

available at [www.sciencedirect.com](http://www.sciencedirect.com)

Clinical Immunology

[www.elsevier.com/locate/yclim](http://www.elsevier.com/locate/yclim)



# Nrf2 is closely related to allergic airway inflammatory responses induced by low-dose diesel exhaust particles in mice

Ying Ji Li<sup>a,d</sup>, Hajime Takizawa<sup>b</sup>, Arata Azuma<sup>c</sup>, Tadashi Kohyama<sup>e</sup>,  
Yasuhiro Yamauchi<sup>e</sup>, Satoru Takahashi<sup>f</sup>, Masayuki Yamamoto<sup>f,1</sup>,  
Tomoyuki Kawada<sup>a</sup>, Shoji Kudoh<sup>c</sup>, Isamu Sugawara<sup>d,\*</sup>

<sup>a</sup> Department of Hygiene and Public Health, Nippon Medical School, Tokyo, Japan

<sup>b</sup> Fourth Department of Internal Medicine, Teikyo University, School of Medicine, Kawasaki, Japan

<sup>c</sup> Department of Pulmonary Medicine/Infection and Oncology, Nippon Medical School, Tokyo, Japan

<sup>d</sup> The Research Institute of Tuberculosis, Kiyose, Japan

<sup>e</sup> Department of Respiratory Medicine, University of Tokyo, School of Medicine, Tokyo, Japan

<sup>f</sup> Institute of Basic Medical Sciences, University of Tsukuba, Ibaragi, Japan

Received 15 May 2010; accepted with revision 31 July 2010

## KEYWORDS

Nrf2-knockout mouse;  
Low-dose diesel exhaust  
particles;  
Allergic airway  
inflammation;  
Anti-oxidant stress

**Abstract** We have recently reported that disruption of nuclear erythroid 2 P45-related factor 2 (Nrf2) enhances susceptibility to airway inflammatory responses induced by low-dose diesel exhaust particles (DEP) in mice. C57BL/6 Nrf2 knockout (Nrf2<sup>-/-</sup>) mice and wild-type (Nrf2<sup>+/+</sup>) mice were further exposed to low-dose DEP for 7 h/day, 5 days/week, for a maximum of 8 weeks. After exposure to DEP for 5 weeks, allergic airway inflammation was generated in the mice by intraperitoneal sensitization with OVA followed by intranasal challenge. Nrf2<sup>-/-</sup> mice exposed to relatively low-dose DEP showed significantly increased percentage changes relative to the OVA alone group in terms of airway hyperresponsiveness (AHR) and inflammatory cells, levels of IL-5 and thymus and activation regulated chemokine (TARC) in bronchoalveolar lavage (BAL) fluid than did Nrf2<sup>+/+</sup> mice. Lung tissues of Nrf2<sup>-/-</sup> mice after DEP exposure showed inflammatory cell infiltrates, and increased PAS staining-positive mucus cell hyperplasia. In contrast, the percentage changes relative to the OVA group in the reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio in whole blood was higher in Nrf2<sup>+/+</sup> mice than in Nrf2<sup>-/-</sup> mice. By using Nrf2<sup>-/-</sup> mice, it was shown for the first time that relatively low-dose DEP exposure induces oxidant stress, and that host anti-oxidant responses play a key role in the development of DEP-induced exacerbation of allergic airway inflammation.

© 2010 Elsevier Inc. All rights reserved.

\* Corresponding author. Fax: +81 424 92 4600.

E-mail address: [s6671112@sea.plala.or.jp](mailto:s6671112@sea.plala.or.jp) (I. Sugawara).

<sup>1</sup> Present address: Department of Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Japan.

## 1. Introduction

PM<sub>2.5</sub> has been especially singled out for its harmful effects on health. Diesel exhaust particles (DEP) are the major component of PM<sub>2.5</sub>, and therefore the relationship between PM<sub>2.5</sub> or PM<sub>10</sub> and various diseases has been investigated [1–3]. On the basis of *in vitro* experimental studies, it has been postulated that reactive oxygen species (ROS) generated by exposure to DEP play a role in the subsequent oxidative stress response [4–9]. These findings suggest that DEP induce activation of transcription factors and that ROS may play an important role in these processes. We have shown that continuous exposure to a low level of DEP (100 µg/m<sup>3</sup> for 7 h/day, 5 days/week) significantly augments AHR and Th2-type cytokine/chemokine gene expression in murine models of asthma [10]. Studies using two different mouse strains have demonstrated a difference in susceptibility to DEP exposure, and that the genes of certain antioxidant enzymes may play a role in this susceptibility, C57BL/6 mice being more sensitive to low-dose DEP exposure than BALB/c mice [11,12].

On the other hand, nuclear erythroid 2 P45-related factor 2 (Nrf2) is a redox-sensitive basic leucine zipper transcription factor that is involved in the transcriptional regulation of many antioxidant genes. The Nrf2-regulated genes in the lungs include almost all of the relevant antioxidants, such as HO-1 and several members of the GST family [13]. Therefore, to clarify whether oxidative stress and host antioxidant defenses play a central role in the pathogenesis of lung disease, several studies, such as those focusing on ovalbumin (OVA)-induced asthma in Nrf2<sup>-/-</sup> mice, have been reported [14]. It has been suggested that oxidative stress is involved in the development of DEP-induced airway inflammation [4–9] and that Nrf2 is also a key transcription factor that regulates antioxidants and defense against the proinflammatory and oxidizing effects of DEP *in vitro* [7,9]. Recently, we reported that disruption of Nrf2 enhances susceptibility to airway inflammatory responses induced by low-dose DEP in mice [15]. However, no previous study examining the pathogenesis of allergic airway inflammatory responses induced by DEP in Nrf2<sup>-/-</sup> mice has been reported.

