

Effects of phenanthraquinone on allergic airway inflammation in mice

K. Hiyoshi^{*†}, H. Takano[†], K.-I. Inoue[†], T. Ichinose[‡], R. Yanagisawa[†], S. Tomura^{*} and Y. Kumagai^{§¶}

^{*}Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ibaraki, Japan; [†]Inhalation Toxicology and Pathophysiology Research Team, National Institute for Environmental Studies, Ibaraki, Japan; [‡]Department of Health Science, Oita University of Nursing and Health Science, Oita, Japan; [§]Department of Environmental Medicine, Doctoral Programs in Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan and [¶]Southern California Particle Center and Supersite, Institute of the Environment, University of California, Los Angeles, CA 90095, USA

Summary

Background Diesel exhaust particles (DEP) enhance allergic airway inflammation in mice (Takano et al., Am J Respir Crit Care Med 1997; 156: 36–42). DEP consist of carbonaceous nuclei and a vast number of organic chemical compounds. However, it remains to be identified which component(s) from DEP are responsible for the enhancing effects. 9,10-Phenanthraquinone (PQ) is a quinone compound involved in DEP.

Objective To investigate the effects of PQ inoculated intratracheally on allergic airway inflammation related to ovalbumin (OVA) challenge.

Materials and Methods We evaluated effects of PQ on airway inflammation, local expression of cytokine proteins, and allergen-specific immunoglobulin production in mice in the presence or absence of OVA.

Results In the presence of OVA, PQ (2.1 ng/animal) significantly increased the numbers of eosinophils and mononuclear cells in bronchoalveolar lavage fluid as compared with OVA alone. In contrast, the numbers of these cells around the airways were not significantly different between OVA challenge and OVA plus PQ challenge in lung histology. PQ exhibited adjuvant activity for the allergen-specific production of IgG1 and IgE. OVA challenge induced significant increases in the lung expression of IL-4, IL-5, eotaxin, macrophage chemoattractant protein-1, and keratinocyte chemoattractant as compared with vehicle challenge. However, the combination of PQ with OVA did not alter the expression levels of these proteins as compared with OVA alone.

Conclusion These results indicate that PQ can enhance the immunoglobulin production and the infiltration of inflammatory cells into alveolar spaces that are related to OVA, whereas PQ seems to be partially responsible for the DEP toxicity on the allergic airway inflammation.

Keywords airway inflammation, immunoglobulin, ovalbumin, 9,10-phenanthraquinone

Submitted: 29 November 2004; revised 10 April 2005; accepted 1 June 2005

Diesel exhaust particles (DEP) are major contributors to the atmospheric particulate air pollution in metropolitan areas. DEP have been correlated with lung cancer, pulmonary fibrosis, chronic alveolitis [1], and oedematous changes [2]. DEP can also modulate allergic reactions [3, 4] including allergen-specific IgE response [5, 6]. We have previously reported that DEP (100 µg/animal) aggravate allergic airway inflammation induced by intratracheal instillation of ovalbumin (OVA) *in vivo* [7–10].

DEP are complicated particles consisting of carbonaceous nuclei and a vast number of organic chemical compounds. Previous *in vitro* studies have indicated that organic chemicals extracted from DEP results in induction of apoptosis [11], increases oxidative stress [12], and induces inflammatory cells [13–15], through the release of pro-inflammatory molecules *in*

vitro [15, 16]. However, research about the component(s) of DEP responsible for their effects on the respiratory system and/or pulmonary diseases remains unsatisfactory especially *in vivo*.

To date, a variety of quinones have been identified as DEP components [17, 18]. Quinones reportedly have several toxicological properties [19–22]. On the other hand, oxidative stress elicited by DEP has been considered to correlate to organ injuries. 9,10-Phenanthraquinone (PQ) is found to be a major quinone in DEP [18, 22] and has a pro-oxidant role [23]. We have recently shown that single intratracheal administration of PQ induces recruitment of inflammatory cells such as eosinophils and neutrophils into the lung with the lung expression of pro-inflammatory molecules *in vivo* [24]. More recently, we have demonstrated that PQ induces oxidative damages on airway epithelial cells through apoptosis and down-regulation of superoxide dismutase *in vitro* [25]. Previous studies have raised the possibility that PQ may also aggravate allergic airway inflammation or diseases.

