

The role of diesel exhaust particles and their associated polyaromatic hydrocarbons in the induction of allergic airway disease

Diaz-Sanchez D. The role of diesel exhaust particles and their associated polyaromatic hydrocarbons in the induction of allergic airway disease. Allergy 1997; 52 (suppl 38): 52-56. © Munksgaard 1997.

The increase in allergic airway disease has paralleled the increase in the use of fossil fuels. Studies were undertaken to examine whether extracts of polyaromatic hydrocarbons (PAH) from diesel exhaust particles (DEP) (PAH-DEP) acted as mucosal adjuvants to help initiate or enhance immunoglobulin E (IgE) production in response to common inhaled allergens. *In vitro* studies demonstrated that PAH-DEP enhanced IgE production by tonsillar B-cells in the presence of interleukin-4 (IL-4) and CD40 monoclonal antibody, and altered the nature of the IgE produced, i.e. a decrease in the CH4'-CHe5 variant, a marker for differentiation of IgE-producing B-cells, and an increase in the M2' variant. *In vivo* nasal provocation studies using 0.30 mg DEP in saline also showed enhanced IgE production in the human upper respiratory mucosa, accompanied by a reduced CH4'-CHe5 mRNA splice variant. The effects of DEP were also isotype-specific, with no effect on IgG, IgA, IgM, or albumin, but it produced a small increase in the IgG₄ subclass. The ability of DEP to act as an adjuvant to the ragweed allergen *Amb a 1* was examined by nasal provocation in ragweed allergic subjects using 0.3 mg DEP, *Amb a 1*, or both. Although allergen and DEP each enhanced ragweed-specific IgE, DEP plus allergen promoted a 16-times greater antigen-specific IgE production. Nasal challenge with DEP also influenced cytokine production. Ragweed challenge resulted in a weak response, DEP challenge caused a strong but non-specific response, while allergen plus DEP caused a significant increase in the expression of mRNA for TH₀ and TH₂-type cytokines (IL-4, IL-5, IL-6, IL-10, IL-13) with a pronounced inhibitory effect on IFN- γ gene expression. These studies suggest that DEP can enhance B-cell differentiation, and by initiating and elevating IgE production, may play an important role in the increased incidence of allergic airway disease.

D. Diaz-Sanchez

Division of Clinical Immunology and Allergy,
University of California Los Angeles School of
Medicine, Los Angeles, California, USA

Key words: cytokines; diesel exhaust particles;
IgE; polyaromatic hydrocarbons.

Dr David Diaz-Sanchez
Hart and Louise Lyon Laboratory
Division of Clinical Immunology and Allergy
University of California Los Angeles School of
Medicine
10833 Le Conte Avenue, 52-175 CHS, Los
Angeles
CA 90024-1680, USA

Introduction

In a paper entitled 'Hay fever - its causes, diagnosis and treatment', published in 1907 by William Lloyd, it was postulated that the disease was perhaps a result of urbanization and agricultural change. Since that time, many epidemiological and correlation studies have provided indirect evidence for an increased incidence of asthma and atopy linked to airborne pollution (1). It has also been noted that the dramatic increase in expression of human allergic airway disease in the past 200 years has paralleled the increase in the use of fossil fuels. The burning of fossil fuels generates airborne

particulates and polyaromatic hydrocarbons (PAH). The enormity and extent of particulates generated can be appreciated by the fact that global 'particulate haze' may now be reflecting enough sunlight to induce global cooling and counter the potential effects of global warming (2). Because of their ubiquitous nature, these same particulates are universally found in indoor air, often in concentrations equal to or exceeding those found in ambient outdoor air (3). Even non-occupational settings can result in acute exposures to particulates and PAH, as high as 100-times the recommended peak concentrations. Diesel exhaust particles (DEP) are of special research interest because their occurrence

has been reasonably well studied, both over time and quantitatively under a variety of circumstances, and thus can serve as a model for airborne particulates containing PAH. In addition, DEP constitute, on average, 40% of the 10 μ particulate respirable particles (PM10) in a city such as Los Angeles. Since 95% of DEP are smaller than 1 μ m, they persist in the atmosphere for a long period of time and can be inhaled easily (4).