In the present study, C57BL/6 Nrf2<sup>-/-</sup> and Nrf2<sup>+/+</sup> mice were used. After exposure to a low dose of DEP for 5 weeks, allergic airway inflammation was generated in the mice by intraperitoneal sensitization with OVA, followed by intranasal challenge. At 24 h after OVA challenge and the last DEP exposure, we assessed various parameters.

## 2. Materials and methods

### 2.1. Animals

Nrf2-deficient C57BL/6 mice were generated as described by Itoh et al. [16]. Mice were genotyped for Nrf2 status by PCR amplification of genomic DNA extracted from the tail [17]. PCR amplification was performed using three different primers:

Nrf2-sense for both genotypes: 5'-TGGACGGGACTATTGAAGGCTG-3'

Nrf2-antisense for wild-type mice: 5'-GCCGCCTTTTCAGTAGATGGAGG-3'

Nrf2-antisense for LacZ: 5'-GCGGATTGACCGTAATGGGATAGG-3'

Amplification conditions were 96 °C for 20 s, 59 °C for 30 s, 72 °C for 45 s, 30 cycles. The wild-type allele produces a 734-bp band, whereas the knockout allele produces a 449-bp band. Mice were housed under specific pathogen-free (SPF) conditions in a controlled environment (23±2 °C, 12 h light/dark periods). Age-matched 6-week-old female mice from the same litter were placed into chambers under the same controlled conditions and exposed to DEP. All procedures conformed to the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals.

### 2.2. DEP exposure

The *in vivo* DEP exposure system has been described previously [17,18]. The concentration of DEP was monitored and kept at low levels (approximately 100 µg/m<sup>3</sup>). C57BL/6 Nrf2<sup>-/-</sup> and Nrf2<sup>+/+</sup> mice were exposed to DEP for 7 h daily, 5 days per week.

### 2.3. Study design

The study design is shown in Fig. 1. Nrf2<sup>-/-</sup> and Nrf2<sup>+/+</sup> C57BL/6 mice were exposed to low-dose DEP for 7 h/day, 5 days/week, or to clean air, for a maximum of 8 weeks. After DEP or clean air exposure for 5 weeks, allergic airway inflammation was generated in the mice by intraperitoneal sensitization with OVA followed by intranasal challenge. Twenty-four hours after OVA challenge, and the last DEP exposure, we assessed the various parameters. AHR was measured in all experimental groups immediately, and the mice were then sacrificed. We examined the histopathology of the lung tissues and the cell populations in BAL fluid, and also measured the concentrations of inflammatory cytokines and chemokines in the BAL fluid, and the IgE, IgG<sub>1</sub> and IgG<sub>2a</sub> levels in the serum. We also examined the GSH/GSSG ratio in the whole blood.

### 2.4. Determination of AHR

Airway hyperresponsiveness was assessed by whole-body plethysmography with a free-moving application (Buxco Electronics, Troy, NY) [24], according to a previously published procedure [10,11].

### 2.5. Histological analysis

For histologic examination, 10%-formalin-fixed lung tissues were embedded in paraffin and cut into sections, which were then stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS). Histopathological changes were examined using a light microscope (Eclipse E800, Nikon, Tokyo, Japan).

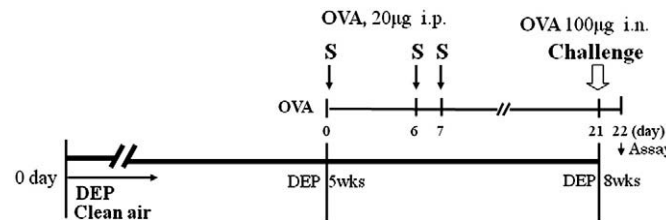


**Figure 1** Experimental design. OVA, ovalbumin; DEP, diesel exhaust particles; i.p., intraperitoneal injection; i.n., intranasal drip.

## 2.6. BAL and cell differentials in BAL fluid

BAL was performed as described previously [11,12]. The total number of cells in the BAL fluid was counted with a hemocytometer. For differential counts of leukocytes in BAL fluid, cytospin smear slides (Lab Systems; Tokyo, Japan) were prepared and stained with Diff-Quick Romanowski stain (Muto Kagaku Co., Tokyo, Japan).

## 2.7. Measurement of cytokines/chemokines in BAL fluid

Immunoreactivity for cytokines (IL-4, IL-5, IL-13, IL-12) and chemokines (monocyte chemoattractant protein, MCP-1; regulated upon activation, normal T expressed and secreted, RANTES; eotaxin, and TARC) in the BAL fluid supernatants was measured with an enzyme-linked immunosorbent assay (ELISA) kit (Biosource International, Inc., Camarillo, CA) in accordance with the manufacturer's instruction sheet. Each sample was assayed in triplicate.

