, as well as by other types of stress (*e.g.*, heme and LPS) colocalized with mitochondria in this cell type. Mitochondrial localization of HO-1 was confirmed by cell fractionation, as well as by electron microscopy and co-immunofluorescence (105). Related studies in the liver model have suggested that HO-1, which localizes in the mitochondrial inner membrane, downregulates the synthesis of mitochondrial hemoproteins by restricting heme bioavailability, although other mechanisms of mitochondria-specific protection remain to be explored (19).

PROTECTIVE EFFECTS OF HO-1/CO IN ASTHMA/AIRWAY INFLAMMATION

Asthma is an inflammatory disease of the upper airways that reduces airflow, which can be exacerbated by inhalation of allergenic substances such as pollen or dusts. Increased airway proinflammatory cell counts, mucus build-up, and bronchoconstriction are major characteristics of asthma. Like other chronic inflammatory diseases, asthma is associated with increased prooxidant burden (89).

HO-1 expression has been examined in alveolar or airway macrophages of human asthmatics (33, 35, 55). Increased HO-1 expression occurred in alveolar macrophages of patients with recent asthma exacerbations relative to healthy controls or patients undergoing steroid treatment (33, 35). This observation was associated with increased bilirubin levels in the induced sputum, as well as increased levels of CO on exhaled breath of non–steroid-treated asthmatics relative to control subjects (35). Patients with exacerbations of asthma, or patients from whom inhaled steroids were discontinued showed higher exhaled CO levels, when compared with steroid-treated asthmatics and healthy controls (132). Furthermore, exhaled CO decreased in parallel with inflammatory markers in response to prolonged therapy with inhaled corticosteroids (132). Higher levels of exhaled CO were also reported in children with persistent asthma relative to healthy controls (120). Furthermore, patients with asthma displayed an increased Hb-CO level at the time of exacerbation, which declined to control levels in response to oral steroid treatment (127). Despite these observations, conflicting reports exist on whether changes in exhaled CO occur in asthmatics, casting uncertainty on the relative usefulness of exhaled CO as a marker of airways inflammation (55, 131). In one negative study, no change in exhaled CO occurred in asthmatics after a 1-month treatment of inhaled corticosteroids, despite decreases in airway eosinophil content and bronchial responsiveness to metacholine (55). The same study also reported no change in HO-1 expression in airway macrophages and epithelium of mild asthmatics relative to normal patients, and no further change with extended inhaled steroid treatment (55).

Given the known antiinflammatory properties of CO, several studies examined the potential for inhalation CO in modulating airway inflammation and reactivity in preclinical asthma models. Mice develop an airway hyperresponsiveness, similar to that seen in human asthma, when challenged with aerosolized ovalbumin after initial sensitization. In this model, ovalbum in challenge increased HO-1 expression in the bronchial subepithelium and in alveolar macrophages (46). CO treatment of ovalbumin-challenged animals reduced inflammatory cell counts, especially of eosinophils and macrophages, in the bronchoalveolar lavage at 24 h after challenge. Exogenous CO administration also significantly reduced interleukin-5 production and proinflammatory mediator levels (IFN-γ, leukotriene B4, and prostaglandin E2) (16). In further studies, an acute dose of CO (500–1,000 ppm; 10 min) reduced metacholine-induced airway resistance in ovalbumin-challenged C57BL/6 mice and in airway-hyperresponsive A/J mice. Repeated administrations of low-dose CO (250–500 ppm) over a 5-day period in both naïve and inflamed A/J mice significantly reduced airway resistance (8). Furthermore, heme preconditioning, which resulted in increased HO-1 expression and activity, reduced airway hyperreactivity and inflammatory cell influx in ovalbumin-challenged mice as well as in guinea pigs (7, 124). In addition to antiinflammatory effects (of HO-1/CO), as previously described, involving enhanced IL-10 production, the heme preconditioning in mice increased the number and activity of $CD4^+CD25(high)$ regulatory T cells (Tregs), which are associated with suppression of inflammation (124). In a murine model of asthma, exogenous bilirubin application was also shown to exert a protective effect by inhibiting VCAM-1–associated airway inflammation and lung leukocyte influx, as well as inhibiting leukocyte migration *in vitro* (41).

Effects of HO-1/CO on respiratory smooth muscle contractility and proliferation

Airway smooth muscle contractility, which can be increased through ROS formation, was inhibited by HO-1 induction. Specifically, the HO inducer and substrate heme decreased, whereas metalloporphyrin inhibitors of HO (*e.g.*, SnPPIX) increased ROS production as well as the contractile response to carbamylcholine in isolated guinea pig tracheal rings (98). Application of exogenous bilirubin likewise reduced ROS production and contractility in this system. These effects were at-

tributed to decreased phosphorylation of the contractile protein myosin light chain, after heme or bilirubin administration (98).

Exogenous CO application was demonstrated in several studies to inhibit the proliferation of cultured human airway smooth muscle cells (HASMCs), through downregulation of ERK1/2 activation (106, 113). Conversely, heme treatment, which increased HO activity, also inhibited HASMC proliferation, which was attributed in this case to increased bilirubin production, rather than to endogenous CO production. The authors demonstrated that exogenous bilirubin application can inhibit HASMC proliferation by downregulating ERK1/2 activation (112).

Antiinflammatory effects of HO-1/CO in cultured airway cells

Primary cultured airway epithelial cells produced a strong HO-1 response when challenged *in vitro* with IL-1β, TNF-α, and IFN-γ (cytomix) or NO donors and were also positive for constitutive HO-2 expression. This observation led the authors to propose that HO activity in the epithelium may represent a significant source of CO production in the airways (22). The antiinflammatory potential of CO has been tested *in vitro*, with respect to the regulation of the granulocyte–macrophage colony-stimulating factor (GM-CSF). Elevated GM-CSF levels appear in asthma and other chronic inflammatory pulmonary diseases. Treatment of cultured human airway smooth muscle cells with cytomix, or IL-1β alone, stimulated the release of GM-CSF, whereas pretreatment of these cells with CO inhibited the induction of GM-CSF protein in response to these stimuli, through a mechanism involving increased cGMP levels and downregulation of the ERK1/2 MAPK pathway (107). CO treatment also attenuated the production of GM-CSF by macrophages in response to LPS treatment, involving downregulation of the NF-κB pathway (99).

PROTECTIVE EFFECTS OF HO-1/CO IN ACUTE LUNG INJURY

Inflammatory lung injury

Bacterial lipopolysaccharide (LPS) exposure in animals represents a model of acute lung injury. LPS challenge promotes a massive inflammatory response associated with lung tissue injury, lung cell apoptosis, and necrosis. Intratracheal administration of LPS into the lungs of mice caused epithelial cell injury within 1 day of application, associated with increases in apoptotic markers in lung macrophages, neutrophils, and in the alveolar wall within 24 h (47). CO exposure can exert a potent antiinflammatory effect in murine models of endotoxemia. CO preconditioning at low concentration (250 ppm) reduced the production of serum TNF-α, IL-β, IL-6, and prolonged survival after LPS challenge (68, 78). Antiinflammatory effects of CO *in vivo*, with respect to modulation of pro- or antiinflammatory cytokine production, were diminished in heat-shock factor-1 knockout (*hsf1*$^{-/-}$) mice, indicating a role of the heat-shock response (45). Furthermore, adenoviral-mediated *ho-1* gene transfer protected against LPS-induced lung injury in mice by enhancing IL-10 production (38) and also protected against acute lung injury after influenza virus infection (34). Administration of biliverdin, a product of HO activity, to rats also protected against systemic inflammation and lung injury, and prolonged survival after exposure to a lethal dose of LPS. The protection afforded by biliverdin was associated with a reduction of proinflammatory cytokines in the serum (*e.g.*, IL-6) and upregulation of serum IL-10 levels, as well as reduction of lung-injury markers. This protection against LPS-induced injury also extended to cultured lung endothelial cells and macrophages (100).

Oxidative lung injury

Mechanical ventilation with high oxygen tension (hyperoxia, $>95\%$ O_2) is frequently used in critical care situations as supportive care for acute, severe respiratory failure. Unfortunately, this treatment can cause cell and organ injury, involving the increased generation of ROS (138). The lung damage resulting from hyperoxia exposure occurs predominantly in the respiratory endothelium and epithelium (51). Rats and mice subjected to hyperoxia ($>95\%$ O_2) develop inflammatory lung injury, characterized by neutrophil influx in the airways, pulmonary edema, pleural effusion, and increased lung cell apoptotic markers. Elevated HO-1 protein expression was reported in lungs of mice subjected to hyperoxia (52). The expression of *ho-1* in rat lungs by intratracheal adenoviral-mediated gene transfer, which increased HO-1 expression in the bronchiolar epithelium, protected against the development of pulmonary damage during hyperoxia exposure (79). Rats infected with *ho-1* before hyperoxia displayed reductions in lung-injury markers, neutrophil infiltration, and apoptosis, and a marked increase in survival against hyperoxic stress when compared with control infected rats (79). Similarly, the inclusion of CO at a concentration of 250 ppm in the hyperoxic environment also prolonged the survival time of rats and mice subjected to a lethal dose of hyperoxia, and dramatically reduced histologic markers of lung injury, including airway neutrophil infiltration, fibrin deposition, alveolar proteinosis, pulmonary edema, and total apoptotic index, relative to animals exposed to hyperoxia alone (80, 81).

