

Diesel exhaust exposure favors T_H2 cell recruitment in nonatopic subjects by differentially regulating chemokine production

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Background: The prevalence of allergic diseases has increased in the last 20 years, and a number of studies have shown that diesel exhaust particle-associated polyaromatic hydrocarbons can exacerbate the allergic reaction. Much less is known about their potential capacity to generate a T_H2-type allergic reaction in nonatopic subjects.

Objective: The aim of this study was to test the hypothesis that diesel exhaust exposure might favor, in nonatopic donors, T_H2-type cell recruitment, either through increased production of T_H2-attracting chemokines or decreased production of T_H1-attracting chemokines.

Methods: PBMCs from nonatopic donors were incubated with diesel exhaust particle-polyaromatic hydrocarbons, and the supernatants were evaluated for the presence of pro-T_H1 chemokines (IFN- γ -induced protein 10 and monokine Induced by IFN- γ) and pro-T_H2 chemokines (macrophage-derived chemokine, I-309, and pulmonary and activation-regulated chemokine) by means of ELISA. The functional effect was evaluated by using chemotaxis assays with polarized T_H1 and T_H2 cells.

Results: Diesel exhaust exposure of PBMCs from nonatopic donors induced a late increase after 48 hours in pulmonary and activation-regulated chemokine mediated by IL-13 and a decrease in IFN- γ -induced protein 10 levels selectively at both the protein and mRNA levels. The functional effect of these chemokine variations resulted in an enhanced chemotaxis of T_H2, but not T_H1, cells.

Conclusion: These findings show that diesel exhaust exposure might be involved in the genesis of allergic diseases by differentially regulating chemokines favoring the recruitment of T_H2 cells in nonatopic subjects.

Clinical implications: Environmental factors, especially air pollution, might favor the genesis of allergic diseases. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Diesel, chemokines, allergy, asthma, air pollution

Epidemiologic studies have linked the increased incidence of allergic diseases in part to the increase in exposure to atmospheric pollutants.¹⁻⁴ Air pollutants, including diesel exhaust particles (DEPs) and in particular their associated polyaromatic hydrocarbons (DEP-PAHs), have been shown to affect allergic responses. In allergic subjects DEPs are reported to potentiate or facilitate the allergic inflammatory response. DEP-PAHs act as an adjuvant by potentiating both total and specific IgE production by committed B cells. DEP-PAHs can also act directly on many effector cells, such as lymphocytes, monocytes, macrophages, neutrophils, and eosinophils, by inducing their recruitment and by triggering the release of pro-inflammatory mediators.⁵⁻⁸ In particular, in response to common allergens, DEPs can enhance T_H2 cytokine⁹ and pro-T_H2 chemokine^{8,10} production. Moreover, DEPs have been shown to induce sensitization to a neoallergen toward a T_H2 response in allergic subjects.¹¹

Some studies have evaluated the effects of DEP-PAHs in healthy nonatopic subjects. Some inflammatory changes in the airways have been observed in healthy human subjects exposed to DEPs.¹²⁻¹⁶ In particular, cytokine release of IL-8 and growth-related oncogene- α has been observed in the bronchial tissue¹⁷ and bronchial epithelial cells,¹⁸⁻²⁰ leading to chemoattraction of inflammatory cells, such as macrophages and neutrophils. Other authors have shown modifications in nonatopic subjects that could be relevant in the context of the potential induction of allergic reactions, in particular recruitment of B cells, induction of histamine release in airway lavage,²¹ switch in Ig production toward IgE,²² and induction of histamine and IL-4 production by human basophils.²³ In the laboratory we have shown that diesel exposure induces the production of IL-8 and RANTES by PBMCs from nonatopic donors, leading to an increased recruitment of neutrophils and eosinophils.²⁴

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Abbreviations used

- DEP: Diesel exhaust particle
 DEP-PAH: Diesel exhaust particles–polyaromatic hydrocarbons
 IP-10: IFN- γ -induced protein 10
 MDC: Macrophage-derived chemokine
 MIG: Monokine induced by IFN- γ
 PARC: Pulmonary and activation-regulated chemokine

Along with adhesion molecules, chemokines and their receptors are believed to be essential for inflammatory cell recruitment.²⁵ Certain chemokines and their corresponding receptors have been shown to be associated with type 1- and type 2-mediated immune responses. Type 1 T cells preferentially express the chemokine receptor CXCR3 and are attracted by its ligands IFN- γ -induced protein 10 (IP-10/CXCL10) and monokine induced by IFN- γ (MIG/CXCL11), whereas type 2 T cells preferentially express CCR3, CCR4, and CCR8 and are attracted, respectively, by eotaxin (CCL11), macrophage-derived chemokine (MDC/CCL22), and I-309 (CCL1).²⁶ More recently, the chemokine pulmonary and activation-regulated chemokine (PARC/CCL18) has been shown to be involved in allergic asthma through the recruitment of T_H2 cells, although its receptor is still unknown.²⁷

The production of chemokines involved in the recruitment of T_H1 or T_H2 cells was investigated in PBMCs from healthy nonatopic donors to evaluate the potential effects of diesel exhaust exposure in the genesis of allergic reactions.

METHODS

Generation of diesel exhaust and extraction of DEP-PAHs

DEPs were obtained from a light-duty diesel-powered passenger car (Renault, Pollution Department, Boulogne-Billancourt, France), and DEP-PAHs were extracted in dichloromethane (CH₂Cl₂) solvent. The resulting extract was then analyzed by means of HPLC to determine the concentration of its main organic constituents, as previously described.¹⁵

The stock solution (1 mg of DEP-PAH/mL of CH₂Cl₂) was stored at 4°C in the dark and was diluted freshly each time in RPMI 1640 medium (Sigma, St Louis, Mo) before use.