## 2.8. Measurement of IgG<sub>1</sub>, IgG<sub>2a</sub> and IgE in serum

Immunoreactivity for IgG<sub>1</sub>, IgG<sub>2a</sub> (Cygnus Technologies, Inc., Southport, NC) and IgE (Bethyl Laboratories, Inc., Montgomery, TX) in the serum was measured with an ELISA kit in accordance with the manufacturer's instruction sheet. Each sample was assayed in triplicate.

## 2.9. GSH/GSSG ratio in whole blood

Determination of the GSH/GSSG ratio in whole blood was performed using a BIOXYTECH GSH/GSSG-412 kit in accordance with the manufacturer's instructions.

## 2.10. Statistical analysis

Results are shown as means  $\pm$  standard error (SE). Differences between groups were examined by Student's *t*-test using the Stat Mate III software package (ATMS Digital Medical Station, Tokyo, Japan). Differences at *P* < 0.05 were considered significant.

## 3. Results

### 3.1. Assessment of changes in AHR in response to DEP exposure

To examine airway responses to low-dose DEP in Nrf2<sup>-/-</sup> mice after OVA challenge, we first assessed AHR (as expressed in Penh) by using whole-body plethysmography. The percentage changes relative to the OVA only group in Nrf2<sup>+/+</sup> and Nrf2<sup>-/-</sup> mice respectively were used to evaluate the effect of DEP exposure. Exposure to DEP significantly increased the airway reactivity to methacholine (12 and 24 mg/ml) in Nrf2<sup>-/-</sup> mice relative to that in Nrf2<sup>+/+</sup> mice (Fig. 2).

### 3.2. Lung histopathology

Tissue sections were prepared from dissected mouse lungs and observed for histological changes by light microscopy. In OVA-sensitized and challenged mice, peribronchial and perivascular infiltration of inflammatory cells was evident in both Nrf2<sup>+/+</sup> (Fig. 3A, a) and Nrf2<sup>-/-</sup> (Fig. 3A, b) mice. DEP exposure did not increase the number of peribronchial

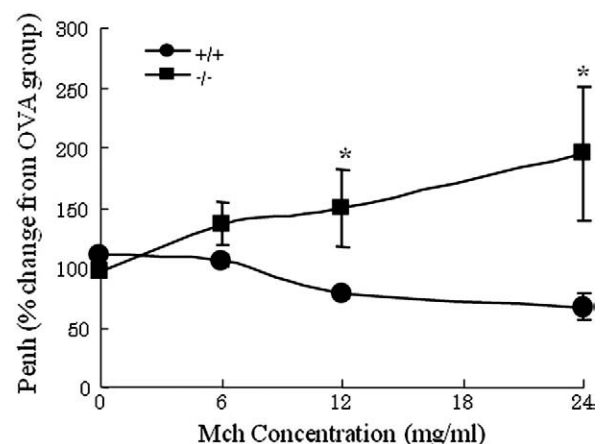


**Figure 2** Airway hyperresponsiveness was assessed by whole-body plethysmography with a free-moving application and then evaluated in terms of Penh values in response to inhaled aerosolized methacholine (0, 6, 12, 24 mg/ml). The horizontal axis shows the concentration of methacholine (mg/ml); the vertical axis shows percentage changes in the Penh values relative to the OVA only group. +/+ : wild-type mice; -/- : Nrf2-knockout mice. Results are means  $\pm$  SEM of data in each group (*n* = 6). \**p* < 0.05 Nrf2<sup>-/-</sup> mice compared with Nrf2<sup>+/+</sup> mice at each methacholine concentration.