Previous studies have shown correlations between total air pollution, pulmonary function, and allergic airway disease, and have suggested that pollutants act by non-specific mucosal inflammatory effects. Since increased immunoglobulin E (IgE) production is the hallmark of allergic disease, our studies have focused on whether DEP and their PAH act as mucosal adjuvants to help initiate or enhance IgE production in response to common inhaled allergens in humans.

***In vitro* studies: enhanced IgE production by PAH-DEP**

The potential for PAH to alter immune function has been well established, but most of the studies performed have investigated 'suppressive' effects of high levels of PAH. Our initial experiments tested the ability of extracts of PAH from DEP (PAH-DEP) to enhance human IgE production from either purified blood or tonsillar B-cells (5). In the absence of co-stimulants, PAH-DEP could not induce *de novo* production of IgE from B-cells. However, addition of PAH-DEP to purified B-cell cultures simultaneously stimulated with interleukin-4 (IL-4) and CD40 monoclonal antibody, resulted in enhanced IgE production (20–360%) (Fig. 1). In contrast, PAH-DEP could not increase production of either IgG or IgM.

Delaying the addition of PAH-DEP until 2–5 days after the initiation of cultures further increased its ability to enhance IgE production. This suggested that PAH-DEP was enhancing ongoing IgE production. This was supported by the fact that no epsilon germ line transcripts could be detected from fresh peripheral blood mononuclear cells stimulated with PAH-DEP in the absence of IL-4. In addition, PAH-DEP could not synergize with IL-4 alone to induce IgE switching from purified B-cells that were undergoing germ line transcription. PAH-DEP also enhanced IgE production by an Epstein-Barr virus transformed human B-cell line (2C4/F3) that produces productive epsilon mRNA and IgE protein spontaneously (6). In this cell line, while IL-4 could induce the non-expressed Ig allele to produce germ line epsilon transcripts, this could not be achieved by stimulation with PAH-DEP.

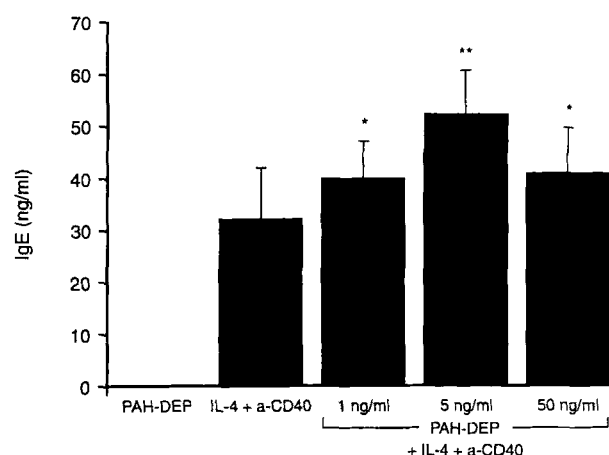


Fig. 1. Enhancing effect of PAH-DEP on IL-4 plus CD40 monoclonal antibody-driven IgE production. PBL (2×10^5 cells/well) were cultured for 14 days in the presence of IL-4 (200 U/ml) plus CD40 monoclonal antibody (0.1 μ g/ml) plus different combinations of PAH-DEP. The mean of five experiments is shown. * $P < 0.05$ and ** $P < 0.01$ compared with IL-4 plus a-CD40 only (Mann-Whitney).

In addition to the quantitative effects on IgE, PAH-DEP also qualitatively altered the nature of the IgE produced. Alternative 3' splicing of the epsilon heavy-chain gene results in different splice variants for epsilon mRNA, and these variants code for IgE proteins of different lengths. Addition of PAH-DEP to IL-4 plus CD40-stimulated B-cells induced a selective 200-fold increase in the production of one particular variant (M2') and a marked selection against another variant (CH4'-CHe5). A similar shift was also observed in the 2C4/F3 cell line after stimulation with PAH-DEP. It is interesting to note that this relative decrease in the CH4'-CHe5 mRNA splice variant also seems to be a marker for differentiation of IgE-producing B-cells (7).