Subjects

Venous blood was collected from 12 healthy nonatopic subjects (35 $\pm$ 11 years old). None of the subjects had a personal history of allergic diseases, but 2 had a family history of allergy. Results of skin prick tests to 15 common aeroallergens, including pollens, house dust mites, animal dander, and molds, and RAST results were negative for all subjects. Total IgE concentration was 13.1 $\pm$ 5.4 kU/mL. All subjects signed an informed consent form.

Cell culture

PBMCs were prepared from blood collected on heparin. Briefly, after removing the platelets, the blood was diluted and layered on a Ficoll-Hypaque gradient (Pharmacia, Uppsala, Sweden). The RPMI

1640 medium used for culture was supplemented with 2 mmol/L glutamine, 100 IU/mL penicillin, 100 μ g/mL streptomycin, and 10% FCS. PBMCs (2×10^6 cells/well) were cultured in 24-well, flat-bottomed microculture plates (Nunc, Roskilde, Denmark). Experiments were performed at 18, 24 and 48 hours with DEP-PAH concentrations of 25, 50, and 100 ng/mL and CH₂Cl₂ alone. No toxic effect was detected (viability >97% for all conditions), irrespective of the time point. The addition of CH₂Cl₂ in cell cultures had no significant effect on the production of chemokines and cytokines compared with RPMI 1640 medium alone. In some experiments neutralizing anti-IL-4, anti-IL-10, and anti-IL-13 mAbs (R&D Systems, Minneapolis, Minn) were used at final concentrations of 10, 1, and 10 μ g/mL, respectively. The corresponding isotypes were used as negative controls. In other experiments PBMCs were stimulated with 5 and 10 pg/mL rhIL-13 (Pepro Tech, London, United Kingdom) to check whether small doses of IL-13 were able to induce PARC production. The LPS content in CH₂Cl₂, DEP-PAHs, antibodies, isotypes, and rhIL-13 was less than 0.1 EU/mL, as determined by using the limulus amebocyte lysate assay (Cambrex, Walkersville, Md).

Generation of polarized human T_H1 and T_H2 cells

T cells were polarized *in vitro* toward a T_H1 or T_H2 cytokine profile, as previously described.^{28,29} CD4⁺ CD45RA⁺ naive T cells were obtained from human PBMCs by means of negative selection with the CD4⁺ T cell isolation kit and CD45RO microbeads (Milenyi Biotec, Paris, France). Cells were activated with coated anti-CD3 (10 ng/mL; PharMingen, San Diego, Calif) in the presence of 2 ng/mL IL-12 (R&D Systems) and 200 ng/mL anti-IL-4 (R&D Systems) for induction of T_H1 cells or 10 ng/mL IL-4 (R&D Systems) and 2 μ g/mL anti-IL-12 (R&D Systems) for induction of T_H2 cells. After 3 days, 10 ng/mL IL-2 (TEBU, Le Perray en Yvelines, France) was added to the cultures. At day 10, cells were restimulated with coated anti-CD3 (2 μ g/mL) and soluble anti-CD28 (2 μ g/mL, PharMingen) for 5 hours: concentrations of IFN- γ and IL-4 were measured by means of ELISA, according to the manufacturer's recommendations, and expressed in picograms per milliliter (Diacclone Research, Besançon, France).

Quantification of chemokine levels in cell culture supernatants

Concentrations of PARC, MDC, I-309, MIG (R&D Systems, Abington, United Kingdom), IP-10 (Biosource International, Camarillo, Calif), IL-4, IL-10, and IL-13 (Diacclone Research, Besançon, France) were measured by means of ELISA, according to the manufacturer's recommendations, and expressed in picograms per milliliter. The level of sensitivity was 7.81 pg/mL for PARC, MDC, I-309, and IP-10; 31.2 pg/mL for MIG; 1.1 pg/mL for IL-4; 12.5 pg/mL for IL-10; and 1.56 pg/mL for IL-13. Results were expressed as means $\pm$ SEM.

Semiquantitative RT-PCR

After removal of the supernatants, PBMCs were resuspended in TRIzol reagent (Gibco BRL Life Technologies, Gaithersburg, Md), and total cellular RNA was extracted according to the manufacturer's procedure. RT-PCR was performed as previously described.¹⁰

T-cell chemotaxis assays

Chemotaxis assays were performed on *in vitro*-polarized T_H1 and T_H2 cells to evaluate the resulting biologic chemotactic activity of the supernatants.^{29,30} Human T_H1 and T_H2 cells were harvested and resuspended in RPMI at a concentration of 2×10^6 cells/mL. The chemotaxis protocol was performed with a 48-well microchemotaxis chamber (Neuro Probe, Cabin John, Md) using 5- μ m-pore

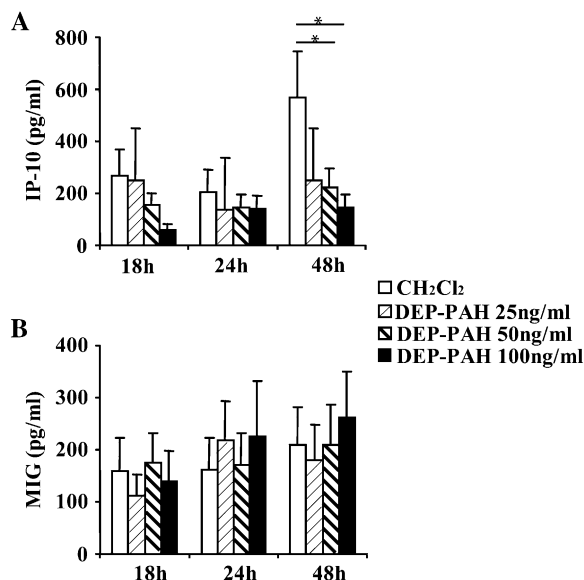


FIG 1. Kinetics of production and dose response of the T_H1 -associated chemokines IP-10 (A) and MIG (B) by PBMCs from healthy donors after incubation with various concentrations of DEP-PAHs. Results are expressed as means $\pm$ SEM for 8 subjects. * $P < .05$.

