

In Vivo Nasal Challenge with Diesel Exhaust Particles Enhances Expression of the CC Chemokines Rantes, MIP-1 α , and MCP-3 in Humans

David Diaz-Sanchez,* Minna Jyrala,* David Ng,* Andre Nel,*† and Andrew Saxon*†‡

*The Hart and Louise Lyon Laboratory, Division of Clinical Immunology/Allergy, Department of Medicine,

†The Jonsson Comprehensive Cancer Center Institute, and ‡The Molecular Biology Institute,

UCLA School of Medicine, University of California, Los Angeles, California 90095-1680

Diesel exhaust particles (DEP) enhance allergic inflammation by increasing *in vivo* IgE and cytokine production in the human upper respiratory mucosa. CC chemokines have been shown to play an important role in inflammation. We examined whether DEP could alter the production of CC chemokines by cells residing in the human nasal mucosa. At both 6 and 24 h following intranasal DEP challenge, the levels of nasal RANTES, MIP-1 α , and MCP-3 were significantly elevated compared to baseline. In contrast, DEP did not enhance levels of Eotaxin at any time, demonstrating that the action of DEP was not simply a global effect on all CC chemokines. Challenge with saline resulted in no significant change in expression of any chemokine at any time. Challenge with DEP also resulted in an increase in total cell counts in nasal lavage fluids. Increases in lymphocyte, monocyte/macrophage, and neutrophil cells were observed but there was no change in eosinophil cell numbers. In contrast, there was a significant enhancement of ECP protein levels in washes performed 6 to 24 h after DEP challenge. Elevated specific nasal chemokine expression following exposure to DEP likely participates in the inflammation, cellular infiltration, and increase in IgE observed in the absence of allergen. © 2000 Academic Press

Key Words: diesel; pollution; chemokines; allergy; inflammation.

INTRODUCTION

One of the hallmarks of allergic airway disease is infiltration of the airways with inflammatory cells, such as eosinophils, basophils, mast cells, and mononuclear cells. A group of chemotactic cytokines have been identified, termed “chemokines,” and categorized into several families according to a cysteine signature motif (C, CC, CXC, and CX₃C) (1, 2). The CC chemokine family is so named since it has two adjacent N-terminal-conserved cysteine residues (3); members of this family include RANTES, MCP, MIP-1, and

Eotaxin (3–6). CC chemokines are thought to be especially important in allergic inflammation since they are involved in the chemotaxis of mononuclear cells, activation of eosinophils, mediator release from basophils and mast cells, and augmentation of T cell proliferation and cytokine production (1, 7). In addition, elevated expression of CC chemokines has been reported in allergic asthmatic patients (8) and in nasal or bronchial secretions of allergic subjects following challenge with allergen (9).

In this study, we demonstrate for the first time enhanced expression of CC chemokines *in vivo* in response to the model pollutant diesel exhaust particle aggregates (DEP). We have previously shown that DEP challenge, in the absence of allergen, will cause a local increase in total IgE responses in human subjects *in vivo* in parallel with increased cytokine production (10, 11). In addition, allergic subjects challenged with both DEP and allergen produce a heightened TH2-type cytokine response and elevated levels of allergen-specific IgE (12). We have also observed an increase in the number of inflammatory cell types which appear in the nasal mucosa in response to DEP exposure. In this study, therefore, we extended our previous findings to investigate the effect of DEP challenge on chemotactic cytokines. Here, we show that *in vivo* challenge of human subjects with DEP alone will induce or increase expression of RANTES, MIP-1 α , and MCP-3 in nasal secretions.

MATERIALS AND METHODS

Subjects

Ten healthy, nonsmoking volunteers (3 men and 7 women), ranging in age from 23 to 31 years old, were recruited in Los Angeles, California. Eight had no history of asthma, allergic rhinitis, or any allergic or chronic respiratory illness. Two had a history of seasonal allergies but were asymptomatic and did not take any medication 3 days prior to or at any time during

the duration of the study. All atopic subjects were outside their allergy season. None of the volunteers had any out of the ordinary exposure to air pollutants. All studies were approved by the Human Subject Protection Committee of the University of California at Los Angeles.