***In vivo* studies: DEP induces local IgE production**

Seminal studies by Miyamoto and colleagues at the University of Tokyo demonstrated a direct effect of DEP on IgE antibody production in mice *in vivo* (8, 9). They showed that intraperitoneal injection of DEP increased the IgE response to bystander antigens such as ovalbumin and Japanese cedar pollen. Even more compelling, intranasal inoculation of mice with DEP plus antigen markedly enhanced the IgE response while not increasing the haemagglutination titre (IgG response) to the same degree. This result contrasted with the adjuvant effect of alum or *Bordetella pertussis*, which increased the IgG but not the IgE response. The investigators suggested that the particulate nature of DEP may provide for antigen

adsorption and retention in the host. Subsequent studies in mice demonstrated that pyrene (a PAH found in diesel exhaust) could also act as an adjuvant, suggesting that mechanisms other than simple antigen adsorption to the diesel particles may be involved (10).

We extended these findings from animal models by demonstrating that DEP can enhance *in vivo* IgE production in the human upper respiratory mucosa. An attempt was made to establish a human model to simulate acute exposure to DEP (11). Nasal challenge was performed by spraying either saline or 0.30 mg of DEP in saline into the nostrils of subjects. Although 0.30 mg of DEP is equivalent to total exposure on 1–3 average days in Los Angeles (12), this level of exposure can occur in certain non-occupational settings, such as sitting at a busy bus stop or in an express highway tunnel. Immunoglobulin levels were measured in the supernatants of nasal lavages performed prior to and at several timepoints after nasal challenge. Albumin levels in the lavage fluids were also determined, which served as a control for protein derived from vascular leakage.

Although no change in IgE was seen after saline challenge, DEP induced a significant increase in IgE production (Fig. 2). Enhanced nasal IgE levels could be observed after as little as 24 h in some individuals, though generally, peak production was observed 4 days after DEP challenge. By day 7, IgE levels had returned to baseline values. The increase in protein levels following DEP challenge was reflected by a 25-fold increase in the levels of epsilon mRNA from cells recovered in the nasal lavage fluid at day 4. In addition, this was accompanied by a qualitative change in the nature of the IgE produced. By day 4, there was a marked relative decrease in the CH4'-CHe5 mRNA splice

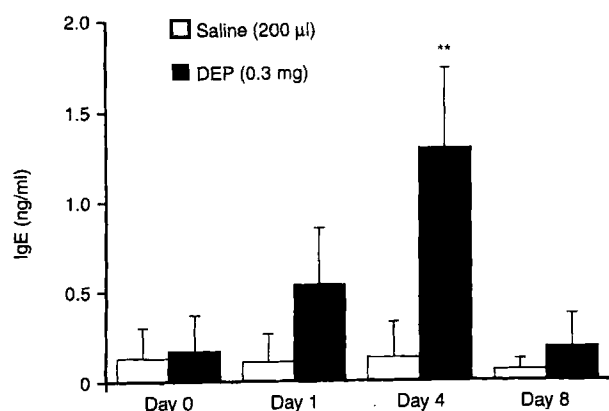


Fig. 2. DEP enhances *in vivo* nasal IgE responses. Mean levels of IgE in 12 subjects in nasal washes performed immediately before or 1, 4 or 8 days following nasal challenge with 0.3 mg DEP or 200 µl saline. ** $P < 0.01$ compared with saline challenge (paired *t*-test).

variant, a feature characteristic of IgE-producing B-cells that have undergone differentiation.

The effects of DEP seemed to be somewhat isotype-specific, since in contrast to the IgE results, DEP challenge had no effect on the levels of either IgG, IgA, IgM or albumin. In addition, DEP induced a concomitant, though somewhat small, increase in IgG₄, but did not alter IgG₃ protein levels. Expression of the IgG₄ subclass has often been linked with IgE production. The possibility that DEP was selectively enhancing local production of IgE was demonstrated by enumeration of Ig-secreting cells recovered from nasal washes. Whereas the number of IgA-secreting cells in lavage fluid (as determined by enzyme-linked immunosorbent assay [ELISA-SPOT]) remained unchanged after DEP challenge, the number of IgE-secreting cells rose dramatically from less than 1 in 2,000,000 to more than 1 in 100,000.