Correspondence: Hirohisa Takano, MD, PhD, Inhalation Toxicology & Pathophysiology Research Team, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, 305-8506, Japan.
E-mail: htakano@nies.go.jp

The aim of the present study was to elucidate the effects of PQ (2.1 ng/animal) on allergic airway inflammation, the local expression of IL-4, IL-5, and eotaxin, and the production of allergen-specific IgG1 and IgE.

Materials and methods

Animals

Male ICR mice 6–7 weeks of age and weighing 29–33 g (Japan Clea Co., Tokyo, Japan) were used in all experiments. They were fed a commercial diet (Japan Clea Co.) and given water *ad libitum*. Mice were housed in an animal facility that was maintained at 24–26 °C with 55–75% humidity and a 12 h/12 h light/dark cycle.

Study protocol

Mice were divided into four groups (Fig. 1). The vehicle group received phosphate-buffered saline (PBS) at pH 7.4 (Nissui Pharmaceutical Co., Tokyo, Japan) containing 0.05% Tween 80 (Nacalai Tesque, Kyoto, Japan) once a week for 6 weeks. The OVA group received 1 µg of OVA (Sigma Chemical, St Louis, MO, USA) dissolved in the same vehicle bi-weekly for 6 weeks. The PQ group received 0.01 nmol (2.1 ng)/animal of PQ dissolved in 100 µL of the same vehicle every week for 6 weeks. We selected the dose (2.1 ng) of PQ, since 2.1 ng of PQ is involved in 100 µg of DEP, with which we have previously investigated the enhancing effects of DEP on the same model [7, 8]. The PQ + OVA group received the combined treatment in the same protocol as the PQ and the OVA groups. In each group, vehicle, OVA, PQ, or OVA + PQ was dissolved in 0.1 mL aliquots, and inoculated intratracheally as previously described [8, 26]. The animals were studied 24 h after the last intratracheal administration with lung histology, bronchoalveolar lavage (BAL), protein levels of cytokines in the total lung supernatants, and with allergen-specific immunoglobulins. The studies adhered to the National Institutes of Health guidelines for the experimental use of animals. All animal studies were approved by the Institutional Review Board.

Blood retrieval and analysis

Mice were anaesthetized with diethyl ether. The chest and abdominal walls were opened, and blood was retrieved by

cardiac puncture. Plasma was prepared and frozen at –80 °C until assayed for allergen-specific IgG1 and IgE.

Bronchoalveolar lavage, histological evaluation, and measurement of cytokines and chemokines

BAL and cell counts were conducted as previously described [8]. The lungs were fixed and stained with haematoxylin and eosin as previously described [8]. All bronchi of the whole sections of randomly selected lung were examined, and the intensity of inflammation was graded in blinded fashion as previously described [10]. The lungs were homogenized and centrifuged as previously described. ELISA for IL-4 (Amersham, Buckinghamshire, UK), IL-5, granulocyte/macrophage colony-stimulating factor (GM-CSF), IL-2, IFN-γ (Endogen, Cambridge, MA, USA), eotaxin, and regulated on activation and normal T cells expressed and secreted (RANTES) (R&D systems, Minneapolis, MN, USA) in the lung tissue supernatants were conducted according to the manufacturer's instruction. Minimum detectable dose of IL-4, IL-5, GM-CSF, IL-2, IFN-γ, eotaxin, or RANTES was 5, 5, 5, 3, 10, 3, and 2 pg/mL, respectively.

Allergen-specific immunoglobulin determination

Allergen-specific IgE antibody was measured by IgE-capture ELISA as described previously [8]. Allergen-specific IgG1 antibodies were measured by ELISA with solid-phase antigen as previously described [8].

Statistical analysis

Data were reported as mean ± SEM. Differences among groups were analysed by ANOVA followed by Fisher's PLSD test (Stat view version 4.0; Abacus Concepts, Inc, Berkeley, CA, USA) as described previously [8]. Significance was assigned to *P*-values smaller than 0.05.