Nasal Challenge and Cell Counts

DEP were obtained from light duty diesel passenger cars and processed as previously described (10). Nasal washes and provocation challenges were performed as previously described (10, 12, 13). Briefly, 5 ml of normal saline was delivered into each nostril of the subjects and after 10 s, the wash fluid was collected. The initial wash was discarded. The subjects then performed up to eight subsequent nasal washes at 10-min intervals. The combined lavage fluids were centrifuged at 350g for 10 min at 4°C, and the aqueous supernatants separated from the cell pellets. The total number of cells at each time was counted with a modified Neubauer hemocytometer (American Optical Corp., Buffalo, NY). Differential staining was performed using a modified Giemsa stain. Ten minutes following the last wash, subjects were challenged with either 200 μ l saline or 0.3 mg DEP in 200 μ l saline. The dose of DEP used had previously been shown to produce optimal IgE production *in vivo*. Nasal washes were then repeated, as above, on subjects 2, 4, 6, and 24 h after challenge.

Measurement of Chemokines and Mediators

Protein levels were measured in nasal washes using commercially available ELISA kits for RANTES, Eotaxin (Intergen, Purchase, NY), MIP-1 α (Endogen, Woburn, MA), and MCP-3 (Pharmingen, San Diego, CA) and were performed following manufacturers' instructions. The amounts of ECP in the nasal washes were determined using a sensitive radioimmunoassay (Pharmacia Diagnostics, Inc., Fairfield, NJ).

Statistical Analysis

The Statview II computer package (Abacus Concepts, Berkeley, CA) for the Macintosh was used for all analysis. Comparisons within groups were done using paired *t* tests. Comparisons between groups were performed using unpaired *t* tests.

RESULTS

Elevated cell counts in nasal lavages were detected in subjects following *in vivo* challenge with DEP. Figure 1 shows that the total number of cells rose 2 h after

