

RAPID COMMUNICATION

Diesel Exhaust-Associated Gas Components Enhance Chemokine Production by Cervical Lymph-Node Cells from Mice Immunized with Sugi Basic Proteins

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Previously, we showed that exposure to diesel exhaust (DE) increased inflammatory cells in the airway and cytokine production from local lymph-node cells after antigen stimulation. To clarify the role of particle-free diesel gas components in induction of allergic inflammation, we compared the effect of DE and gas components on pollen-antigen-stimulated chemokine production by cervical lymph nodes (CLN) cells in BALB/c mice. Groups of mice were exposed to 0 (control), 1.0 mg diesel exhaust particles (DEP)/m³ (DE), or filtered 1.0 mg DEP/m³ DE (gas) for 12 h daily for 5 wk. Each group of mice was injected intraperitoneally with sugi basic protein (SBP), a major allergen of Japanese cedar pollen, immediately before their exposure to DE or gas. On days 14 and 35, each mouse received an additional SBP intranasally. Exposure to DE or gas did not affect the lymphocyte subpopulations of CLN. Culture supernatants of CLN cells from DE-exposed, SBP-immunized mice had significantly increased levels of monocyte chemoattractant protein-1. Exposure to gas significantly increased the amount of thymus- and activation-regulated chemokine and macrophage inflammatory proteins-1 α in the CLN cells from SBP-immunized mice. These results suggest that Gas components as well as DEP may differentially regulate production of chemokines at local sites.

Airborne particulates can affect immune defense mechanisms. Diesel exhaust (DE), for example, induced hyperreactivity in the airway that resulted in the accumulation of inflammatory cells in the respiratory and chronic respiratory diseases (Nel et al., 1998; Salvi & Holgate, 1999). The intranasal instillation of diesel exhaust particles (DEP) and Japanese cedar pollen allergen enhanced antigen-specific immunoglobulin E (IgE) responses in mice (Takafuji et al., 1987) and increased

interleukin 4 (IL-4) production in murine cervical lymph node (CLN) cells, but interferon (IFN)- γ production was significantly decreased (Fujimaki et al., 1995). In humans, intranasal instillation of DEP increased nonspecific IgE antibody production in nasal lavage fluid and induced IgE isotype switching (Fujieda et al., 1998). When DEP were instilled with ragweed allergen in both allergic and normal volunteers, the allergic patients manifested increased allergen-specific IgE antibody and Th2-type cytokine production in cells collected from nasal lavage fluid (Diaz-Sanchez et al., 1997). DEP enhanced expression of CC chemokines in the cells collected from nasal lavage fluids following intranasal challenge (Diaz-Sanchez et al., 2000). These findings suggest that exposure to DE or DEP enhances the immune response to pollen allergens and induces or aggravates allergic rhinitis. However, the roles of gas components in the DE-associated enhancement of the allergic response at upper respiratory sites remain unclear.

In this study, we exposed BALB/c mice to DE or particle-free diesel gas (gas) and compared the functions and cellularity of CLNs.

MATERIALS AND METHODS

Male BALB/c mice (3 wk old) were purchased from Japan SLC, Inc. (Shizuoka, Japan); 4-wk-old mice were used in all experiments. Food and water were given ad libitum. Groups of mice ($n = 7$) were exposed to 0 (control), 1.0 mg DEP/m³ (DE), or filtered 1.0 mg DEP/m³ DE (gas) for 12 h daily for 5 wk. For particle-free gas inhalation, diluted DE was passed through HEPA filters, and 96% of the particulate matter was excluded before introduction of the fumes into the exposure chambers. Mean concentrations of major chamber components during experiments (35 d) were shown in Table 1. The mass median aerodynamic diameter of DEP is 0.4 μ m. The details on DE inhalation were previously described (Fujimaki et al., 1997). Each group of mice was injected intraperitoneally with sugi basic protein (SBP; presented by Dr. T. Endo), a major allergen of Japanese cedar pollen composed of Cry j I and Cry j II proteins, immediately before mouse exposure to DE or gas.

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TABLE 1
Major components of diesel exhaust

Group	SPM		NO _x (ppm)	NO ₂ (ppm)	NO (ppm)	SO ₂ (ppm)	CO ₂ (ppm)
	(cpm)	(mg/m ³)					
C	6.44 ± 0.04	0.01 ± 0.0001	<0.01	0	0	0	475.9 ± 6.4
DE	705.2 ± 3.2	1.01 ± 0.005	12.09 ± 0.15	1.99 ± 0.02	10.02 ± 0.12	0.18 ± 0.002	1769.2 ± 13.2
Gas	27.28 ± 0.30	0.04 ± 0.0004	11.93 ± 0.13	2.93 ± 0.06	8.91 ± 0.09	0.11 ± 0.003	1838.8 ± 15.3

On days 14 and 35, each mouse received an additional SBP intranasally.

