

DJ-1 Modulates Nuclear Erythroid 2–Related Factor-2–Mediated Protection in Human Primary Alveolar Type II Cells in Smokers

Karim Bahmed^{1,2*}, Elise M. Messier^{3*}, Wenbo Zhou⁴, Rubin M. Tuder⁵, Curt R. Freed⁴, Hong Wei Chu³, Steven G. Kelsen^{1,2}, Russell P. Bowler³, Robert J. Mason³, and Beata Kosmider^{1,2,3,6}

¹Department of Thoracic Medicine and Surgery, ²Center for Inflammation, Translational and Clinical Lung Research, and ⁶Department of Physiology, Temple University, Philadelphia, Pennsylvania; ³Department of Medicine, National Jewish Health, Denver, Colorado; ⁴Department of Medicine, Division of Clinical Pharmacology, University of Colorado Denver, Aurora, Colorado; and ⁵Department of Medicine, Division of Pulmonary Sciences and Critical Care Medicine, University of Colorado School of Medicine, Aurora, Colorado

Abstract

Cigarette smoke (CS) is a main source of oxidative stress and a key risk factor for emphysema, which consists of alveolar wall destruction. Alveolar type (AT) II cells are in the gas exchange regions of the lung. We isolated primary ATII cells from deidentified organ donors whose lungs were not suitable for transplantation. We analyzed the cell injury obtained from nonsmokers, moderate smokers, and heavy smokers. DJ-1 protects cells from oxidative stress and induces nuclear erythroid 2–related factor-2 (Nrf2) expression, which activates the antioxidant defense system. In ATII cells isolated from moderate smokers, we found DJ-1 expression by RT-PCR, and Nrf2 and heme oxygenase (HO)-1 translocation by Western blotting and immunocytofluorescence. In ATII cells isolated from heavy smokers, we detected Nrf2 and HO-1 cytoplasmic localization. Moreover, we found high oxidative stress, as detected by 4-hydroxynonenal (4-HNE) (immunoblotting), inflammation by IL-8 and IL-6 levels by ELISA, and apoptosis by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay in ATII cells obtained from heavy smokers. Furthermore, we detected early DJ-1 and late Nrf2 expression after ATII cell treatment with CS extract. We also overexpressed DJ-1 by

adenovirus construct and found that this restored Nrf2 and HO-1 expression and induced nuclear translocation in heavy smokers. Moreover, DJ-1 overexpression also decreased ATII cell apoptosis caused by CS extract *in vitro*. Our results indicate that DJ-1 activates the Nrf2-mediated antioxidant defense system. Furthermore, DJ-1 overexpression can restore the impaired Nrf2 pathway, leading to ATII cell protection in heavy smokers. This suggests a potential therapeutic strategy for targeting DJ-1 in CS-related lung diseases.

Keywords: DJ-1; nuclear erythroid 2–related factor-2; alveolar type II cells; cigarette smoke

Clinical Relevance

Cigarette smoke is a major risk factor for emphysema development. Our results demonstrate that DJ-1 can restore the impaired antioxidant defense system regulated by nuclear erythroid 2–related factor-2 in human primary alveolar type II cells in heavy smokers. This may lead to potential therapeutic strategies against emphysema development.

Cigarette smoke (CS) contains mutagens, carcinogens, and toxic substances (1, 2). It has been linked to a variety of chronic lung disorders and is a major cause of

morbidity and mortality. CS is also a main risk factor for emphysema development through generation of severe oxidative stress and chronic inflammation (3, 4).

Oxidative stress is a state of imbalance between the production of free radicals and their clearance by the cellular antioxidant defense system (5, 6). Recent evidence also

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*These authors contributed equally to this work.

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Correspondence and requests for reprints should be addressed to Beata Kosmider, Ph.D., Department of Physiology, Department of Thoracic Medicine and Surgery, Center for Inflammation, Translational and Clinical Lung Research (CILR), 3500 North Broad Street, MERB, Room 1155, Philadelphia, PA 19140. E-mail: beata.kosmider@temple.edu

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indicates that the apoptosis of alveolar epithelial cells is involved in the pathogenesis of pulmonary emphysema, characterized by the loss of the alveolar wall (7). Alveolar type (AT) II cells are in the gas exchange regions of the lung (8). They have a distinct morphology with apical microvilli and characteristic lamellar bodies. ATII cells make and secrete pulmonary surfactant. They also proliferate to restore the epithelium after lung injury.

DJ-1 is a small, conserved, multifunctional protein (~20 kD) reported to be involved in diverse cellular processes, ranging from antioxidant defense to control of protein-RNA interaction to the oxidative stress response (9, 10). It has been detected in lung tissue (11) and reported to protect against reactive oxygen species (12, 13) and apoptosis in different cell types (14–17). It is expressed in the cytoplasm and nucleus. DJ-1 may possess chaperone-like activity; however, its physiological function is unclear (18).

The nuclear erythroid 2-related factor-2 (Nrf2) transcription factor is a key

regulator of the cellular redox status and phase II detoxifying enzymes in the lung (6, 19). Nrf2 also regulates the expression of other antioxidant genes, such as heme oxygenase (HO)-1. We previously reported that Nrf2 protects human and murine ATII cells against injury by CS *in vitro* and *in vivo* (20–22). However, the role of DJ-1 in the activation of the Nrf2-mediated antioxidant defense system in the lung is not fully understood.

We hypothesize that ATII cells obtained from heavy smokers will have more injury than from moderate smokers or nonsmokers. Furthermore, we expect that DJ-1 will positively regulate the Nrf2-mediated antioxidant defense system against CS-induced oxidative stress in ATII cells. There is no report on this activation in human primary alveolar epithelial cells. We also anticipate that DJ-1 overexpression by adenovirus (Ad) DJ-1 construct will activate the Nrf2 pathway, which will provide protection against ATII cell injury by CS *in vitro*. To our knowledge, this is the first study of primary ATII cells isolated from nonsmokers, moderate smokers, and

heavy smokers. This approach provides an excellent system to study the effects of ATII cell exposure to CS both *ex vivo* and *in vitro*.