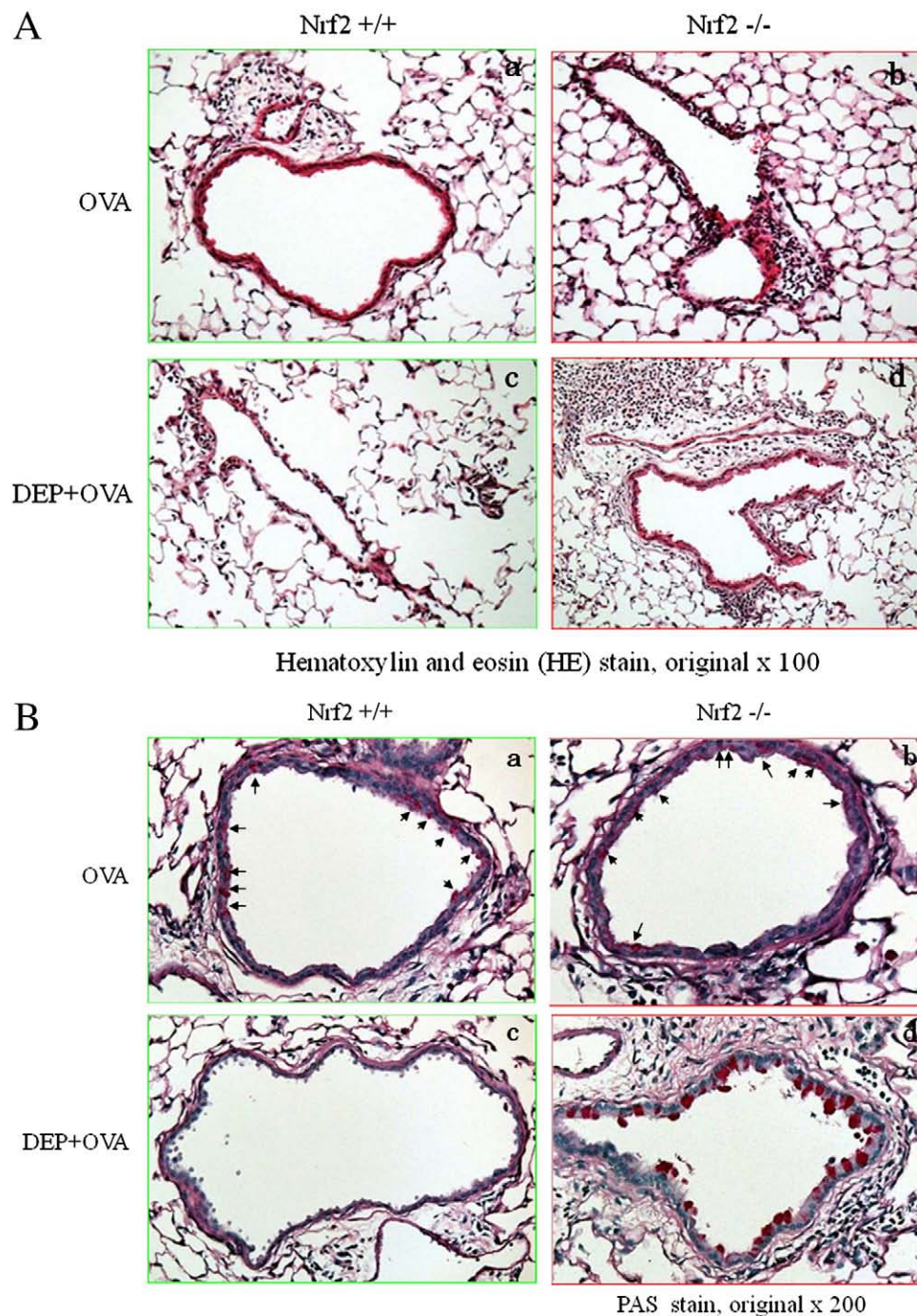


**Figure 3** Histopathology of the lung tissues. Lung sections were stained with hematoxylin and eosin and examined by light microscopy (100 $\times$ ) (A). Lung sections were stained with PAS and examined by light microscopy (200 $\times$ ) (B). The arrows indicate the cells positive for periodic acid-Schiff staining.

inflammatory cells in Nrf2<sup>+/+</sup> mice (Fig. 3A, c). In contrast, there was intense infiltration of peribronchial inflammatory cells in Nrf2<sup>-/-</sup> mice (Fig. 3A, d). In OVA-sensitized and challenged mice, an increased number of cells were positive for mucin staining (PAS-positive cells) in the airways of both Nrf2<sup>+/+</sup> (Fig. 3B, a) and Nrf2<sup>-/-</sup> (Fig. 3B, b) mice. Although DEP exposure had no effect on the number of PAS-positive cells, a decrease was evident in Nrf2<sup>+/+</sup> mice (Fig. 3B, c). In contrast, the number of PAS-positive cells were markedly increased in Nrf2<sup>-/-</sup> mice (Fig. 3B, d).

### 3.3. BAL cell differentials

Examination of the total number of cells and the differential cell count in BAL fluid expressed as a percentage relative to the OVA only group showed that the total numbers of cells and lymphocytes were significantly more increased in Nrf2<sup>-/-</sup> mice than in Nrf2<sup>+/+</sup> mice. The percentage changes relative to the OVA only group in the numbers of neutrophils and eosinophils were greater after DEP exposure in Nrf2<sup>-/-</sup>

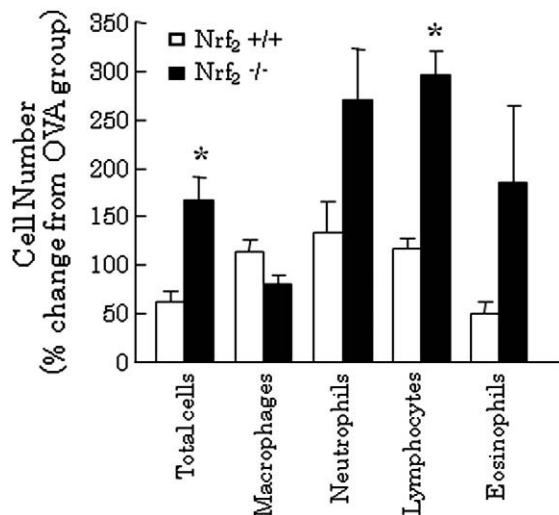


**Figure 4** Changes in total and differential inflammatory cell populations in BAL fluid. BAL fluid smears were stained with Diff-Quick Romanowski stain. The vertical axis shows percentage changes in cell number relative to the OVA only group. +/+ : wild-type mice; -/- : Nrf2-knockout mice. Results are means  $\pm$  SEM of data in each group ( $n=6$ ). \* $p<0.05$  Nrf2<sup>-/-</sup> mice compared with Nrf2<sup>+/+</sup> mice.

mice than in Nrf2<sup>+/+</sup> mice. In contrast, the percentage change in the number of macrophages was decreased more

after DEP exposure in Nrf2<sup>-/-</sup> mice than in Nrf2<sup>+/+</sup> mice (Fig. 4).