In the mouse model, hyperoxia exposure induced the expression of proinflammatory cytokines including TNF-α, IL-1β, and IL-6, and activated major MAPK in lung tissue, including the extracellular regulated kinase-1/2 (ERK1/2), c-Jun amino terminal kinase (JNK), as well as p38 MAPK and its upstream kinases MKK3/6. The protection afforded by CO treatment against the lethal effects of hyperoxia correlated with the inhibited release of the proinflammatory cytokines in bronchoalveolar lavage fluid. Genetic studies in the mice revealed that the antiinflammatory effect of CO was associated primarily with the upregulation of p38β MAPK and its upstream regulator, mitogen-activated protein kinase kinase (MKK3) (81). Deletion of MKK3 (*mkk3*$^{-/-}$) accelerated the expression of proinflammatory cytokines in response to hyperoxia. CO failed to inhibit the expression of proinflammatory cytokines or protect against hyperoxia-induced lung injury and death, in *mkk3*$^{-/-}$ mice. Similar results were reported in wild-type mice treated with the selective chemical inhibitor of p38α/β MAPK (81). Recent studies demonstrate comparable antiinflammatory effects of inhaled CO in rats subjected to experimental venti-

lator-induced lung injury (21). Rats ventilated with an injurious ventilator setting in the presence of intraperitoneal LPS exhibited increased expression of HO-1 in the lung. The inclusion of CO (250 ppm) in the ventilation protocol reduced the inflammatory cell count in bronchoalveolar lavage fluid. In the absence of significant cardiovascular effects, CO dose-dependently decreased TNF-α and increased IL-10 in the bronchoalveolar lavage. Lung tissue extracts displayed increased activation of p38 MAPK after ventilation with CO, whereas chemical inhibition of p38 MAPK *in vivo* attenuated IL-10 production. These experiments suggested that mechanical ventilation in the presence of CO may provide protection in animal models of ventilator-induced lung injury (21).

Hyperoxia induces the HO-1 response *in vitro*, as demonstrated in several cell types including endothelial cells and fibroblasts (26, 52). The protective effects of HO-1/CO against hyperoxia-induced lung cell injury and death have been studied *in vitro*. For example, HO-1 overexpression protected A549 alveolar epithelial cells against cell killing by exposure to hyperoxia (53). CO treatment of A549 epithelial cells inhibited hyperoxia-induced cell death. Treatment with the p38 MAPK inhibitor or transient transfection with dominant negative mutants of p38β or MKK3 abolished the cytoprotective effect of CO against hyperoxia (81). Recent studies have added STAT3 as a candidate mediator of CO-dependent antiapoptotic protection in hyperoxic lung cell injury (136).

Recently, we showed that low-dose CO (250 ppm) specifically inhibited the initiation and propagation of extrinsic apoptosis pathways in mouse lung endothelial cells (MLECs) subjected to hyperoxia (122). CO cotreatment inhibited hyperoxia-induced plasma membrane assimilation of the death-inducing signal complex (DISC) as well as downstream caspase-8 and Bid activation. Antiapoptotic cytoprotection culminated with the inhibition of caspase-9/3 activation and protection against cell death. Inhibitors of NADPH oxidase activation, as well as CO, diminished hyperoxia-induced ROS production, as well as the activation of the DISC, its association with the epidermal growth factor receptor, and its plasma membrane assimilation. Furthermore, the protective effects of CO in this model depended on downregulation of the ERK1/2 MAPK pathway, which regulated ROS production in this cell type during hyperoxia (122).

PROTECTIVE EFFECTS OF HO-1/CO IN LUNG ISCHEMIA/REPERFUSION INJURY

Pulmonary dysfunction may arise as a result of ischemia/reperfusion (I/R) injury to the lung sustained during cardiac surgeries. I/R, which generates cytotoxic ROS and promotes the recruitment of inflammatory leukocytes, causes lung injury and cell death involving both necrosis and apoptosis. Endothelial cells appear to represent the primary target for ROS-dependent injury during I/R. Latent but potentially lethal ischemic damage may cause cells in different regions of the lung to sustain reperfusion injury on restitution of blood flow. Cell death may also arise as a secondary consequence of inflammation (74).

Homozygous *ho-1* null mice (*ho-1*$^{-/-}$) were highly susceptible to lung I/R injury. CO inhalation compensated for the HO-1 deficiency in *ho-1*$^{-/-}$ mice and improved survival after lung I/R (28). The protection provided by CO involved the stimulation of fibrinolysis by the cGMP-dependent inhibition of plasminogen activator inhibitor-1, a macrophage-derived activator of smooth muscle cell proliferation (28). CO also inhibited fibrin deposition and improved circulation in an ischemic lung model (64). These protective effects were recently associated with downregulated expression of EGR-1, a multifunctional transcription factor, with the concomitant downregulation of EGR-1 target genes, many of which contribute to inflammatory or prothrombotic processes. The downregulation of EGR-1 depended on the enhancement of cGMP signaling by CO treatment, leading to the downregulation of the ERK1/2 MAPK pathway (64). In addition to inflammation and thrombosis, pulmonary I/R induced biochemical features of apoptosis in lung tissue (133). The protective effect of CO pretreatment on mice subjected to lung I/R injury *in vivo* was associated with the inhibition of apoptosis markers, including caspase-3 activation, and depended on activation of p38α MAPK (133, 137). Recent studies indicate that *ho-1* knockdown using siRNA dramatically increased lung apoptosis during I/R (135).

Oxidative lung cell injury (hypoxia/reoxygenation)

In vitro experiments using pulmonary endothelial cells demonstrated that exogenously applied CO at low concentrations inhibited anoxia/reoxygenation–induced apoptosis, associated with the CO-dependent activation of the p38α MAPK isoforms, with parallel suppression of ERK and JNK activation (133, 137). Chemical inhibition of p38 MAPK, or the use of the *mkk3*$^{-/-}$ mice, abolished the antiapoptotic effects of CO in this model (133). In addition to activation of p38α MAPK and its upstream MAPK kinase (MKK3), the antiapoptotic effect of CO involved inhibition of Fas/FasL expression, and other apoptosis-related factors, including caspases (-3, -8, -9), mitochondrial cytochrome *c* release, Bcl-2 proteins, and poly(ADP-ribose) polymerase cleavage (133). Further studies demonstrated that antiapoptotic functions of CO after anoxia/reoxygenation *in vitro* also depended on activation of the PI3K/Akt and the p38α MAPK-dependent STAT3 pathway (134).

PROTECTIVE EFFECTS OF HO-1/CO IN INTERSTITIAL LUNG DISEASE (FIBROSIS)

Idiopathic pulmonary fibrosis (IPF) is a terminal disease characterized by scarring or thickening of lung tissues associated with fibroblast hyperproliferation and extracellular matrix remodeling, with no known etiology or effective treatment (102). IPF affects primarily the lower respiratory tract, resulting in compromised efficiency of alveolar gas exchange (102). What is currently known about the role of HO-1/CO in IPF is limited to experimental models of fibrotic lung injury (Fig. 9). Bleomycin, a redox cycling compound that generates O_2^- and H_2O_2, causes lesions in mouse lung after intratracheal administration, similar to IPF. Tsuburai *et al*. (117) demonstrated that HO-1 expression by adenovirus-mediated gene transfer to the lung protected against bleomycin-induced pulmonary fibrosis

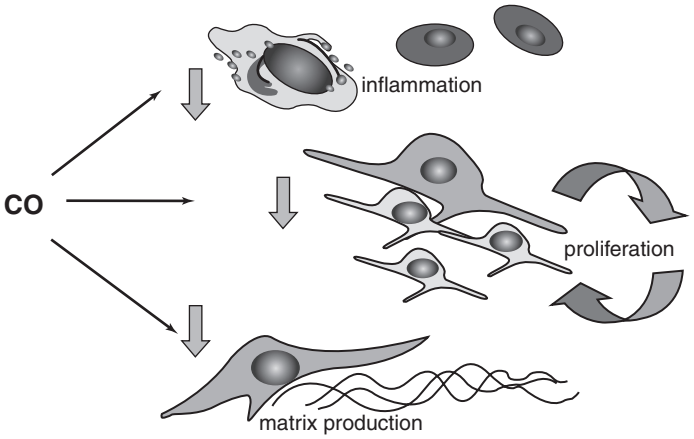

FIG. 9. Possible mechanisms of the antifibrotic effects of CO. CO inhibits bleomycin-induced lung injury (fibrosis) *in vivo.* Several mechanisms have been proposed (141), including general antiinflammatory protection to the lung, as well as the inhibition of fibroblast proliferation and matrix deposition.

in the mouse model. The protection was associated with decreased epithelial cell apoptosis and increased IFN-γ production.

Exogenous CO treatment can also provide protection against bleomycin-induced fibrotic lung injury in mice (141). In mice treated with bleomycin intratracheally and then exposed to CO or ambient air, the lungs from CO-treated animals displayed reduced lung hydroxyproline, collagen, and fibronectin levels relative to air-treated bleomycin-injured controls. The protective effect of CO in this model was associated with an antiproliferative effect of CO on fibroblast proliferation associated with the upregulation of $p21^{Waf1/Cip1}$ and inhibition of cyclins A/D expression (141).