Results

Phenanthraquinone partly accelerate allergen-induced airway inflammation

To evaluate the effects of PQ on allergic airway inflammation, we investigated the cellular profile of BAL fluid and lung histology in four groups of mice 24 h after the last intratracheal instillation.

The number of BAL eosinophils was significantly greater in the PQ + OVA group than in the vehicle, the PQ group or in the OVA group ($P < 0.01$, Fig. 2). The vehicle group showed no eosinophil in BAL fluid. The number of mononuclear cells was significantly greater in the OVA group ($P < 0.05$) or in the PQ + OVA group ($P < 0.01$) than in the vehicle group. The number was greater in the PQ + OVA group than in the PQ group ($P < 0.05$) or in the OVA group (without significance).

To quantitate the infiltration of inflammatory cells around the airways, we estimated the magnitude of the histopathological changes in the lung specimens stained with Diff-Quik (Table 1). Instillation of OVA or OVA + PQ significantly increased the numbers of infiltrated eosinophils or neutrophils + mononuclear cells compared with vehicle or PQ

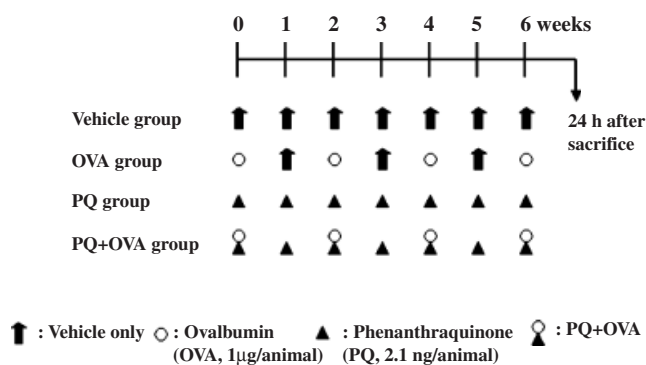


Fig. 1. Experimental design.

exposure. However, no significant differences were found in the numbers between the OVA and OVA + PQ group.

Effects of phenanthraquinone on the increase in goblet cells in the airways after allergen challenge

To evaluate bronchial epithelial injury and mucus hypersecretion, lung sections were stained with PAS. Instillation of OVA or OVA + PQ significantly increased the number of goblet cells compared with vehicle or PQ instillation (Table 1). However, no significant difference was found in the numbers between the OVA and the OVA + PQ groups.

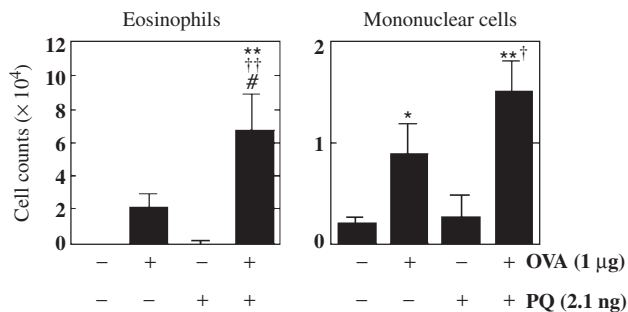


Fig. 2. Cellular profile in bronchoalveolar lavage fluid. Four groups of mice were intratracheally administered vehicle, ovalbumin (OVA), phenanthraquinone (PQ), or a combination of OVA and PQ for 6 weeks. Bronchoalveolar lavage (BAL) was conducted 24 h after the last intratracheal instillation. Differential cell counts were assessed with Diff-Quik-staining. Results are presented as mean $\pm$ SEM. * P < 0.05 vs. vehicle. ** P < 0.001 vs. vehicle. † P < 0.05 vs. PQ. ‡ P < 0.01 vs. PQ. # P < 0.01 vs. OVA.