polycarbonate filters (Nucleopore Corp, Pleasanton, Calif) for 2 hours at 37°C in 5% CO₂ and using supernatants from cells exposed to CH₂Cl₂ solvent and to different concentrations of DEP-PAHs. T cells that had migrated through the filter and reached the bottom well were counted under a light microscope with a magnification of 500-fold by using a Thoma cell. Each condition was performed in triplicate. The results were expressed as the subtraction of the mean number of migrating T cells with complete RPMI medium alone from the mean number of T_H1 or T_H2 cells that migrated specifically in response to chemokines present in the culture supernatants. For positive controls, human recombinant MDC and IP-10 were used for T_H2 and T_H1 , respectively, at a concentration of 10^{-7} mol/L (Tebu, Le Perray-en-Yvelines, France), whereas human recombinant IP-10 and MDC were used for T_H2 and T_H1 for negative controls, respectively. For neutralization assays, samples were incubated for 1 hour at 37°C before the chemotaxis assay with anti-PARC-neutralizing polyclonal antibodies (R&D Systems) at a final concentration of 2 μ g/mL. A chemokinesis effect was ruled out, as previously described.³⁰ Results corresponding to the chemotaxis assays were expressed as means $\pm$ SEM.

Statistical analysis

Statistical analysis for production of chemokines and cytokines was first performed within the subgroups by using the ANOVA test and, when significant, followed by the post hoc Bonferroni-Dunn test. The paired t test was performed for chemotaxis assays. P values of less than .05 were regarded as statistically significant. Statistical analysis was performed with the Statview 5.1 software (Abacus Concepts, Berkeley, Calif) on a Macintosh.

RESULTS

DEP-PAHs decrease T_H1 -associated IP-10, but not MIG, chemokine production

DEP-PAH effects were evaluated on the production of T_H1 -associated chemokines by PBMCs from nonatopic

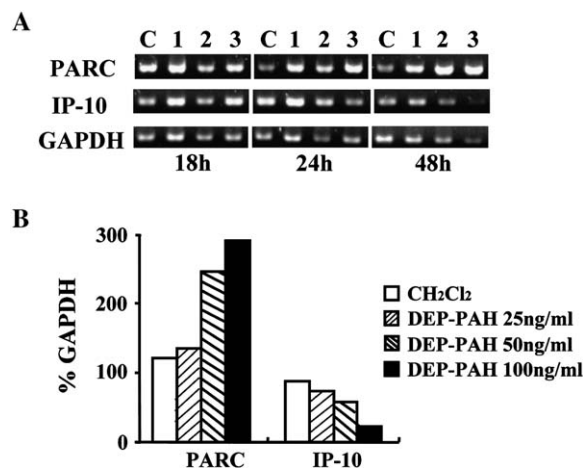


FIG 2. A, Kinetics of mRNA expression and dose response of PARC, IP-10, and human glyceraldehyde-phosphate dehydrogenase (GAPDH) by PBMCs from a healthy donor after incubation with various concentrations of DEP-PAHs (25 ng/mL [1], 50 ng/mL [2], and 100 ng/mL [3]) compared with CH₂Cl₂ (C). B, Densitometric analysis of chemokine mRNA expression normalized against transcripts for human GAPDH at 48 hours' stimulation with DEP-PAHs.

donors. A spontaneous production of IP-10 was observed in supernatants from unstimulated PBMCs. Exposure of PBMCs from healthy nonatopic subjects to both 50 and 100 ng/mL DEP-PAH induced a late (48 hours) decrease in the T_H1 -attracting chemokine IP-10 compared with the control (Fig 1, A). A similar decrease was observed at the mRNA level (Fig 2). No significant difference was observed between cell cultures exposed to DEP-PAH and control before 48 hours. For the other pro- T_H1 chemokine, MIG, no variation was observed, irrespective of the time point or DEP-PAH dose used (Fig 1, B).

DEP-PAHs selectively induce T_H2 -associated PARC, but not MDC and I-309, chemokine production

DEP-PAH effects were next evaluated on the production of T_H2 -associated chemokines by PBMCs from nonatopic donors. No significant difference was observed in MDC and I-309 levels in the supernatants from PBMCs exposed to diesel extracts between the different conditions of cell stimulation, irrespective of the time point (Fig 3, A and B). When a panel of DEP-PAH concentrations ranging from 25 to 100 ng/mL was used in the cell cultures, an increase of PARC levels in PBMC supernatants was observed for 100 ng/mL DEP-PAH compared with the CH₂Cl₂ control 48 hours after stimulation but not at 18 or 24 hours (Fig 3, C). This was confirmed at the mRNA level, which was increased at the corresponding time points (Fig 2).

All together, these results show that DEP-PAHs upregulate PARC and decrease IP-10 release by PBMCs from healthy nonatopic donors, reflecting a pro- T_H2 pattern.