Furthermore, rats that were infused with bilirubin, an HO pathway end product, were also resistant to bleomycin-induced pulmonary fibrosis relative to control animals. The bilirubin-treated rats displayed reduced lung damage in response to bleomycin, as indicated by hydroxyproline content, and reduced proinflammatory cell counts, as well as transforming growth factor-β levels in bronchoalveolar lavage fluid (121).

Protective effects of HO-1 have also been proposed in particle-induced fibrosis, or pneumoconiosis (*e.g.*, asbestosis, silicosis). In rats acutely exposed to crocidolite asbestos by intratracheal instillation, HO-1 increased in the lung and alveolar macrophages (70). Increased HO-1 expression was detected in the serum of patients with silicosis relative to age-matched healthy normal patients. In silicosis patients, the expression level of serum HO-1 positively correlated to lung function and negatively correlated to DNA-damage markers, suggesting a beneficial role for HO-1 in this type of injury. Elevated HO-1 expression also occurred in the lungs of mice after prolonged silica exposure, coincident with the focal sites of particle accumulation (101). Preconditioning the mice with heme reduced silica-induced lung inflammation, whereas treatment with tin-protoporphyrin-IX, a competitive inhibitor of HO activity, exacerbated the injury (101).

PROTECTIVE EFFECTS OF HO-1/CO IN CYSTIC FIBROSIS

Cystic fibrosis is an inherited disorder characterized by abnormal mucus buildup in the airways, which can cause pulmonary damage associated with secondary infections. In cystic fibrosis patients, elevated HO-1 levels were detected in macrophages. Consistent with a protective role of HO-1 in this disease state, cultured airway epithelial cells overexpressing HO-1 were resistant to apoptosis and injury caused by *Pseudomonas aeruginosa* infection (140).

PROTECTIVE EFFECTS OF HO-1/CO IN PULMONARY HYPERTENSION

Pulmonary arterial hypertension (PAH) is a terminal disease characterized by a progressive increase in pulmonary vascular resistance, leading to right ventricular failure. A number of studies have demonstrated that HO enzymes or CO, or both, can exert a protective function against the formation of pulmonary hypertension and in the regulation of hypoxic pulmonary vasoconstriction. The *ho-1*$^{-/-}$ null mice displayed an exaggerated response to chronic hypoxia relative to wild-type mice, in particular, dramatic right heart hypertrophy, which included right ventricular infarcts and the formation of mural thrombi (130). The induction of HO-1 protein with chemical inducing agents such as heme and $NiCl_2$ prevented the development of PAH in the rat lung in response to chronic hypoxia treatment (17). Furthermore, transgenic mice with the targeted overexpression of HO-1 in the lung, displayed reduced lung inflammation, pulmonary hypertension, and vascular hypertrophy as a function of treatment for chronic hypoxia, relative to wild-type mice (63). In monocrotaline-induced hypertension, protective effects were observed by treatment with the antiproliferative agent rapamcyin, associated with the induction of HO-1 (139). *In vitro*, the antiproliferative effect of rapamycin on smooth muscle cells, also depended in part on HO-1 expression, as it was diminished in smooth muscle cells derived from *ho-1*$^{-/-}$ mice (139).

CO provided protection in rodent models of monocrotaline-induced and hypoxia-induced PAH (Fig. 10). Exposure to CO (1 h/day) reversed established PAH and right ventricular hypertrophy, and restored right ventricular and pulmonary arterial pressures, as well as pulmonary vascular morphology, to that of controls. The ability of CO to reverse PAH required endothelial nitric oxide synthase (NOS3) and NO generation, because CO failed to reverse chronic hypoxia-induced PAH in

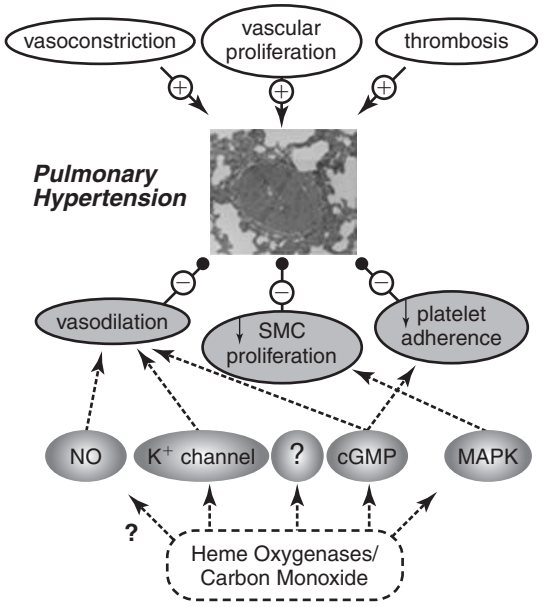

FIG. 10. Potential role of HO-1/CO in pulmonary arterial hypertension. HO-1 and CO have been demonstrated to exert protective effects against the development of pulmonary hypertension, as discussed in recent studies (17, 63, 139, 142). Exogenous or HO-derived CO potentially exerts these effects through vasodilatory effects, inhibition of thrombosis, and inhibition of smooth muscle cell (SMC) proliferation. The vasoactive effects of CO in PAH have been assigned to cGMP-dependent effects, modulation of potassium (K^+) channel activities, and secondary effects on NO generation (23, 142). The antiproliferative effects and antithrombotic effects of CO involve cGMP, and/or MAPK signaling (67, 142). Although the precise mechanisms for the protective effects of CO likely involve a combination of these mechanisms, further work is needed to clarify these relations.

eNOS-deficient (*nos3*$^{-/-}$) mice. The protective effect of CO was endothelial cell dependent, and associated with increased apoptosis and decreased cellular proliferation of vascular smooth muscle cells (142). Recent studies have demonstrated that CO decreased pulmonary artery vascular resistance and inhibited hypoxic vasoconstriction, by mechanisms potentially involving cGMP, and the hyperpolarization of potassium channels (23).

Interestingly, antiproliferative effects of biliverdin/bilirubin on vascular smooth muscle have been described, and these may also have therapeutic benefit in hyperproliferative disorders (72, 77).

THERAPEUTIC IMPLICATIONS OF HO-1 AND HEME METABOLITES IN THE LUNG

Elevated expression of HO-1, an inducible response to oxidative stress, can occur in the context of several pulmonary disease states involving lung inflammation, including chronic smoke exposure and asthma. In such cases, HO-1 elevation is generally regarded as an endogenous defense against oxidative or inflammatory injury. Conversely, in COPD, which is associated with persistent inflammation, in which antioxidant responses may be compromised, a reduced or impaired HO-1 expression may contribute to disease progression. Direct protective effects of HO-1 expression by gene transfer in the lung have been observed in several animal models, including endotoxemia, I/R injury, oxidative lung injury, and more recently in fibrotic lung diseases and emphysema. In this light, gene-therapy approaches have been proposed for manipulating HO-1 expression for therapeutic gain. As an alternative to genetic approaches, preconditioning with chemical inducers of HO-1 can be used with similar intent. Unfortunately, many agents used experimentally for HO-1 preconditioning, (*e.g.*, cobalt protoporphyrin, hemin) are not well suited for clinical application. Therefore, further development of nontoxic and specific agonists may be required.

The mechanisms by which HO-1 confers tissue protection remain incompletely understood, but likely involve downstream effects of the reaction products on intracellular signaling pathways. Recent studies demonstrate that the pharmacologic application of the heme metabolites to cells and animals can confer tissue protection in stress/injury models. Protective effects of biliverdin or bilirubin, involving antiinflammatory and antiproliferative effects, have been reported in lung endotoxinemia, vascular injury, as well as in I/R injury of various other organs (95). The protective properties of CO have been demonstrated in lung and other organ I/R injury models, lung and vascular transplantation, endotoxemia, and recently, in specialized lung-disease models such as ventilator-induced lung injury and bleomycin-induced pulmonary fibrosis. These relate to antiinflammatory and antiapoptotic (as in endotoxinemia, oxidative lung injury, and I/R injury) or antiproliferative effects (as in vascular injury or PAH), as well as possible vasodilatory effects (as in I/R injury).

The demonstrated efficacy of low-dose CO as a therapeutic agent in multiple models of tissue injury and disease continues to herald a possible therapeutic use in humans, with applications ranging from the improved efficacy of organ transplantation to the critical care of terminal diseases. Although some concerns of safety or therapeutic efficiency have been raised (25, 61), a recent study demonstrated that the application of CO to animals at low concentrations approximating cigarette-smoke exposure caused no apparent lung pathology (108). Like all new potential therapeutic modalities, we must continue to pursue with commitment and excitement the novel therapeutic application of inhaled CO in human diseases. The recently developed carbon monoxide–releasing molecules (CORMs) (*i.e.*, transition metal carbonyls) may provide an alternative vehicle for the localized pharmacologic delivery of CO (69). Currently progressing and future "Investigational New Drug" approved clinical trials will determine the future of this gas and its donor compounds as clinically useful therapeutic tools.

ACKNOWLEDGMENTS

This work was supported by awards from the American Heart Association to S. W. Ryter (AHA 0335035N), and H. P. Kim (AHA 0525552U), a Veterans Administration Career Devel-

opment Award to D. Morse, and NIH grants R01-HL60234, R01-HL55330, R01-HL079904, and P01-HL70807 awarded to A. M. K. Choi.