Table 1. Degree of infiltrations of inflammatory cells around the airways and goblet cells in the bronchial epithelium

Group	<i>n</i>	Neutrophils and mononuclear cells	Eosinophils	Goblet cells
Vehicle	8	0.38 $\pm$ 0.21	0.25 $\pm$ 0.25	0.50 $\pm$ 0.38
OVA	7	2.63 $\pm$ 0.25*	2.75 $\pm$ 0.28*	2.44 $\pm$ 0.26*
PQ	8	1.71 $\pm$ 0.24*	0.86 $\pm$ 0.21	0.29 $\pm$ 0.18
OVA + PQ	9	2.94 $\pm$ 0.21*†‡	2.78 $\pm$ 0.19*†	2.39 $\pm$ 0.45*†

Animals received intratracheal instillation of vehicle, OVA, PQ, or OVA + PQ for 6 weeks. Lungs were removed and fixed 24 h after the last intratracheal administration. Sections were stained with haematoxylin and eosin for measurement of inflammatory cells around the airways or with PAS for goblet cells in the bronchial epithelium. Degree of infiltrations of inflammatory cells and goblet cells was estimated. Values are mean $\pm$ SEM.

* P < 0.05 vs. vehicle. ** P < 0.001 vs. vehicle. † P < 0.001 vs. PQ. OVA, ovalbumin; PQ, 9,10-phenanthraquinone.

Phenanthraquinone did not affect local expression of T helper type 2 cytokines and eotaxin, RANTES, GM-CSF and IL-2 in the presence of allergen

To explore the role of local expression of Th2 cytokines in the enhancing effects of PQ on allergen-induced airway inflammation, we quantitated protein levels of IL-4 and IL-5 in the lung tissue supernatants 24 h after the last intratracheal instillation (Table 2). The OVA group and the OVA + PQ group showed increases in IL-5 (P < 0.01 for the OVA group, and without significance for the OVA + PQ group) as compared with the vehicle group. No significant difference in the level of IL-5 was found between OVA and OVA + PQ treatment. The levels of IL-4 in the lung tissue were not different among the four experimental groups.

To investigate the local expression of eotaxin, RANTES, GM-CSF, IL-2, and IFN- γ , we measured protein levels of these cytokines and chemokine in the lung tissue 24 h after the last intratracheal instillation (Table 2). The OVA group and OVA + PQ group showed a significant increase in the levels of eotaxin and RANTES as compared with vehicle group or PQ group (P < 0.01). However, the levels were not significantly different between the OVA group and the OVA + PQ group. No significant differences in the levels of GM-CSF, IL-2, and IFN- γ were found among the experimental groups.

Phenanthraquinone have adjuvant activity for allergen-specific production of immunoglobulins G1 and E

To examine whether PQ has adjuvant activity for allergen-specific IgS production, we measured allergen-specific IgG1 and IgE 24 h after the last intratracheal instillation. The titres of allergen-specific IgG1 was significantly greater in the OVA + PQ group than in the other groups (P < 0.01 vs. each group) (Fig. 3a). The combination of OVA plus PQ significantly increased allergen-specific production of IgE as compared with OVA alone (P < 0.05) (Fig. 3b).

Discussion

The present study has demonstrated that PQ (2.1 ng/animal) administered by intratracheal route, at least partially, enhances allergic airway inflammation in mice. Also, PQ enhances allergen-specific production of IgG1 and IgE.

We have previously shown that intratracheal administration of DEP enhances airway inflammation related to allergen. The enhancement in the airway inflammation is

Table 2. Protein levels of cytokines and chemokines in the lung (pg/total lung supernatants)

Group	<i>n</i>	IFN- γ	IL-2	IL-4	IL-5	Eotaxin	GM-CSF	RANTES
Vehicle	7	1128.5 $\pm$ 124.1	226.6 $\pm$ 14.3	N.D	3.5 $\pm$ 1.4	34.9 $\pm$ 6.6	15.1 $\pm$ 0.9	264.7 $\pm$ 16.0
OVA	8	1320.3 $\pm$ 136.5	252.5 $\pm$ 13.6	N.D	75.0 $\pm$ 23.6**	460.0 $\pm$ 141.5*	13.9 $\pm$ 0.5	334.9 $\pm$ 68.0
PQ	7	1412.4 $\pm$ 300.7	223.4 $\pm$ 9.0	N.D	4.7 $\pm$ 1.7	58.4 $\pm$ 7.2	14.7 $\pm$ 0.8	243.1 $\pm$ 35.3
OVA + PQ	8	1093.9 $\pm$ 85.2	229.1 $\pm$ 17.2	N.D	43.9 $\pm$ 22.2	336.1 $\pm$ 189.2	13.1 $\pm$ 0.4	285.4 $\pm$ 44.5

