**Mycoplasma pneumoniae** Infection and Environmental Tobacco Smoke Inhibit Lung Glutathione Adaptive Responses and Increase Oxidative Stress

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Received 30 January 2008/Returned for modification 7 March 2008/Accepted 16 June 2008

Chronic cigarette smoking evokes a lung glutathione (GSH) adaptive response that results in elevated GSH levels in the lung epithelial lining fluid (ELF). Currently, little is known about how the lung regulates or maintains steady-state levels of ELF GSH. Pathogens such as *Mycoplasma pneumoniae* can exacerbate airway inflammation and oxidative stress. The present study examined whether *M. pneumoniae* infections synergize with environmental tobacco smoke (ETS) to disrupt lung GSH adaptive responses. Mice were exposed separately and in combination to ETS and *M. pneumoniae* for 16 weeks. ETS exposure resulted in a doubling of ELF GSH levels, which was blocked in the *M. pneumoniae*-exposed mice. In addition, the ETS-plus-*M. pneumoniae*-exposed mice had elevated levels of oxidized glutathione (GSSG), resulting in a dramatic change in the ELF redox state that corresponded with an increase in lung tissue DNA oxidation. Similar findings were observed in human lung epithelial cells in vitro. Cells exposed separately or in combination to cigarette smoke extract and *M. pneumoniae* for 48 h had elevated apical levels of GSH compared to control cells, and these increases were blocked by *M. pneumoniae* and were also associated with increased cellular DNA oxidation. Further studies showed that *M. pneumoniae* exposure blocked ETS-induced increases in GSH reductase, an enzyme that recycles GSSG back to GSH, both in vitro and in vivo. These studies suggest that *M. pneumoniae* infection synergizes with ETS and suppresses the lung's ability to respond appropriately to environmental challenges leading to enhanced oxidative stress.

The lung's large surface area makes it susceptible to potential adverse effects of airborne environmental agents (13). Agents such as cigarette smoke, ozone, nitrogen oxides, and fuel emissions react with the lung epithelium to generate reactive species (RS) (17, 45). These RS can directly or indirectly damage lung proteins, lipids, and DNA, contributing to lung oxidative stress. Environmental tobacco smoke (ETS) contains more than 4,700 chemical entities, generating approximately $10^{14}$ RS per puff (36). These RS are found in both the gas and tar phases. Cigarette smoking is implicated as a primary risk factor for the development of many lung diseases, including chronic obstructive pulmonary disease (COPD) (38, 42) and cancer (48, 50). However, even in the presence of the chronic oxidative burden of chronic cigarette smoking, only 20% of smokers develop COPD. We speculate that lung antioxidant adaptive responses may be an important protective factor in the 80% of chronic smokers who do not develop COPD. Although it has been hypothesized that the imbalance between oxidants and antioxidants plays a prominent role in the development of COPD, only a limited number of studies have explored whether agents that exacerbate lung disease may affect the lung's ability to mount and sustain antioxidant adaptive responses.

The lung is continually exposed to mixtures of pollutants and to airborne pathogens such as *Pseudomonas aeruginosa* and *Mycoplasma pneumoniae* (26). Previous studies suggest that *M. pneumoniae* infection not only causes respiratory diseases but is also responsible for extrapolmonary complications (8). *M. pneumoniae* has been shown to increase oxidative stress in epithelial cells (1, 2, 21). In addition, *M. pneumoniae* is also known to increase COPD exacerbations, and a subset of COPD patients have chronic *M. pneumoniae* infections (32). *M. pneumoniae* is difficult to culture, which makes this organism hard to detect (14), and *M. pneumoniae* infections are likely underreported.

The lung is well defended against the deleterious effects of environmental oxidants by a number of potent antioxidants. The apical fluid bathing airway epithelium is also known as the epithelial lining fluid (ELF) and is the first to encounter these environmental agents. The ELF contains numerous enzymatic and nonenzymatic antioxidants, such as ascorbic acid, uric acid, glutathione (GSH), superoxide dismutase, catalase, GSH peroxidase, and plasma proteins (45). A major antioxidant found in the ELF is GSH. ELF GSH protects the lung by scavenging exogenously generated RS and is an essential cofactor for many cellular detoxification pathways. The normal concentration of GSH in the ELF is 10 to 100 times that found in the plasma (6). The cystic fibrosis transmembrane conductance regulator (CFTR) protein is currently the only known apical epithelial GSH transporter in the lungs but is responsible for regulating only 50% of basal ELF GSH levels (47).