ABBREVIATIONS

cGMP, guanosine 3′,5′-monophosphate; CO, carbon monoxide; COPD, chronic obstructive pulmonary disease; CS, cigarette smoke; CSE, cigarette smoke extract; DISC, death-inducing signal complex; ERK1/2 MAPK, extracellular regulated kinase-1/2 (mitogen-activated protein kinase); GM-CSF, granulocyte–macrophage colony-stimulating factor; Hb-CO, carboxyhemoglobin; HO-1, heme oxygenase-1; HO-2, heme oxygenase-2; IFN-γ interferon-γ IL-1β interleukin-1β IL-6, interleukin-6; IL-10, interleukin-10; IPF, idiopathic pulmonary fibrosis; I/R, ischemia/reperfusion; JNK, c-jun-NH2-terminal kinase; p38 MAPK, p38 mitogen-activated protein kinase; MKK3, mitogen-activated protein kinase kinase-3; MLEC, mouse lung endothelial cells; NF-κB, nuclear factor kappa-B; Nrf2, NF-E2–related factor 2; PAH, pulmonary arterial hypertension; ROS, reactive oxygen species; TNF-α tumor necrosis factor-α.

REFERENCES

1. Alam J and Cook J. How many transcription factors does it take to turn on the heme oxygenase-1 gene? *Am J Respir Cell Mol Biol* 36: 166–174, 2006.
2. Alam J, Cai J, and Smith A. Isolation and characterization of the mouse heme oxygenase-1 gene: distal 5′ sequences are required for induction by heme or heavy metals. *J Biol Chem* 269: 1001–1009, 1994.
3. Alam J, Camhi S, and Choi AM. Identification of a second region upstream of the mouse heme oxygenase-1 gene that functions as a basal level and inducer-dependent transcription enhancer. *J Biol Chem* 270: 11977–11984, 1995.
4. Alam J, Igarashi K, Immenschuh S, Shibahara S, and Tyrrell RM. Regulation of heme oxygenase-1 gene transcription: recent advances and highlights from the International Conference (Uppsala, 2003) on Heme Oxygenase. *Antioxid Redox Signal* 6: 924–933, 2004.
5. Alam J, Stewart D, Touchard C, Boinapally S, Choi AM, and Cook JL. Nrf2, a Cap'n'Collar transcription factor, regulates induction of the heme oxygenase-1 gene. *J Biol Chem* 274: 26071–26078, 1999.
6. Alam J, Wicks C, Stewart D, Gong P, Touchard C, Otterbein S, Choi AM, Burow ME, and Tou J. Mechanism of heme oxygenase-1 gene activation by cadmium in MCF-7 mammary epithelial cells: role of p38 kinase and Nrf2 transcription factor. *J Biol Chem* 275: 27694–27702, 2000.
7. Almolki A, Taille C, Martin GF, Jose PJ, Zedda C, Conti M, Megret J, Henin D, Aubier M, and Boczkowski J. Heme oxygenase attenuates allergen-induced airway inflammation and hyperreactivity in guinea pigs. *Am J Physiol Lung Cell Mol Physiol* 287: L26–L34, 2004.
8. Ameredes BT, Otterbein LE, Kohut LK, Gligonic AL, Calhoun WJ, and Choi AM. Low-dose carbon monoxide reduces airway hyperresponsiveness in mice. *Am J Physiol Lung Cell Mol Physiol* 285: L1270–L1276, 2003.
9. Bach FH. Heme oxygenase-1 and transplantation tolerance. *Hum Immunol* 2006 67: 430–432, 2006.
10. Barnes PJ, Shapiro SD, and Pauwels RA. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. *Eur Respir J* 22: 672–688, 2003.
11. Bowler RP, Barnes PJ, and Crapo JD. The role of oxidative stress in chronic obstructive pulmonary disease. *COPD* 1: 255–277, 2004.
12. Brouard S, Berberat PO, Tobiasch E, Seldon MP, Bach FH, and Soares MP. Heme oxygenase-1-derived carbon monoxide requires the activation of transcription factor NF-kappa B to protect endothelial cells from tumor necrosis factor-alpha-mediated apoptosis. *J Biol Chem* 277: 17950–17961, 2002.
13. Brouard S, Otterbein LE. Anrather J, Tobiasch E, Bach FH, Choi AM, and Soares MP. Carbon monoxide generated by heme oxygenase-1 suppresses endothelial cell apoptosis. *J Exp Med* 192: 1015–1026, 2000.
14. Camhi SL, Alam J, Wiegand GW, Chin BY, and Choi AM. Transcriptional activation of the HO-1 gene by lipopolysaccharide is mediated by 5′ distal enhancers: role of reactive oxygen intermediates and AP-1. *Am J Respir Cell Mol Biol* 18: 226–334, 1998.
15. Celli BR, MacNee W, Agusti A, Anzueto A, Berg B, Buist AS, Calverley PMA, Chavannes N, Dillard T, Fahy B, Fein A, Heffner J, Lareau S, Meek P, Martinez F, McNicholas W, Muris J, Austegard E, Pauwels R, Rennard S, Rossi A, Siafakas N, Tiep B, Vestbo J, Wouters E, and ZuWallack R. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J* 23: 932–946, 2004.
16. Chapman JT, Otterbein LE, Elias JA, and Choi AM. Carbon monoxide attenuates aeroallergen-induced inflammation in mice. *Am J Physiol Lung Cell Mol Physiol* 281: L209–L216, 2001.
17. Christou H, Morita T, Hsieh CM, Koike H, Arkonac B, Perrella MA, and Kourembanas S. Prevention of hypoxia-induced pulmonary hypertension by enhancement of endogenous heme oxygenase-1 in the rat. *Circ Res* 86: 1224–1229, 2000.
18. Coburn RF, Williams WJ, White P, and Kahn SB. The production of carbon monoxide from hemoglobin in vivo. *J Clin Invest* 46: 346–356, 1967.
19. Converso DP, Taille C, Carreras MC, Jaitovich A, Poderoso JJ, and Boczkowski J. HO-1 is located in liver mitochondria and modulates mitochondrial heme content and metabolism. *FASEB J* 20: 1236–1238, 2006.
20. Deveci SE, Deveci F, Acik Y, and Ozan AT. The measurement of exhaled carbon monoxide in healthy smokers and non-smokers. *Respir Med* 98: 551–556, 2004.
21. Dolinay T, Szilasi M, Liu M, and Choi AMK. Inhaled carbon monoxide confers anti-inflammatory effects against ventilator-induced lung injury. *Am J Respir Crit Care Med* 170: 613–620, 2004.
22. Donnelly LE and Barnes PJ. Expression of heme oxygenase in human airway epithelial cells. *Am J Respir Cell Mol Biol* 24: 295–303, 2001.
23. Dubuis E, Potier M, Wang R, and Vandier C. Continuous inhalation of carbon monoxide attenuates hypoxic pulmonary hypertension development presumably through activation of BKCa channels. *Cardiovasc Res* 65: 751–761, 2005.
24. Durante W, Johnson FK, and Johnson RA. Role of carbon monoxide in cardiovascular function. *J Cell Mol Med* 10: 672–686, 2006.
25. Favory R, Lancel S, Tissier S, Mathieu D, Decoster B, and Neviere R. Myocardial dysfunction and potential cardiac hypoxia in rats induced by carbon monoxide inhalation. *Am J Respir Crit Care Med* 174: 320–325, 2006.
26. Fogg S, Agarwal A, Nick HS, and Visner GA. Iron regulates hyperoxia-dependent human heme oxygenase 1 gene expression in pulmonary endothelial cells. *Am J Respir Cell Mol Biol* 20: 797–804, 1999.
27. Fu WP, Sun C, Dai LM, Yang LF, and Zhang YP. Relationship between COPD and polymorphisms of HOX-1 and mEPH in a Chinese population. *Oncol Rep* 17: 483–488, 2007.
28. 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.
29. Fukano Y, Yoshimura H, and Yoshida T. Heme oxygenase-1 gene expression in human alveolar epithelial cells (A549) following exposure to whole cigarette smoke on a direct in vitro exposure system. *Exp Toxicol Pathol* 57: 411–418, 2006.