Four groups of mice were intratracheally inoculated with vehicle, OVA, PQ, or the combination of OVA + PQ for 6 weeks. Lungs were removed and frozen 24 h after the last intratracheal administration. Protein levels in the lung tissue supernatants were analysed using ELISA. Results are shown as mean $\pm$ SEM.

* P < 0.05 vs. vehicle. ** P < 0.05 vs. vehicle. OVA, ovalbumin; PQ, 9,10-phenanthraquinone; GM-CSF, granulocyte/macrophage colony-stimulating factor.

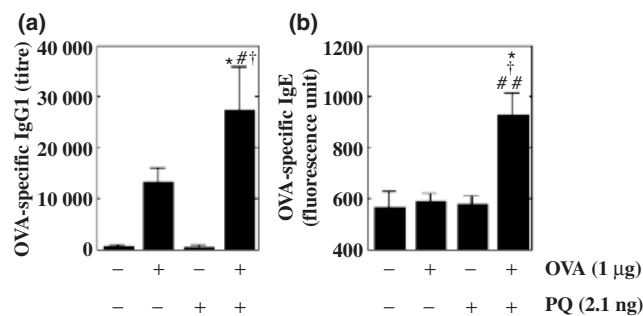


Fig. 3. Ovalbumin (OVA)-specific immunoglobulin titres. Four groups of mice were intratracheally administered vehicle, OVA, phenanthraquinone (PQ), or the combination of OVA and PQ for 6 weeks. Plasma samples were retrieved 24 h after the last intratracheal instillation. OVA-specific immunoglobulins were analysed using ELISA. Results are expressed as mean $\pm$ SEM. (a) OVA-specific IgG1; (b), OVA-specific IgE. * $P < 0.001$ vs. vehicle. # $P < 0.05$ vs. OVA. ## $P < 0.001$ vs. OVA. † $P < 0.001$ vs. PQ.

associated with an increased production of allergen-specific antibodies [7, 8]. DEP consist of carbonaceous nuclei and a vast number of organic chemical compounds such as polyaromatic hydrocarbons, aliphatic hydrocarbons, heterocycles, and quinones. Previous *in vitro* studies have indicated that exposure of macrophages to organic chemicals extracted from DEP results in induction of apoptosis [11] and increases the gene expression of the oxidative stress-inducible protein heme oxygenase-1 [12]. Organic chemicals in DEP can also affect inflammatory effector cells including neutrophils, eosinophils, and macrophages [13–15], and trigger the release of pro-inflammatory molecules such as IL-1, IL-8, TNF- α , and RANTES *in vitro* [15, 16]. We have recently shown that intratracheal instillation of organic chemicals in DEP enhances the neutrophilic lung inflammation related to endotoxin, especially in BAL fluid [27]. However, there is no study identifying the component(s) of DEP responsible for its enhancing effects on the airway inflammation induced by allergen challenge *in vivo*.

Quinones have been identified as DEP components [17, 18]. Quinones have toxicological properties to serve as alkylating agents and to interact with, for example, flavoproteins to generate reactive oxygen species (ROS), which can induce biological injury [19–22]. Also, we have recently shown that single intratracheal administration of PQ can induce recruitment of inflammatory cells such as eosinophils and neutrophils with the local expression of pro-inflammatory molecules such as IL-5 and eotaxin *in vivo* [24]. Our previous results suggest that PQ may also enhance airway inflammation related to allergen challenge. We have previously examined the effects of DEP on allergic airway inflammation using 100 μ g of DEP *in vivo* [7–9]. Based on the previous studies from our laboratory, we choose the dosage of PQ (2.1 ng/animal), which is involved in 100 μ g of DEP. Furthermore, we examined the effects of PQ at higher doses (21 or 210 ng/animal) on the current model. As a result, the three doses of PQ had similar enhancing effects on the model. However, the effects were not significantly different among the doses (data not shown). In the present study, the numbers of BAL eosinophils and mononuclear cells were significantly greater in the OVA + PQ group than in the OVA group. The results indicate that PQ can exacerbate airway inflammation related