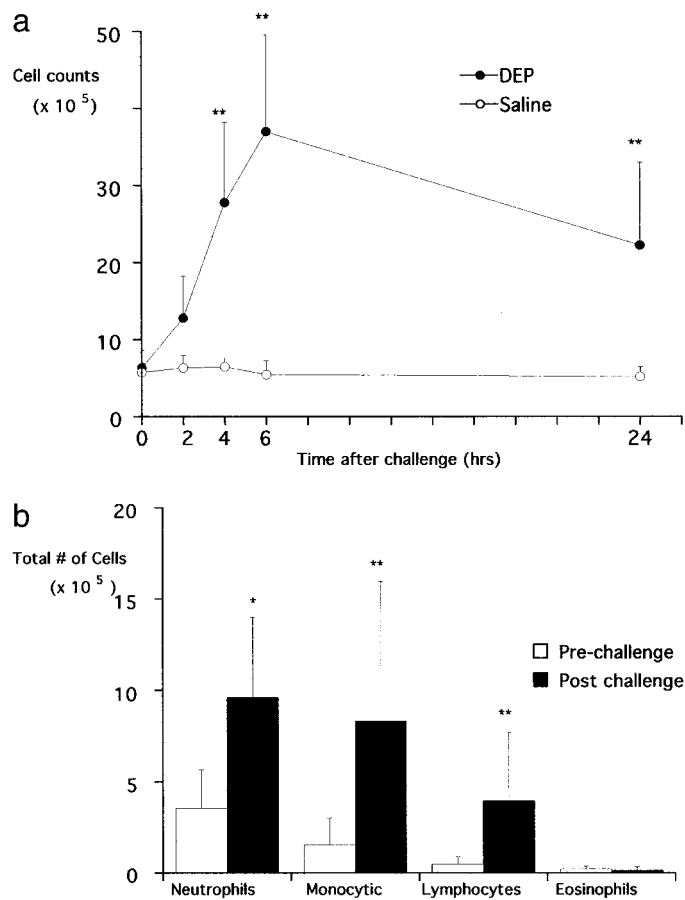


FIG. 1. Changes in cell counts in nasal wash fluids. (a) The number of nonepithelial cells and mononuclear cells following challenge with 0.3 mg DEP or 200 μ l saline. (b) The total numbers of neutrophils, monocyte/macrophages, lymphocytes, and eosinophils in nasal washes performed immediately before or 24 h after challenge with 0.3 mg DEP. The mean plus 1 SD of 10 subjects is shown. **P* < 0.05, ***P* < 0.05, compared to baseline (time 0).

the challenge and continued to increase up to a peak at 6 h. The numbers of lymphocytes, monocyte/macrophages, and neutrophils were all elevated 24 h after challenge (*P* < 0.01, *P* < 0.01, *P* < 0.05, respectively) but there was no change in eosinophil cell numbers. In contrast, challenge with saline did not result in any change in non-epithelial cell numbers at any time (data not shown).

Challenge of subjects with DEP resulted in an increase in RANTES protein in nasal washes (Fig. 2a). Prior to challenge RANTES protein could be detected in washes from 8 of the 10 subjects. There was no increase in RANTES production at any time after challenge with saline. In contrast, RANTES was detectable and mean levels were significantly elevated compared to prechallenge levels at 4, 6, and 24 h after challenge with DEP. Mean maximal expression was reached 6 h after challenge. There was also a significant increase in

