

# Chemical constituents of diesel exhaust particles induce IL-4 production and histamine release by human basophils

Gilles Devouassoux, MD,<sup>a</sup> Andrew Saxon, MD,<sup>b</sup> Dean D. Metcalfe, MD,<sup>c</sup>  
Calman Prussin, MD,<sup>c</sup> Maurice G. Colomb, MD,<sup>d</sup> Christian Brambilla, MD,<sup>a</sup> and  
David Diaz-Sanchez, PhD<sup>b</sup> *Los Angeles, Calif, Bethesda, Md, and Grenoble, France*

**Background:** An epidemiologic relationship between airway allergic diseases and exposure to atmospheric pollutants has been demonstrated and suggested to be one factor in the increasing prevalence of asthma. Diesel exhaust particles (DEPs) have been shown to participate in the development of allergic airway inflammation, in which the targets include macrophages, B and T cells, epithelial cells, and mast cells. In addition to the adjuvant effect of DEPs on total and allergen-specific IgE production, DEPs also act to induce chemokines and cytokines and may play a key role in primary sensitization. **Objective:** DEPs have been shown to increase local IL-4-containing Kit<sup>+</sup> cells soon after in vivo nasal challenge. The aim of this study was to examine the effects of DEPs on human basophils, a key source of IL-4.

**Methods:** Peripheral blood leukocytes from allergic and control subjects were cultured in the presence of organic extracts of DEP (DEPex) with or without allergen. The cultures were analyzed for IL-4-containing cells by using multiparameter flow cytometry, IL-4 secretion with ELISA, and histamine release. **Results:** Basophils, when exposed in vitro to DEPex, expressed IL-4 and released histamine significantly ( $P < .01$ ) more than with antigen activation. DEPex did not synergize with allergen in cytokine production and histamine release. DEPex-induced basophil IL-4 expression peaked at 2 hours and persisted through 20 hours, in contrast to allergen-induced IL-4, which was transient. The effect of DEPex on basophil cytokine expression and histamine release was dose dependent and occurred with cells from both allergic and nonallergic subjects. DEPex induced IL-4 expression and histamine release in high-

ly enriched basophil populations, suggesting it acts directly on basophils. Other peripheral blood leukocytes, including T cells, did not contribute to this cytokine expression. Preincubation with N-acetylcysteine completely abrogated DEPex-driven basophil IL-4 expression.

**Conclusions:** Basophils are a direct target for DEPex, inducing IL-4 expression and histamine release in an IgE-allergen independent fashion. N-acetylcysteine inhibition of DEPex-driven IL-4 expression provides evidence that generation of reactive oxygen species is required for the effects observed. The capability of DEPex to activate basophils in both allergic and non-allergic subjects suggests a potential role of this pollutant in the increasing prevalence of allergic diseases. (*J Allergy Clin Immunol* 2002;109:847-53.)

**Key words:** Basophil, IL-4, histamine, diesel exhaust particles, allergy, pollution

Epidemiologic studies have linked the increased incidence of allergic diseases in part to an increase in exposure to atmospheric pollutants.<sup>1-3</sup> The effect of diesel exhaust particles (DEPs) on the pathophysiology of allergic airway disease has been extensively analyzed in both animals and human subjects by using in vitro and in vivo models.<sup>4-8</sup> These data have clearly identified a relationship between DEP challenge and activation of an IgE-dependent allergic inflammatory response. Particular attention has been given to the effects of DEPs and their major organic components.<sup>9-11</sup>

DEPs, which are major components of atmospheric pollutants, are primarily composed of small particles having a diameter of less than 2.5  $\mu\text{m}$ . These accumulate and are retained in lung-associated lymph nodes,<sup>12,13</sup> allowing interactions with a wide range of cells involved in the immune response, including macrophages, epithelial cells, mast cells, and B and T cells.<sup>10,14-19</sup> DEPs have been shown in vivo in human subjects to act as an adjuvant, enhancing IgE production and T<sub>H</sub>2 cytokine production in response to common allergens<sup>10,15,20</sup> and exacerbating local mucosa inflammation.<sup>5,6,18,21</sup> DEP exposure has also been shown to be capable of driving sensitization to a neoantigen toward a T<sub>H</sub>2 response.<sup>22</sup> After mucosa exposure to DEPs, rapid enhancement of local IL-4 production has been observed in vivo, and this early IL-4 has been postulated to play a key role in the T<sub>H</sub>2 adjuvant effects of DEPs.<sup>16</sup>