to allergen challenge. On the other hand, the estimation for the magnitude of inflammation around the airways in the lung specimens revealed no significant differences between the OVA and OVA + PQ groups. The discrepant results between the BAL and histologic findings may be explained by the strength of the efficiency of PQ. Intratracheal exposure to PQ might affect within alveolar spaces, but might be too weak to affect beyond alveolar spaces to interstitial spaces. In contrast, our previous study has shown that DEP exaggerated allergic airway inflammation in both BAL and histopathological findings [8]. Therefore, our results suggest that PQ may not be the single or dominant contributor to the toxicological properties of DEP at least in allergic airway inflammation. On the other hand, we have previously demonstrated that PQ affects endothelial cells through the inhibition of nitric oxide activity *in vitro* [28]. It is also likely that PQ exaggerated epithelial–endothelial integrity, resulting dominantly in the aggravated BAL cellularity rather than in histopathology. Further detailed examinations about the other component(s) of DEP including organic chemical components and particles are needed in the future.

Allergic asthma is often associated with activation of IL-4, IL-5, and GM-CSF gene cluster, a pattern compatible with predominant activation of Th2-like T lymphocyte population. Among chemokines, eotaxin is essential for eosinophil recruitment in allergen-related airway inflammation [29, 30]. RANTES is a strong chemotactic and activating factor for eosinophils, and can modulate eosinophil adhesion [31, 32]. On the other hand, IL-2 has been reported to increase allergen-specific IgG and airway inflammation [33]. In fact, our previous studies have confirmed that the exaggerated allergic airway inflammation induced by DEP paralleled the local elevation of the inflammatory proteins [7, 8]. Therefore, we measured local expression of the cytokines and chemokines also in the present study. However, PQ did not significantly enhance the expression of these proteins in the presence of allergen. The results indicate that the enhancing effects of PQ on allergic airway inflammation may not be mediated via the enhanced local expression of the cytokines and chemokines. Also, 2.1 ng of PQ should be partly responsible for the enhancing effects of DEP on the pathogenesis of allergic airway inflammation.

Allergen-specific antibodies are thought to contribute to inflammatory cell accumulation after allergen challenge via IgE-mediated degranulation of mast cells [34]. On the other hand, IgG with allergen is a strong agonist for eosinophil degradation *in vitro* [35]. Furthermore, late asthmatic reactions are associated with IgG antibody [36]. The previous studies have reported that DEP with allergen demonstrate adjuvant activity for IgE production *in vivo* [6] and that those without antigen induce non-specific IgE response in humans [37, 38]. In addition, we have reported that DEP enhance allergen-specific production of IgE and IgG induced by intratracheal challenge with allergen *in vivo* [7, 8]. In the present study, the combined intratracheal administration of PQ and allergen induced a significant greater increase in both allergen-specific IgG1 and IgE than the other administration. It is likely that PQ plays a role in the enhancing effects of DEP on Igs production related to allergen exposure. In addition, deteriorated epithelial–endothelial integrity induced by intratracheal exposure to PQ might allow allergen to

penetrate easily into circulation, resulting in the enhancement of IgS production. Also, the enhancement in the allergen-specific IgS production by PQ can induce the enhanced release of a variety of inflammatory mediators such as histamine, leukotrienes, prostaglandin D₂, platelet activating factor, and thromboxane, or can activate complement system, resulting in aggravated manifestations of allergic asthma.

In conclusion, the present study has shown that PQ can enhance allergic airway inflammation. Furthermore, PQ enhances allergen-specific production of IgE and IgG1. These results suggest that environmental quinones are implicated in the increasing prevalence of allergic asthma in recent years and may play, at least in part, a role in the adverse effects of DEP on allergic airway diseases such as asthma.