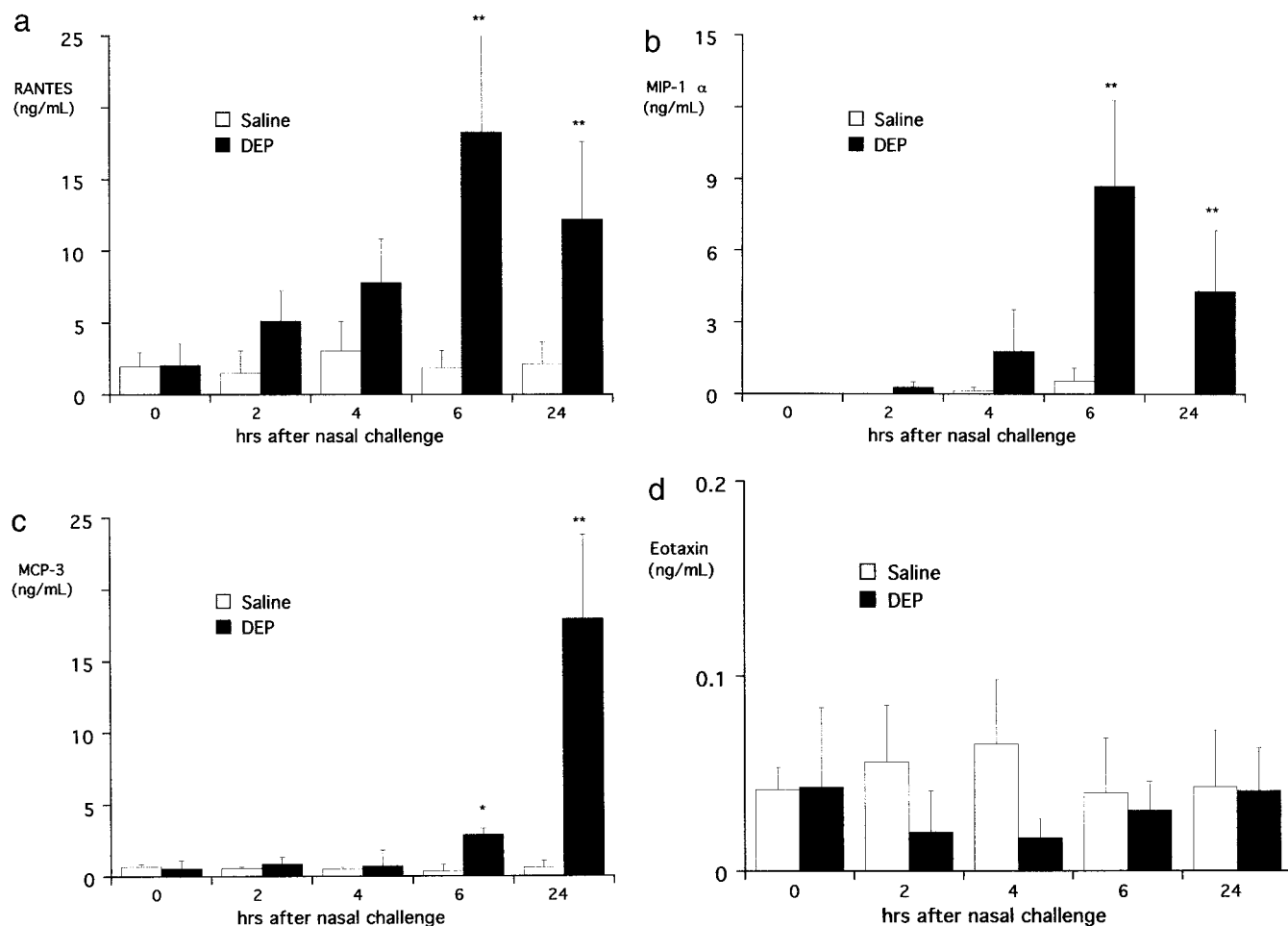


FIG. 2. DEP-induced changes in nasal chemokine production. Time course of RANTES (a), MIP-1 α (b), MCP-3 (c), and Eotaxin (d) secretion in nasal washes following challenge with 0.3 mg DEP ($n = 10$). The mean and 1 SD are shown. * $P < 0.05$ compared to baseline.

the levels of MIP-1 α following *in vivo* challenge with DEP. Both chemokines had a similar time course of induction; maximal MIP-1 α mRNA expression was observed 6 h postchallenge and remained elevated at 24 h after challenge ($P < 0.01$ for both times compared to prechallenge levels) (Fig. 2b). MIP-1 α could not be detected in cells obtained from lavages performed prior to challenge or after challenge with saline in any subject. MCP-3 production was also enhanced by DEP challenge. In contrast to MIP-1 α detectable levels of MCP-3 were measured in nearly all subjects (9/10) prior to any challenge. The time course of DEP-induced MCP-3 production clearly differed from that of RANTES and MIP-1 α (Fig. 2c). Thus, maximal MCP-3 levels were detected at 24 h after challenge, although significantly elevated MCP-3 levels were observed 6 h after *in vivo* challenge. Again, MCP-3 levels were not altered by challenge with saline. Although eotaxin could be detected in 3 of the 10 subjects prior to challenge, *in vivo* challenge with DEP did not enhance its production or

secretion at any time (Fig. 2d). Thus there was no difference at any time in eotaxin levels between washes performed after challenge with DEP and those done after saline challenge ($P > 0.05$).

An important factor in allergic inflammation is the activation of eosinophils (14). Therefore, the release of specific granule proteins by eosinophils was monitored by measuring ECP levels in the nasal washes at the different times (Fig. 3). Compared to baseline levels there was a significant increase in ECP levels at 6 and 24 h following challenge with DEP ($P < 0.01$ for both times). Moreover, challenge with saline did not result in any change in ECP levels at any time.