### 3.4. Cytokine/chemokine levels in BAL fluid

Examination of the cytokine/chemokine levels in BAL fluid showed that the percentage changes relative to the OVA only group in the levels of IL-5 were significantly greater in Nrf2<sup>-/-</sup> mice than in Nrf2<sup>+/+</sup> mice after DEP exposure (Fig. 5A, b). The percentage changes in the levels of IL-4 and IL-13 were greater in Nrf2<sup>-/-</sup> mice than in Nrf2<sup>+/+</sup> mice after DEP exposure (Fig. 5A, a, d). The percentage change in the level of TARC was significantly greater in Nrf2<sup>-/-</sup> mice than in Nrf2<sup>+/+</sup> mice after DEP exposure (Fig. 5B, d). The percentage changes in the levels of MCP-1, RANTES and Eotaxin in the BAL fluid showed no significant differences between the Nrf2<sup>+/+</sup> and the Nrf2<sup>-/-</sup> mice after DEP exposure (Fig. 5B, a, b and c).

### 3.5. IgG<sub>1</sub>, IgG<sub>2a</sub> and IgE levels in serum

There were no significant changes, expressed as a percentage relative to the OVA only group, in the levels of IgE, IgG<sub>1</sub> and IgG<sub>2a</sub> in serum between Nrf2<sup>+/+</sup> and Nrf2<sup>-/-</sup> mice after DEP exposure (Fig. 6).

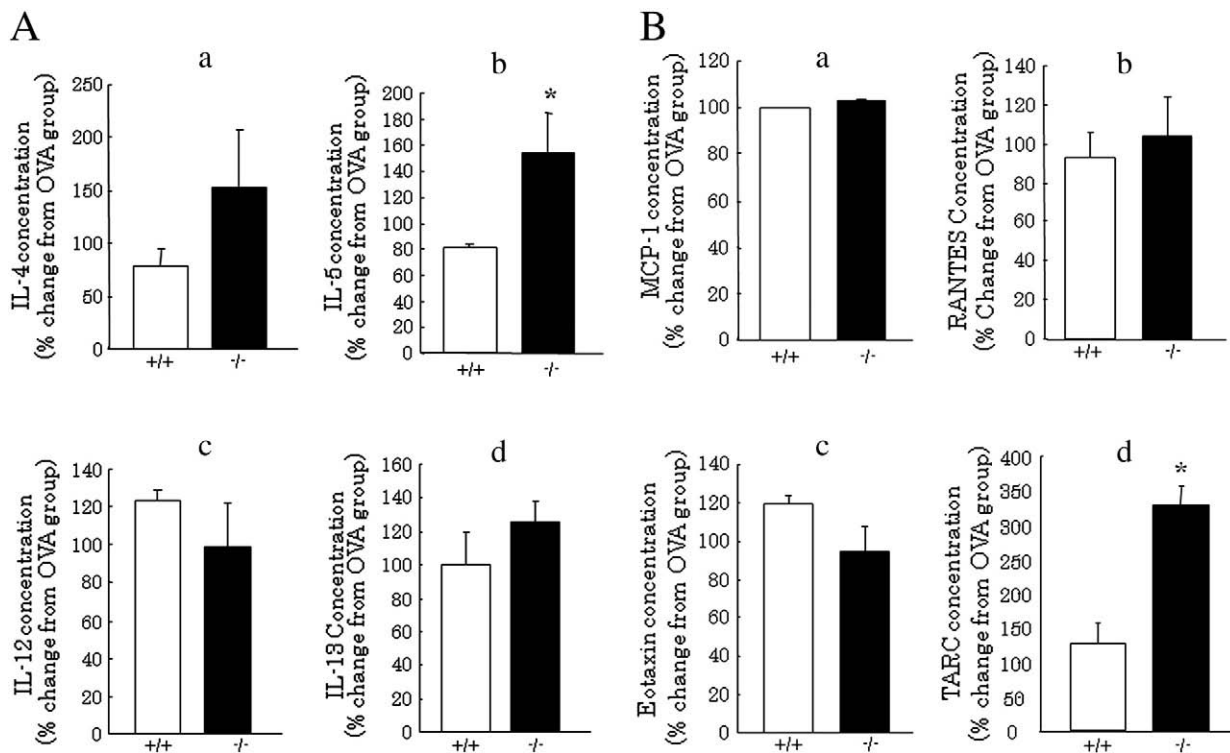


**Figure 5** Levels of cytokines (A) and chemokines (B) in the BAL fluid as evaluated by ELISA. The vertical axis shows percentage changes in cytokine or chemokine concentrations relative to the OVA only group. +/+ , wild-type mice; -/- , Nrf2-knockout mice. Results are means  $\pm$  SEM of data in each group ( $n=6$ ). \* $p<0.05$  Nrf2<sup>-/-</sup> mice compared with Nrf2<sup>+/+</sup> mice.