References

- McClellan RO. Health effects of exposure to diesel exhaust particles. *Annu Rev Pharmacol Toxicol* 1987; 27:279–300.
- Ichinose T, Furuyama A, Sagai M. Biological effects of diesel exhaust particles (DEP). II. Acute toxicity of DEP introduced into lung by intratracheal instillation. *Toxicology* 1995; 99: 153–67.
- Nikasinovic L, Momas I, Just J. A review of experimental studies on diesel exhaust particles and nasal epithelium alterations. *J Toxicol Environ Health B Crit Rev* 2004; 7:81–104.
- Takizawa H. Diesel exhaust particles and their effect on induced cytokine expression in human bronchial epithelial cells. *Curr Opin Allergy Clin Immunol* 2004; 4:355–9.
- Muranaka M, Suzuki S, Koizumi K et al. Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. *J Allergy Clin Immunol* 1986; 77:616–23.
- Takafuji S, Suzuki S, Koizumi K et al. Diesel-exhaust particulates inoculated by the intranasal route have an adjuvant activity for IgE production in mice. *J Allergy Clin Immunol* 1987; 79:639–45.
- Miyabara Y, Yanagisawa R, Shimajo N et al. Murine strain differences in airway inflammation caused by diesel exhaust particles. *Eur Respir J* 1998; 11:291–8.
- Takano H, Yoshikawa T, Ichinose T, Miyabara Y, Imaoka K, Sagai M. Diesel exhaust particles enhance antigen-induced airway inflammation and local cytokine expression in mice. *Am J Respir Crit Care Med* 1997; 156:36–42.
- Takano H, Ichinose T, Miyabara Y, Yoshikawa T, Sagai M. Diesel exhaust particles enhance airway responsiveness following allergen exposure in mice. *Immunopharmacol Immunotoxicol* 1998; 20:329–36.
- Ichinose T, Takano H, Miyabara Y, Sagai M. Long-term exposure to diesel exhaust enhances antigen-induced eosinophilic inflammation and epithelial damage in the murine airway. *Toxicol Sci* 1998; 44:70–9.
- Hiura TS, Kaszubowski MP, Li N, Nel AE. Chemicals in diesel exhaust particles generate reactive oxygen radicals and induce apoptosis in macrophages. *J Immunol* 1999; 163:5582–91.
- Li N, Venkatesan MI, Miguel A et al. Induction of heme oxygenase-1 expression in macrophages by diesel exhaust particle chemicals and quinones via the antioxidant-responsive element. *J Immunol* 2000; 165:3393–401.
- Kanemitsu H, Nagasawa S, Sagai M, Mori Y. Complement activation by diesel exhaust particles (DEP). *Biol Pharm Bull* 1998; 21:129–32.
- Terada N, Maesako K, Hiruma K et al. Diesel exhaust particulates enhance eosinophil adhesion to nasal epithelial cells and cause degranulation. *Int Arch Allergy Immunol* 1997; 114:167–74.
- Yang HM, Ma JY, Castranova V, Ma JK. Effects of diesel exhaust particles on the release of interleukin-1 and tumor necrosis factor- α from rat alveolar macrophages. *Exp Lung Res* 1997; 23: 269–84.
- Fahy O, Tsicopoulos A, Hammad H, Pestel J, Tonnel AB, Wallaert B. Effects of diesel organic extracts on chemokine production by peripheral blood mononuclear cells. *J Allergy Clin Immunol* 1999; 103:1115–24.
- Schuetzle D, Lee FS, Prater TJ. The identification of polynuclear aromatic hydrocarbon (PAH) derivatives in mutagenic fractions of diesel particulate extracts. *Int J Environ Anal Chem* 1981; 9:93–144.
- Schuetzle D. Sampling of vehicle emissions for chemical analysis and biological testing. *Environ Health Perspect* 1983; 47: 65–80.
- Monks TJ, Hanzlik RP, Cohen GM, Ross D, Graham DG. Quinone chemistry and toxicity. *Toxicol Appl Pharmacol* 1992; 112:2–16.