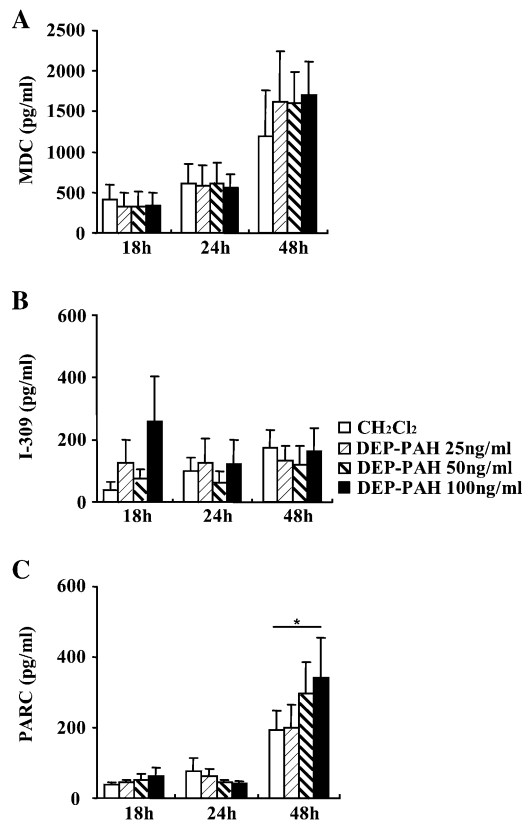


FIG 3. Kinetics of production and dose response of the T_H2-associated chemokines MDC (A), I-309 (B), and PARC (C) by PBMCs from healthy donors after incubation with various concentrations of DEP-PAHs. Results are expressed as means $\pm$ SEM for 8 to 12 subjects. * $P < .05$.

DEP-PAH-stimulated PBMCs from nonatopic subjects exhibit a T_H2 chemotactic activity

Supernatant chemotactic activity was evaluated on *in vitro* polarized T_H1 and T_H2 cells to investigate whether chemokines produced by PBMCs from nonatopic subjects stimulated with DEP-PAH were functional. Positive and negative controls were, respectively, MDC and IP-10 for T_H2 cells and IP-10 and MDC for T_H1 cells. Forty-eight-hour supernatants from DEP-PAH-stimulated PBMCs exhibited no ability to recruit polarized T_H1 cells (Fig 4, A). In contrast, the same supernatants powerfully attracted polarized T_H2 cells for the 50 and 100 ng/mL DEP-PAH concentrations (Fig 4, B). The addition of neutralizing PARC antibody dose-dependently inhibited T_H2 cell migration, with a maximal inhibition of 69.5% at a DEP-PAH concentration of 100 ng/mL (Fig 4, C).

IL-13, but not IL-4 and IL-10, mediates PARC production by PBMCs exposed to DEP-PAHs

The possible involvement of T_H2 cytokines known to induce PARC production³¹ was assessed to identify the mechanism involved in PARC enhancement by DEP-

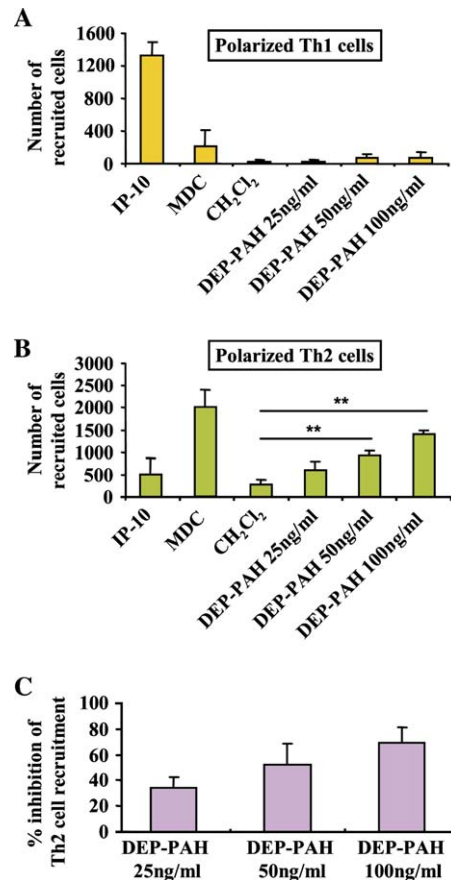


FIG 4. Capacity of PBMCs from nonatopic patients stimulated with DEP-PAHs to induce T_H1 and T_H2 cell chemotaxis. PBMC supernatants were harvested at 48 hours and used in a chemotaxis assay with either T_H1 (A) or T_H2 (B) cells. ** $P < .01$. C, Inhibitory effect of anti-PARC-neutralizing antibody on T_H2 cell recruitment induced by PBMC supernatants. Results are expressed as means $\pm$ SEM (n = 5).

PAH exposure. We assayed the presence of IL-4, IL-10, and IL-13 and the effect of neutralizing mAbs in PBMC cultures. DEP-PAHs did not induce production of IL-4 and IL-10 in PBMCs from nonatopic subjects (data not shown), and neutralizing antibodies against IL-4 and IL-10 had no effect on PARC production (Fig 5, A). In contrast, IL-13 production was transiently but significantly increased by exposure to 100 ng/mL DEP-PAH compared with exposure to the CH₂Cl₂ control at the 24-hour time point ($P < .01$; Fig 5, B), although at low levels. Neutralizing anti-IL-13 mAb inhibited DEP-PAH-induced PARC production at 50 and 100 ng/mL DEP-PAH (70.4% and 66.6% inhibition, respectively) at the 48-hour time point compared with the isotype control (21.7% and 19.5% inhibition, respectively; Fig 5, A). PBMCs were stimulated with 5 and 10 pg/mL IL-13 for 24 hours to check that small doses of IL-13 were indeed able to activate PARC production. As shown in Fig 5, C, 10 pg/mL IL-13 was able to double PARC baseline production. Therefore IL-13 is involved in the DEP-PAH-induced PARC production from nonatopic PBMCs.