The purpose of the current study was to examine the effect...
of potential interactions between ETS and *M. pneumoniae* infection on lung GSH adaptive responses. We report that ETS stimulates increases in the ELF GSH steady-state levels that are only partially dependent upon CFTD and that chronic ETS-mediated increases in GSH levels are impaired in *M. pneumoniae*-infected mice. Furthermore, our study also suggests that *M. pneumoniae* infections interfere with the lung’s ability to keep the GSH in its reduced form and that this is associated with increased lung oxidative stress. We observed similar effects in cultured human lung epithelial cells, where *M. pneumoniae* infections blunted cigarette smoke extract (CSE)-induced increases in apical GSH levels. We have also discovered that *M. pneumoniae* infection inhibits ETS-induced GSH reductase (GR) levels, which correspond with elevated levels of oxidized GSH (GSSG) and increased markers of oxidative stress. These studies are the first to provide a mechanistic explanation for how *M. pneumoniae* may exacerbate lung disease in smokers by interfering with lung GSH adaptive responses and enhancing oxidative stress.

**MATERIALS AND METHODS**

**Animals.** Pathogen-free 8- to 10-week-old BALB/c female mice were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were fed Purina mouse chow (5010) and autoclaved tap water ad libitum. Mice were allowed to acclimate in the vivarium for at least 1 week before use. All animal procedures were approved by the IACUC committee at National Jewish Medical and Research Center. *M. pneumoniae* preparation and inoculation. *Mycoplasma pneumoniae* (strain FH; ATCC 15531) was cultured in SP-4 broth for approximately 10 days at 37°C, the IACUC committee at National Jewish Medical and Research Center. 

**Cell culture.** Human lung bronchial epithelial cells either sufficient (C38) or deficient (IB3) in CFTR activity were used (16). The IB3 cells were derived from bronchial epithelial cells from a cystic fibrosis patient and were immortalized by viral transformation. These cells possess both the ΔF508 and W128X mutations in the CFTD gene. This cell line was also stably transfected with cDNA encoding wild-type human CFTD (C38). The cells were obtained from ATCC (by P. Zeitlin, John Hopkins University). Cells were cultured in a transwell plate, using 1.3 cm-9 filter containing 10% fetal bovine serum without antibiotics, and were maintained at 37°C with humidified air containing 5% CO₂. Before treatment with 10 CFU/cell of *M. pneumoniae* and/or 10% CSE, the cells were replenished with fresh medium without the addition of antibiotics. Primary human small airway epithelial cells (SAEC) were purchased from Cambrex (Baltimore, MD). Cells were cultured using a basal medium (SAGM) containing bovine pituitary extract, human recombinant epidermal growth factor, retinoic acid, transferrin, insulin, pyridoxamine, and ascorbic acid. The SAEC were fed ETS-free medium for 3 days and were then exposed to cigarette smoke. Exposure conditions. In order to estimate actual ELF concentrations of soluble antioxidants from BALF, a dilution factor was derived from the difference between serum and BALF urea concentrations. This method is based on the assumption that urea concentrations in the vascular and ELF compartments are equivalent because of the freely diffusible nature of urea (10). A dilution factor was obtained by dividing the serum urea concentration by the BALF urea concentration. Three 1-ml aliquots of room temperature sterile PBS at a pH of 7.4 were instilled and collected by gentle aspiration. The BAL fluid (BFLF) was pooled together and centrifuged at 4,000 × g for 10 min at 4°C to isolate BAL cells. An aliquot of supernatant (75 μl) was used to analyze GSH levels in the ELF, using high-performance liquid chromatography (HPLC) coupled with fluorescence detection as described below.