- O'Brien PJ. Molecular mechanisms of quinone cytotoxicity. *Chem Biol Interact* 1991; 80:1–41.
- Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ. Role of quinones in toxicology. *Chem Res Toxicol* 2000; 13:135–60.
- Cho AK, Di Stefano E, Ying Y et al. Determination of four quinines in diesel exhaust particles, SRM 1649a, and atmospheric PM_{2.5}. *Aerosol Sci Technol* 2004; 38:68–81.
- Kumagai Y, Koide S, Taguchi K et al. Oxidation of proximal protein sulfhydryls by phenanthraquinone, a component of diesel exhaust particles. *Chem Res Toxicol* 2002; 15:483–9.
- Hiyoshi K, Takano H, Inoue K et al. Effects of a single intratracheal administration of phenanthraquinone on murine lung. *J Appl Toxicol* 2005; 25:47–51.
- Sugimoto R, Kumagai Y, Nakai Y, Ishii T. 9,10-phenanthraquinone in diesel exhaust particles downregulates Cu, Zn-SOD and HO-1 in human pulmonary epithelial cells: intracellular iron scavenger 1, 10-phenanthroline affords protection against apoptosis. *Free Radical Biol Med* 2005; 38:388–95.
- Takano H, Yanagisawa R, Ichinose T et al. Diesel exhaust particles enhance lung injury related to bacterial endotoxin through expression of proinflammatory cytokines, chemokines, and intercellular adhesion molecule-1. *Am J Respir Crit Care Med* 2002; 165:1329–35.
- Yanagisawa R, Takano H, Inoue K et al. Enhancement of acute lung injury related to bacterial endotoxin by components of diesel exhaust particles. *Thorax* 2003; 58:605–12.
- Kumagai Y, Hayashi T, Miyauchi T et al. Phenanthraquinone inhibits eNOS activity and suppresses vasorelaxation. *Am J Physiol Regul Integr Comp Physiol* 2001; 281:R25–30.
- Quackenbush EJ, Wershil BK, Aguirre V, Gutierrez-Ramos JC. Eotaxin modulates myelopoiesis and mast cell development from embryonic hematopoietic progenitors. *Blood* 1998; 92: 1887–97.
- Humbles AA, Conroy DM, Marleau S et al. Kinetics of eotaxin generation and its relationship to eosinophil accumulation in allergic airways disease: analysis in a guinea pig model in vivo. *J Exp Med* 1997; 186:601–12.
- Alam R, Stafford S, Forsythe P et al. RANTES is a chemotactic and activating factor for human eosinophils. *J Immunol* 1993; 150:3442–8.
- Kakazu T, Chihara J, Saito A, Nakajima S. Effect of RANTES on eosinophil adhesion to plates coated with recombinant soluble intercellular adhesion molecule-1 and expression of beta 2-integrin adhesion molecules on eosinophils. *Int Arch Allergy Immunol* 1995; 108:9–11.
- Renzi PM, Sapienza S, Waserman S et al. Effect of interleukin-2 on the airway response to antigen in the rat. *Am Rev Respir Dis* 1992; 146:163–9.

- 34 Zweiman B. The late-phase reaction: role of IgE, its receptor and cytokines. *Curr Opin Immunol* 1993; 5:950–5.
- 35 Kaneko M, Swanson MC, Gleich GJ, Kita H. Allergen-specific IgG1 and IgG3 through Fc gamma RII induce eosinophil degranulation. *J Clin Invest* 1995; 95:2813–21.
- 36 Durham SR, Lee TH, Cromwell O et al. Immunologic studies in allergen-induced late-phase asthmatic reactions. *J Allergy Clin Immunol* 1984; 74:49–60.
- 37 Diaz-Sanchez D, Dotson AR, Takenaka H, Saxon A. Diesel exhaust particles induce local IgE production in vivo and alter the pattern of IgE messenger RNA isoforms. *J Clin Invest* 1994; 94:1417–25.
- 38 Diaz-Sanchez D, Tsien A, Casillas A, Dotson AR, Saxon A. Enhanced nasal cytokine production in human beings after in vivo challenge with diesel exhaust particles. *J Allergy Clin Immunol* 1996; 98:114–23.