DISCUSSION

Recent studies have highlighted the importance of CC chemokines in both allergic inflammation and histamine release (1). In this study we show that some of the CC chemokines can be induced and up-regulated by

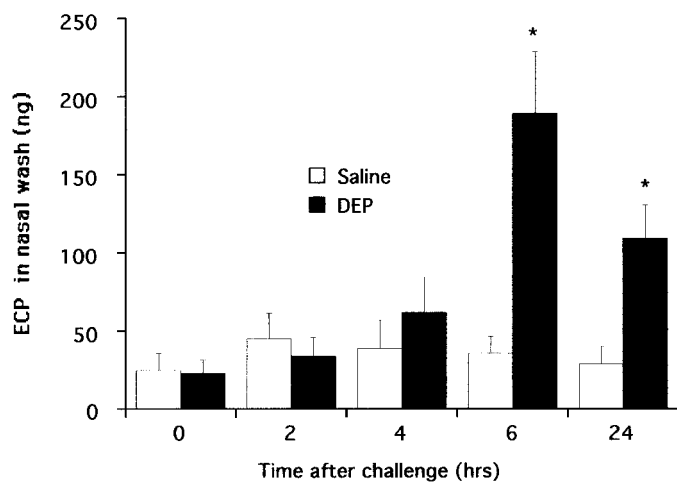


FIG. 3. DEP-induced changes in nasal ECP. The amount of ECP increased after challenge with 0.3 mg DEP but did not change after challenge with saline. The mean and 1 SD are shown. * $P < 0.01$ compared to baseline.

DEP as model pollutant *in vivo*. Challenge of human subjects with DEP induced expression of MIP-1 α , RANTES, and MCP-3 but did not alter Eotaxin production. These results are important in light of previous studies that show that DEP will both initiate (15) and augment (10–12) allergen-induced responses and may provide part of the explanation of how DEP can increase IgE production in the absence of allergen.

Previous work by our group (10–12) and others (16, 17) studying the effect the prototypic particulate matter pollutant DEP have focused on its effect on allergic inflammation, that is, inflammation enhancing the TH2 cytokine profile and IgE antibody responses that lead to increased sensitization. DEP and the polycyclic aromatic hydrocarbons (PAH) they contain can enhance both human and murine IgE responses *in vivo* and *in vitro*. Additionally, DEP can act *in vivo* to potentiate cytokine production at the human upper respiratory mucosal surface. In the presence of allergen, DEP will enhance allergen-specific IgE and induce a TH2 cytokine profile. Additionally, DEP can induce allergic sensitization, causing the formation of IgE to a neoallergen. In this study we have extended these findings to show that DEP may also have a role in acute inflammation *in vivo*. Many studies have implicated ambient air pollution as a major health problem, especially in its ability to exacerbate airway inflammation and asthma (18–21). The ability of pollutants such as ozone to induce acute inflammation and cause cellular infiltration of the airways has also been shown (22). Recently Fahy *et al.* showed that the organic extract from DEP could directly act on peripheral blood mononuclear cells to increase secretion of RANTES and the CXC chemokine IL-8 *in vitro* (23). These studies sup-

port our view that pollutants may cause cellular infiltration and an inflammatory response in the absence of allergen by induction of certain chemokines.

Chemokines are a family of homologous chemotactic cytokines which are released as a response to injury, allergens antigens, and microorganisms. CC chemokines are chemotactic for monocytes and lymphocytes, with the exception of Eotaxin, which seems to act exclusively on eosinophils (1, 24). This family of chemokines is of particular interest in allergic airway disease since many of its members have other nonchemotactic activities, (1, 7) in particular the ability to release histamine from basophils in an IgE-independent fashion (1, 5, 25). DEP increased expression of MCP-3, MIP-1 α , and RANTES, all of which are potent histamine-releasing factors and all of which can augment T cell proliferation and cytokine production. In addition, RANTES has been shown to activate eosinophils and cause the secretion of eosinophil cationic protein (ECP) (1, 7). Increased RANTES expression may, therefore, explain the elevated ECP levels observed in nasal washes following challenge with DEP.