**Serum and BALF urea concentrations.** In order to estimate actual ELF concentrations of soluble antioxidants from BALF, a dilution factor was derived from the difference between serum and BALF urea concentrations. This method is based on the assumption that urea concentrations in the vascular and ELF compartments are equivalent because of the freely diffusible nature of urea (10). A dilution factor was obtained by dividing the serum urea concentration by the BALF urea concentration. Both mobile phases were adjusted for a pH of 4.25 by using ammonium hydroxide. The detector excitation and emission wavelengths were set at 390 and 480 nm, respectively. The detector excitation and emission wavelengths were set at 390 and 480 nm, respectively.
Analysis of DNA oxidation in lung tissue and cells. DNAs from mouse lung tissue and IB3 and C38 cells were extracted using a DNA WB extractor kit (Wako). The purified DNAs were then hydrolyzed to nucleosides with 4 units of HPLC coupled with UV and electrochemical detection (CoulArray model 5600; ESA Inc., Chelmford, MA), respectively. Sample analysis was performed using a 4.6- by 150-mm C18 reverse-phase column (YMClassic; YMC Inc., Wilmington, NC). The mobile phases consisted of 0.1 M sodium acetate at a pH of 5.2 and 10% methanol. 8-OHdG was detected by UV, 30 min. The samples were then analyzed for 2-deoxyguanosine (2dG) and 8-hydroxy-2-deoxyguanosine (8OH2dG) by HPLC coupled with UV and electrochemical detection (CoulArray model 5600; ESA Inc., Chelmsford, MA), respectively. Sample analysis was performed using a 4.6- by 150-mm C18 reverse-phase column (YMClassic; YMC Inc., Wilmington, NC). The mobile phases consisted of 0.1 M sodium acetate at a pH of 5.2 and 10% methanol. 8-OHdG was detected by UV, 30 min. The samples were then analyzed for 2-deoxyguanosine (2dG) and 8-hydroxy-2-deoxyguanosine (8OH2dG) by HPLC coupled with UV and electrochemical detection (CoulArray model 5600; ESA Inc., Chelmsford, MA), respectively.

Real-time reverse transcription-PCR analyses of GR. Total RNA was extracted by using a Qiagen RNeasy Mini kit following treatment of cells with CSE and/or M. pneumoniae infection for 48 h to detect mRNAs for GR and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The total RNA concentration was determined with a nanodrop (ND-1000) UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE), adjusted to 80 ng/μl, and added to standard TaqMan reagents (Applied Biosystems, Branchburg, NJ). To compare the effects of treatments on mRNA levels, all mRNA measurements were normalized with GAPDH mRNA levels contained in total RNA pools derived from each culture. The RNAs were transcribed to produce cDNA templates that were then amplified by PCRs (high-capacity cDNA reverse transcription kit; Applied Biosystems), using a 9800 series thermal cycler (Applied Biosystems) and the following conditions: 10 min at 25°C, 2 h at 37°C, and 85°C for 5 s. Gene-specific TaqMan primers and probes were used in real-time PCRs to detect GR and GAPDH. To control for possible RNA or DNA contamination during processing, non-reverse transcriptase and nontemplate controls were run in parallel with the other samples.

Western blotting of GR. Cells were homogenized with a protease inhibitor cocktail containing EDTA and centrifuged at 500 × g for 10 min following 48 h of treatment with M. pneumoniae and/or CSE. The pellet was resuspended in 250 μl of buffer (300 mM sucrose, 10 mM HEPES, 40 μg/ml phenylmethysulfonyl fluoride, pH 7.5). A 12.5% polyacrylamide–sodium dodecyl sulfate gel was loaded with 17 μg of protein per well. Samples were run at 150 V for 60 min and transferred to a PVDF Plus membrane (Osmonics, Westborough, MA) at 100 V for 1 h. Membranes were blocked for 1 h at room temperature in 5% powdered milk dissolved in 1× Tris-buffered saline–Tween 20 (TBST). Rabbit polyclonal immunoglobulin G (IgG) antibody against GR (1:2,000) (Abcam Inc., Cambridge, MA) was applied for 2.5 h with gentle rocking. Secondary antibody (peroxidase-conjugated Affini-Pure goat anti-rabbit IgG; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) was diluted 1:35,000 in TBST and applied for 30 min with gentle rocking. All wash steps were performed in triplicate for 10 min in TBST. GR apoprotein was detected using ECL Plus Western blotting detection reagents (Amersham Biosciences, Buckinghamshire, United Kingdom). A PVDF Plus membrane was probed for GAPDH by use of rabbit polyclonal IgG (1:2,500) (Abcam Inc., Cambridge, MA) with 2.5 h with gentle rocking.