30. Furchgott RF and Jothianandan D. Endothelium-dependent and independent vasodilation involving cyclic GMP: relaxation induced by nitric oxide, carbon monoxide and light. *Blood Vessels* 28: 52–61, 1991.
31. Gorman D, Drewry A, Huang YL, and Sames C. The clinical toxicology of carbon monoxide. *Toxicology* 187: 25–38, 2003.
32. Guenegou A, Leynaert B, Benessiano J, Pin I, Demoly P, Neukirch F, Boczkowski J, and Aubier M. Association of lung function decline with the heme oxygenase-1 gene promoter microsatellite polymorphism in a general population sample: results from the European Community Respiratory Health Survey (ECRHS), France. *J Med Genet* 43: e43, 2006.
33. Harju T, Soini Y, Paakko R, and Kinnula VL. Up-regulation of heme oxygenase-I in alveolar macrophages of newly diagnosed asthmatics. *Respir Med* 96: 418–423, 2002.
34. Hashiba T, Suzuki M, Nagashima Y, Suzuki S, Inoue S, Tsuburai T, Matsuse T, and Ishigatubo Y. Adenovirus-mediated transfer of heme oxygenase-1 cDNA attenuates severe lung injury induced by the influenza virus in mice. *Gene Ther* 8: 1499–1507, 2001.
35. Horvath I, Donnelly LE, Kiss A, Paredi P, Kharitonov SA, and Barnes PJ. Raised levels of exhaled carbon monoxide are associated with an increased expression of heme oxygenase-1 in airway macrophages in asthma: a new marker of oxidative stress. *Thorax* 53: 668–672, 1998.
36. Horvath I, MacNee W, Kelly FJ, Dekhuijzen PN, Phillips M, Doring G, Choi AM, Yamaya M, Bach FH, Willis D, Donnelly LE, Chung KF, and Barnes PJ. "Haemoxygenase-1 induction and exhaled markers of oxidative stress in lung diseases," summary of the ERS Research Seminar in Budapest, Hungary, September 1999. *Eur Respir J* 18: 420–430, 2001.
37. Hoshino Y, Mio T, Nagai S, Miki H, Ito I, and Izumi T. Cytotoxic effects of cigarette smoke extract on an alveolar type II cell-derived cell line. *Am J Physiol* 281: L509–L516, 2001.
38. Inoue S, Suzuki M, Nagashima Y, Suzuki S, Hashiba T, Tsuburai T, Ikehara K, Matsuse T, and Ishigatsubo Y. Transfer of heme oxygenase 1 cDNA by a replication-deficient adenovirus enhances interleukin 10 production from alveolar macrophages that attenuates lipopolysaccharide-induced acute lung injury in mice. *Hum Gene Ther* 12: 967–979, 2001.
39. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, and Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun* 236: 313–322, 1997.
40. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, and Yamamoto M. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev* 13: 76–86, 1999.
41. Keshavan P, Deem TL, Schwemberger SJ, Babcock GF, Cook-Mills JM, and Zucker SD. Unconjugated bilirubin inhibits VCAM-1 mediated transendothelial leukocyte migration. *J Immunol* 174: 3709–3718, 2005.
42. Keyse SM and Tyrrell RM. Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. *Proc Natl Acad Sci U S A* 86: 99–103, 1989.
43. Kim HP, Ryter SW, and Choi AM. CO as a cellular signaling molecule. *Annu Rev Pharmacol Toxicol* 46: 411–449, 2006.
44. Kim HP, Wang X, Nakao A, Kim SI, Murase N, Choi ME, Ryter SW, and Choi AMK. Caveolin-1 expression by means of p38β mitogen activated protein kinase mediates the antiproliferative effect of carbon monoxide. *Proc Natl Acad Sci U S A* 102: 11319–11324, 2005.
45. Kim HP, Wang X, Zhang J, Suh GY, Benjamin I, Ryter SW, and Choi AM. Heat shock factor-1 mediates the cytoprotective effect of carbon monoxide. *J Immunol* 175: 2622–2629, 2005.
46. Kitada O, Kodama T, Kuribayashi K, Ihaku D, Fujita M, Matsuyama T, and Sugita M. Heme oxygenase-1 protein induction in a mouse model of asthma. *Clin Exp Allergy* 31: 1470–1477, 2001.
47. Kitamura Y, Hashimoto S, Mizuta N, Kobayashi A, Kooguchi K, Fujiwara I, and Nakajima H. Fas/FasL-dependent apoptosis of alveolar cells after lipopolysaccharide-induced lung injury in mice. *Am J Respir Crit Care Med* 163: 762–769, 2001.
48. Kitamuro T, Takahashi K, Ogawa K, Udono-Fujimori R, Takeda K, Furuyama K, Nakayama M, Sun J, Fujita H, Hida W, Hattori T, Shirato K, Igarashi K and Shibahara S. Bach1 functions as a hypoxia-inducible repressor for the heme oxygenase-1 gene in human cells. *J Biol Chem* 278: 9125–9133, 2003.
49. Knorr-Wittmann C, Hengstermann A, Gebel S, Alam J, and Muller T. Characterization of Nrf2 activation and heme oxygenase-1 expression in NIH3T3 cells exposed to aqueous extracts of cigarette smoke. *Free Radic Biol Med* 39: 1438–1448, 2005.
50. Kobayashi A, Kang MI, Okawa H, Ohtsuji M, Zenke Y, Chiba T, Igarashi K, and Yamamoto M. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol Cell Biol* 24: 7130–7139, 2004.
51. Lee PJ and Choi AMK. Pathways of cell signaling in hyperoxia. *Free Radic Biol Med* 35: 341–350, 2003.
52. Lee PJ, Alam J, Sylvester SL, Inamdar N, Otterbein L, and Choi AM. Regulation of heme oxygenase-1 expression in vivo and in vitro in hyperoxic lung injury. *Am J Respir Cell Mol Biol* 14: 556–568, 1996.
53. 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
54. Li N, Venkatesan MI, Miguel A, Kaplan R, Gujuluva C, Alam J, and Nel A. Induction of heme oxygenase-1 expression in macrophages by diesel exhaust particle chemicals and quinones via the antioxidant-responsive element. *J Immunol* 165: 3393–3401, 2000.
55. Lim S, Groneberg D, Fischer A, Oates T, Caramori G, Mattos W, Adcock I, Barnes PJ, and Chung KF. Expression of heme oxygenase isoenzymes 1 and 2 in normal and asthmatic airways: effect of inhaled corticosteroids. *Am J Respir Crit Care Med* 162: 1912–1918, 2000.
56. Liu X, Conner H, Kobayashi T, Kim H, Wen F, Abe S, Fang Q, Wang X, Hashimoto M, Bitterman P, and Rennard SI. Cigarette smoke extract induces DNA damage but not apoptosis in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 33: 121–129, 2005.
57. Maestrelli P, El Messlemani AH, De Fina O, Nowicki Y, Saetta M, Mapp C, and Fabbri LM. Increased expression of heme oxygenase (HO)-1 in alveolar spaces and HO-2 in alveolar walls of smokers. *Am J Respir Crit Care Med* 164: 1508–1513, 2001.
58. Maestrelli P, Paska C, Saetta M, Turato G, Nowicki Y, Monti S, Formichi B, Miniati M, and Fabbri LM. Decreased haem oxygenase-1 and increased inducible nitric oxide synthase in the lung of severe COPD patients. *Eur Respir J* 21: 971–976, 2003.
59. Maines MD. The heme oxygenase system: update 2005. *Antioxid Redox Signal* 7: 1761–1766, 2006.
60. Mathew R, Huang J, Shah M, Patel K, Gewitz M, and Sehgal PB. Disruption of endothelial-cell caveolin-1alpha/raft scaffolding during development of monocrotaline-induced pulmonary hypertension. *Circulation* 110: 1499–1506, 2004.
61. Mayr FB, Spiel A, Leitner J, Marsik C, Germann P, Ullrich R, Wagner O , and Jilma B. Effects of carbon monoxide inhalation during experimental endotoxemia in humans. *Am J Respir Crit Care Med* 171: 354–360, 2005.
62. Menzel DB and Amdur MO. Toxic response of the respiratory system. In: Casarett and Doull's toxicology, the basic science of poisons. 3rd ed., edited by Klaassen K, Amdur MO, and Doull J. New York: Macmillan, 1986, pp. 330–358.
63. Minamino T, Christou H, Hsieh CM, Liu Y, Dhawan V, Abraham NG, Perrella MA, Mitsialis SA, and Kourembanas S. Targeted expression of heme oxygenase-1 prevents the pulmonary inflammatory and vascular responses to hypoxia. *Proc Natl Acad Sci U S A* 98: 8798–8803, 2001.
64. Mishra S, Fujita T, Lama VN, Nam D, Liao H, Okada M, Minamoto K, Yoshikawa Y, Harada H, and Pinsky DJ. Carbon monoxide rescues ischemic lungs by interrupting MAPK-driven expression of early growth response 1 gene and its downstream target genes. *Proc Natl Acad Sci U S A* 13: 5191–5196, 2006.