The rapid time course of expression of these chemokines anticipates the increase in cell numbers and ECP levels which are elevated within 6 h of challenge with DEP. The increase in chemokine levels suggest that DEP-induced enhanced chemotaxis may occur and result in infiltration of the airway by monocytes, lymphocytes, and other inflammatory cells.

Interestingly, the CC chemokine response to nasal challenge with DEP mimics that observed after nasal challenge with allergen (9). Allergen challenge increases recovery of MCP-1, MIP-1 α , and RANTES from nasal secretions. This is in keeping with a growing body of evidence suggesting a role for these molecules in allergic disease and asthma. Elevated levels of CC chemokines are detected in the bronchoalveolar lavage fluid obtained from patients with mild allergic asthma (8) and allergen challenge will increase RANTES levels in these subjects. Elevated levels of RANTES are also observed in patients with atopic dermatitis and injection of allergen into these subjects results in cutaneous mRNA expression of MCP-3 and RANTES (26).

In this study, while challenge of subjects with DEP resulted in the increased expression of MCP-3, RANTES, and MIP-1 α , it did not result in increased Eotaxin production. It appears that in our model Eotaxin is regulated differently from the other chemokines studied. In contrast, in animal models, aerosolized allergen will result in the accumulation of Eotaxin mRNA in lung tissue (27). It is possible, though unlikely, that we are simply failing to recover those cells that are producing Eotaxin. A significant proportion of cells recovered from nasal lavages are epithelial cells, cells now reported to produce Eotaxin (28). Current studies are directed at finding the source of the chemo-

kines. The inability of DEP to induce Eotaxin may explain why eosinophil cell numbers fail to increase after DEP challenge, while other cell types do.

Another consequence of elevated DEP-induced CC chemokine expression may be in the regulation of IgE. As noted, we have previously shown that in subjects challenged with DEP in the absence of allergen, there is an increase in IgE and IgG4 protein but not in other immunoglobulins, such as IgA, IgM, or total IgG1. Simultaneously, there was an increase in IgE-secreting cell numbers and total epsilon mRNA recovered from nasal washes. Kimata *et al.* (29) have shown that, both *in vivo* and *in vitro*, both RANTES and MIP-1 α will selectively enhance IgE and IgG4. They showed that the enhancement was due to direct stimulation of sIgE⁺ and sIgG4⁺ B cells, suggesting that these chemokines may play a role in IgE production by and growth of IgE-committed B cells. Additionally, we have previously noted that enhanced IgE and IgG4 production appears to result, at least in part, from DEP expanding a preexisting population of IgE-committed memory B cells present in the nasal mucosa (10). This expansion may occur via increased production of RANTES and MIP-1 α . The action of these chemokines may explain the IgE and IgG4 isotype-specific effect observed after challenge with DEP.

The results presented here have several important clinical implications. First, they demonstrate that the pollutant DEP can mimic some of the effects of allergen in inducing CC chemokines. This could lead to infiltration of the airway with lymphocytes, monocytes, and possibly eosinophils. Second, our findings suggest that in allergic subjects, inflammation resulting from exposure to DEP will be of a dual nature; that is, there will be both IgE-mediated and IgE-independent histamine-releasing factors present. Third, increased chemokine expression may lead to enhanced IgE production from sIgE⁺ and sIgG4⁺ B cells, thereby heightening future allergic inflammation.

ACKNOWLEDGMENTS

This work was supported by United States Public Health Service Grant AI-34567 (UCLA Asthma, Allergy, and Immunologic Disease Center), funded by the NIAID and the NIEHS. We are also indebted to Dr. Shigeru Takafuji, Department of Medicine and Physical Therapy, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo, Japan, and to Dr. Hiroshi Takenaka, Department of Otorhinolaryngology, Kyoto Prefectural University of Medicine, Kawaramachi, Hirokoji Kamigyoku, Kyoto, 602 Japan, for their kind gifts of diesel exhaust particles.