We have demonstrated that basophils account for the majority of IL-4-producing cells in peripheral blood from allergic subjects after specific antigen activation in vitro. Cytokine production by basophils peaked at 2

From <sup>a</sup>the Department of Respiratory Medicine, and INSERM EMI 9924, and <sup>b</sup>the Division of Clinical Immunology, Department of Medicine, University of California, Los Angeles; <sup>c</sup>the Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda; and <sup>d</sup>the Department of Immunology, Hôpital A. Michallon, CHU de Grenoble, and Institut A. Bonniot, Université J. Fourrier, Grenoble.

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Reprint requests: Gilles Devouassoux, MD, Department of Respiratory Medicine, DMAS, Hôpital A. Michallon, CHU de Grenoble, BP 217, 38043 Grenoble Cedex 09, France.

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**Abbreviations used**

DEP: Diesel exhaust particle  
 DEPex: Organic extracts of diesel exhaust particles  
 DMSO: Dimethyl sulfoxide  
 NAC: N-acetylcysteine

hours, providing evidence for basophil involvement in initiation and amplification of the specific IgE-dependent allergic inflammatory response.<sup>23</sup> Although an increase in IL-4-containing Kit<sup>+</sup> tissue mast cells has been reported after DEP-associated antigen nasal challenge in human subjects,<sup>16</sup> the potential effects of DEPs on cytokine production and histamine release from human basophils remain unknown. Blood mononuclear cells from allergic and nonallergic subjects were incubated with the organic extract of DEPs (DEPex) to address this issue. The resulting IL-4 production and histamine release were analyzed. Additionally, we investigated the effect of antigen plus DEPex on both cytokine production and histamine release. Finally, because the cellular redox state is involved in macrophage cytokine production related to DEPs,<sup>24</sup> we tested the effect of a thiol-reducing agent, N-acetylcysteine (NAC), on DEPex-induced basophil IL-4 production. The results show that the chemicals present in DEPs can directly induce basophils to produce IL-4, and this can be inhibited by the antioxidant NAC.

**METHODS****Reagents and mAbs**

Ionomycin, brefeldin A, paraformaldehyde, propidium iodide, Histopaque 1083, dimethyl sulfoxide (DMSO), and NAC (Sigma Chemical Co); saponin (Fluka Biochemika); and standardized cat hair (10,000 AU/mL) and *Dermatophagoides pteronyssinus* (30,000 AU/mL) allergenic extracts (Hollister-Stier Laboratories) were purchased.

DEPs were collected from a light-duty diesel source<sup>25</sup> and were kindly provided by Dr Marasu Sagai (National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan). DEPex was prepared as previously described.<sup>26</sup> Briefly, 100 mg of DEPs was suspended in 50 mL of methanol and vortexed, sonicated for 2 minutes on ice, and centrifuged for 10 minutes at 2500 rpm. After methanol evaporation under nitrogen gas, the tubes were reweighed to determine the extracted amount of DEPs. The methanol-extractable component comprised 36% of the dry particle weight. The dried extracts were resuspended in DMSO to provide the stock concentration of 100 µg/mL and stored in the dark at -20°C until use. For control experiments, DMSO was added to the cell culture in the absence of DEPex. The DEP extract, rather than the DEPs themselves, was used for in vitro experiments because the presence of particulates in DEPs in vitro was believed to be a marked departure from what cells are exposed to in vivo, and pyrene, a major organic constituent of DEPs, elicits DEP-like effects in vivo.<sup>27</sup>

Phycoerythrin/cyanin 5-conjugated mouse anti-CD14, anti-CD16 (Caltag Laboratories), anti-CD2, anti-CD19, phycoerythrin-conjugated rat anti-IL-4 (clone MP4-25D2), allophycocyanin-conjugated streptavidin, phycoerythrin-conjugated rat anti-IgG1 (Pharmingen), and biotin-conjugated goat anti-human IgE (Biosource) were obtained commercially.