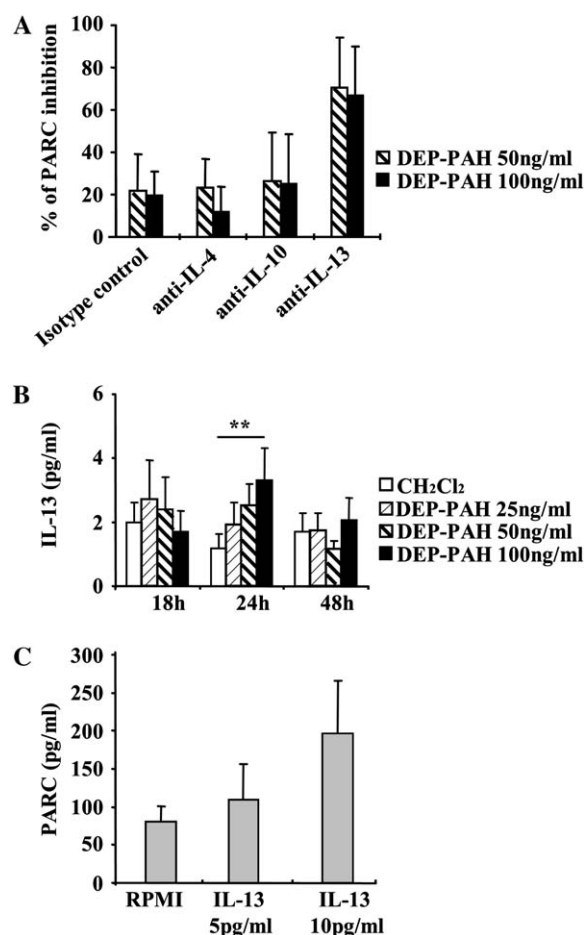


FIG 5. IL-13 mediates increased PARC production by DEP-PAH-stimulated PBMCs from healthy donors. **A**, Inhibitory effect of neutralizing anti- T_H2 cytokine mAbs on DEP-PAH-induced PARC enhancement ($n = 5$ subjects). **B**, Kinetics of production and dose response of IL-13 release by PBMCs ($n = 8$ subjects, $**P < .01$). **C**, Effect of IL-13 on PARC production at 24 hours ($n = 4$ subjects). All results are expressed as means $\pm$ SEM.

DISCUSSION

A number of studies, both epidemiologic and biologic, have shown that diesel pollution might be involved in the exacerbation and amplification of allergic diseases, but it is still not clear whether diesel exhaust might influence allergic reactions in nonatopic subjects.

In a previous study we have shown that diesel exposure of PBMCs from allergic patients modifies chemokine production, favoring the recruitment of T_H2 cells. The costimulation by the allergen was required for increased production of MDC, whereas diesel alone was sufficient to stimulate the production of I-309 and PARC and to decrease the constitutive synthesis of IP-10.^{8,10} We therefore wondered whether diesel exposure of PBMCs from nonatopic subjects would also affect the production of those chemokines.

MIG (CXCL9) is a related member of the CXC chemokine subfamily. Like IP-10, MIG binds to the CXCR3

receptor and is produced by monocytes,³² macrophages,³³ neutrophils,³⁴ and human umbilical vein endothelial cells³⁵ in response to IFN- γ .^{34,36} IP-10 is produced by monocytes-macrophages and T cells and is constitutively expressed by human keratinocytes³⁷ and epithelial cells.³⁸ MIG and IP-10 are chemotactic for T_H1 lymphocytes, natural killer cells, and eosinophils.³⁹ For both chemokines, a baseline production was observed in unstimulated PBMCs from nonatopic subjects. Interestingly, compared with our previous studies, in which we evaluated these chemokines in allergic subjects, in the present study the baseline levels of MIG¹⁰ and IP-10⁸ were higher in nonatopic subjects. These observations suggest that MIG and IP-10 might play a role in the maintenance of a T_H1 -dominated response in nonatopic subjects, as shown in another study.⁴⁰ In the present study diesel exhaust exposure did not modify MIG production by PBMCs, irrespective of the dose and the kinetics. In contrast, it induced a 5-fold dose-dependent decrease of IP-10, almost reaching the low baseline levels observed in allergic patients. Corresponding variations of mRNA expression confirmed that DEP-PAHs affected its production at the transcriptional level. However, this effect was observed after 48 hours of incubation, whereas in the previous study on allergic patients, it was obtained at 24 hours,⁸ suggesting that a longer exposure to diesel exhaust is necessary in nonatopic subjects to observe effects similar to those in allergic subjects. These results suggest that in nonatopic subjects chronic diesel exhaust exposure might favor a type 2-dominated response by decreasing the release of the pro- T_H1 chemokine IP-10. These data are in agreement with recent studies showing that diesel exhaust exposure in healthy individuals inhibits the T_H1 response by decreasing IL-12 and IFN- γ production.^{41,42}

MDC is mainly produced by monocytes-macrophages, as well as T cells, and binds selectively to CCR4, expressed on type 2 T cells and basophils.⁴³ The CC chemokine I-309 (CCL1) binds to CCR8, also expressed on type 2 T cells,^{44,45} and is produced by activated monocytes and by T cells.⁴⁶ Accordingly, these chemokines have been shown to attract selectively T_H2 cells^{29,47,48} and to be involved in the T_H2 response, in particular in allergic reactions,⁴⁹ although expression of I-309 was not observed in the airway mucosa or in the bronchial epithelial cells of atopic asthmatic subjects, whereas MDC and CCR8 immunoreactivities were found.⁵⁰ In allergic patients MDC was induced by diesel exposure but only when allergen costimulation was provided⁸; therefore in the present study it was not surprising that diesel exhaust did not induce MDC production by nonatopic PBMCs. In contrast, I-309 production, although increased by diesel exposure in allergic patients,¹⁰ was not modified in nonatopic subjects. These data suggest that diesel-induced I-309 production is not related to DEP-PAHs themselves but to the atopic status of the PBMC donors.

