HO-1 expression in type II pneumocytes after transpulmonary gene delivery

YI-HAO WENG, ARTHUR TATAROV, BLYTHE P. BARTOS, CHRISTOPHER H. CONTAG, AND PHYLLIS A. DENNERY
Division of Neonatal and Developmental Medicine, Department of Pediatrics, Stanford University School of Medicine, Palo Alto, California 94304

Weng, Yi-Hao, Arthur Tatarov, Blythe P. Bartos, Christopher H. Contag, and Phyllis A. Dennery. HO-1 expression in type II pneumocytes after transpulmonary gene delivery. Am J Physiol Lung Cell Mol Physiol 278: L1273–L1279, 2000.—Somatic cell gene transfer is a potentially useful strategy to alter lung function. However, achieving efficient transfer to the alveolar epithelium, especially in smaller animals, has not been demonstrated. In this study, mouse heme oxygenase-1 (HO-1) gene was delivered to the lungs of neonatal mice via transpulmonary injection. A bidirectional promoter construct coexpressing both HO-1 and a luciferase reporter gene was used so that in vivo gene expression patterns could be monitored in real time. HO-1 expression levels were also modulated with doxycycline and assessed in vivo with bioluminescent light transmitted through the tissues from the coregulated luciferase reporter. As a model of oxidative stress and HO-1-mediated protection, groups of animals were exposed to hyperoxia. After gene transfer, elevated levels of HO-1 were detected predominantly in alveolar type II cells by immunocytochemistry. With overexpression of HO-1, increased oxidative injury was observed. Furthermore, this model demonstrated a cell-specific effect of lung HO-1 overexpression in oxidative stress. Specific control of expression for therapeutic genes is possible in vivo. The transpulmonary approach may prove useful in targeting gene expression to cells of the alveolar epithelium or to circumscribed areas of the lung.

SOMATIC CELL GENE TRANSFER has been shown to modify lung function in several models (6, 7). Because the primary targets of oxygen toxicity are the alveolar cells (11), finding ways to specifically alter gene expression in these cells would yield maximal physiological effects in the lung. Although gene delivery via the systemic route is achievable, low levels of gene expression in the lung and delivery to secondary targets may occur (15). As to intratracheal administration, this method has not resulted in heme oxygenase (HO)-1 gene transfer to the alveoli but rather to the larger airways (18). Therefore, alveolar HO-1 gene delivery via this route would be an even less likely occurrence in neonatal animals where the airways are narrow. Perhaps a method to target the alveoli would be through transpulmonary delivery.

Herein, the gene encoding rat HO-1 and the reporter gene luciferase were used to test transpulmonary gene delivery. HO is rate limiting in the degradation of heme to bilirubin in a reaction that releases ferrous iron and carbon monoxide (CO). The HO-1 isoform is readily induced by oxidative stresses, including hyperoxia (13, 25), and has been shown to play a role in protection against oxygen toxicity in several transfection models (1, 14, 24). In human pulmonary epithelial cells (14) and in rat fetal lung cells (24), increased resistance to hyperoxia was seen with HO-1 overexpression. Nonetheless, little is known about the effect of HO-1 overexpression in neonatal lungs.

The neonatal animals have higher levels of lung HO-1 than adult animals (8); therefore, HO-1 overexpression in neonates may result in different effects than in adults. The goal of this study was to increase HO-1 activity by transfecting rat HO-1 cDNA into the lungs of neonatal mice and to determine whether this leads to protection against oxygen toxicity. A construct containing HO-1 and the luciferase gene as a reporter was injected into the right lungs of neonatal mice, and in vivo gene expression was assessed in real time through the use of transmitted light detected with a sensitive photon-counting camera. A doxycycline-regulatable promoter was used to express HO-1, and modulation of expression was monitored in living mice.