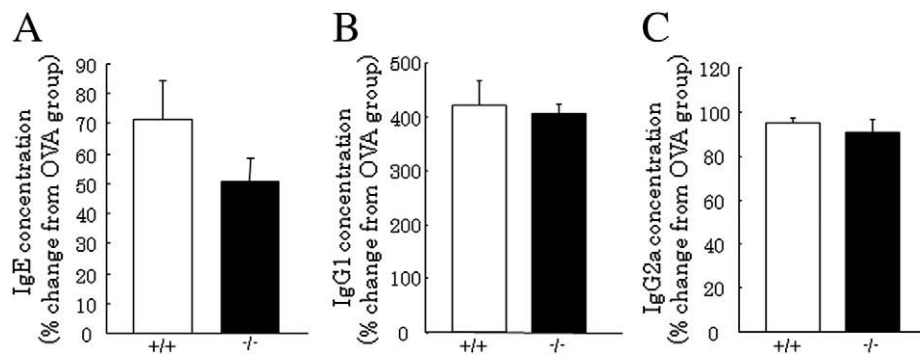


**Figure 6** Immunoglobulin levels in serum, as evaluated by ELISA. The vertical axis shows percentage changes in the immunoglobulin concentration relative to the OVA only group. +/+, wild-type mice; -/-, Nrf2-knockout mice. Results are means  $\pm$  SEM of data in each group ( $n=6$ ).

### 3.6. GSH/GSSG ratio in whole blood

Examination of the GSH/GSSG ratio in whole blood expressed as a percentage relative to the OVA only group showed that the ratio was significantly higher in Nrf2<sup>+/+</sup> mice than in Nrf2<sup>-/-</sup> mice (Fig. 7).

## 4. Discussion

In the present study, using Nrf2-knockout mice, we demonstrated for the first time that disruption of Nrf2 enhances susceptibility to allergic airway inflammatory responses induced by low-dose diesel exhaust particles in mice. The Nrf2<sup>+/+</sup> and Nrf2<sup>-/-</sup> C57BL/6 mice were exposed to low-dose DEP (i.e., at concentrations similar to those inhaled by humans outdoors) for 7 h/day, 5 days per week, for a maximum of 8 weeks. After 5 weeks of DEP exposure, allergic airway inflammation was generated in mice by intraperitoneal sensitization with OVA followed by intranasal challenge, then 24 h after OVA challenge and the final exposure to DEP, we assessed the various parameters. We found for the first time that Nrf2<sup>-/-</sup> mice showed an increase of airway

responsiveness, allergic inflammation and cytokine/chemokine levels when exposed to low-dose DEP.

We assessed airway hyperresponsiveness (as expressed by enhanced Pause values in Penh) by using whole-body plethysmography. Penh has been used in an experimental mouse model for the evaluation of airway hyperresponsiveness [19–21]. In OVA-sensitized and challenged mice, the percentage changes relative to the OVA only group in airway reactivity to methacholine showed a significant increase in Nrf2<sup>-/-</sup> mice compared with Nrf2<sup>+/+</sup> mice after DEP exposure. Our previous study showed that Nrf2<sup>-/-</sup> mice had only PAS-positive mucus cell hyperplasia, but no destruction, of airway epithelial cells in lung tissues [15]. Although it has been confirmed that AHR is increased by oxidative stress induced by exposure to DEP, the mechanism by which AHR increases in Nrf2<sup>-/-</sup> mice is still not clear. In the present study, Nrf2<sup>-/-</sup> mice after DEP exposure showed intense peribronchial inflammatory cell infiltration, and the number of PAS-positive cells was markedly increased. We consider that, in this experimental system, the changes in AHR caused by low-dose DEP may be dependent on allergic airway inflammation. However, the relationship between inflammation and AHR is complex, and there is no clear correlation between the severity of inflammation and the severity of AHR.

It has been reported that OVA-sensitized and challenged Nrf2<sup>-/-</sup> mice show increased AHR and airway inflammation [14]. However, in the present study, there were no remarkable changes in mice that were OVA-sensitized and challenged without exposure to DEP. As we used Nrf2<sup>-/-</sup> mice, the allergic effect of airway inflammation induced by OVA sensitization and challenge may have been mild.

Eosinophil recruitment into inflammatory sites is a complex process regulated by a number of cytokines, including IL-5 [22]. Our findings suggest that IL-5 may have been involved in the recruitment of eosinophils into the airways of Nrf2<sup>-/-</sup> mice after exposure to DEP in our experimental system. We found a significant increase in the level of TARC, but not that of eotaxin or RANTES, in the Nrf2<sup>-/-</sup> mice after DEP exposure. Dias-Sanchez et al. have suggested that the effects of DEP on cytokine/chemokine expression are not global or non-specific [23]. Among these CC chemokines, TARC was the first to have been shown to selectively chemoattract T lymphocytes [24]. TARC was

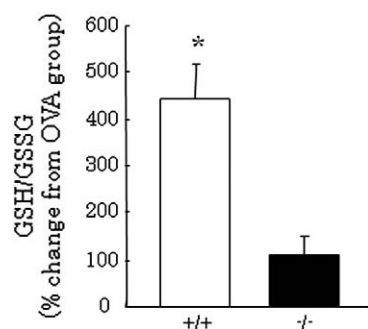


**Figure 7** GSH/GSSG ratio in whole blood. The vertical axis shows percentage changes in the GSH/GSSG ratio in whole blood relative to the OVA only group. +/+, wild-type mice; -/-, Nrf2-knockout mice. Results are means  $\pm$  SEM of data in each group ( $n=6$ ). \* $p < 0.05$  Nrf2<sup>+/+</sup> mice compared with Nrf2<sup>-/-</sup> mice.

subsequently found to induce chemotaxis of T cells, especially Th2-type CD4<sup>+</sup> T lymphocytes [25]. TARC is a pivotal chemokine in the development of Th2-dominated experimental asthma with eosinophilia and AHR [26]. Our results indicate that TARC is also a pivotal chemokine for the development of Th2-dominated oxidative stress-induced allergic airway inflammation after exposure to DEP, being consistent with our previous results [15].