Material and Methods

Human Primary ATII Cells

Human lungs from organ donors were obtained from the International Institute for the Advancement of Medicine. We selected donors without a history of chronic lung disease, with the ratio of partial pressure arterial oxygen and fraction of inspired oxygen of greater than 225, X-ray and clinical history not indicative of infection. Nonsmokers were individuals who never smoked, moderate smokers who smoked 5–10 cigarettes, and heavy smokers who smoked 25–40 cigarettes per day for at least 3 years (23). The Committee for the Protection of Human Subjects at National Jewish Health (Denver, CO) approved this research. ATII cells were isolated and cultured as we previously published (21, 22, 24, 25).

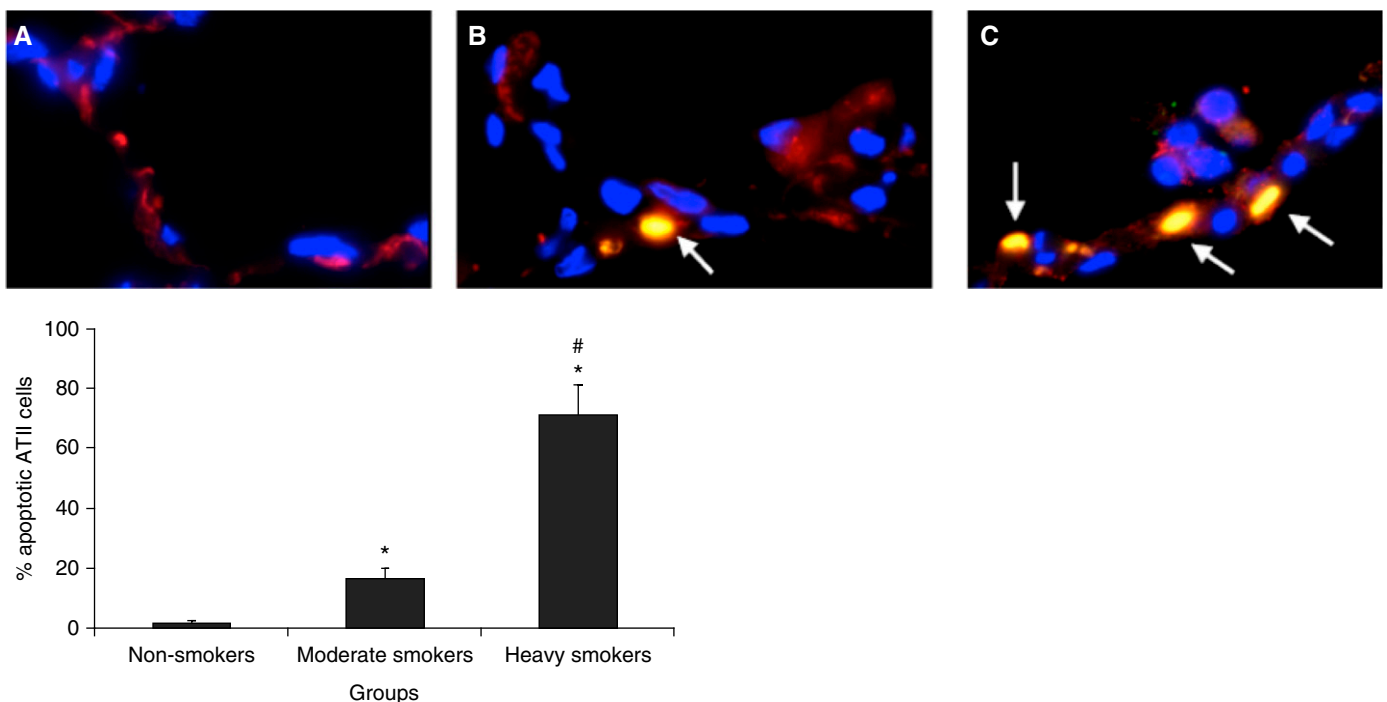


Figure 1. Cigarette smoke (CS) induces apoptosis in alveolar type (AT) II cells isolated from heavy smokers *ex vivo*. (A) nonsmokers; (B) moderate smokers; (C) heavy smokers. ATII cells in the lung tissue sections were identified by staining with prosurfactant protein-C (red), and apoptotic cells were detected by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, blue). Apoptotic ATII cells are shown by arrows. *Statistically significant increase compared with control ($P < 0.05$); #statistically significant increase compared with moderate smokers ($n = 6$). Data are shown as the mean (\pm SEM).

Infection with AdDJ-1

AdDJ-1 and AdGFP were prepared and used as we previously described (12, 25). We used virus diluted to a multiplicity of infection of 200 plaque forming unit (pfu)/cell in PBS for ATII cell infection. Cells were allowed to express transgenes for 24 hours before usage. All infected cell cultures were examined for adequate infection efficiency, as assessed by green fluorescence protein (GFP) fluorescence (91%) and by Western blotting for DJ-1.

ATII Cell Treatment with CS Extract *In Vitro*

The CS extract (CSE) was prepared as we previously described (21, 22). Briefly, the smoke of one 3R4F cigarette (Kentucky Tobacco Research and Development Center, Lexington, KY) was drawn into 12.5 ml Dulbecco's modified Eagles medium with a peristaltic pump (Manostat 72-310-000; Barrington, IL). A 3% CSE concentration was selected for our study based on the viability assay.

Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling Assay

We used a combination of staining for antiprosurfactant protein C (proSP-C) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay as previously described (26–28). The percentages of TUNEL-positive apoptotic ATII cells were calculated per 10 high-power fields (magnification, 10×40 objective) (29). The same protocol (without proSP-C staining) was used to determine apoptosis by TUNEL assay in cultured ATII cells treated with CSE *in vitro*.

Western Blotting

Protein expression in ATII cells was measured by Western blotting, as we described previously (24). We used anti-HO-1, -lamin B1, -Nrf2, -DJ-1, I-kappa-B alpha (-I κ B- α), -IL-8, and 4-hydroxynonenal (-4-HNE) antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA).