Measurement of GR activity in cells. Cells were homogenized in 250 μl of buffer (50 mM potassium phosphate and 1 mM EDTA, pH 7.5) and centrifuged at 8,500 × g for 10 min at 4°C. Supernatant was retained for GR analysis. GR activities were measured spectrophotometrically (340 nm) from the rate of NADPH consumption by GR during the reduction of GSSG, using a commercially available kit (Oxis International). GR activity is expressed in mU/mg of protein sample, where a unit is defined as 1 μmol of NADPH consumed per minute (46).

Statistical analysis. Data are expressed as means ± standard deviations. Analysis of variance (ANOVA), Tukey’s multiple comparison test, Pearson correlation, and Student’s t test statistical analyses were performed using Prism, version 5 (GraphPad, San Diego, CA). P values of <0.05 were considered statistically significant.

RESULTS

Mycoplasma infection disrupts ELF GSH responses in ETS-exposed mice. Groups of mice were exposed to ETS for 6 h a day 5 days a week for 16 weeks. A subgroup of mice was also inoculated with M. pneumoniae (∼108 CFU) 2, 10, and 16 weeks after the start of ETS exposure. Mice exposed to ETS for 16 weeks had a twofold increase in their steady-state ELF GSH levels compared to those exposed to FA (Fig. 1A). Mice infected with M. pneumoniae for 4 months showed a small but significant decrease in their ELF GSH levels compared to the FA group. Interestingly, the mice treated with both ETS and
M. pneumoniae had the lowest levels of ELF GSH among all of the groups. We also measured GSSG in all of the groups. Mice that were exposed to ETS had similar ELF GSSG levels to those in mice that were exposed to FA (Fig. 1B). However, mice that were exposed solely to M. pneumoniae infection had significantly higher levels of GSSG in their ELF than those in mice that were exposed to FA or ETS alone. Interestingly, mice exposed to both ETS and M. pneumoniae had the highest ELF levels of GSSG, and these ELF GSSG changes were inversely correlated with changes in ELF GSH levels ($r^2 = 0.8941; P < 0.0001$).

**Effects of Mycoplasma on markers of lung oxidative stress in vivo.** Oxidative stress occurs as a result of imbalances between oxidants and antioxidants and was assessed in mice exposed to ETS and M. pneumoniae. A common method to evaluate the redox environment is to examine the ratio of GSH to GSSG. Lower ratios correspond to a more oxidative environment. We observed that the ETS-exposed mice had far less oxidative stress than all the other groups (Fig. 1C), which corresponded with their higher ELF GSH levels (Fig. 1A). This result illustrates the lung's large capacity to mount and maintain an adequate antioxidant adaptive response to a chronic oxidant exposure. On the other hand, mice exposed to M. pneumoniae infection, by itself or in combination with ETS, had significantly lower GSH/GSSG ratios, indicating of higher levels of oxidative stress than those in mice exposed to FA or ETS alone (Fig. 1C). We also measured a lung tissue marker of DNA oxidation, 8OH2dG (Fig. 1D). The ETS/M. pneumoniae group had the highest levels of lung DNA oxidation, which corresponded with the lowest GSH/GSSG ratio.