The ability of DEP to act as an adjuvant to bystander antigen was further dissected by performing nasal provocation challenges with DEP or the ragweed allergen *Amb a 1*, or both simultaneously, in a group of ragweed-sensitive subjects (13). Subjects were identified as being short ragweed allergic by history and skin prick test. The antigen employed in the challenge studies (short ragweed) is not present in the Los Angeles area and the cross-reacting western ragweed is a minor allergen. All challenges were performed outside the ragweed season. Subjects were challenged with increasing doses of *Amb a 1* until symptoms first appeared, up to a maximum of 1,000 AU of antigen. Nasal washes were undertaken 18 h after challenge (day 1), and on days 4 and 8. After a minimum of 60 days, subjects underwent a combined challenge with the same dose of *Amb a 1* previously given, plus 0.3 mg of DEP in 200 µl saline.

When subjects were challenged with ragweed alone there was an increase in both IgE and IgG₄ in nasal lavage fluid, which was slightly greater than that seen with DEP. In addition, ragweed-specific IgE was detectable in the lavage fluid after challenge. Similar levels of total IgE and IgE-secreting cell numbers were detected following challenge with either ragweed alone or DEP plus ragweed. In contrast, ragweed-specific IgE was 16-times higher following ragweed plus DEP challenge compared with ragweed alone (Fig. 3). As stated previously, challenge with either ragweed or DEP alone preferentially selected against the CH4'-CHe5 mRNA splice variant, but again, when subjects were challenged with both ragweed and DEP, they synergized to accentuate this effect.

While DEP alone can enhance total IgE, DEP in the presence of allergen specifically enhanced

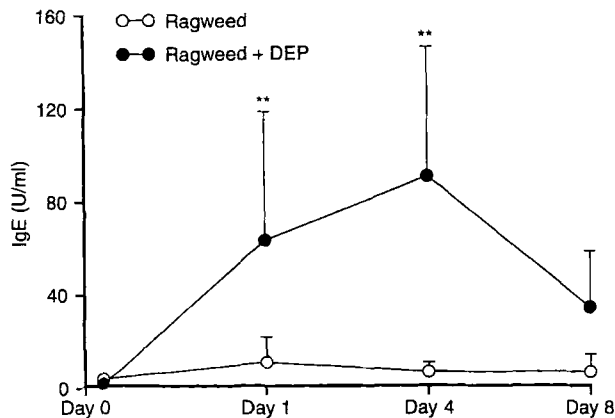


Fig. 3. DEP enhances ragweed-induced IgE responses. Ragweed-sensitive subjects were challenged nasally with ragweed allergen (up to 1,000 U/ml) or with both ragweed and 0.3 mg DEP. The mean level of 13 subjects is shown. ** $P < 0.01$ compared with challenge with ragweed alone (paired t -test).

antigen-specific IgE. In contrast, both total and antigen-specific IgG₄ levels were boosted by the presence of DEP, suggesting that regulation of mucosal ragweed IgE and IgG₄ production are under similar but not identical control mechanisms. IgG levels remained unaltered by challenge with DEP plus antigen. As a control, subjects were challenged with ragweed as above, and 60 days later were challenged with ragweed alone. In this group, ragweed-specific IgE production was not enhanced after the second challenge, indicating that there was no carryover or 'priming' effect.

DEP influences the cytokine environment

The direct effects of DEP on B-cells are evident from the *in vitro* studies. However, it is likely that DEP also affects other cells that regulate the IgE response. Therefore, we examined whether DEP could alter the production of cytokines by cells residing in the nasal mucosa. Cytokine mRNA was measured by a reverse transcriptase PCR technique from cells obtained both before and 18 h after intranasal challenge of ragweed-allergic subjects with either saline, DEP, ragweed antigen, or a combination of ragweed and DEP (14).