MATERIALS AND METHODS

Animals. Litters of C57BL/6 mice with their dams were obtained from Simonsen (Gilroy, CA). The animals were kept in a 12:12-h light-dark cycle and allowed to feed ad libitum until the time of experimentation. Animals were 5 days old when experiments began. In some experiments, neonatal mice and their mothers were exposed to either air or hyperoxic atmosphere (>95% O2) as previously described (8). At this time, dams that had been in one atmosphere (i.e., air or hyperoxia) were changed to the other (air to hyperoxia and vice versa) every 24 h and allowed to nurture the new litter to obviate the effects of hyperoxia on the dams. Some animals transfected with pBl-L/HO-1 and pTet-Off or pTet-On were injected intraperitoneally with doxycycline (1 mg/kg) 24 and 36 h after transfection, and the bioluminescent signal was then evaluated 48 h later.

Plasmids and in vivo DNA delivery. The full-length rat HO-1 cDNA was inserted at the HindIII and EcoR V restriction sites of a plasmid vector containing a bidirectional tetracycline (Tet) vector (pBl-L, Clontech, Palo Alto, CA) to

http://www.ajplung.org 1040-0605/00 $5.00 Copyright © 2000 the American Physiological Society
create the plasmid pBI-L/HO-1. Ten microliters of lipofectin and 12.5 µg of pBI-L/HO-1 with 12.5 µg of either the reporter plasmid pTet-Off or pTet-On were incubated at room temperature for 1 h, then injected transcutaneously into the right lungs of neonatal mice in the midaxillary region. As a control, a plasmid encoding a modified luciferase gene (pGL3, Promega, Madison, WI) with the immediate-early gene promoter from human cytomegalovirus (pGL3-CMV) without HO-1 cDNA was similarly injected into neonatal mice.

Determination of in vivo luciferase expression. After anesthesia with ketamine (5 mg/kg), d-luciferin (150 mg/kg), the substrate for luciferase, was injected into the peritoneal cavity 10 min before imaging (6). With an intensified charge-coupled device camera (model C2400-32, Hamamatsu, Japan), a pseudocolor image representing light intensity was generated and superimposed over a gray-scale whole body reference image as previously described (5). Light intensity from the right lung area was measured every 24 h to determine the duration of gene expression.

Tissue protein content. Lung tissue was collected, homogenized in 0.01 M sodium phosphate buffer, pH 7.4, containing 0.0062 M Na2HPO4 and 0.0038 M NaH2PO4 and centrifuged at 12,500 g. The protein content was quantified in the supernatants with the Bradford reagent (Sigma, St. Louis, MO) and read in a spectrophotometer at an absorbance of 595 nm.

Histological sections and immunocytochemistry. Whole lungs were inflated with 4% paraformaldehyde in PBS, immersed in increasing concentrations of sucrose, allowed to freeze in liquid nitrogen, and then stored at −80°C. The samples were later cut at a thickness of 6 µm and placed onto positively charged glass slides. To detect HO-1 protein by immunohistochemistry, 6-µm tissue sections were fixed onto glass slides in ice-cold acetone (100%). The cells in the tissue sections were permeabilized in 0.3% saponin in PBS, and then the tissue sections were incubated with a 1:20 dilution of rabbit anti-rat HO-1 antibody (gift from Dr. Michael Beers, University of Pennsylvania, Philadelphia, PA). The sections were then stained with a solution containing a 1:50 dilution of Texas Red-conjugated goat anti-rabbit antibody (Molecular Probes, Eugene, OR) in PBS. The coverslips were placed over the tissue sections in glycerol buffer containing an antifade reagent (Slowfade, Molecular Probes), and viewed with a fluorescence microscope/confocal laser scanning unit (model 2010, Molecular Dynamics, Sunnyvale, CA) as previously described (9). Staining for p53, a marker of apoptosis, was performed with a 1:20 dilution of rabbit p53 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at room temperature for 2 h followed by incubation with a 1:50 dilution of Texas Red-conjugated anti-rabbit antibody as described above.