Although it is reported that DEP-induced oxidative stress can initiate and exacerbate airway allergic responses through enhanced IgE production [27,28], our low-dose DEP exposure system elicited no remarkable changes in IgE production in the serum of Nrf2<sup>-/-</sup> mice.

GSH is a major intracellular thiol antioxidant that acts directly as a ROS scavenger. The GSH redox system plays a critical role in determining intracellular redox balance and antioxidant function [29]. Measurement of the GSSG level or determination of the GSH/GSSG ratio is a useful indicator of oxidative stress and can be used to monitor the effectiveness of antioxidant intervention strategies. The GSH/GSSG ratio was significantly increased in Nrf2<sup>+/-</sup> mice relative to Nrf2<sup>-/-</sup> mice, strongly suggesting that DEP-induced oxidant stress and host anti-oxidant responses play a key role in the development of DEP-induced allergic airway inflammatory disease.

## Acknowledgment

This study was supported in part by the Pollution-related Health Damage Compensation and Prevention Association of Japan.

## References

- [1] D.W. Dockery, C.A. Pope, X. Xu, J.D. Spengler, J.H. Ware, M.E. Fay, B.G. Ferris, F.E. Speizer, An association between air pollution and mortality in six U.S. cities, *N. Engl. J. Med.* 329 (1993) 1753–1759.
- [2] J.M. Samet, F. Dominici, F.C. Curriero, I. Coursac, S.L. Zeger, Fine particulate air pollution and mortality in 20 U.S. cities, 1987–1994, *N. Engl. J. Med.* 343 (2000) 1742–1749.
- [3] G. Hoek, B. Brunekreef, S. Goldbohm, P. Fischer, P.A. van den Brandt, Association between mortality and indicators of traffic-related air pollution in the Netherlands: a cohort study, *Lancet* 360 (2002) 1203–1209.
- [4] H. Takizawa, T. Ohtoshi, S. Kawasaki, T. Kohyama, M. Desaki, T. Kasama, K. Kobayashi, K. Nakahara, K. Yamamoto, K. Matsushima, S. Kudoh, Diesel exhaust particles induce NF-kappa B activation in human bronchial epithelial cells in vitro: importance in cytokine transcription, *J. Immunol.* 162 (1999) 4705–4711.
- [5] H. Takizawa, S. Abe, H. Okazaki, T. Kohyama, I. Sugawara, Y. Saito, T. Ohtoshi, S. Kawasaki, M. Desaki, K. Nakahara, K. Yamamoto, K. Matsushima, M. Tanaka, M. Sagai, S. Kudoh, Diesel exhaust particles upregulate eotaxin gene expression in human bronchial epithelial cells via nuclear factor-kappa B-dependent pathway, *Am. J. Physiol. Lung. Cell. Mol. Physiol.* 284 (2003) L1055–L1062.
- [6] S. Hashimoto, Y. Gon, I. Takeshita, K. Matsumoto, I. Jibiki, H. Takizawa, S. Kudoh, T. Horie, Diesel exhaust particles activate p38 MAP kinase to produce interleukin 8 and RANTES by human bronchial epithelial cells and N-acetylcysteine attenuates p38 MAP kinase activation, *Am. J. Respir. Crit. Care. Med.* 161 (2000) 280–285.
- [7] G.G. Xiao, M. Wang, N. Li, J.A. Loo, A.E. Nel, Use of proteomics to demonstrate a hierarchical oxidative stress response to diesel exhaust particle chemicals in a macrophage cell line, *J. Biol. Chem.* 278 (2003) 50781–50790.
- [8] N. Li, M.I. Venkatesan, A. Miguel, R. Kaplan, C. Gujuluva, J. Alam, A. Nel, Induction of heme oxygenase-1 expression in macrophages by diesel exhaust particle chemicals and quinones via the antioxidant-responsive element, *J. Immunol.* 165 (2000) 3393–3401.
- [9] N. Li, J. Alam, M.I. Venkatesan, A. Eiguren-Fernandez, D. Schmitz, E. Di Stefano, N. Slaughter, E. Killeen, X. Wang, A. Huang, M. Wang, A.H. Miguel, A. Cho, C. Sioutas, A.E. Nel, Nrf2 is a key transcription factor that regulates antioxidant defense in macrophages and epithelial cells: protecting against the proinflammatory and oxidizing effects of diesel exhaust chemicals, *J. Immunol.* 173 (2004) 3467–3481.
- [10] A. Matsumoto, K. Hiramatsu, Y.J. Li, A. Azuma, S. Kudoh, H. Takizawa, I. Sugawara, Repeated exposure to low-dose diesel exhaust after allergen challenge exaggerates asthmatic responses in mice, *Clin. Immunol.* 121 (2006) 227–235.
- [11] Y.J. Li, T. Kawada, A. Matsumoto, A. Azuma, S. Kudoh, H. Takizawa, I. Sugawara, Airway inflammatory responses to oxidative stress induced by low-dose diesel exhaust particle exposure differ between mouse strains, *Exp. Lung. Res.* 33 (2007) 227–244.
- [12] Y.J. Li, T. Kawada, H. Takizawa, A. Azuma, S. Kudoh, I. Sugawara, Y. Yamauchi, T. Kohyama, Airway inflammatory responses to oxidative stress induced by prolonged low-dose diesel exhaust particle exposure from birth differ between mouse BALB/c and C57BL/6 strains, *Exp. Lung. Res.* 34 (2008) 125–139.
- [13] T. Nguyen, P.J. Sherratt, C.B. Pickett, Regulatory mechanisms controlling gene expression mediated by the antioxidant response element, *Annu. Rev. Pharmacol. Toxicol.* 43 (2003) 233–260.
- [14] T. Rangasamy, J. Guo, W.A. Mitzner, J. Roman, A. Singh, A.D. Fryer, M. Yamamoto, T.W. Kensler, R.M. Tuder, S.N. Georas, S. Biswal, Disruption of Nrf2 enhances susceptibility to severe airway inflammation and asthma in mice, *J. Exp. Med.* 202 (2005) 47–59.
- [15] Y.J. Li, H. Takizawa, A. Azuma, T. Kohyama, Y. Yamauchi, S. Takahashi, M. Yamamoto, T. Kawada, S. Kudoh, I. Sugawara, Disruption of Nrf2 enhances susceptibility to airway inflammatory responses induced by low-dose diesel exhaust particles in mice, *Clin. Immunol.* 128 (2008) 366–373.
- [16] K. Itoh, T. Chiba, S. Takahashi, T. Ishii, K. Igarashi, Y. Katoh, T. Oyake, N. Hayashi, K. Satoh, I. Hatayama, M. Yamamoto, Y. Nabeshima, An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements, *Biochem. Biophys. Res. Commun.* 236 (1997) 313–322.
- [17] Y. Saito, A. Azuma, S. Kudoh, H. Takizawa, I. Sugawara, Long-term inhalation of diesel exhaust affects cytokine expression in murine lung tissues: comparison between low- and high-dose diesel exhaust exposure, *Exp. Lung. Res.* 28 (2002) 493–506.
- [18] K. Hiramatsu, A. Azuma, S. Kudoh, M. Desaki, H. Takizawa, I. Sugawara, Inhalation of diesel exhaust for three months affects major cytokine expression and induces bronchus-associated lymphoid tissue formation in murine lungs, *Exp. Lung. Res.* 29 (2003) 607–622.
- [19] S. Finotto, G.T. De Sanctis, H.A. Lehr, U. Herz, M. Buerke, M. Schipp, B. Bartsch, R. Atreya, E. Schmitt, P.R. Galle, H. Renz, M.F. Neurath, Treatment of allergic airway inflammation and hyperresponsiveness by antisense-induced local