Nuclear and Cytoplasmic Fraction Isolation

We used the NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's recommendations to isolate nuclear and cytoplasmic fractions from ATII cells for subsequent Western

blotting. We used lamin B1 and I κ B- α as markers of nuclear and cytoplasmic fractions, respectively, as previously reported (30).

Immunocytofluorescence

We analyzed Nrf2 translocation in freshly isolated ATII cells. ATII cell cytoplasts were incubated with rabbit anti-Nrf2 antibody. The secondary antibody, Alexa Fluor 594 IgG, was applied for 1 hour. Cells were mounted with Vectashield medium (Burlingame, CA) containing 4',6-diamidino-2-phenylindole.

ELISA

IL-8 and IL-6 levels were measured in freshly isolated ATII cells by ELISA (ELISA Tech., Aurora, CO) according to the manufacturer's recommendations.

We used a MicroQuant microplate spectrophotometer (BioTek, Winooski, VT) and analyzed data with KCjunior Data Analysis Software (BioTek).

Real-Time PCR

Total RNA was isolated using the RNeasy Mini kit (Qiagen, Germantown, MD). Assay On-Demand DJ-1 probe (Hs00994896_g1) was purchased from Applied Biosystems (Thermo Fisher Scientific, Waltham, MA). Gene expression levels were calculated as a ratio to the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Hs99999905_m1) expression.

Statistical Analysis

One-way ANOVA by GraphPad Prism 4 (GraphPad Software, Inc., La Jolla, CA) was

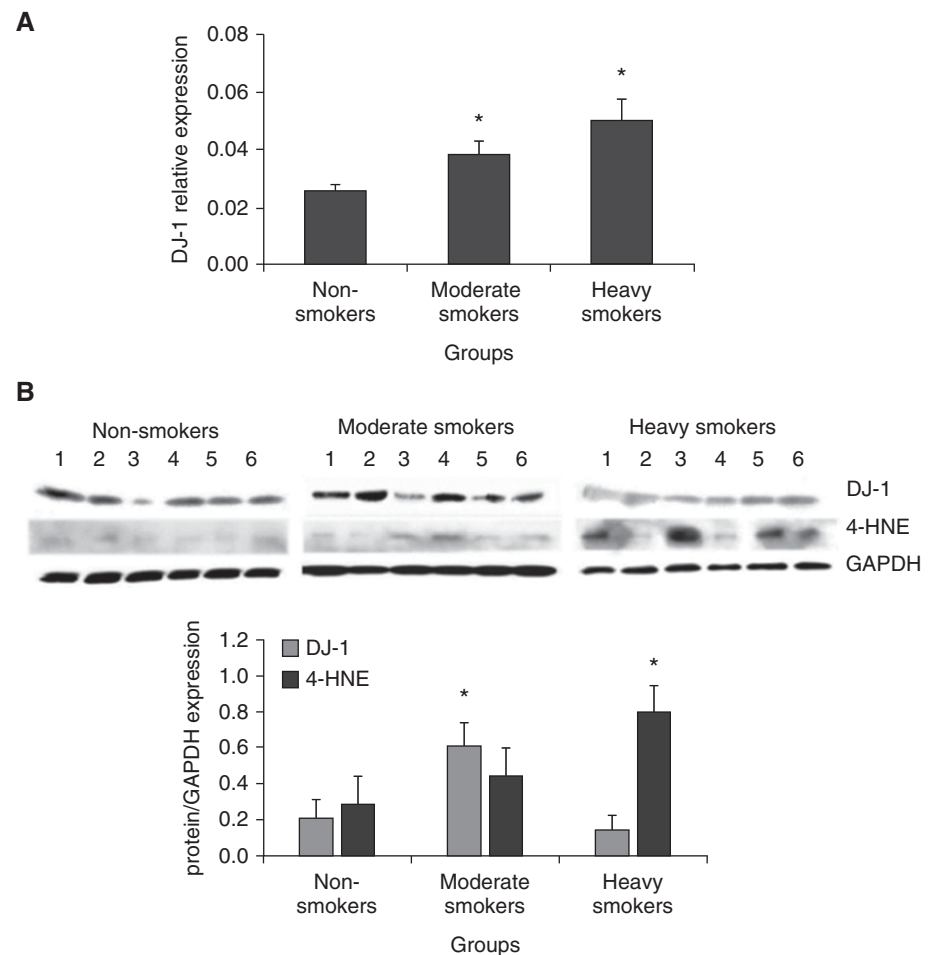


Figure 2. DJ-1 mRNA and protein expression in ATII cells *ex vivo*. DJ-1 mRNA expression by RT-PCR (A) and protein (B) levels (immunoblotting) were analyzed from freshly isolated ATII cells obtained from nonsmokers, moderate smokers, and heavy smokers. Relative expression is also shown. *Statistically significant increase in comparison with control ($P < 0.05$; $n = 6$). Data are shown as the mean (\pm SEM). 4-HNE, 4-hydroxynonenal.