Determination of HO-1-immunoreactive protein (Western analysis). Proteins extracted from 20 µg of lung tissue homogenates were separated by electrophoresis on 12% polyacrylamide gels and then transferred overnight to polyvinylidene difluoride (PVDF) membranes. These membranes were briefly washed in PBS, then incubated for 1 h at 37°C with rabbit anti-rat HO-1 antibody that had been diluted 1:500 in PBS. Blots were washed with PBS containing Tween 20 (PBS-T) and incubated for 1 h at 37°C with a horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:5,000 dilution; Caltag Laboratories, South San Francisco, CA). Antigen-antibody complexes were visualized with the HRP chemiluminescence assay by incubating for 1 min with enhanced chemiluminescence Western blotting detection reagent (Amersham, Piscataway, NJ). Equal loading of protein in each lane was verified by Coomassie blue staining of the polyacrylamide gels, and quantification was performed with Molecular Analyst image-analysis software (Bio-Rad, Hercules, CA).

Tissue HO activity. Assays were conducted in subdued lighting. Twenty microliters of lung 12,500-g supernatants were reacted with NADPH and hemin in a septum-sealed, amber-colored vial at 37°C. Vials were purged with CO-free air and allowed to incubate for 15 min in the dark. The
reaction was stopped with dry ice, and CO generation in the vial gas headspace was analyzed by gas chromatography (27). Determination of protein carbonyls. Protein oxidation was measured by detecting carbonyl groups with the OxyBlot Kit (Oncor, Gaithersburg, MD). Briefly, protein extracted from 20-µg aliquots of lung supernatants was incubated with 2,4-dinitrophenyhydrazine (DNPH) for 15 min at room temperature. The samples were then neutralized by neutralization solution (Oncor) and electrophoresed on a 12% polyacrylamide gel. After transfer to PVDF membranes, blots were washed in blocking buffer for 1 h, then incubated for 1 h at room temperature with rabbit anti-dinitrophenyhydrazine IgG diluted 1:150 in blocking buffer. Blots were washed in PBS-T and incubated for 1 h at room temperature with a 1:300 dilution of HRP-conjugated goat anti-rabbit IgG. Antigen-antibody complexes were visualized with the HRP chemiluminescence system as above, and quantification was performed.

Determination of 8-isoprostanes. Lung samples were weighed and homogenized. Alkaline hydrolysis was performed by incubating with an equal volume of 15% KOH. Samples were then purified with a C18 solid-phase extraction cartridge (Bakerbond, J. T. Baker) and eluted with ethyl acetate containing 1% methanol. Extracts were vacuum centrifuged and reconstituted. Fifty-microliter aliquots of each sample were then assayed by enzyme immunoassay for 8-isoprostanes (Cayman Chemical, Ann Arbor, MI).

Iron staining. Six-micrometer frozen lung sections were fixed in 0.5% glutaraldehyde in PBS for 10 min. After being rinsed in PBS, the slides were incubated with Perl’s solution (2% HCl and 2% potassium ferrocyanide, 1:1 vol/wt) at room temperature for 30 min. Sections were then rinsed in PBS and incubated in diamino-benzidine and urea-H2O (Sigma) in deionized water for 20 min. The reaction was stopped by rinsing in PBS. Lung sections were then counterstained with Gill no. 3 hematoxylin (Sigma) and mounted with Cytoseal (Stephens Scientific, Riverdale, NJ).

Statistical analyses. For comparison between treatment groups, the null hypothesis that there was no difference between treatment means was tested by ANOVA for multiple groups or unpaired t-test for two groups (Statview 4.02; Abacus Concepts, Berkeley, CA). Significance (P < 0.05) between and within groups was determined by means of Fisher’s method of multiple comparison.