One day after the final SBP challenge, mice were euthanized under ether anesthesia, and CLN samples were collected and cultured (Fujimaki et al., 1995). Culture supernatants were collected 24 and 48 h after SBP stimulation, centrifuged, and frozen at -70°C until assayed. We quantitated the amount of macrophage inflammatory proteins (MIP)-1 α , MIP-2, monocyte chemoattractant protein (MCP)-1, and thymus- and activation-regulated chemokine (TARC) in the supernatants from cultured CLN by using commercially available mouse enzyme-linked immunosorbent assays (ELISAs) (R&D Systems, Minneapolis, MN). We analyzed the immunophenotypes of the CLN cells from each group of mice by using flow cytometry (Fujimaki et al., 2001). Differences between group means were analyzed using analysis of variance (ANOVA) with a post hoc test (Bonferroni/Dunn). A p value of $<.05$ was considered to be significant.

RESULTS

CLN is a draining lymph node that plays an important role in the mucosal immune response to antigen invasion. The percentages of CD45R/B220⁺, CD45R/B220⁻, CD4⁺, CD8⁺, CD3⁺TCR- $\alpha\beta$ ⁺, and CD3⁺TCR- $\gamma\delta$ ⁺ cells in the CLN cells from DE- and gas-exposed mice were similar to controls (data not shown).

At 24 h after restimulation with SBP, culture supernatants of CLN cells from DE- and gas-exposed mice contained significantly more MCP-1 than SBP-stimulated controls (Figure 1a). Restimulation with SBP significantly increased MCP-1 production in culture supernatants of CLN cells from DE- and gas-exposed mice compared with each no-SBP group. Forty-eight hours after restimulation, MCP-1 production was significantly increased in the supernatants of CLN cells from all groups of mice. The production of MCP-1 was significantly increased in culture supernatants of CLN cells from SBP-restimulated, DE-exposed mice compared with the DE-exposed, no-SBP group, and the SBP-restimulated control group. A modest, early increase in the levels of MIP-1 α 24 h after restimulation with SBP was seen in the culture supernatant of CLN cells from gas-exposed mice (Figure 1b). Forty-eight hours after restimulation with SBP, MIP-1 α production was significantly increased

in the supernatants of CLN cells from gas-exposed mice compared with the gas-exposed no-SBP group. In no-SBP groups, exposure to gas markedly increased MIP-1 α production.

There are no significant differences in culture supernatants from exposed and control groups for TARC and MIP-2 levels 24 h after restimulation with SBP (data not shown). Forty-eight hours after restimulation with SBP, the production of TARC was significantly enhanced in the CLN culture supernatants from gas-exposed mice compared with the control group and gas-exposed, no-SBP group (Figure 2a). Restimulation with SBP significantly increased MIP-2 production in control group. However, MIP-2 production in the CLN culture supernatants from DE- or gas-exposed mice remained at control levels (Figure 2b).

DISCUSSION

In this study, exposure to DE significantly enhanced SBP-induced MCP-1 production in the culture supernatants of CLN cells. On the other hand, exposure to gas components significantly increased SBP-induced MIP-1 α and TARC. Increased production of chemokines by SBP stimulation was more prominent in gas components than in DE inhalation. These results suggest that gas components as well as DEP may differentially regulate production of chemokines at local sites.

We previously showed that intranasal instillation of DEP and antigen induced increased IL-4 production in murine CLN cells, but interferon (IFN)- γ production was significantly decreased (Fujimaki et al., 1995). This finding suggests that DEP modulate the Th1/Th2 cytokine balance to promote the production of Th2-type cytokines. Both MIP-1 α and MCP-1 are regulatory chemokines for Th1/Th2 balance by virtue of their abilities to enhance antigen-stimulated T-cell activation. MIP-1 α is a chemokine that is associated with Th1 cytokine responses, and MCP-1 is associated with Th2 cytokine responses (Gu et al., 2000). Thymus- and activation-regulated chemokine (TARC) facilitates the recruitment, activation, and development of Th2-polarized cells (Lieberman & Förster, 1999). MIP-1 α and MIP-1 β regulate T-lymphocyte migration from the circulation into lymph nodes during an immune response and contribute to the immunologic outcome (Tedla et al., 1998). Chemokines are believed to play an important role during T-cell priming by facilitating T-cell migration into and within lymph nodes and by