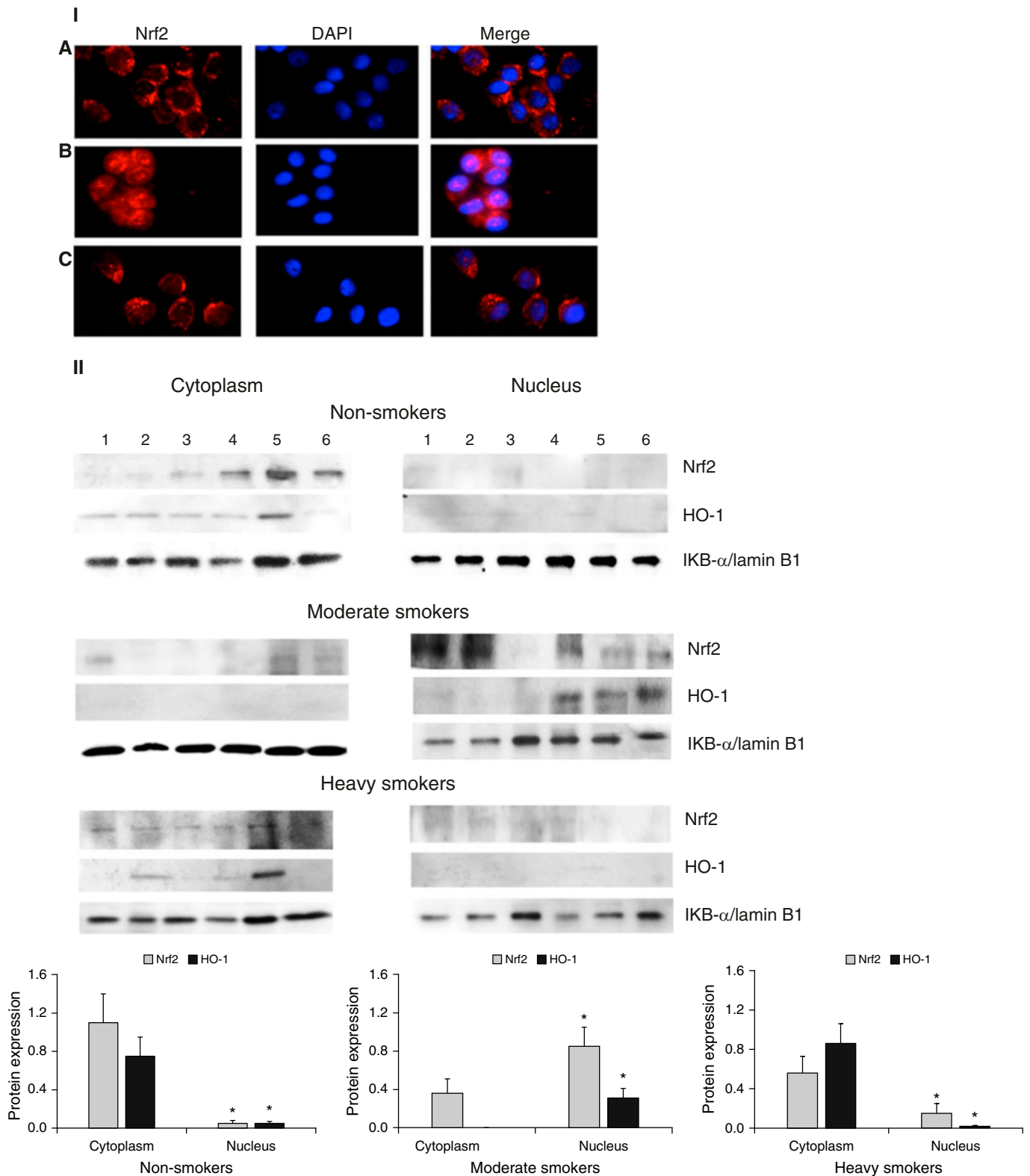


Figure 3. Nuclear erythroid 2-related factor-2 (Nrf2) localization in ATII cells isolated from moderate smokers *ex vivo*. *Panel I*: representative pictures of Nrf2 localization in ATII cell cytoplasm on the day of isolation. (A) Nonsmoker; (B) moderate smoker; (C) heavy smoker (immunocytofluorescence). *Panel II*: Nrf2 and heme oxygenase (HO)-1 localization in nuclear and cytoplasmic fractions in freshly isolated ATII cells obtained from nonsmokers, moderate smokers, and heavy smokers (immunoblotting). I κ B- α and lamin B1 were used for cytoplasmic and nuclear fractions, respectively. Relative expression is also shown. Data are shown as the mean (\pm SEM), $n = 6$, * $P < 0.05$.

used. A Dunnett's test was applied ($P < 0.05$). Data are shown here as the mean (\pm SEM) from at least three independent experiments.

Results

CS Induces ATII Cell Apoptosis in Heavy Smokers *Ex Vivo*

We have previously shown that CSE generates oxidative stress, DNA damage, and ATII cell injury *in vitro* and *in vivo* (20, 22). Here, we wanted to determine ATII cell injury induced by CS in moderate smokers and heavy smokers compared with nonsmokers *ex vivo*. We used a combination of TUNEL assay and staining for proSP-C to identify apoptotic ATII cells in lung tissue. We found the highest percentage of apoptotic ATII cells (71.2%) in heavy smokers in comparison with moderate smokers (16.2%) and nonsmokers (1.5%) (Figure 1). These results indicate that, first, CS induces apoptosis in ATII cells *ex vivo*, and, second, the percent of apoptotic ATII cells correlates with the smoking status of lung donors.

DJ-1 Expression in ATII Cells Obtained from Nonsmokers, Moderate Smokers, and Heavy Smokers *Ex Vivo*

Because DJ-1 is involved in protection against oxidative stress, we wanted to determine its expression at the transcription and protein levels by RT-PCR and Western blotting, respectively. We found higher DJ-1 mRNA levels in ATII cells isolated from heavy smokers in comparison with moderate smokers or nonsmokers (Figure 2A). We also wanted to determine DJ-1 protein expression, and found lower DJ-1 levels in heavy smokers in comparison with moderate smokers and nonsmokers by Western blotting (Figure 2B). To determine the discrepancy between DJ-1 protein and gene levels in these lung donors, we measured the oxidative stress by using 4-HNE. We observed higher 4-HNE expression in heavy smokers in comparison with moderate smokers and nonsmokers (Figure 2B). These results suggest DJ-1 degradation during high oxidative stress in these lung donors. They are in agreement with the highest percentage of apoptotic ATII cells detected in heavy smokers. Our results show that DJ-1 is induced by CS. Furthermore, DJ-1 protects ATII cells

against CS-induced oxidative injury in moderate smokers, but not in heavy smokers *ex vivo*.