65. Montuschi P, Kharitonov SA, and Barnes PJ. Exhaled carbon monoxide and nitric oxide in COPD. *Chest* 120: 496–501, 2001.
66. Morita T and Kourembanas S. Endothelial cell expression of vasoconstrictors and growth factors is regulated by smooth muscle cell-derived carbon monoxide. *J Clin Invest* 96: 2676–2682, 1995.
67. Morita T, Mitsialis SA, Koike H, Liu Y, and Kourembanas S. Carbon monoxide controls the proliferation of hypoxic vascular smooth muscle cells. *J Biol Chem* 272: 32804–32809, 1997.
68. Morse D, Pischke SE, Zhou Z, Davis RJ, Flavell RA, Loop T, Otterbein SL, Otterbein LE, and Choi AM. Suppression of inflammatory cytokine production by carbon monoxide involves the JNK pathway and AP-1. *J Biol Chem* 278: 36993–36998, 2003.
69. Motterlini R, Mann BE, and Foresti R. Therapeutic applications of carbon monoxide-releasing molecules. *Expert Opin Invest Drugs* 14: 1305–1318, 2005.
70. Nagatomo H, Morimoto Y, Oyabu T, Hirohashi M, Ogami A, Yamato H, Kuroda K, Higashi T, and Tanaka I. Expression of heme oxygenase-1 in the lungs of rats exposed to crocidolite asbestos. *Inhal Toxicol* 17: 293–296, 2005.
71. Nakahira K, Kim HP, Geng XH, Nakao A, Wang X, Murase N, Drain PF, Wang X, Sasidhar M, Nabel EG, Takahashi T, Lukacs NW, Ryter SW, Morita K, and Choi AM. Carbon monoxide differentially inhibits TLR signaling pathways by regulating ROS-induced trafficking of TLRs to lipid rafts. *J Exp Med* 203: 2377–2389, 2006.
72. Nakao A, Murase N, Ho C, Toyokawa H, Billiar TR, and Kanno S. Biliverdin administration prevents the formation of intimal hyperplasia induced by vascular injury. *Circulation* 112: 587–591, 2005.
73. Nakayama M, Takahashi K, Kitamuro T, Yasumoto K, Katayose D, Shirato K, Fujii-Kuriyama Y, and Shibahara S. Repression of heme oxygenase-1 by hypoxia in vascular endothelial cells. *Biochem Biophys Res Commun* 271: 665–671, 2000.
74. Ng CS, Wan S, and Yim AP. Pulmonary ischaemia-reperfusion injury: role of apoptosis. *Eur Respir J* 25: 356–363, 2005.
75. Ning W, Li CJ, Kaminski N, Feghali-Bostwick CA, Alber SM, Di YP, Otterbein SL, Song R, Hayashi S, Zhou Z, Pinsky DJ, Watkins SC, Pilewski JM, Sciurba FC, Peters DG, Hogg JC, and Choi AM. Comprehensive gene expression profiles reveal pathways related to the pathogenesis of chronic obstructive pulmonary disease. *Proc Natl Acad Sci U S A* 101: 14895–14900, 2004.
76. Ogawa K, Sun J, Taketani S, Nakajima O, Nishitani C, Sassa S, Hayashi N, Yamamoto M, Shibahara S, Fujita H, and Igarashi K. Heme mediates derepression of Maf recognition element through direct binding to transcription repressor Bach1. *EMBO J* 20: 2835–2843, 2001.
77. Ollinger R, Bilban M, Erat A, Froio A, McDaid J, Tyagi S, Csizmadia E, Graca-Souza A, Liloia A, Soares MP, Otterbein LE, Usheva A, Yamashita K, and Bach FH. Bilirubin: a natural inhibitor of vascular smooth muscle proliferation. *Circulation* 112: 1030–1039, 2005.
78. Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, Davis RJ, Flavell RA, and Choi AM. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat Med* 6: 422–428, 2000.
79. 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 hyperoxic lung injury. *J Clin Invest* 103: 1047–1054, 1999.
80. Otterbein LE, Mantell LL, and Choi AM. Carbon monoxide provides protection against hyperoxic lung injury. *Am J Physiol* 276: L688–L694, 1999.
81. Otterbein LE, Otterbein SL, Ifedigbo E, Liu F, Morse DE, Fearns C, Ulevitch RJ, Knicklebein R, Flavell RA, and Choi AM. MKK3 mitogen activated protein kinase pathway mediates carbon monoxide-induced protection against oxidant induced lung injury. *Am J Pathol* 163: 2555–2563, 2003.
82. Otterbein LE, Zuckerbraun BS, Haga M, Liu F, Song R, Usheva A, Stachulak C, Bodyak N, Smith RN, Csizmadia E, Tyagi S, Akamatsu Y, Flavell RJ, Billiar TR, Tzeng E, Bach FH, Choi AM, and Soares MP. Carbon monoxide suppresses arteriosclerotic lesions associated with chronic graft rejection and with balloon injury. *Nat Med* 9: 183–190, 2003.
83. Palozza P, Serini S, Curro D, Calviello G, Igarashi K, and Mancuso C. β-Carotene and cigarette smoke condensate regulate heme oxygenase-1 and its repressor factor Bach1: relationship with cell growth. *Antioxid Redox Signal* 8: 1069–1080, 2006.
84. Petrache I, Natarajan V, Zhen L, Medler TR, Richter AT, Cho C, Hubbard WC, Berdyshev EV, and Tuder RM. Ceramide upregulation causes pulmonary cell apoptosis and emphysema-like disease in mice. *Nat Med* 11: 491–498, 2005.
85. Petrache I, Otterbein LE, Alam J, Wiegand GW, and Choi AM. Heme oxygenase-1 inhibits TNF-alpha-induced apoptosis in cultured fibroblasts. *Am J Physiol Lung Cell Mol Physiol* 278: L312–L319, 2000.
86. Poss KD and Tonegawa S. Heme oxygenase 1 is required for mammalian iron reutilization. *Proc Natl Acad Sci U S A* 94: 10919–10924, 1997.
87. 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.
88. Rahman I and MacNee W. Role of oxidants/antioxidants in smoking-induced lung diseases. *Free Radic Biol Med* 21: 669–681, 1996.
89. Rahman I, Biswas SK, and Kode A. Oxidant and antioxidant balance in the airways and airway diseases. *Eur J Pharmacol* 533: 222–239, 2006.
90. Rangasamy T, Cho CY, Thimmulappa RK, Zhen L, Srisuma SS, Kensler TW, Yamamoto M, Petrache I, Tuder RM, and Biswal S. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. *J Clin Invest* 114: 1248–1259, 2004.
91. Rennard SI. Cigarette smoke in research. *Am J Respir Cell Mol Biol* 31: 479–480, 2004.
92. Rutgers SR, Timens W, Kauffman HF, and Postma DS. Markers of active airway inflammation and remodelling in chronic obstructive pulmonary disease. *Clin Exp Allergy* 31: 193–205, 2001.
93. Ryter S, Morse D, and Choi AM. Carbon monoxide and bilirubin: potential therapies for pulmonary/vascular injury and disease. *Am J Respir Cell Mol Biol* 36: 175–182, 2007.
94. Ryter SW and Choi AMK. Heme oxygenase-1: molecular mechanisms of gene expression in oxygen-related stress. *Antioxid Redox Signal* 4: 625–632, 2002.
95. Ryter SW, Alam J, and Choi AM. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. *Physiol Rev* 86: 583–650, 2006.
96. Ryter SW and Otterbein L. Carbon monoxide in biology and medicine. *Bioessays* 26: 270–280, 2004.
97. Ryter SW, Si M, Lai CC, and Su CY. Regulation of endothelial heme oxygenase activity during hypoxia is dependent on chelatable iron. *Am J Physiol Heart Circ Physiol* 279: H2889–H2897, 2000.
98. Samb A, Taille C, Almolki A, Megret J, Staddon JM, Aubier M, and Boczkowski J. Heme oxygenase modulates oxidant-signaled airway smooth muscle contractility: role of bilirubin. *Am J Physiol Lung Cell Mol Physiol* 283: L596–603, 2002.
99. Sarady J, Otterbein SL, Liu F, Otterbein LE, and Choi AMK. Carbon monoxide modulates endotoxin-induced production of granulocyte macrophage colony-stimulating factor in macrophages. *Am J Respir Cell Mol Biol* 27: 739–745, 2002.
100. Sarady-Andrews JK, Liu F, Gallo D, Nakao A, Overhaus M, Ollinger R, Choi AM, and Otterbein LE. Biliverdin administration protects against endotoxin-induced acute lung injury in rats. *Am J Physiol Lung Cell Mol Physiol* 289: L1131–L1137, 2005.
101. Sato T, Takeno M, Honma K, Yamauchi H, Saito Y, Sasaki T, Morikubo H, Nagashima Y, Takagi S, Yamanaka K, Kaneko T, and Ishigatsubo Y. Heme oxygenase-1, a potential biomarker of chronic silicosis, attenuates silica-induced lung injury. *Am J Respir Crit Care Med* 174: 906–914, 2006.
102. Selman M, King TE, Pardo A, American Thoracic Society, European Respiratory Society, and American College of Chest Physicians. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med* 134: 136–151, 2001.
103. Shinohara T, Kaneko T, Nagashima Y, Ueda A, Tagawa A, and Ishigatsubo Y. Adenovirus-mediated transfer and overexpression