RESULTS

A model for real-time, noninvasive monitoring of transgene expression. Transgene expression is traditionally monitored ex vivo in postmortem samples. This limits detection to a single point in time and does not allow for a complete understanding of gene regulation in living animals. The pBI-L/HO-1 plasmid was designed to coexpress luciferase and HO-1 and enable the use of luciferase as a transcriptional reporter to monitor HO-1 transgene expression. After transcutaneous injection of pBI-L/HO-1 and pTet-Off into the right lung and luciferin intraperitoneally, a uniform bioluminescent signal was observed in the area of the right lung (Fig. 1A). The light intensity collected from the injected lungs was significantly increased 24 h after injection. Thereafter, the light intensity remained significantly elevated but declined gradually over the course of 8 days (Fig. 1B).

The animals tolerated the procedure well. The overall mortality was <10%.

Transgene expression is regulated by doxycycline and upregulated by hyperoxia. In vitro, when cotransfected with the Tet-Off plasmid, pBI-L/HO-1 allows for maximal expression of HO-1 and luciferase in the absence and minimal expression in the presence of doxycycline. Conversely, with the Tet-On plasmid, pBI-L/HO-1 allows for minimal expression of HO-1 and luciferase in the absence and maximal expression in the presence of doxycycline (3). Twenty-four hours after administration of doxycycline, the bioluminescent signal from the pBI-L/HO-1-injected lung decreased nearly 10.8 times in the pTet-Off cotransfected lungs and increased 10.6
times in the pTet-On cotransfected lungs (Fig. 2A) compared with control lungs not treated with doxycycline. This demonstrated that HO-1 gene expression could be regulated in vivo by doxycycline with either the Tet-Off or Tet-On series of plasmid vectors.

To determine whether hyperoxia would influence transgene expression from the CMV-based promoters, groups of transfected animals were exposed to >95% O2 for 48 h. With hyperoxic exposure, the bioluminescent signal increased 1.9 times in the lungs transfected with pBI-L/HO-1 plus pTet-Off compared with the transfected lungs exposed to air. However, the bioluminescent signal also increased 2.8 times in the lungs transfected with pGL3-CMV (a vector containing luciferase but not HO-1 cDNA) compared with the transfected lungs exposed to air (Fig. 2B). There was no difference in bioluminescent signal intensity between the animals transfected with pGL3-CMV and the animals transfected with pBI-L/HO-1 plus pTet-Off with the same exposure (air or hyperoxia). These data indicated that hyperoxia induced transgene expression but that this effect was likely attributable to the hyperoxic regulation of the CMV promoter (4).

HO-1 overexpression is achieved in alveolar type II cells. The distribution of HO-1 protein was verified in the transfected and untransfected lungs. In the animals exposed to air, the highest level of HO-1 protein was observed in the middle lobe of the right lung where the pBI-L/HO-1 construct had been injected. Transfection with pGL3/CMV did not result in increased HO-1-immunoreactive protein in the lung (Fig. 3A). In the animals exposed to hyperoxia, the HO-1 protein level in the left (untransfected) lung was increased 2.7 times compared with that in the animals exposed to air. Furthermore, the highest level of HO-1 protein remained in the middle lobe of the right lung after hyperoxia (Fig. 3A).