Nrf2 Protects ATII Cells against Injury Induced by CS in Moderate Smokers *Ex Vivo*

We found that DJ-1 is involved in ATII cell protection against injury induced by CS in moderate smokers. It has been reported that the DJ-1 pathway is widely associated with the antioxidant pathway induced by Nrf2 (11). Therefore, next, we wanted to determine whether DJ-1 expression correlates with Nrf2 levels, a key regulator of the antioxidant defense system. First, we analyzed cytopspins of freshly isolated ATII cells from nonsmoker, moderate smoker, and heavy smoker lung donors. We found Nrf2 cytoplasmic localization in ATII cells isolated from nonsmokers and heavy smokers, and nuclear Nrf2 translocation in moderate smokers (Figure 3, *panel I*). This suggests Nrf2 activation in these latter lung donors. We also isolated nuclear and cytoplasmic fractions from ATII cells obtained from nonsmokers,

moderate smokers and heavy smokers to determine expression of Nrf2 and its downstream target HO-1 by Western blotting. We found that both Nrf2 and HO-1 have mainly nuclear localization in moderate smokers and predominant cytoplasmic localization in heavy smokers and nonsmokers (Figure 3, *panel II*). Our results indicate that: first, the Nrf2 pathway is involved in the protection of ATII cells against injury by CS in moderate smokers *ex vivo*; second, nuclear localization of Nrf2 induces expression of phase 2 enzymes, such as HO-1 in ATII cells in moderate smokers; and third, Nrf2 and HO-1 cytoplasmic expression in heavy smokers indicates an impaired antioxidant defense system. These results are in agreement with the higher percentage of apoptotic ATII cells and oxidative stress observed in heavy smokers compared with moderate smokers and nonsmokers.

CSE Induces Early DJ-1 and Late Nrf2 Expression in ATII Cells *In Vitro*

We identified that both DJ-1 and Nrf2 protect ATII cells against injury by CS in

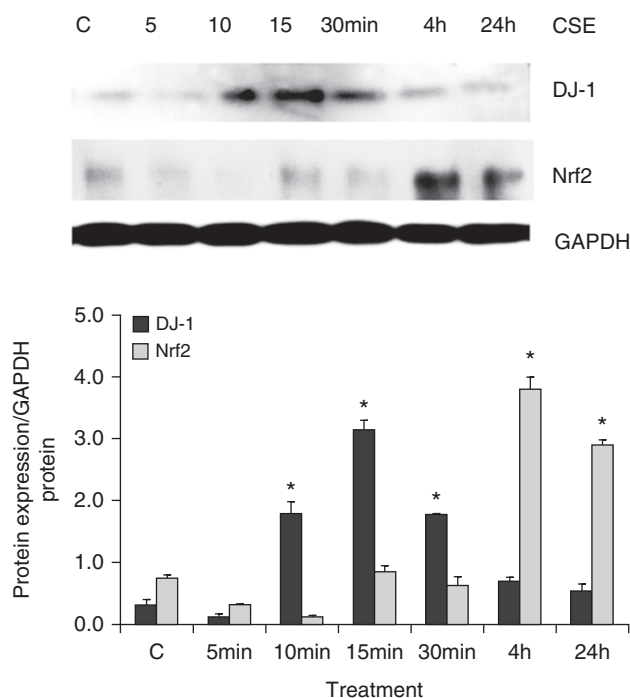


Figure 4. CS extract (CSE) induces early DJ-1 and late Nrf2 expression in ATII cells *in vitro*. ATII cells were isolated from nonsmokers, cultured, and treated with 3% CSE *in vitro*, as described in the MATERIALS AND METHODS section for different times (immunoblotting; C, control). Relative expression of these proteins is also shown. *Statistically significant increase in comparison with control ($P < 0.05$; $n = 3$). Data are shown as the mean (\pm SEM).

moderate smokers. Next, we sought to determine the time frame of their expression *in vitro*. We cultured ATII cells as described in the MATERIALS AND METHODS section, followed by treatment with 3% CSE for 5 minutes, 10 minutes, 15 minutes, 30 minutes, 4 hours, and 24 hours to analyze DJ-1 and Nrf2 expression in a time-dependent manner. We found early DJ-1 expression with a maximum level at 15 minutes (Figure 4). Nrf2 levels increased later, with a maximum expression at 4 hours after CSE treatment. These data indicate that DJ-1 is involved in an early response to the oxidative stress response, whereas Nrf2 is activated later (Figure 4). In addition, these results suggest that DJ-1 may regulate the Nrf2 pathway.

High IL-8 and IL-6 Levels in ATII Cells Isolated from Heavy Smokers *Ex Vivo*

Next, we sought to determine whether low DJ-1 and Nrf2 expressions in heavy smokers contribute to the inflammatory response in these lung donors. We focused on CS-mediated regulation of IL-8 and IL-6, as these cytokines are known contributors to inflammation in emphysema (31). We found the highest IL-8 and IL-6 levels in ATII cells isolated from heavy smokers compared with moderate smokers or nonsmokers (Figure 5). These results suggest that impairment of the antioxidant defense system correlates with high proinflammatory response in heavy smokers *ex vivo*. Our data indicate that DJ-1 protects ATII cells against CS-induced injury, and may play an important role in modulating susceptibility to lung diseases.

AdDJ-1 Can Restore Nrf2-Mediated Protection in Heavy Smokers

We found low DJ-1 and Nrf2 expression in ATII cells in heavy smokers *ex vivo*. Therefore, we wanted to examine whether DJ-1 overexpression using AdDJ-1 construct can activate Nrf2 levels in ATII cells *in vitro*. This is an important approach to determine whether we can restore the impaired Nrf2 pathway in ATII cells in heavy smokers. We observed Nrf2 induction by treatment with 3% CSE for 24 hours. We also found Nrf2 and HO-1

activation in ATII cells infected with AdDJ-1 after 24 hours (Figure 6, *panel I*). However, Nrf2 expression was decreased after infection with AdDJ-1 followed by treatment with CSE in comparison with CSE alone.