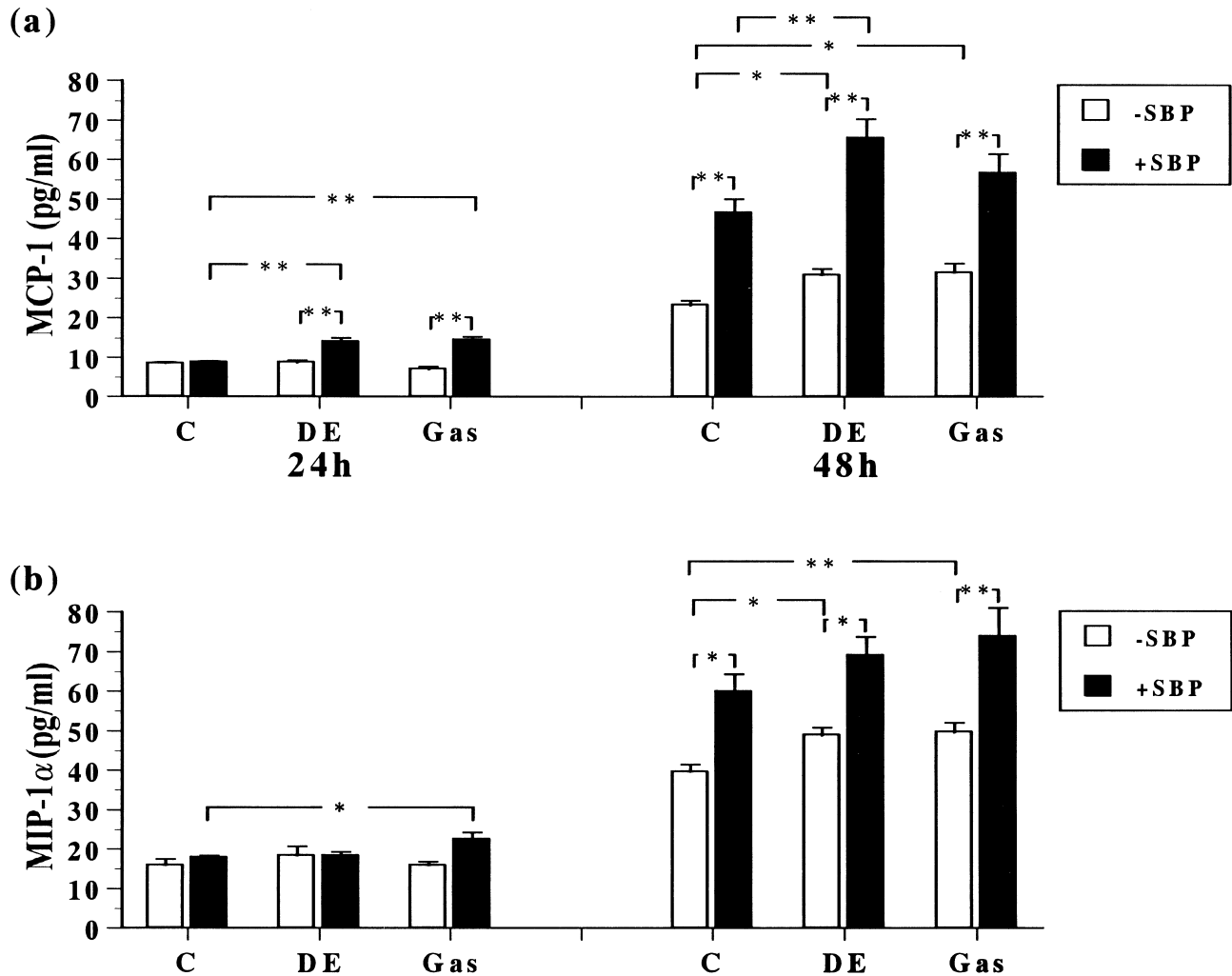


FIG. 1. (a) MCP-1 and (b) MIP-1 α production in culture supernatants of CLN cells from DE- or gas-exposed mice. CLN cells from DE-exposed (DE), gas-exposed (Gas), and control (C) mice were cultured for 24 h and 48 h in the presence (solid bars) or absence (open bars) of SBP; then the chemokine levels in the supernatants were determined by ELISA. Data are presented as the mean $\pm$ standard error ($n = 7$). Asterisk indicates significant difference at $p < .05$; double asterisk, $p < .01$.

regulating the balance of Th1/Th2 immune responses. In our study, the production of MCP-1 in culture supernatants 24 h after restimulation with SBP or 48 h after nonstimulation was significantly increased in both DE- and gas-exposed mice (Figure 1a), suggesting that DE-associated gas components cause these effects. Perhaps DEP or gas components cause a Th1/Th2 imbalance by affecting chemokine production in the local draining lymph nodes. We believe that the present study is the first analysis of the effects of diesel exhaust gas components inhalation on the production of chemokine in mice. Our study can provide a new insight necessary for clarifying environmental factors responsible for increased allergic rhinitis.