Fig. 3. HO-1 expression in lung. A: immunoreactive HO-1 protein levels were determined in neonatal mice that had been transfected with pBI-L/HO-1+pTet-Off after hyperoxic exposure. Top: representative Western analysis. U, right upper lung; M, right middle lung; L, right lower lung; L’, left lung. Equal loading was verified by Coomassie blue staining. Bottom: densitometric evaluation of HO-1-immunoreactive protein. Open bars, pGL3-CMV in air; hatched bars, pBI-L/HO-1+pTet-Off in air; solid bars, pBI-L/HO-1+pTet-Off in hyperoxia. Values are means ± SE of relative density from 4 separate experiments. *P < 0.05 vs. air exposure, similar transfection and similar pulmonary lobe; †P < 0.05 vs. right middle lobe transfected with pGL3-CMV. B: colocalization of both immunoreactive HO-1 and surfactant protein (SP) C in tissue sections. a: Alveoli of left (untransfected) lung at ×200. b: Alveoli of right middle (transfected) lung at ×200 showing HO-1 localizing in alveolar type II cells (yellow arrow). c: Blood vessels of right middle lung at ×400 showing HO-1 signal in smooth muscle cells of blood vessels (green arrow). d: Bronchiolar epithelium of right middle lobe at ×400 showing little HO-1-immunoreactive protein. Red signal, SP-C in alveolar type II cells; green signal, HO-1-immunoreactive protein; yellow signal, colocalization of HO-1 and SP-C. C: lung HO activity in neonatal mice that were transfected with pBI-L/HO-1+pTet-Off or pGL3-CMV. Open bar, left (nontransfected) lung; hatched bar, right middle lobe transfected with pGL3-CMV; solid bar, right middle lung transfected with pBI-L/HO-1+pTet-Off. Values are means ± SE of HO activity from 5 separate experiments. *P < 0.05 vs. left (untransfected) lung. †P < 0.05 vs. pGL3-CMV-transfected lungs.
To determine which cell type was expressing HO-1, immunohistochemistry was performed. Forty-eight hours after gene transfer, HO-1 protein was detected in right middle lung and predominantly in the alveolar type II cells (Fig. 3B). Some of the HO-1-immunoreactive signal was also seen in alveolar macrophages and smooth muscle cells of blood vessels. The left (untransfected) lung and the bronchiolar epithelium had little or no evidence of HO-1 protein.

Lung HO activity was measured by gas chromatography to determine whether the exogenous HO-1 was functionally active. After 48 h of air exposure, HO activity increased 1.6 times in the right middle lungs transfected with pBI-L/HO-1 plus pTet-Off compared with that in the lungs transfected with pGL3-CMV (Fig. 3C), indicating that the increased HO activity likely resulted from transfection with the plasmid encoding the HO-1.

HO-1 overexpression in the lung of neonatal mice worsens oxidative stress. To study the effect of exogenous HO-1 administration on oxidative injury in the neonatal lung, several markers of oxidative damage were measured. The presence of protein carbonyls has become a widely accepted measurement for oxidative injury (22). The highest carbonyl content was in the right middle lobe where HO-1 was maximally expressed (Fig. 4A). In contrast, transfection with the pGL3/CMV vector did not result in increased protein oxidation. These results indicated that oxidative injury was not due to the transfection itself but likely to the increased HO-1 expression. To further investigate whether HO-1 would protect against oxygen toxicity, protein carbonyls were measured after hyperoxic exposure. Protein carbonyls were enhanced in the hyperoxia-exposed lungs, indicating that hyperoxia increased oxidative injury. However, the right middle lung had the most protein carbonyls, indicating that HO-1 further exacerbated oxidative injury in hyperoxia.

Lipid peroxidation was measured by detecting the 8-isoprostanes (8-epi-PGF$_2\alpha$), created by nonenzymatic, free radical-catalyzed lipid peroxidation of arachidonic acid. The 8-isoprostanes are specific markers of oxidative stress in vivo and are considered superior to other assays for lipid peroxidation (17). The 8-isoprostanes were increased in the lungs transfected with pBI-L/HO-1 plus pTet-Off either in hyperoxia or in air compared with those in the lungs transfected with pGL3-CMV (Fig. 4B), indicating that HO-1 increased oxidative stress to membrane lipids.

Because oxidative stress has been well documented to induce apoptosis in vivo in lung cells (12), p53 protein was measured by immunocytochemistry as an index of apoptosis (21). Forty-eight hours after gene transfer, p53 protein was increased in the lung transfected with the HO-1 expression plasmid compared with that in the untransfected lung (data not shown).