**Role for CFTR in cigarette smoke-induced adaptive GSH responses in vitro.** Human lung bronchial epithelial cells sufficient (C38) and deficient (IB3) were exposed to different concentrations of CSE for 48 h to assess the role of CFTR in CSE-induced GSH efflux responses. CSE exposure for 48 h increased apical GSH steady-state levels in both CFTR-sufficient and -deficient cells, in a concentration-dependent manner (Fig. 2A). There were significant increases in apical GSH levels at 10% CSE without cytotoxicity, as measured by the release of lactate dehydrogenase (Fig. 2B). These data suggest that CFTR partly contributes to the CSE-induced GSH transport response and suggest the involvement of other transporters. For all further experiments, we used 10% CSE.

**Effects of Mycoplasma and CSE on adaptive GSH responses in vitro.** Primary human SAEC and C38 and IB3 cells were grown in transwells and exposed to 10 CFU/cell M. pneumoniae and/or 10% CSE for 48 h. The 10% CSE caused increases in the steady-state apical GSH levels (Fig. 3A). The highest responses were observed in the SAEC, and the lowest responses were observed in the CFTR-deficient IB3 cells. M. pneumoniae infection alone did not show any statistically significant difference in apical GSH levels compared to the control group for all three cell systems. Interestingly, M. pneumoniae infection in combination with CSE significantly blunted the CSE-induced increase in GSH steady-state levels in all three cell systems (Fig. 3A). This effect was similar to that observed in mice exposed to a combination of M. pneumoniae and ETS for 16 weeks (Fig. 1A). These changes in GSH steady-state levels were inversely correlated with changes in ELF GSSG levels ($r^2 = 0.8941; P < 0.0001$).
state levels corresponded to changes in the level of DNA oxidation (Fig. 3B). It was interesting that *M. pneumoniae* infection by itself did not cause DNA oxidation in SAEC but that it did so in C38 cells.

*Mycoplasma inhibits ETS-mediated GR response.* GSH steady-state levels are controlled by rates of GSH synthesis, recycling, utilization, transport, and degradation (38, 39). Given the large change in the ratio of GSH to GSSG seen in vivo and that GR recycles GSSG back into GSH, we examined the effects of *M. pneumoniae* on GR at the transcription and translation levels, as well as enzymatic activity. To better understand how *M. pneumoniae* could disrupt normal CSE-induced GSH adaptive responses, we also compared *M. pneumoniae* effects to those of a known GR inhibitor, 1,3-bis(2-chloroethy1)-1-nitrosourea (BCNU). Cells were exposed to CSE alone or in combination with the GR inhibitor. Apical and intracellular GSH levels were determined 48 h later. It was interesting that BCNU significantly blocked the CSE-induced increases in apical and intracellular GSH levels (Fig. 4A and B).

We were interested in understanding the mechanism(s) behind *M. pneumoniae* infection-induced decreases in GSH levels in the apical compartment. Using GR-specific TaqMan probes, we examined the mRNA levels in CFTR-sufficient cells. CSE caused significant increases in the mRNA level of GR (Fig. 5A). *M. pneumoniae* infection by itself did not alter the mRNA level of GR; however, in combination with CSE, it significantly abolished the CSE-induced increases in GR mRNA levels (Fig. 5A). CSE-induced increases in mRNA levels of GR were associated with increases in GR protein expression, as determined by Western blotting (Fig. 5B). *M. pneumoniae* infection in combination with CSE significantly attenuated increases in GR protein expression (Fig. 5B). We also examined the GR activity in vitro and in vivo. Human epithelial cells (SAEC and C38 cells) were exposed to 10 CFU/cell *M. pneumoniae* and 10% CSE. After 48 h of exposure, intracellular GR activity was measured. Interestingly, we saw increases in GR activity in CSE-exposed cells which corresponded to increases in mRNA and protein expression for GR and in apical GSH levels (Fig. 5D).
Furthermore, we also observed that *M. pneumoniae* infection significantly blunted these CSE-induced increases in intracellular GR activity (Fig. 5C). To test this observation in vivo, we examined lung tissue GR activity in mice exposed to ETS for 16 weeks. We observed similar effects to those seen in the in vitro studies, as ETS-exposed mice had higher lung tissue GR activities than those in groups exposed to FA. These ETS-induced increases in GR activity were completely blocked by *M. pneumoniae* infection (Fig. 5D).