This correlates with lower HO-1 levels. Moreover, we detected that AdDJ-1 induced Nrf2 and HO-1 nuclear translocation in ATII cells isolated from heavy smokers (Figure 6, *panel II*). We also performed TUNEL assay to determine the protective effect of DJ-1 overexpression against cell death. We found decreased apoptosis in ATII cells infected with AdDJ-1 followed by treatment with CSE *in vitro* (Figure 6, *panel III*). Our results indicate that: first, DJ-1 overexpression induces Nrf2

and HO-1 expression, which protects ATII cells against CSE-induced injury; second, DJ-1 overexpression induces Nrf2 and HO-1 nuclear translocation in ATII cells obtained from heavy smokers, which can restore antioxidant defense systems in these donors; and third, DJ-1 overexpression decreased ATII cell apoptosis induced by CSE.

AdDJ-1 Inhibits 4-HNE and IL-8 Expression Induced by CSE in ATII Cells *In Vitro*

We found that AdDJ-1 decreases apoptosis of ATII cells induced by CSE (Figure 6, *panel III*). Next, we sought to determine the mechanism by which DJ-1 protects

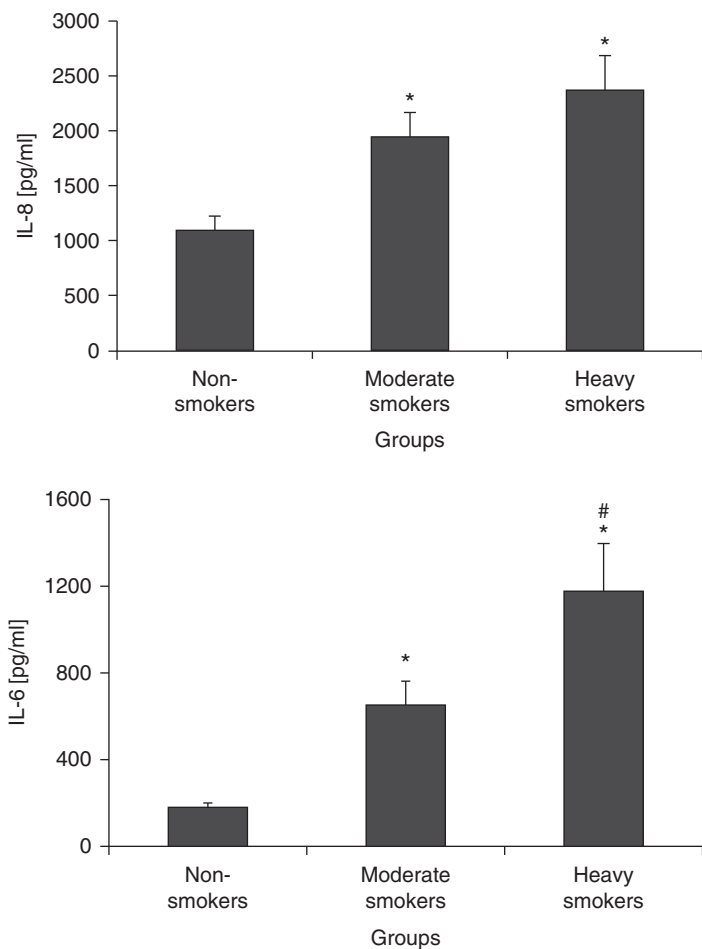


Figure 5. High IL-8 and IL-6 levels in ATII cells isolated from heavy smokers *ex vivo*. IL-8 and IL-6 levels were analyzed in freshly isolated ATII cells obtained from nonsmokers, moderate smokers, and heavy smokers by ELISA. *Statistically significant increase compared with nonsmokers; #statistically significant increase compared with moderate smokers ($P < 0.05$; $n = 6$). Data are shown as the mean (\pm SEM).