- blockade of GATA-3 expression, *J. Exp. Med.* 193 (2001) 1247–1260.
- [20] A.S. Leme, C. Hubeau, Y. Xiang, A. Goldman, K. Hamada, Y. Suzaki, L. Kobzik, Role of breast milk in a mouse model of maternal transmission of asthma susceptibility, *J. Immunol.* 165 (2006) 762–769.
- [21] P. Depuydt, G.F. Joos, R.A. Pauwels, Ambient ozone concentrations induce airway hyperresponsiveness in some rat strains, *Eur. Respir. J.* 38 (1999) 14125–14131.
- [22] M.E. Rothenberg, Eosinophilia, *N. Engl. J. Med.* 338 (1998) 1592–1600.
- [23] D. Diaz-Sanchez, M. Jyrala, D. Ng, A. Nel, A. Saxon, In vivo nasal challenge with diesel exhaust particles enhances expression of the CC chemokines rantes, MIP-1 $\alpha$ , and MCP-3 in humans, *Clin. Immunol.* 97 (2000) 140–145.
- [24] T. Imai, M. Baba, M. Nishimura, M. Kakizaki, S. Takagi, O. Yoshie, The T cell-directed CC chemokine TARC is a highly specific biological ligand for CC chemokine receptor 4, *J. Biol. Chem.* 272 (1997) 15036–15042.
- [25] T. Imai, D. Chantry, C.J. Raport, C.L. Wood, M. Nishimura, R. Godiska, O. Yoshie, P.W. Gray, Macrophage-derived chemokine is a functional ligand for the CC chemokine receptor 4, *J. Biol. Chem.* 273 (1997) 1764–1768.
- [26] S. Kawasaki, H. Takizawa, H. Yoneyama, T. Nakayama, R. Fujisawa, M. Izumizaki, T. Imai, O. Yoshie, I. Homma, K. Yamamoto, K. Matsushima, Intervention of thymus and activation-regulated chemokine attenuates the development of allergic airway inflammation and hyperresponsiveness in mice, *J. Immunol.* 166 (2001) 2055–2062.
- [27] M.J. Whitekus, N. Li, M. Zhang, M. Wang, M.A. Horwitz, S.K. Nelson, L.D. Horwitz, N. Brechun, D. Diaz-Sanchez, A.E. Nel, Thiol antioxidants inhibit the adjuvant effects of aerosolized diesel exhaust particles in a murine model for ovalbumin sensitization, *J. Immunol.* 168 (2002) 2560–2567.
- [28] J. Wan, D. Diaz-Sanchez, Phase II enzyme induction blocks the enhanced IgE production in B cells by diesel exhaust particles, *J. Immunol.* 177 (2006) 3477–3483.
- [29] A. Meister, Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy, *Pharmacol. Ther.* 51 (1991) 155–194.