Prior to challenge, mRNA was readily detectable in most subjects for interferon- γ (IFN- γ), IL-2 and IL-13. However, mRNA for IL-4, IL-5, IL-6, IL-10, and lymphotoxin could not be detected in the majority of subjects. Following challenge with ragweed, no consistent pattern of cytokine alteration was observed between subjects and no significant elevation of any cytokine mRNA. However, after DEP challenge there was a significant increase in both the number of subjects from whom various cytokine mRNAs could be detected

and in the levels of mRNA for all of the cytokines studied (Fig. 4). This enhancement was far greater than that observed following challenge with ragweed alone in allergic subjects. Although levels of cytokine proteins are normally too low to be detected in nasal fluid, increased IL-4 protein was detectable in the post-DEP challenge lavage fluid. It is likely that the enhanced cytokine production by nasal mucosal cells following DEP challenge was due to interaction with cells in the mucosal tissue, since peripheral blood mononuclear cells from the same subjects, cultured alone or in the presence of the organic extract of DEP, showed no significant increase in the production of mRNA for any of the cytokines measured.

In contrast to the weak response seen after ragweed challenge, and the strong but non-specific

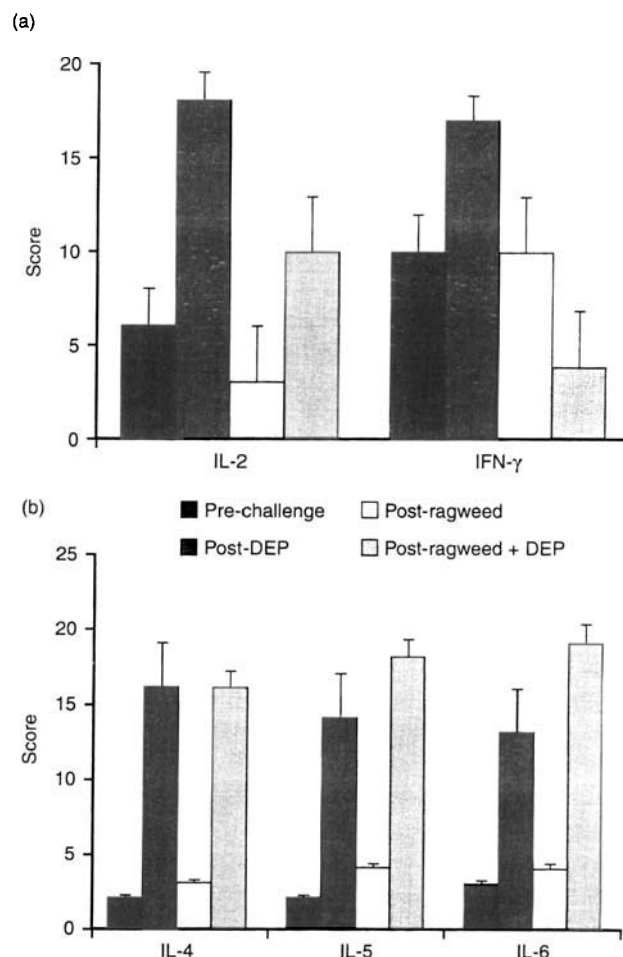


Fig. 4. DEP enhances cytokine mRNA levels. The levels of cytokine mRNA were measured by reverse transcriptase PCR from mRNA recovered from nasal washes performed either on day 0 (pre-challenge), or 18 h after challenge with either 0.3 mg DEP, up to 1,000 U/ml ragweed, or both ragweed and DEP. The levels were scored according to the number of subjects from whom message could be detected and the levels of mRNA detected. The mean scores are shown for six subjects for IL-1 and IFN- γ (a) and for IL-4, IL-5, and IL-6 (b).

response in cytokine mRNA production seen after DEP challenge, nasal provocation challenge with combined allergen plus DEP resulted in a strong increase in specific cytokines. In almost all subjects, expression of mRNA for TH₀ and TH₂-type cytokines (IL-4, IL-5, IL-6, IL-10, and IL-13) was significantly enhanced (Fig. 4). Although ragweed challenge alone resulted in virtually no change in IFN- γ gene expression, challenge with ragweed plus DEP had a pronounced inhibitory effect. IFN- γ expression could be detected in 85% of subjects after allergen challenge, but this fell to 38% if DEP was also present. Furthermore, even in those subjects who still had detectable levels of IFN- γ mRNA following DEP plus allergen challenge, the level of expression was notably decreased. Similar results were seen with lymphotoxin mRNA expression and to a lesser extent IL-2. Therefore, while DEP alone induces a non-TH₁, non-TH₂ specific pattern of cytokine production, DEP plus ragweed selects against TH₁-type cytokines.