**DISCUSSION**

These studies found that (i) the lung increases ELF GSH steady-state levels when chronically exposed to ETS, and this increase is largely independent of CFTR activity; (ii) *M. pneumoniae* infection synergizes with ETS and is associated with enhanced oxidative stress; and (iii) *M. pneumoniae* causes mild oxidative stress and also inhibits ETS-induced induction of GR activity. These studies are the first to demonstrate that the common respiratory pathogen *M. pneumoniae* produces lung oxidative stress which synergizes with ETS through inhibition of GR and potentiates lung oxidative stress.

Epidemiological studies have shown that approximately 80% of COPD patients are current or former smokers, but only 20% of smokers actually develop COPD (40). It remains unclear why only some smokers develop COPD. Paradoxically, we and others have found that chronic ETS actually raises antioxidant steady-state levels in the lung, which can be attributed to a robust adaptive response to chronic oxidant exposure (6). We propose that factors which interfere with the lung’s ability to adapt to ETS-induced challenges can make individuals more susceptible to chronic lung diseases. The present study was undertaken to investigate how respiratory pathogens such as *M. pneumoniae*, which produces oxidative stress and is known to exacerbate lung disease, may interfere with the lung’s ability to adapt to ETS. Previous studies have shown that COPD patients may respond differently to community-acquired infections (28, 31).

*M. pneumoniae* infections have been found in both asthma and COPD subjects (9, 18, 20, 27, 43). In addition, *M. pneumoniae* infection is known to produce hydrogen peroxide via NAD (NADH₂) oxidase activity (29). We observed that *M. pneumoniae* infection had a mild effect by itself on ELF GSH steady-state levels. However, *M. pneumoniae* in combination with ETS blunted the ETS-induced GR adaptive response and prevented the lungs from maintaining adequate scavenging of ETS oxidants. Rahman and colleagues have shown that CSE can modulate the intracellular GSH level after 24 h of CSE treatment in human alveolar epithelial cells (37). We observed similar results for human lung epithelial cells after exposure to CSE. However, *M. pneumoniae* infection interfered significantly with both the intracellular and extracellular GSH steady-state levels, signifying the importance of GSH synthesis in maintaining GSH in both compartments. In fact, our studies suggest that alterations in GSH transport and recycling pathways can contribute to the loss of adaptive GSH responses to ETS.

A number of studies have shown that high ELF GSH concentrations similar to those found in smokers protect cultured epithelial cells against oxidative stress (6, 33, 34). We found that mice exposed to 16 weeks of ETS had increased ELF GSH steady-state levels that were blocked in the *M. pneumoniae* group. ETS has been shown to acutely depress ELF GSH levels, which return or rebound within 2 to 6 h (3, 12, 23). These changes have also been shown to correlate with the elevation of transcription factors known to regulate genes involved in GSH synthesis (51). In human alveolar epithelial cells, CSE exposure for 24 h caused an increase in GSH levels due to increases in γ-glutamylcysteine ligase activity (37). In the present study, we also observed that CSE increased GSH levels both in vivo and in vitro. ELS is the first point of contact between the lung and oxidants derived from cigarette smoke, and it is not surprising to see that the lungs are able to maintain high levels of antioxidants in the ELF as part of an adaptive mechanism. Cantin and coworkers recently reported that exposure to cigarette smoke decreased CFTR mRNA expression in calu-3 cells and that this was associated with increases in cellular GSH levels, but they did not measure the extracellular GSH levels (4, 5). It is interesting that in our in vitro studies, the CFTR-deficient (IB3) cells also responded to CSE by increasing the extracellular GSH level, suggesting that there is another unidentified GSH transporter(s) responsible for the efflux of GSH, thus allowing the lung to maintain higher steady-state levels of GSH in the apical compartment (25, 47).