REFERENCES

1. Alam, R., Chemokines in allergic inflammation. *J. Allergy Clin. Immunol.* **99**, 273–277, 1997.
2. Bazan, J. F., Bacon, K. B., Hardiman, G., Wang, W., Soo, K., Rossi, D., Greaves, D. R., Zlotnik, A., and Schall, T. J., A new class of membrane-bound chemokine with a CX3C motif. *Nature* **385**, 640–644, 1997.
3. Oppenheim, J. J., Zachariae, C. O., Mukaida, N., and Matsu-shima, K., Properties of the novel proinflammatory supergene “intercrine” cytokine family. *Annu. Rev. Immunol.* **9**, 617–648, 1991.
4. Opendakker, G., Fiten, P., Nys, G., Froyen, G., Van Roy, N., Spelman, F., Laureys, G., and Van Damme, J., The human MCP-3 gene (SCYA7): Cloning, sequence analysis, and assignment to the C-C chemokine gene cluster on chromosome 17q11.2–q12. *Genomics* **21**, 403–408, 1994.
5. Alam, R., Forsythe, P. A., Stafford, S., Lett-Brown, M. A., and Grant, J. A., Macrophage inflammatory protein-1 alpha activates basophils and mast cells. *J. Exp. Med.* **176**, 781–786, 1992.
6. Bartels, J., Schluter, C., Richter, E., Noso, N., Kulke, R., Christophers, E., and Schroder, J. M., Human dermal fibroblasts express eotaxin: Molecular cloning, mRNA expression, and identification of eotaxin sequence variants. *Biochem. Biophys. Res. Commun.* **225**, 1045–1051, 1996.
7. Homey, B., and Zlotnik, A., Chemokines in allergy. *Curr. Opin. Immunol.* **11**, 626–634, 1999.
8. Berkman, N., Krishnan, V. L., Gilbey, T., Newton, R., O'Connor, B., Barnes, P. J., and Chung, K. F., Expression of RANTES mRNA and protein in airways of patients with mild asthma. *Am. J. Respir. Crit. Care Med.* **154**, 1804–1811, 1996.
9. Sim, T. C., Reece, L. M., Hilsmeier, K. A., Grant, J. A., and Alam, R., Secretion of chemokines and other cytokines in allergen-induced nasal responses: Inhibition by topical steroid treatment. *Am. J. Respir. Crit. Care Med.* **152**, 927–933, 1995.
10. Diaz-Sanchez, D., Dotson, A. R., Takenaka, H., and Saxon, A., Diesel exhaust particles induce local IgE production *in vivo* and alter the pattern of IgE messenger RNA isoforms. *J. Clin. Invest.* **94**, 1417–1425, 1994.
11. Diaz-Sanchez, D., Tsien, A., Casillas, A., Dotson, A. R., and Saxon, A., Enhanced nasal cytokine production in human beings after *in vivo* challenge with diesel exhaust particles. *J. Allergy Clin. Immunol.* **98**, 114–123, 1996.
12. Diaz-Sanchez, D., Tsien, A., Fleming, J., and Saxon, A., Combined diesel exhaust particulate and ragweed allergen challenge markedly enhanced *in vivo* nasal ragweed-specific IgE and skews cytokine production to a TH2-type pattern. *J. Immunol.* **158**, 2406–2413, 1997.
13. Diaz-Sanchez, D., Tsien, A., Fleming, J., and Saxon, A., Effect of topical Fluticasone propionate on the mucosal allergic response induced by ragweed allergen and diesel exhaust particle challenge. *Clin. Immunol.* **90**, 313–322, 1999.
14. Busse, W. W., Calhoun, W. F., and Sedgwick, J. D., Mechanism of airway inflammation in asthma. *Am. Rev. Respir. Dis.* **147**, S20–S24, 1993.
15. Diaz-Sanchez, D., Penichet-Garcia, M., Wang, M., Jyrala, M., and Saxon, A., Nasal challenge with diesel exhaust particles can induce sensitization to a neoallergen in the human mucosa. *J. Allergy Clin. Immunol.* **104**, 1183–1188, 1999.
16. Muranaka, M., Suzuki, S., Koizumi, K., Takafuji, S., Miyamoto, T., Ikemori, R., and Tokiwa, H., Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. *J. Allergy Clin. Immunol.* **79**, 639–645, 1986.
17. Takafuji, S., Suzuki, S., Koizumi, K., Tadokoro, K., Miyamoto, T., Ikemori, R., and Muranaka, M., Diesel-exhaust particulates inoculated by the intranasal route have an adjuvant activity for IgE production in mice. *J. Allergy Clin. Immunol.* **79**, 639–645, 1987.
18. Ishizaki, T., Koizumi, K., Ikemori, R., Ishiyama, Y., and Kushibiki, E., Studies of prevalence of Japanese cedar pollinosis