The last chemokine that we assessed was PARC. This chemokine is preferentially expressed in the lung,⁵¹ is induced by T_H2 -type cytokines,³¹ and is mainly produced by antigen-presenting cells, such as activated

macrophages, monocytes, and dendritic cells. Its receptor is still unknown, but PARC chemoattracts naive CD45RA⁺ T cells but not monocytes or granulocytes.⁵¹⁻⁵³ PARC has been found in atheromatosis⁵⁴ and in contact hypersensitivity reactions⁵⁵ and subsequently in atopic dermatitis⁵⁶ and allergic asthma.²⁷ In a previous study we have shown that both diesel exhaust and allergen were able to induce PARC release from PBMCs from allergic patients, becoming significant at late time points (48 hours),¹⁰ suggesting that PARC might participate in the chronicity of allergic reactions. In the present study PARC was also induced at both translational and transcriptional levels after 48-hour diesel exhaust exposure of PBMCs from nonatopic subjects. Interestingly, T_H2 chemoattraction by supernatants from diesel-stimulated PBMCs was inhibited by a neutralizing antibody directed against PARC. This result suggests that PARC is able to chemoattract T_H2 cells, which has been recently demonstrated in our laboratory.²⁷ This result also suggests the presence of a receptor for the chemokine PARC at the surface of T_H2 cells. Moreover, diesel exhaust induces PARC expression by PBMCs from both atopic and nonatopic subjects, suggesting that PARC might represent one of the pathways used by diesel pollutants to initiate a T_H2 response in nonatopic healthy subjects.

The involvement of the T_H2 cytokine pathway in DEP-PAH-induced PARC production was then investigated. No variation was observed in production levels of IL-4 and IL-10 by PBMCs from nonallergic subjects, and neutralizing antibodies against these cytokines had no effect. Similar results have been obtained for IL-4 in healthy subjects⁴² after DEP exposure of activated T cells. However, in another study exposure of basophils from healthy donors resulted in IL-4 induction.²³ In our PBMC cultures, basophils were present, but their numbers were probably too low to allow a detectable IL-4 production. For IL-10, the results published in different human studies addressing healthy subjects are contradictory, with some of them finding a decrease⁴² or no change⁵⁷ in IL-10 production after DEP exposure and the differences probably reflecting the cell types studied (T cells vs bronchial epithelial cells, respectively). In contrast, induction of IL-13 was observed in the present study. IL-13 expression preceded PARC production in PBMCs exposed to DEP-PAHs, and its neutralization inhibited PARC production, suggesting that IL-13 plays a key role in PARC induction by DEP-PAHs. This result is in agreement with previous studies performed both in atopic and nonatopic subjects, in which induction of IL-13 by DEPs has been reported in nasal provocation tests² and in the bronchial epithelium,⁵⁸ respectively. Because IL-13 is involved in T_H2 cell development, the induction of IL-13 by DEP-PAHs might contribute to T_H2 differentiation in nonatopic subjects.⁵⁹

Altogether, these results suggest that diesel pollution participates in the genesis of allergic reactions by favoring T_H2-mediated responses through an enhancement of IL-13 and PARC secretion and a decreased IP-10 production. Through these modifications, diesel exhaust exposure might shift the immune response in nonatopic individuals

toward an allergic pattern of response and might contribute to the increased prevalence of allergic disorders.

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REFERENCES

1. Bascom R, Kulle T, Kagey-Sobotka A, Proud D. Upper respiratory tract environmental tobacco smoke sensitivity. *Am Rev Respir Dis* 1991;143:1304-11.
2. Riedl M, Diaz-Sanchez D. Biology of diesel exhaust effects on respiratory function. *J Allergy Clin Immunol* 2005;115:221-8.
3. Bernstein JA, Alexis N, Barnes C, Bernstein IL, Bernstein JA, Nel A, et al. Health effects of air pollution. *J Allergy Clin Immunol* 2004;114:1116-23.
4. Peden DB. The epidemiology and genetics of asthma risk associated with air pollution. *J Allergy Clin Immunol* 2005;115:213-9.
5. 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.
6. Diaz-Sanchez D, Tsien A, Fleming J, Saxon A. Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. *J Immunol* 1997;158:2406-13.
7. Wang M, Saxon A, Diaz-Sanchez D. Early IL-4 production driving Th2 differentiation in a human in vivo allergic model is mast cell derived. *Clin Immunol* 1999;90:47-54.
8. Fahy O, Senechal S, Pene J, Scherpereel A, Lassalle P, Tonnel AB, et al. Diesel exposure favors Th2 cell recruitment by mononuclear cells and alveolar macrophages from allergic patients by differentially regulating macrophage-derived chemokine and IFN-gamma-induced protein-10 production. *J Immunol* 2002;168:5912-9.
9. Diaz-Sanchez D. The role of diesel exhaust particles and their associated polycyclic aromatic hydrocarbons in the induction of allergic airway disease. *Allergy* 1997;52:52-8.
10. Senechal S, de Nadai P, Ralainirina N, Scherpereel A, Vorng H, Lassalle P, et al. Effect of diesel on chemokines and chemokine receptors involved in Th1/Th2 recruitment in asthmatics. *Am J Respir Crit Care Med* 2003;168:215-21.
11. Diaz-Sanchez D, Garcia MP, Wang M, Jyräla M, Saxon A. Nasal challenge with diesel exhaust particles can induce sensitization to a neoallergen in the human mucosa. *J Allergy Clin Immunol* 1999;104:1183-8.
12. Bayram H, Devalia JL, Sapsford RJ, Ohtoshi T, Miyabara Y, Sagai M, et al. The effect of diesel exhaust particles on cell function and release of inflammatory mediators from human bronchial epithelial cells in vitro. *Am J Respir Cell Mol Biol* 1998;18:441-8.
13. Nightingale JA, Maggs R, Cullinan P, Donnelly LE, Rogers DF, Kinnersley R, et al. Airway inflammation after controlled exposure to diesel exhaust particulates. *Am J Respir Crit Care Med* 2000;162:161-6.
14. Sydbom A, Blomberg A, Parnia S, Stenfors N, Sandstrom T, Dahlen SE. Health effects of diesel exhaust emissions. *Eur Respir J* 2001;17:733-46.
15. Holgate ST, Sandstrom T, Frew AJ, Stenfors N, Nordenhall C, Salvi S, et al. Health effects of acute exposure to air pollution. Part I: Healthy and asthmatic subjects exposed to diesel exhaust. *Res Rep Health Eff Inst* 2003;112:1-30.
16. Behndig AF, Mudway IS, Brown JL, Stenfors N, Helleday R, Duggan ST, et al. Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans. *Eur Respir J* 2006;27:359-65.
17. Salvi SS, Nordenhall C, Blomberg A, Rudell B, Pourazar J, Kelly FJ, et al. Acute exposure to diesel exhaust increases IL-8 and GRO-alpha production in healthy human airways. *Am J Respir Crit Care Med* 2000;161:550-7.
18. Steerenberg PA, Zonnenberg JA, Dormans JA, Joon PN, Wouters IM, van Bree L, et al. Diesel exhaust particles induced release of interleukin 6 and 8 by (primed) human bronchial epithelial cells (BEAS 2B) in vitro. *Exp Lung Res* 1998;24:85-100.
19. Ohtoshi T, Takizawa H, Okazaki H, Kawasaki S, Takeuchi N, Ohta K, et al. Diesel exhaust particles stimulate human airway epithelial cells to produce cytokines relevant to airway inflammation in vitro. *J Allergy Clin Immunol* 1998;101:778-85.