To determine which cell type was expressing HO-1, immunohistochemistry was performed. Forty-eight hours after gene transfer, HO-1 protein was detected in right middle lung and predominantly in the alveolar type II cells (Fig. 3B). Some of the HO-1-immunoreactive signal was also seen in alveolar macrophages and smooth muscle cells of blood vessels. The left (untransfected) lung and the bronchiolar epithelium had little or no evidence of HO-1 protein.

Lung HO activity was measured by gas chromatography to determine whether the exogenous HO-1 was functionally active. After 48 h of air exposure, HO activity increased 1.6 times in the right middle lungs transfected with pBI-L/HO-1 plus pTet-Off compared with that in the lungs transfected with pGL3-CMV (Fig. 3C), indicating that the increased HO activity likely resulted from transfection with the plasmid encoding the HO-1.

HO-1 overexpression in the lung of neonatal mice worsens oxidative stress. To study the effect of exogenous HO-1 administration on oxidative injury in the neonatal lung, several markers of oxidative damage were measured. The presence of protein carbonyls has become a widely accepted measurement for oxidative injury (22). The highest carbonyl content was in the right middle lobe where HO-1 was maximally expressed (Fig. 4A). In contrast, transfection with the pGL3/CMV vector did not result in increased protein oxidation. These results indicated that oxidative injury was not due to the transfection itself but likely to the increased HO-1 expression. To further investigate whether HO-1 would protect against oxygen toxicity, protein carbonyls were measured after hyperoxic exposure. Protein carbonyls were enhanced in the hyperoxia-exposed lungs, indicating that hyperoxia increased oxidative injury. However, the right middle lung had the most protein carbonyls, indicating that HO-1 further exacerbated oxidative injury in hyperoxia.

Lipid peroxidation was measured by detecting the 8-isoprostanes (8-epi-PGF$_2\alpha$), created by nonenzymatic, free radical-catalyzed lipid peroxidation of arachidonic acid. The 8-isoprostanes are specific markers of oxidative stress in vivo and are considered superior to other assays for lipid peroxidation (17). The 8-isoprostanes were increased in the lungs transfected with pBI-L/HO-1 plus pTet-Off either in hyperoxia or in air compared with those in the lungs transfected with pGL3-CMV (Fig. 4B), indicating that HO-1 increased oxidative stress to membrane lipids.

Because oxidative stress has been well documented to induce apoptosis in vivo in lung cells (12), p53 protein was measured by immunocytochemistry as an index of apoptosis (21). Forty-eight hours after gene transfer, p53 protein was increased in the lung transfected with the HO-1 expression plasmid compared with that in the untransfected lung (data not shown).
Accumulation of iron is associated with HO-1 overexpression. Iron deposition was evaluated in the transfected lungs because this molecule is a toxic by-product of the HO reaction (30). In hyperoxia, lungs transfected with HO-1 demonstrated increased evidence of iron staining in the alveoli and blood vessels compared with that in nontransfected lungs (Fig. 5), indicating that HO-1 overexpression may be associated with increased iron deposition in the lung.

**DISCUSSION**

In these experiments, we demonstrated a method of gene transfer with a biological effect in living animals into the lungs of neonatal animals. Furthermore, we demonstrated that expression of the transgene was efficiently regulated in vivo by doxycycline with plasmids that contain both the Tet-On and Tet-Off promoters. In this study, HO-1 was used as a model for in vivo transfection, and increased expression of the HO-1 gene was predominantly found in the alveolar type II cells. This is an important finding because intratracheal HO-1 gene delivery previously resulted in HO-1 expression only in the bronchial epithelium (18). Because oxidative injury manifests predominantly in the alveolar epithelial cells (11), it may be more relevant to target these cells than other cell types.