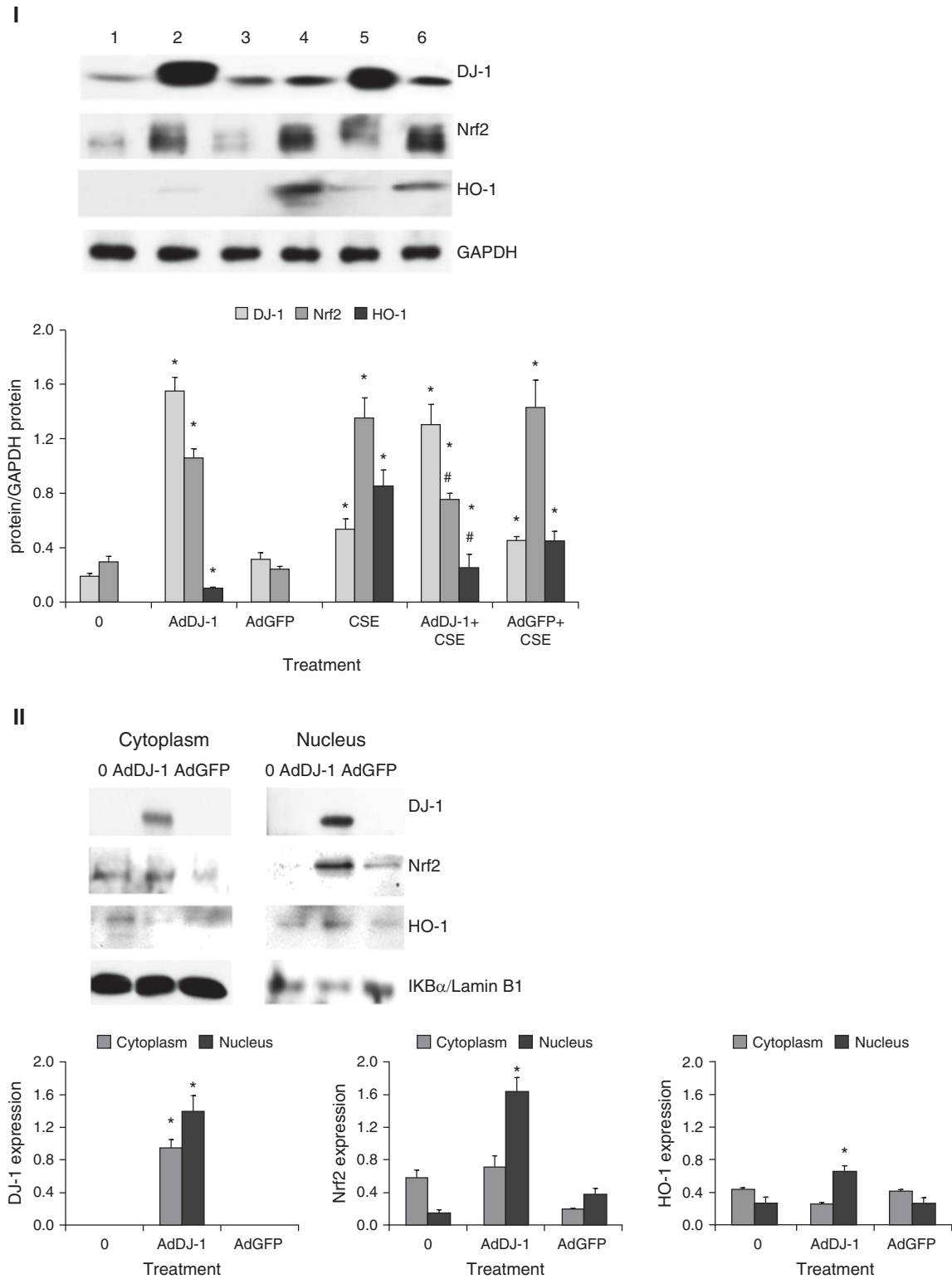


Figure 6. DJ-1 overexpression activates Nrf2 and decreases apoptosis of ATII cells induced by CSE *in vitro*. ATII cells were cultured, infected with adenovirus (Ad) DJ-1 or AdGFP, and treated with 3% CSE for 24 hours, as described in the MATERIALS AND METHODS section. *Panel I*: AdDJ-1 increases Nrf2 and HO-1 expression. *Lane 1*, control; *lane 2*, AdDJ-1; *lane 3*, AdGFP; *lane 4*, CSE; *lane 5*, AdDJ-1 + CSE; *lane 6*, AdGFP + CSE. Relative expression is also shown. *Statistically significant increase in comparison with control. #Statistically significant decrease compared with CSE alone ($P < 0.05$; $n = 3$). *Panel II*: AdDJ-1 restores Nrf2 and HO-1 nuclear expression in ATII cells isolated from heavy smokers. Relative expression of these proteins is shown. *Statistically significant increase in comparison with control ($P < 0.05$; $n = 6$). *Panel III*: AdDJ-1 decreases

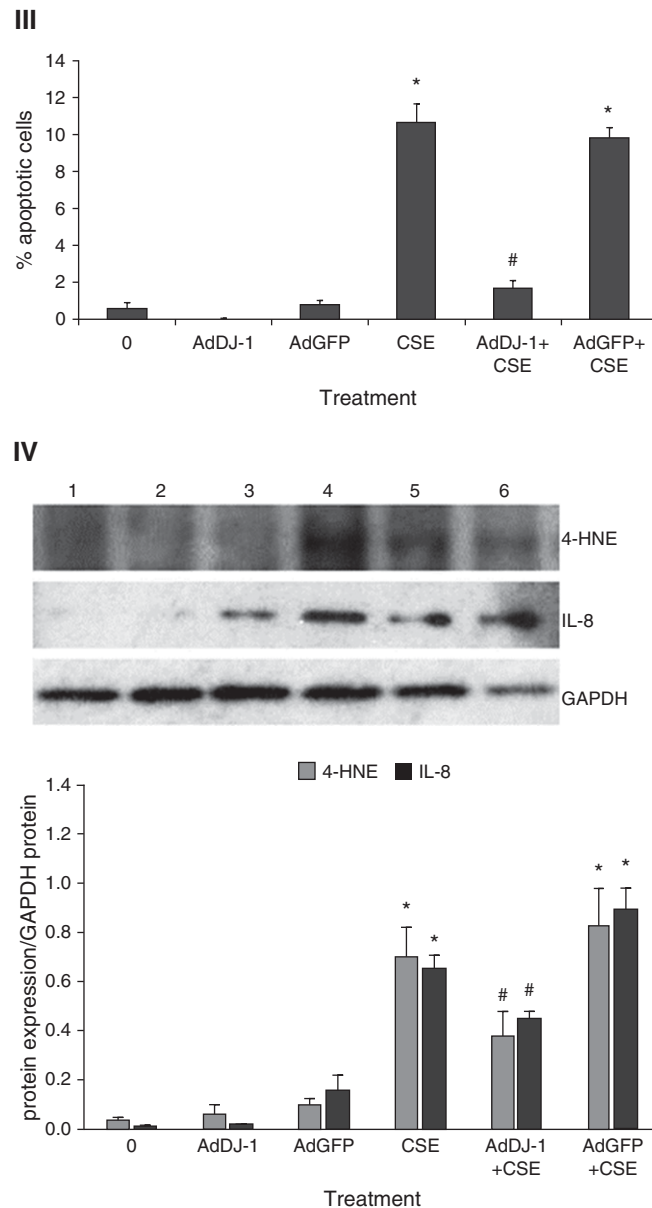


Figure 6. (Continued). apoptosis of ATII cells induced by 3% CSE *in vitro*. Cells were infected with AdDJ-1 followed by treatment with 3% CSE, as described in the MATERIALS AND METHODS section. Apoptosis was analyzed by TUNEL assay. *Statistically significant increase in comparison with control. #Statistically significant decrease compared with CSE alone ($P < 0.05$; $n = 3$). **Panel IV:** AdDJ-1 decreases oxidative stress and inflammatory response induced by 3% CSE in ATII cells as measured by 4-HNE and IL-8 expression, respectively. *Lane 1*, control; *lane 2*, AdDJ-1; *lane 3*, AdGFP; *lane 4*, CSE; *lane 5*, AdDJ-1 + CSE; *lane 6*, AdGFP + CSE. Relative expression is also shown. *Statistically significant increase in comparison with control. #Statistically significant decrease compared with CSE alone ($P < 0.05$; $n = 3$). Data are shown as the mean (\pm SEM).

these cells. We infected ATII cells with AdDJ-1, followed by exposure to 3% CSE. We found that AdDJ-1 significantly lowers 4-HNE and IL-8 expression in cells exposed to CSE compared with CSE alone (Figure 6, *panel IV*). Our results suggest that DJ-1 overexpression decreases oxidative stress

and proinflammatory response induced by CSE in ATII cells.