20. Jimenez LA, Drost EM, Gilmour PS, Rahman I, Antonicelli F, Ritchie H, et al. PM(10)-exposed macrophages stimulate a proinflammatory response in lung epithelial cells via TNF- α . *Am J Physiol Lung Cell Mol Physiol* 2002;282:L237-48.
21. Salvi S, Blomberg A, Rudell B, Kelly F, Sandstrom T, Holgate ST, et al. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. *Am J Respir Crit Care Med* 1999;159:702-9.
22. 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.
23. Devouassoux G, Saxon A, Metcalfe DD, Prussin C, Colomb MG, Brambilla C, et al. Chemical constituents of diesel exhaust particles induce IL-4 production and histamine release by human basophils. *J Allergy Clin Immunol* 2002;109:847-53.
24. Fahy O, Tscipoulos 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.
25. Kim CH. Chemokine-chemokine receptor network in immune cell trafficking. *Curr Drug Targets Immune Endocr Metabol Disord* 2004;4:343-61.
26. Kaplan AP. Chemokines, chemokine receptors and allergy. *Int Arch Allergy Immunol* 2001;124:423-31.
27. de Nadaï P, Charbonnier AS, Chenivresse C, Sénéchal S, Fournier C, Gilet J, et al. Involvement of CCL18 in allergic asthma. *J Immunol* 2006;176:6286-93.
28. Asselin S, Conjeaud H, Minty A, Fradelizi D, Breban M. Stable polarization of peripheral blood T cells towards type 1 or type 2 phenotype after polyclonal activation. *Eur J Immunol* 1998;28:532-9.
29. Imai T, Nagira M, Takagi S, Kakizaki M, Nishimura M, Wang J, et al. Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int Immunol* 1999;11:81-8.
30. Fahy O, Hammad H, Senechal S, Pestel J, Tonnel AB, Wallaert B, et al. Synergistic effect of diesel organic extracts and allergen Der p 1 on the release of chemokines by peripheral blood mononuclear cells from allergic subjects: involvement of the MAP kinase pathway. *Am J Respir Cell Mol Biol* 2000;23:247-54.
31. Politz O, Kodelja V, Guillot P, Orfanos CE, Goerd S. Pseudoxons and regulatory elements in the genomic sequence of the beta-chemokine, alternative macrophage activation-associated CC-chemokine (AMAC)-1. *Cytokine* 2000;12:120-6.
32. Farber JM. Mig and IP-10: CXC chemokines that target lymphocytes. *J Leukoc Biol* 1997;61:246-57.
33. Flier J, Boersma DM, van Beek PJ, Nieboer C, Stoof TJ, Willemze R. Differential expression of CXCR3 targeting chemokines CXCL10, CXCL9, and CXCL11 in different types of skin inflammation. *J Pathol* 2001;94:398-405.
34. Gasperini S, Marchi M, Calzetti F, Laudanna C, Vicentini L, Olsen H, et al. Gene expression and production of the monokine induced by IFN- γ (MIG), IFN-inducible T cell alpha chemoattractant (I-TAC), and IFN- γ -inducible protein-10 (IP-10) chemokines by human neutrophils. *J Immunol* 1999;162:4928-37.
35. Piali L, Weber C, LaRosa G, Mackay CR, Springer TA, Clark-Lewis I, et al. The chemokine receptor CXCR3 mediates rapid and shear-resistant adhesion-induction of effector T lymphocytes by the chemokines IP10 and Mig. *Eur J Immunol* 1998;28:961-72.
36. Fujieda S, Diaz-Sanchez D, Saxon A. Combined nasal challenge with diesel exhaust particles and allergen induces in vivo IgE isotype switching. *Am J Respir Cell Mol Biol* 1998;19:507-12.
37. Boersma DM, Flier J, Sampat S, Ottevanger C, de Haan P, Hooft L, et al. Chemokine IP-10 expression in cultured human keratinocytes. *Arch Dermatol Res* 1998;290:335-41.
38. Sauty A, Dziejman M, Taha RA, Iarossi AS, Neote K, Garcia-Zepeda EA, et al. The T cell-specific CXC chemokines IP-10, Mig, and I-TAC are expressed by activated human bronchial epithelial cells. *J Immunol* 1999;162:3549-58.
39. Jinquan T, Jing C, Jacobi HH, Reimert CM, Millner A, Quan S, et al. CXCR3 expression and activation of eosinophils: role of IFN- γ -inducible protein-10 and monokine induced by IFN- γ . *J Immunol* 2000;165:1548-56.
40. Gangur V, Simons FE, HayGlass KT. IP-10 mediated reinforcement of human type 1 cytokine synthesis to environmental allergens among non-atopic subjects. *Int Arch Allergy Immunol* 1999;118:387-90.