The GSH/GSSG ratio is routinely used as a marker of oxidative stress (15, 24). Mice exposed to *M. pneumoniae* infection alone or in combination with ETS were under severe oxidative stress, as evidenced by their low ELF GSH/GSSG ratios. The presence of oxidative stress in vivo and in vitro was also confirmed by increased levels of lung tissue DNA oxidation. It was interesting that mice who received both ETS and *M. pneumoniae* had significantly higher levels of lung DNA oxidation. A similar trend was also observed in human lung epithelial cells, and this corresponded with lower levels of apical GSH. *M. pneumoniae* may disrupt the lung’s ability to maintain and modulate ELF GSH steady-state levels, resulting in an imbalance between oxidants and antioxidants, facilitating lung oxidative stress by producing reactive oxygen species such as hydrogen peroxide. However, it has been shown that ETS alone can also cause DNA oxidation in the tissues of mice and rats (22, 35). A possible explanation for these contradictory results may be due to the fact that Howard and colleagues used a single short-term ETS exposure. In fact, it is well known that immediately following ETS exposure, there is a sudden drop in tissue GSH levels (7). Hence, it is not surprising to see that in their experimental model, ETS-exposed mice had high levels of DNA oxidation. However, in the long-term multiple exposure system, it is possible that over time the lung can actually adapt itself against the oxidant burden by increasing steady-state GSH levels and repair systems and thus can limit oxidative stress.

When human lung epithelial cells were exposed to 10% CSE in the presence or absence of a GR enzyme inhibitor, it was evident that not only did the inhibitor interfere with the CSE-induced increases in intracellular GSH levels, but it also decreased the extracellular GSH levels. This novel finding illustrates the importance of this pathway in maintaining GSH adaptive responses to environmental oxidants such as ETS. A number of studies have shown that *M. pneumoniae* infections significantly decrease the host cell’s catalase activity.
and increase hydrogen peroxide levels, resulting in oxidative damage (2). They also demonstrated that this was followed by an increase in extracellular GSSG level and a decreased GSH/GSSG ratio, but the possible mechanism(s) was not investigated (1). Our data are in agreement with their studies, since we also observed increases in ELF GSSG levels in M. pneumoniae-infected mice and in ETS-treated and M. pneumoniae-infected mice which were associated with decreases in the GSH/GSSG ratio and increases in DNA oxidation. Many transcription factors require a proper redox state to function (30). It may be possible that M. pneumoniae interferes with the ETS adaptive response through this type of mechanism. We observed that M. pneumoniae infections alone did not cause any changes in intracellular or extracellular GR activity. These data are in agreement with a previous study published by Almagor and colleagues where they demonstrated with human fibroblasts that M. pneumoniae infections did not cause changes in the GR activity (1). Recently, Singh and coworkers showed that ETS can induce GPX-2 in the lungs via the NF-E2-related factor (Nrf2) pathway. They also showed that Nrf2−/− mice were more resistant to ETS-induced oxidative stress than Nrf2+/− animals were, which was attributed to increases in GPX-2 expression as a part of the lung’s adaptive response (41). We also observed that CSE caused increases in the GR activity and that M. pneumoniae blocked this adaptive response. Thus, it is interesting to speculate that M. pneumoniae may abrogate the lung’s adaptive response at the transcriptional level.

It has been hypothesized that environmental factors play an important role in predisposing smokers to the development of progressive lung diseases. Atypical bacterial infections, such as Chlamydia pneumoniae and M. pneumoniae, have been implicated in the exacerbation of progressive lung diseases (9, 49). For the first time, we have shown that an environmental pathogen, Mycoplasma pneumoniae, can interfere with the lung adaptive response to cigarette smoke-derived oxidants. We have also shown that M. pneumoniae infection interferes with GR activation, an important GSH adaptive enzyme, and prevents the lung from maintaining steady-state GSH levels, leading to oxidative stress (Fig. 6). The results presented here show that atypical bacterial infection can modulate the ELF GSH levels and enhance oxidative stress. Future medical therapies aimed at clearing bacterial infection in smokers and/or restoring ELF GSH levels may help in minimizing the oxidative stress and preventing the progression of chronic lung diseases.

ACKNOWLEDGMENTS

This work was supported in part by NIH grants HL075523 (B.J.D.), HL084469 (B.J.D.), and HL073907 (R.J.M.).

REFERENCES