The mechanisms underlying gas components-associated immunomodulation remain unknown. Gas components consist of oxidant gases such as NO₂ and SO₂. The diffusibility and the behavior of inhaled airborne gases depend on their reactivity,

solubility, and vapor pressure. Gas components can directly affect the tissues of the respiratory tract or indirectly induce the release of cytokines and chemokines from impaired tissues and enhance the reactivity of local lymph nodes for antigenic stimulation. Farone et al. (1995) demonstrated the induction of KC and MIP-2 mRNA expression in rat trachea after acute exposure to SO₂. However, little is known about the effect of these gases on allergen-induced chemokine production. CLNs regulate the induction of immunologic tolerance in regional sites to prevent extra-immune responses to intranasally inhaled antigens (Wolvers et al., 1999). DE exposure may affect the function of CD8⁺ cells to modulate cytokine and chemokine production (Fujimaki et al., 2001). We speculated that gas components might suppress downregulation in immune response of CLN. Although $\gamma\delta$ T cells can negatively regulate airway responsiveness (Lahn et al., 1999), we found that the percentages of CD8⁺ and

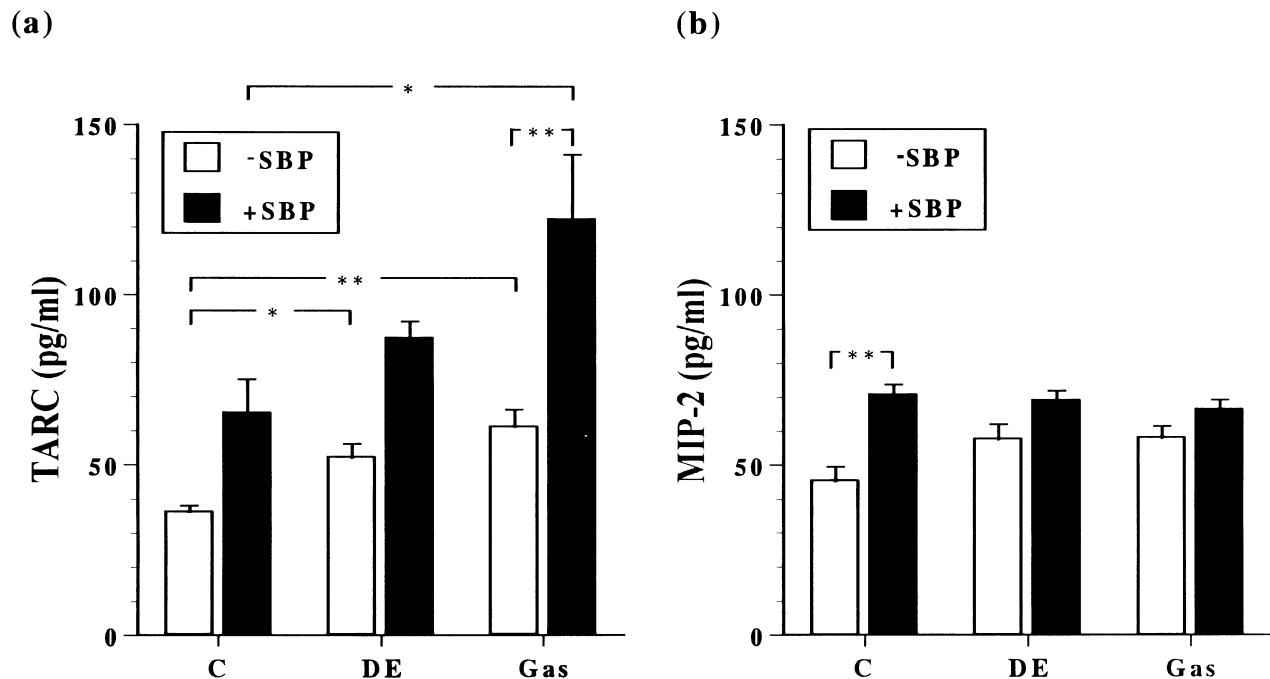


FIG. 2. (a) TARC and (b) MIP-2 production in culture supernatants of CLN cells from DE- or gas-exposed mice. CLN cells from DE-exposed (DE), gas-exposed (Gas), and control (C) mice were cultured for 48 h; then the chemokine levels in the supernatants were determined by ELISA. Data are presented as the mean $\pm$ standard error ($n = 7$). Asterisk indicates significant difference at $p < .05$; double asterisk, $p < .01$.

CD3⁺TCR- $\gamma\delta$ ⁺ in the CLN cells from DE-exposed mice did not differ from those of control or gas-exposed animals. Since nanoparticles may be found in gas components in DE inhalation (Tobias et al., 2001), further study is needed on the effect of each gaseous fraction on chemokine production from draining lymph nodes.

In summary, gas components as well as DEP in diesel exhaust enhance chemokine production from CLN in SBP-stimulated mice and may contribute to enhancement of allergic inflammation.

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