41. Nilsen AM, Hagemann R, Eikas H, Egeberg K, Norkov T, Sundan A. Reduction of IL-12 p40 production in activated monocytes after exposure to diesel exhaust particles. *Int Arch Allergy Immunol* 2003;131:201-8.
42. Ohtani T, Nakagawa S, Kurosawa M, Mizuashi M, Ozawa M, Aiba S. Cellular basis of the role of diesel exhaust particles in inducing Th2-dominant response. *J Immunol* 2005;174:2412-9.
43. Chvatchko Y, Hoogewerf AJ, Meyer A, Alouani S, Juillard P, Buser R, et al. A key role for CC chemokine receptor 4 in lipopolysaccharide-induced endotoxic shock. *J Exp Med* 2000;191:1755-64.
44. Inngjerdingen M, Damaj B, Maghazachi AA. Human NK cells express CC chemokine receptors 4 and 8 and respond to thymus and activation-regulated chemokine, macrophage-derived chemokine, and I-309. *J Immunol* 2000;164:4048-54.
45. Haque NS, Fallon JT, Taubman MB, Harpel PC. The chemokine receptor CCR8 mediates human endothelial cell chemotaxis induced by I-309 and Kaposi sarcoma herpesvirus-encoded vMIP-1 and by lipoprotein (a)-stimulated endothelial cell conditioned medium. *Blood* 2001;97:39-45.
46. Iellem A, Colantonio L, Bhakta S, Sozzani S, Mantovani A, Sinigaglia F, et al. Inhibition by IL-12 and IFN- α of I-309 and macrophage-derived chemokine production upon TCR triggering of human Th1 cells. *Eur J Immunol* 2000;30:1030-9.
47. Andrew DP, Chang MS, McNinch J, Wathen ST, Rihaneck M, Tseng J, et al. STCP-1 (MDC) CC chemokine acts specifically on chronically activated Th2 lymphocytes and is produced by monocytes on stimulation with Th2 cytokines IL-4 and IL-13. *J Immunol* 1998;161:5027-38.
48. Zingoni A, Soto H, Hedrick JA, Stoppacciaro A, Storlazzi CT, Sinigaglia F, et al. The chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells. *J Immunol* 1998;161:547-51.
49. Bochner BS, Hudson SA, Xiao HQ, Liu MC. Release of both CCR4-active and CXCR3-active chemokines during human allergic pulmonary late-phase reactions. *J Allergy Clin Immunol* 2003;112:930-4.
50. Panina-Bordignon P, Papi A, Mariani M, Di Lucia P, Casoni G, Belletato C, et al. The C-C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. *J Clin Invest* 2001;107:1357-64.
51. Hieshima K, Imai T, Baba M, Shoudai K, Ishizuka K, Nakagawa T, et al. A novel human CC chemokine PARC that is most homologous to macrophage-inflammatory protein-1 α /LD78 α and chemotactic for T lymphocytes, but not for monocytes. *J Immunol* 1997;159:1140-9.
52. Adema GJ, Hartgers F, Verstraten R, de Vries E, Marland G, Menon S, et al. A dendritic-cell-derived C-C chemokine that preferentially attracts naive T cells. *Nature* 1997;387:713-7.
53. Pardo A, Smith KM, Abrams J, Coffman R, Bustos M, McClanahan TK, et al. CCL18/DC-CK-1/PARC up-regulation in hypersensitivity pneumonitis. *J Leukoc Biol* 2001;70:610-6.
54. Reape TJ, Rayner K, Manning CD, Gee AN, Barnette MS, Burnand KG, et al. Expression and cellular localization of the CC chemokines PARC and ELC in human atherosclerotic plaques. *Am J Pathol* 1999;154:365-74.
55. Goebeler M, Trautmann A, Voss A, Brocker EV, Toksoy A, Gillitzer R. Differential and sequential expression of multiple chemokines during elicitation of allergic contact hypersensitivity. *Am J Pathol* 2001;158:431-40.
56. Gunther C, Bello-Fernandez C, Kopp T, Kund J, Carballido-Perrig N, Hinteregger S, et al. CCL18 is expressed in atopic dermatitis and mediates skin homing of human memory T cells. *J Immunol* 2005;174:1723-8.
57. Stenfors N, Nordenhall C, Salvi SS, Mudway I, Soderberg M, Blomberg A, et al. Different airway inflammatory responses in asthmatic and healthy humans exposed to diesel. *Eur Respir J* 2004;23:82-6.
58. Pourazar J, Frew AJ, Blomberg A, Helleday R, Kelly FJ, Wilson S, et al. Diesel exhaust exposure enhances the expression of IL-13 in the bronchial epithelium of healthy subjects. *Respir Med* 2004;98:821-5.
59. McKenzie GJ, Emson CL, Bell SE, Anderson S, Fallon P, Zurawski G, et al. Impaired development of Th2 cells in IL-13-deficient mice. *Immunology* 1998;94:23-32.