of heme oxygenase 1 cDNA in lungs attenuates elastase-induced pulmonary emphysema in mice. *Hum Gene Ther* 16: 318–327, 2005.
104. Slebos DJ, Kerstjens HA, Rutgers SR, Kauffman HF, Choi AM, and Postma DS. Haem oxygenase-1 expression is diminished in alveolar macrophages of patients with COPD. *Eur Respir J* 23: 652–653, 2004.
105. Slebos DJ, Ryter SW, van der Toorn M, Liu F, Guo F, Baty CJ, Karlsson JM, Watkins SC, Kim HP, Wang X, Lee JS, Postma DS, Kauffman HF, and Choi AM. Mitochondrial localization and function of heme oxygenase-1 in cigarette smoke-induced cell death. *Am J Respir Cell Mol Biol* 2006 Nov 1; [Epub ahead of print].
106. Song R, Mahidhara RS, Liu F, Ning W, Otterbein LE, and Choi AM. Carbon monoxide inhibits human airway smooth muscle cell proliferation via mitogen-activated protein kinase pathway. *Am J Respir Cell Mol Biol* 27: 603–610, 2002.
107. Song R, Ning W, Liu F, Ameredes BT, Calhoun WJ, Otterbein LE, and Choi AM. Regulation of IL-1β-induced GM-CSF production in human airway smooth muscle cells by carbon monoxide. *Am J Physiol Lung Cell Mol Physiol* 284: L50–L56, 2002.
108. Sorhaug S, Steinshamn S, Nilsen OG, Waldum HL. Chronic inhalation of carbon monoxide: effects on the respiratory and cardiovascular system at doses corresponding to tobacco smoking. *Toxicology* 228: 280–290, 2006.
109. Soti C, Nagy E, Giricz Z, Vigh L, Csermely P, and Ferdinandy P. Heat shock proteins as emerging therapeutic targets. *Br J Pharmacol* 146: 769–780, 2005.
110. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, and Ames BN. Bilirubin is an antioxidant of possible physiological importance. *Science* 235: 1043–1046, 1987.
111. Sun J, Hoshino H, Takaku K, Nakajima O, Muto A, Suzuki H, Tashiro S, Takahashi S, Shibahara S, Alam J, Taketo MM, Yamamoto M, and Igarashi K. Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene. *EMBO J* 21: 5216–5224, 2002.
112. Taille C, Almolki A, Benhamed M, Zedda C, Megret J, Berger P, Leseche G, Fadel E, Yamaguchi T, Marthan R, Aubier M, and Boczkowski J. Heme oxygenase inhibits human airway smooth muscle proliferation via a bilirubin-dependent modulation of ERK1/2 phosphorylation. *J Biol Chem* 278: 27160–27168, 2003.
113. Taille C, El-Benna J, Lanone S, Boczkowski J, and Motterlini R. Mitochondrial respiratory chain and NAD(P)H oxidase are targets for the antiproliferative effect of carbon monoxide in human airway smooth muscle. *J Biol Chem* 280: 25350–25360, 2005.
114. Taille C, El-Benna J, Lanone S, Dang MC, Ogier-Denis E, Aubier M, and Boczkowski J. Induction of heme oxygenase-1 inhibits NAD(P)H oxidase activity by down-regulating cytochrome b558 expression via the reduction of heme availability. *J Biol Chem* 279: 28681–28688, 2004.
115. Tenhunen R, Marver HS, and Schmid R. Microsomal heme oxygenase: characterization of the enzyme. *J Biol Chem* 244: 6388–6394, 1969.
116. 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.
117. Tsuburai T, Suzuki M, Nagashima Y, Suzuki S, Inoue S, Hasiba T, Ueda A, Ikehara K, Matsuse T, and Ishigatsubo Y. Adenovirus-mediated transfer and overexpression of heme oxygenase 1 cDNA in lung prevents bleomycin-induced pulmonary fibrosis via a Fas-Fas ligand-independent pathway. *Hum Gene Ther* 13: 1945–1960, 2002.
118. Tuder RM, Yoshida T, Arap W, Pasqualini R, and Petrache I. State of the art: cellular and molecular mechanisms of alveolar destruction in emphysema: an evolutionary perspective. *Proc Am Thorac Soc* 3: 503–510, 2006.
119. Tuder RM, Zhen L, Cho CY, Taraseviciene-Stewart L, Kasahara Y, Salvemini D, Voelkel NF, and Flores SC. Oxidative stress and apoptosis interact and cause emphysema due to vascular endothelial growth factor receptor blockade. *Am J Respir Cell Mol Biol* 29: 88–97, 2003.
120. Uasuf CG, Jatakanon A, James A, Kharitonov SA, Wilson NM, and Barnes PJ. Exhaled carbon monoxide in childhood asthma. *J Pediatr* 135: 569–574, 1999.
121. Wang HD, Yamaya M, Okinaga S, Jia YX, Kamanaka M, Takahashi H, Guo LY, Ohrui T, and Sasaki H. Bilirubin ameliorates bleomycin-induced pulmonary fibrosis in rats. *Am J Respir Crit Care Med* 165: 406–411, 2002.
122. Wang X, Wang Y, Kim HP, Nakahira K, Ryter SW, and Choi AM. Carbon monoxide protects against hyperoxia-induced endothelial cell apoptosis by inhibiting reactive oxygen species formation. *J Biol Chem* 282: 1718–1726, 2007.
123. Wang XM, Zhang Y, Kim HP, Zhou Z, Feghali-Bostwick CA, Liu F, Ifedigbo E, Xu X, Oury TD, Kaminski N, and Choi AM. Caveolin-1: a critical regulator of lung fibrosis in idiopathic pulmonary fibrosis. *J Exp Med* 203: 2895–2906, 2006.
124. Xia ZW, Zhong WW, Xu LQ, Sun JL, Shen QX, Wang JG, Shao J, Li YZ, and Yu SC. Heme oxygenase-1-mediated CD4+CD25 high regulatory T cells suppress allergic airway inflammation. *J Immunol* 177: 5936–5945, 2006.
125. Yachie A, Niida Y, Wada T, Igarashi N, Kaneda H, Toma, Ohta K, Kasahara Y, and Koizumi S. Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. *J Clin Invest* 103: 129–135, 1999.
126. Yamada N, Yamaya M, Okinaga S, Nakayama K, Sekizawa K, Shibahara S, and Sasaki H. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am J Hum Genet* 66: 187–195, 2000.
127. Yasuda H, Yamaya M, Yanai M, Ohrui T, and Sasaki H. Increased blood carboxyhaemoglobin concentrations in inflammatory pulmonary diseases. *Thorax* 57: 779–783, 2002.
128. Yasuda H, Yamaya M, Nakayama K, Ebihara S, Sasaki T, Okinaga S, Inoue D, Asada M, Nemoto M, and Sasaki H. Increased arterial carboxyhemoglobin concentrations in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 171: 1246–1251, 2005.
129. Yee EL, Pitt BR, Billiar TR, and Kim YM. Effect of nitric oxide on heme metabolism in pulmonary artery endothelial cells. *Am J Physiol* 271: L512–L518, 1996.
130. Yet SF, Perrella MA, Layne MD, Hsieh CM, Maemura K, Kobzik L, Wiesel P, Christou H, Kourembanas S, and Lee ME. Hypoxia induces severe right ventricular dilatation and infarction in heme oxygenase-1 null mice. *J Clin Invest* 103: R23–R29, 1999.
131. Zanconato S, Scollo M, Zaramella C, Landi L, Zacchello F, and Baraldi E. Exhaled carbon monoxide levels after a course of oral prednisone in children with asthma exacerbation. *J Allergy Clin Immunol* 109: 440–445, 2002.
132. Zayasu K, Sekizawa K, Okinaga S, Yamaya M, Ohrui T, and Sasaki H. Increased carbon monoxide in exhaled air of asthmatic patients. *Am J Respir Crit Care Med* 156: 1140–1143, 1997.
133. Zhang X, Shan P, Alam J, Davis RJ, Flavell RA, and Lee PJ. Carbon monoxide modulates Fas/Fas ligand, caspases, and Bcl-2 family proteins via the p38alpha mitogen-activated protein kinase pathway during ischemia-reperfusion lung injury. *J Biol Chem* 278: 22061–22070, 2003.
134. Zhang X, Shan P, Alam J, Fu XY, and Lee PJ. Carbon monoxide differentially modulates STAT1 and STAT3 and inhibits apoptosis via a phosphatidylinositol 3-kinase/Akt and p38 kinase-dependent STAT3 pathway during anoxia-reoxygenation injury. *J Biol Chem* 280: 8714–8721, 2005.
135. Zhang X, Shan P, Jiang D, Noble PW, Abraham NG, Kappas A, and Lee PJ. Small interfering RNA targeting heme oxygenase-1 enhances ischemia-reperfusion-induced lung apoptosis. *J Biol Chem* 279: 10677–10684, 2004.
136. Zhang X, Shan P, Jiang G, Zhang SS, Otterbein LE, Fu XY, and Lee PJ. Endothelial STAT3 is essential for the protective effects of HO-1 in oxidant-induced lung injury. *FASEB J* 20: 2156–2158, 2006.
137. Zhang X, Shan P, Otterbein LE, Alam J, Flavell RA, Davis RJ, Choi AM, and Lee PJ. Carbon monoxide inhibition of apoptosis during ischemia-reperfusion lung injury is dependent on the p38 mitogen-activated protein kinase and involves caspase-3. *J Biol Chem* 278: 1248–1258, 2003.
138. Zhang X, Shan P, Sasidhar M, Chupp GL, Flavell RA, Choi AM, and Lee PJ. Reactive oxygen species and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase mediate hyperoxia-induced cell death in lung epithelium. *Am J Respir Cell Mol Biol* 28: 305–315, 2005.

139. Zhou H, Liu H, Porvasnik SL, Terada N, Agarwal A, Cheng Y, and Visner GA. Heme oxygenase-1 mediates the protective effects of rapamycin in monocrotaline-induced pulmonary hypertension. *Lab Invest* 86: 62–71, 2006.
140. Zhou H, Lu F, Latham C, Zander DS, and Visner GA. Heme oxygenase-1 expression in human lungs with cystic fibrosis and cytoprotective effects against *Pseudomonas aeruginosa* in vitro. *Am J Respir Crit Care Med* 170: 633–640, 2004.
141. Zhou Z, Song R, Fattman CL, Greenhill S, Alber S, Oury TD, Choi AM, and Morse D. Carbon monoxide suppresses bleomycin-induced lung fibrosis. *Am J Pathol* 166: 27–37, 2005.
142. Zuckerbraun BS, Chin BY, Wegiel B, Billiar TR, Czsimadia E, Rao J, Shimoda L, Ifedigbo E, Kanno S, and Otterbein LE. Carbon monoxide reverses established pulmonary hypertension. *J Exp Med* 203: 2109–2119, 2006.