Increased indexes of oxidative stress were noted after HO-1 gene transfer. Several other studies have reported that overexpression of HO-1 was associated with protection against hyperoxia in vitro (1, 14, 24) and in vivo (18). Recent evidence, however, shows that HO-1 protects against oxygen toxicity at low-to-moderate levels of HO-1 overexpression but exacerbates oxygen toxicity at higher levels of expression (10, 23). HO-1 gene transfection and expression did not protect against oxygen toxicity in oxygen-resistant hamster fibroblasts with higher basal levels of HO-1 expression than parental cells (HA-1) (10). In addition, HO activity in HA-1 cells resulted in protection at the lower range (<5-fold) but susceptibility at the higher range (>15-fold) of HO-1 overexpression (23). Because higher HO-1 expression has been observed in neonatal animals (8), perhaps pulmonary levels of HO-1 in the neonatal mice are already optimal to protect against oxidative stress. Therefore, exogenous administration of excess HO-1 may exceed the protective threshold and lead to oxidative stress.

To further corroborate these observations, transgenic mice with overexpression of HO-1 in alveolar type II cells demonstrated increased oxidative injury in hyperoxia (26). These findings are in contrast to increased resistance to hyperoxia with exogenous administration of HO-1 in human lung carcinoma cells (A549) (14) and rat fetal lung fibroblasts (24) and intratracheal administration of HO-1 to bronchial epithelium (18).

In this model, HO-1 was targeted to alveolar type II cells. These cells are more resistant to oxygen toxicity than alveolar type I cells, and some alveolar type II cells will proliferate and differentiate to alveolar type I cells to restore the disrupted alveolar epithelium (2, 29). These cells also synthesize surfactant, which can be inactivated in oxidative stress (19). Abundant HO-1 in the type II pneumocytes may negatively affect the function of these cells and lead to worsened oxidative injury. Overall, this suggests that the protective effect of HO-1 overexpression is cell specific in the lung. Perhaps this may be related to the relative abundance of HO-1 in each cell type, but this remains to be determined.

In the reaction of HO-1, ferrous iron is released. Recently, this molecule was shown to result in the prooxidant effects of HO-1 overexpression (23). In the present study, iron was increased in the lung after HO-1 transfection, further substantiating that this molecule may determine the protective or deleterious effects of HO-1 in the lung. Nonetheless, the distribution of the accumulated iron did not parallel that of HO-1 but was mainly seen in the blood vessels and bronchial epithelium, suggesting that there may be transport of the iron into these cells.

In this model, HO-1 overexpression was further enhanced in hyperoxia. However, the control lungs injected with a plasmid vector not containing HO-1 also showed increased bioluminescent signal, suggesting that expression from the CMV promoter may be induced by hyperoxia. Recently, transcription from the CMV promoter was shown to be induced by a variety of environmental stresses, such as ultraviolet irradiation, osmotic shock, and oxidative stress (4). The CMV promoter contains activator protein-1 and nuclear factor-kB binding sites (20). Both activator protein-1 (13) and nuclear factor-kB (16) are activated after hyperoxia. Therefore, it is not surprising that activity of the CMV promoter was altered by hyperoxia.

In summary, a novel method of gene transfer into alveolar type II cells of the lung of neonatal mice is shown. This system allows for real-time monitoring of transgene expression and for use of small amounts of DNA to achieve significant physiological effects. This model was used to further illustrate the dual nature of HO-1 in protection against oxygen toxicity in vivo.

We thank Winnie Chiu for expert assistance with the cryosections. This study was supported in part by National Heart, Lung, and Blood Institute Grants HL-52701, HL-58752 (P. A. Dennery), and HL-58013-06 (C. H. Contag) and unrestricted gifts from the Mary L. Johnson and Hess Research Funds. Address for reprint requests and other correspondence: P. A. Dennery, Division of Neonatal and Developmental Medicine, Dept. of Pediatrics, Stanford Univ. School of Medicine, 750 Welch Rd. #613, Palo Alto, CA 94304 (E-mail: dennery@leland.stanford.edu). Received 26 October 1999; accepted in final form 11 January 2000.

**REFERENCES**