Our results are in agreement with the finding of enhanced IL-4 production in mediastinal lymph node cells in mice after intra-tracheal instillation of DEP plus antigen, compared with that in mice given antigen alone (15). In addition, the same group recently showed IFN- γ production in cervical lymph node cells to be three times lower following instillation of DEP plus antigen compared with antigen alone (16).

The cellular source of the cytokines induced by DEP challenge is unknown. Recently, we found that the main source of IL-4 from nasal lavage cells after DEP and ragweed challenge was not T-cells. Cells obtained from washes before and 18 h after challenge were intracellularly stained with fluorescein labelled anti-IL-4 monoclonal antibody. In agreement with our previous findings, an increase in the number of IL-4+ cells was detected following DEP plus ragweed challenge; there was also an increase in the intensity of the staining. However, when the surface of the cells was co-stained with anti-CD3 monoclonal antibody, it was clear that the majority of these newly formed IL-4+ cells were also CD3+, suggesting that they were not T-cells.

Conclusion

Diesel exhaust particles are a major source of airborne pollution. They have the ability to induce IgE responses by acting directly on B-cells and indirectly by enhancing cytokine production. In conjunction with allergen, they can generate a TH₂-type cytokine environment and favour the generation of allergen-specific IgE. Our results suggest

that DEP can enhance B-cell differentiation, and by initiating and enhancing IgE production, may play an important role in the increased incidence of allergic airway disease.

References

1. Rusznak C, Devalia JL, Davies RJ. The impact of pollution on allergic disease. *Allergy* 1994;49:21-3.
2. Kerr RA. Study unveils climate cooling caused by pollutant haze. *Science* 1995;268:802.
3. Spangler JD, Samet JM. A perspective on indoor and outdoor air pollution. In: Samet JM, Spangler JD, editors. *Indoor air pollution: a health perspective*. Baltimore: Johns Hopkins University Press, 1991:1-32.
4. McClellan RO. Health effects of exposure to diesel exhaust particles. *Ann Rev Pharmacol Toxicol* 1987;27:279-300.
5. Takenaka H, Zang K, Diaz-Sanchez D, Tsien A, Saxon A. Enhanced human IgE production results from exposure to aromatic hydrocarbons from diesel exhaust: direct effects on B-cell IgE production. *J Allergy Clin Immunol* 1995; 95:103-15.
6. Tsien A, Diaz-Sanchez D, Ma J, Saxon A. The organic component of diesel exhaust particles and phenanthrene, a major polyaromatic hydrocarbon constituent, enhances IgE production by IgE-secreting EBV-transformed human B-cells in vitro. *Toxicol Appl Pharmacol* 1997;142:252-63.
7. Diaz-Sanchez D, Nutman TB, Saxon A. Differential regulation of alternative 3' splicing of epsilon mRNA variants. *J Immunol* 1995;155:1930-41.
8. 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.
9. 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(4):639-45.
10. Suzuki T, Kanoh T, Kanbayashi M, Todome Y, Ohkuni H. The adjuvant activity of pyrene in diesel exhaust on IgE antibody production in mice. *Jpn J Allergol* 1993;42:963-8.
11. 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.
12. Peterson B, Saxon A. Global increases in allergic respiratory disease: the possible role of diesel exhaust particles. *Ann Allergy* 1997;77:263-70.
13. Diaz-Sanchez D, Tsien A, Fleming J, Saxon A. Combined diesel exhaust particulate and ragweed allergen challenge markedly enhanced in vivo nasal ragweed-specific IgE and skews cytokine production to the TH₂-type pattern. *J Immunol* 1997;158:2406-13.
14. 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.
15. Fujimaki H, Nohara O, Ichinose T, Watanabe N, Saito S. IL-4 production in mediastinal lymph node cells in mice intratracheally instilled with diesel exhaust particles and antigen. *Toxicology* 1994;92:261-8.
16. Fujimaki H, Saneyoshi K, Nohara O, Shiraishi F, Imai T. Intranasal instillation of diesel exhaust particles and antigen in mice modulated cytokine productions in cervical lymph node cells. *Int Arch Allergy Immunol* 1995;108:268-73.