Address reprint requests to:
Stefan W. Ryter, Ph.D.
Department of Medicine, Division of Pulmonary, Allergy and Critical Care Medicine
The University of Pittsburgh School of Medicine
MUH 628 NW
3459 Fifth Ave, Pittsburgh, PA 15213

E-mail: Ryters@upmc.edu

Date of first submission to ARS Central, June 30, 2007; date of acceptance, July 2, 2007.

This article has been cited by:

1. Zongmei Wen, Yan Liu, Feng Li, Tao Wen. 2012. Low dose of carbon monoxide intraperitoneal injection provides potent protection against GalN/LPS-induced acute liver injury in mice. *Journal of Applied Toxicology* n/a-n/a. [CrossRef]
2. C. Reboul, J. Thireau, G. Meyer, A. Lucas, P. Obert, O. Cazorla, S. Richard. 2012. Carbon monoxide exposure in the urban environment: An insidious foe for the heart?. *Respiratory Physiology & Neurobiology* . [CrossRef]
3. Guoguang Wang, Wei Li, Xiaohua Lu, Pengju Bao, Xue Zhao. 2012. Luteolin ameliorates cardiac failure in type I diabetic cardiomyopathy. *Journal of Diabetes and its Complications* . [CrossRef]
4. Ju-Fang Liu, Sheng-Mou Hou, Chun-Hao Tsai, Chun-Yin Huang, Wei-Hung Yang, Chih-Hsin Tang. 2012. Thrombin induces heme oxygenase-1 expression in human synovial fibroblasts through protease-activated receptor signaling pathways. *Arthritis Research & Therapy* **14**:2, R91. [CrossRef]
5. Yebin Tao, Liuju Zhong, Xiaoliang Huang, Shou-En Lu, Yi Li, Lingzhen Dai, Yuanhang Zhang, Tong Zhu, Wei Huang. 2011. Acute mortality effects of carbon monoxide in the Pearl River Delta of China. *Science of The Total Environment* **410**:411, 34-40. [CrossRef]
6. Shuang Liu, Wei Hou, Ping Yao, Na Li, Biyun Zhang, Liping Hao, Andreas K. Nüssler, Liegang Liu. 2011. Heme oxygenase-1 mediates the protective role of quercetin against ethanol-induced rat hepatocytes oxidative damage. *Toxicology in Vitro* . [CrossRef]
7. Guo Guang Wang, Xiao Hua Lu, Wei Li, Xue Zhao, Cui Zhang. 2011. Protective Effects of Luteolin on Diabetic Nephropathy in STZ-Induced Diabetic Rats. *Evidence-Based Complementary and Alternative Medicine* **2011**, 1-7. [CrossRef]
8. Meixia Gao, Anju Singh, Kristin Macri, Curt Reynolds, Vandana Singhal, Shyam Biswal, Ernst W Spannhake. 2011. Antioxidant components of naturally-occurring oils exhibit marked anti-inflammatory activity in epithelial cells of the human upper respiratory system. *Respiratory Research* **12**:1, 92. [CrossRef]
9. Réka Gajdócsy, Ildikó Horváth. 2010. Exhaled carbon monoxide in airway diseases: from research findings to clinical relevance. *Journal of Breath Research* **4**:4, 047102. [CrossRef]
10. Hyesun Hyun, Young-Wook Won, Kyung-Min Kim, Jiyoung Lee, Minhyung Lee, Yong-Hee Kim. 2010. Therapeutic effects of a reducible poly (oligo-d-arginine) carrier with the heme oxygenase-1 gene in the treatment of hypoxic-ischemic brain injury. *Biomaterials* **31**:34, 9128-9134. [CrossRef]
11. Stefan W. Ryter, Augustine M. K. ChoiGaseous Therapeutics in Acute Lung Injury . [CrossRef]
12. Fumitoshi Kanagawa, Toru Takahashi, Kazuyoshi Inoue, Hiroko Shimizu, Emiko Omori, Hiroshi Morimatsu, Shigeru Maeda, Hiroshi Katayama, Atsunori Nakao, Kiyoshi Morita. 2010. Protective Effect of Carbon Monoxide Inhalation on Lung Injury After Hemorrhagic Shock/Resuscitation in Rats. *The Journal of Trauma: Injury, Infection, and Critical Care* **69**:1, 185-194. [CrossRef]
13. Xiao-Dong Fang, Fan Yang, Li Zhu, Yue-Liang Shen, Lin-Lin Wang, Ying-Ying Chen. 2009. Curcumin ameliorates high glucose-induced acute vascular endothelial dysfunction in rat thoracic aorta. *Clinical and Experimental Pharmacology and Physiology* **36**:12, 1177-1182. [CrossRef]
14. Matthew W. Lawless, Kenneth J. O'Byrne, Steven G. Gray. 2009. Oxidative stress induced lung cancer and COPD: opportunities for epigenetic therapy. *Journal of Cellular and Molecular Medicine* **13**:9a, 2800-2821. [CrossRef]
15. E. Bargagli, C. Olivieri, D. Bennett, A. Prasse, J. Muller-Quernheim, P. Rottoli. 2009. Oxidative stress in the pathogenesis of diffuse lung diseases: A review. *Respiratory Medicine* **103**:9, 1245-1256. [CrossRef]
16. Chau-Chyun Sheu, Rihong Zhai, Zhaoxi Wang, Michelle N. Gong, Paula Tejera, Feng Chen, Li Su, B. Taylor Thompson, David C. Christiani. 2009. Heme Oxygenase-1 microsatellite polymorphism and haplotypes are associated with the development of acute respiratory distress syndrome. *Intensive Care Medicine* **35**:8, 1343-1351. [CrossRef]
17. I-Ta Lee, Shue-Fen Luo, Chiang-Wen Lee, Shyi-Wu Wang, Chih-Chung Lin, Chia-Chi Chang, Yuh-Lien Chen, Lee-Young Chau, Chuen-Mao Yang. 2009. Overexpression of HO-1 Protects against TNF-#-Mediated Airway Inflammation by Down-Regulation of TNFR1-Dependent Oxidative Stress. *The American Journal of Pathology* **175**:2, 519-532. [CrossRef]
18. Danielle Morse, Ling Lin, Augustine M.K. Choi, Stefan W. Ryter. 2009. Heme oxygenase-1, a critical arbitrator of cell death pathways in lung injury and disease. *Free Radical Biology and Medicine* **47**:1, 1-12. [CrossRef]
19. David S. Fedson. 2009. Confronting the next influenza pandemic with anti-inflammatory and immunomodulatory agents: why they are needed and how they might work. *Influenza and Other Respiratory Viruses* **3**:4, 129-142. [CrossRef]

20. Bo Chen, Lingling Guo, Chunlan Fan, Subhashini Bolisetty, Reny Joseph, Marcienne M. Wright, Anupam Agarwal, James F. George. 2009. Carbon Monoxide Rescues Heme Oxygenase-1-Deficient Mice from Arterial Thrombosis in Allogeneic Aortic Transplantation. *The American Journal of Pathology* **175**:1, 422-429. [CrossRef]
21. X MENG, C NI, L ZHU, Y SHEN, L WANG, Y CHEN. 2009. Puerarin protects against high glucose-induced acute vascular dysfunction: Role of heme oxygenase-1 in rat thoracic aorta. *Vascular Pharmacology* **50**:3-4, 110-115. [CrossRef]
22. Yuki Nakamura, Masanori Miyata, Tetsuro Ohba, Takashi Ando, Kyosuke Hatsushika, Fumiko Suenaga, Naomi Shimokawa, Yuko Ohnuma, Ryohei Katoh, Hideoki Ogawa, Atsuhito Nakao. 2008. Cigarette smoke extract induces thymic stromal lymphopoietin expression, leading to TH2-type immune responses and airway inflammation. *Journal of Allergy and Clinical Immunology* **122**:6, 1208-1214. [CrossRef]
23. Fuzhi Lian, Xiang#Dong Wang. 2008. Enzymatic metabolites of lycopene induce Nrf2#mediated expression of phase II detoxifying/antioxidant enzymes in human bronchial epithelial cells. *International Journal of Cancer* **123**:6, 1262-1268. [CrossRef]
24. K D Vandegriff, M A Young, J Lohman, A Bellelli, M Samaja, A Malavalli, R M Winslow. 2008. CO-MP4, a polyethylene glycol-conjugated haemoglobin derivative and carbon monoxide carrier that reduces myocardial infarct size in rats. *British Journal of Pharmacology* **154**:8, 1649-1661. [CrossRef]
25. G. J. Kato. 2008. Novel Small Molecule Therapeutics for Sickle Cell Disease: Nitric Oxide, Carbon Monoxide, Nitrite, and Apolipoprotein A-I. *Hematology* **2008**:1, 186-192. [CrossRef]
26. Jozef Dulak . 2007. Changing Faces of Heme Oxygenases. *Antioxidants & Redox Signaling* **9**:12, 2043-2048. [Citation] [Full Text PDF] [Full Text PDF with Links]