- among the residents in a densely cultivated area. *Ann. Allergy* **58**, 265–270, 1987.
19. Gilmour, M. I., Interaction of air pollutants and pulmonary allergic responses in experimental animals. *Toxicology* **105**, 335–342, 1995.
 20. Rusznak, C., Devalia, J. L., and Davies, R. J., The impact of pollution on allergic disease. *Allergy* **49**, 21–27, 1994.
 21. Kramer, U., Koch, T., Ranft, U., Ring, J., and Behrendt, H., Traffic-related air pollution is associated with atopy in children living in urban areas. *Epidemiology* **11**, 64–70, 2000.
 22. Peden, D. B., Setzer, R. W. J., and Devlin, R. B., Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics. *Am. J. Respir. Crit. Care Med.* **151**, 1336–1345, 1995.
 23. Fahy, O., Tsicopoulos, A., Hammad, H., Pestel, J., Tonnel, A. B., and Wallaert, B., Effects of diesel organic extracts on chemokine production by peripheral blood mononuclear cells. *J. Allergy Clin. Immunol.* **103**, 1115–1124, 1999.
 24. Kita, H., and Gleich, G. J., Chemokines active on eosinophils: Potential roles in allergic inflammation. *J. Exp. Med.* **183**, 2421–2426, 1996.
 25. Pasmans, S. G., Aalbers, M., Daha, M. R., Knol, E. F., Jansen, H. M., and Aalberse, R. C., Histamine-releasing activity in supernatants of mononuclear cells: Contribution of monocyte chemoattractant protein-1 activity compared with IgE-dependent activity. *J. Allergy Clin. Immunol.* **98**, 962–968, 1996.
 26. Schroder, J. M., Noso, M., Sticherling, M., and Christophers, E., Role of eosinophil-chemotactic C-C chemokines in cutaneous inflammation. *J. Leukocyte Biol.* **59**, 1–5, 1996.
 27. Griffiths-Johnson, D. A., Collins, P. D., Rossi, A. G., Jose, P. J., and Williams, T. J., The chemokine, eotaxin, activates guinea-pig eosinophils in vitro and causes their accumulation into the lung in vivo. *Biochem. Biophys. Res. Commun.* **197**, 1167–1172, 1993.
 28. Lilly, C. M., Nakamura, H., Kesselman, H., Nagler-Anderson, C., Asano, K., Garcia-Zapeda, E. A., Rothenberg, M. E., Drazen, J. M., and Luster, A. D., Expression of eotaxin by human lung epithelial cells: Induction by cytokines and inhibition by glucocorticoids. *J. Clin. Invest.* **99**, 1767–1773, 1997.
 29. Kimata, H., Yoshida, A., Ishioka, C., Fujimoto, M., Lindley, I., and Furosho, K., RANTES and macrophage inflammatory protein 1 alpha selectively enhance immunoglobulin (IgE) and IgG4 production by human B cells. *J. Exp. Med.* **183**, 2397–2402, 1996.

Received July 18, 2000; accepted July 24, 2000