Discussion

CS is a very rich source of oxidants, and has been reported to induce

epithelial cell death in emphysema (32). Nrf2, a key regulator of the antioxidant response system pathway, is critically important for a cellular defense system (33, 34). This work describes the important positive regulatory role of DJ-1 for the Nrf2 pathway, which protects against ATII cell injury by CS. To our

knowledge, there are no studies on the comparison of ATII cell injury in moderate and heavy smokers. We confirmed our hypotheses that ATII cells isolated from heavy smokers have more injury than those obtained from moderate smokers or nonsmokers. We also found that DJ-1 induces Nrf2-mediated protection against ATII cell injury by CS. Our findings show that DJ-1 activation prevents cell injury induced by CS in moderate smokers. The obtained results improve our understanding of the DJ-1–Nrf2 pathway and oxidative stress in ATII cells. Using human primary ATII cells exposed to CS *ex vivo* supports the importance of our studies. Furthermore, this unique approach, including nonsmokers, moderate smokers, and heavy smokers, fills the gap in our knowledge on the mechanism of the impairment of the antioxidant defense system in heavy smokers, which may contribute to emphysema development.

It has been reported that higher apoptosis of ATII cells is associated with CS-induced lung diseases (35). Smoking is a dominating risk factor in the development of emphysema, which is characterized by alveolar wall destruction (36). We detected higher oxidative stress in ATII cells isolated from heavy smokers than in those from moderate smokers. Oxidative stress has been implicated in the initiation of lung inflammatory responses (37). ATII cells can produce inflammatory mediators, such as IL-6 and IL-8 (7, 38). Proinflammatory cytokines are increased in emphysema and appear to amplify inflammation in this disease (39, 40). We observed higher IL-8 and IL-6 levels in ATII cells obtained from heavy smokers than in those from moderate smokers or nonsmokers. Our results are in agreement with those of Garbin and colleagues (23), who observed higher IL-6 and NF- κ B levels in peripheral blood mononuclear cells obtained from heavy smokers than in those from moderate smokers or nonsmokers. Our observations indicate a link between oxidative stress, apoptosis, and inflammation in ATII cells, depending on the smoking status.

Next, we wanted to determine the protective role of DJ-1 against ATII cell

injury by CS. We found high DJ-1 mRNA expression in ATII cells obtained from both moderate and heavy smokers in comparison with nonsmokers. We also checked DJ-1 protein levels and found lower expression in ATII cells isolated from heavy smokers than in those from moderate smokers. This may suggest DJ-1 degradation. Lower DJ-1 levels were also observed in lung tissue obtained from patients with chronic obstructive pulmonary disease compared with that from control smokers (11). Moreover, it was previously reported that DJ-1 can protect cells from cell death *in vitro* (41). In our next approach, we wanted to determine the role of Nrf2 in ATII cell injury by CS. It was shown that Nrf2 activation induces expression of antioxidant genes, such as HO-1 (6). We found Nrf2 and HO-1 nuclear localization in ATII cells obtained from moderate smokers and their cytoplasmic localization in heavy smokers. This indicates Nrf2-mediated protection in the former group. Zhang and Forman (42) showed that HO-1 plays a critical role in resistance to oxidative stress induced by acrolein, a toxicant in CS, in bronchial epithelial cells. Garbin and colleagues (23) also observed higher Nrf2 levels in peripheral blood mononuclear cells obtained from moderate smokers than in those obtained from heavy smokers or nonsmokers. Impaired Nrf2 signaling was reported in lung tissue obtained from patients with chronic obstructive pulmonary disease (43). Our data indicate that low DJ-1 expression may at least partially contribute to Nrf2 decline, high apoptosis, and oxidative stress in ATII cells obtained from heavy smokers.

Our results also suggest a difference in the molecular signature of ATII cells obtained from nonsmokers, moderate smokers, and heavy smokers *in vitro*. Other studies indicate that nasal epithelial cells isolated from nonsmokers and smokers can maintain their different phenotype depending on smoking status during *in vitro* culture (44). We analyzed DJ-1 and Nrf2 expression in ATII cells exposed to CSE *in vitro*. It has been reported that CSE increased DJ-1 levels in A549 cells *in vitro* (45). We found early DJ-1 induction followed by late Nrf2 expression. Our results suggest that

DJ-1 activation decreases oxidative stress and protects ATII cells through induction of the antioxidant response system mediated by Nrf2. This is in agreement with observations that DJ-1 is required to stabilize Nrf2 (17). We continued this approach using the AdDJ-1 construct. We found that DJ-1 overexpression increases Nrf2 and HO-1 expression *in vitro*. Moreover, our results indicate that AdDJ-1 inhibits oxidative stress and proinflammatory response induced by CSE, as measured by 4-HNE and IL-8 expression, respectively. We have previously reported that Nrf2 overexpression protected human ATII cells and ATI-like cells against oxidative stress-induced injury and proinflammatory response (25). It has also been observed that DJ-1 overexpression protects neuronal cells from oxidative stress (12, 46). Furthermore, in the critical experiment, we observed Nrf2 and HO-1 nuclear translocation after transfection of ATII cells obtained from heavy smokers with AdDJ-1. This suggests that DJ-1 overexpression can rescue the impaired Nrf2-mediated antioxidant defense system in these donors. It has been recently found that AdDJ-1 mitigated baseline loss of Nrf2 in bone marrow-derived macrophages obtained from DJ-1 knockout mice (47). However, to our knowledge, there is no report on the role of DJ-1 overexpression in human primary ATII cells.

In conclusion, our results improve our knowledge on the imbalance of the oxidative stress and antioxidant defense system in heavy smokers. The results also indicate the important role of DJ-1 in modulation of Nrf2-mediated protection of ATII cells against injury induced by CS. Our approach may provide a potential therapeutic strategy against CS-induced lung diseases. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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