**Subjects**

Patients had a history of allergic disease and had 3 or more positive skin test responses (>5 mm wheal) to a panel of 8 extracts (cat, elm, ragweed, *Dermatophagoides farinae*, *D pteronyssinus*, cockroach, dog, and rye). Control subjects had no history of allergic disease and did not have skin test responses to the antigen panel.

**Cell culture**

PBMCs were obtained from EDTA-anticoagulated whole blood after separation on a Histopaque gradient at a density of 1.083 g/mL. PBMCs were suspended in RPMI-1640 (Life Technologies) supplemented with 10% FCS, 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 U/mL streptomycin, 25 µg/mL gentamicin, 50 µmol/L 2-mercaptoethanol, 1 µmol/L pyruvate, and nonessential amino acids (Biofluids). PBMCs were incubated at  $2 \times 10^6$  cells/mL in 24-well plates (Costar) at 37°C in the presence of 10 µg/mL brefeldin A. Similarly, purified basophils were incubated with allergen extract (10 biologic allergic units/mL), DEPex (1 µg/mL), or both or ionomycin (1 µmol/L) for 4 hours. Then cells were fixed in 4% paraformaldehyde at room temperature for 5 minutes, washed, and stored in 10% DMSO at -80°C. Where indicated, PBMCs were preincubated with NAC (10 mmol/L) for 1 hour at 37°C.

**Basophil purification**

PBMCs were separated over a discontinuous Percoll gradient (1.080 and 1.069 g/cm<sup>3</sup>) at 400g for 25 minutes at room temperature. The basophil-enriched interface was harvested, washed twice, and further purified by means of negative selection with antibody-coated magnetic beads (anti-CD2, anti-CD7, anti-CD15, anti-CD16, anti-CD36, anti-CD45RO, and anti-HLA-DR; Miltenyi Biotec). The final cell suspension consisted of 20% to 80% basophils, as assessed by means of flow cytometry.

**Flow cytometry**

Cells were stained for cell-surface markers and intracellular cytokines, as previously described.<sup>28</sup> Briefly, the cell pellet were resuspended in PBS with 0.1% saponin and 5% nonfat dry milk for 1 hour to block nonspecific binding. They were then stained with streptavidin allophycocyanin for an additional 30 minutes, washed, resuspended in PBS-BSA 0.1%, and analyzed.

Data were acquired with a 2-laser FACSCALIBUR flow cytometer (Becton Dickinson Immunocytometry Systems), which allows for the simultaneous detection of 4 fluorescent parameters. Typically, 300,000 to 500,000 total events were acquired. List mode files were analyzed with Cellquest software (Becton Dickinson Immunocytometry Systems).

The basophil population was identified with both exclusion (anti-CD2, anti-CD14, anti-CD16, anti-CD19 mAbs [lineage]) and inclusion (anti-IgE) cell-surface markers, as previously described.<sup>23</sup> Basophil intracytoplasmic IL-4 expression was measured by gating on the IgE<sup>high</sup>, lineage<sup>low</sup> cell population.<sup>23</sup> Propidium iodide suspended in PBS was added to the final concentration of 0.5 µg/mL for 5 minutes to evaluate basophil survival.

**Measurement of IL-4 and histamine release**

After purification, basophils were cultured in 96-well plates (Costar) at a density of 500,000 cells/well, with or without DEPex (1 µg/mL) and in the absence of brefeldin A, for 4 hours at 37°C. Cells were then harvested and centrifuged. The supernatant was removed and stored at -80°C. Cell-free supernatants were then assayed for either IL-4 by means of ELISA (R&D Systems) or for histamine (Immunotech), with a sensitivity of 10 pg/mL and 0.5 mmol/L, respectively.

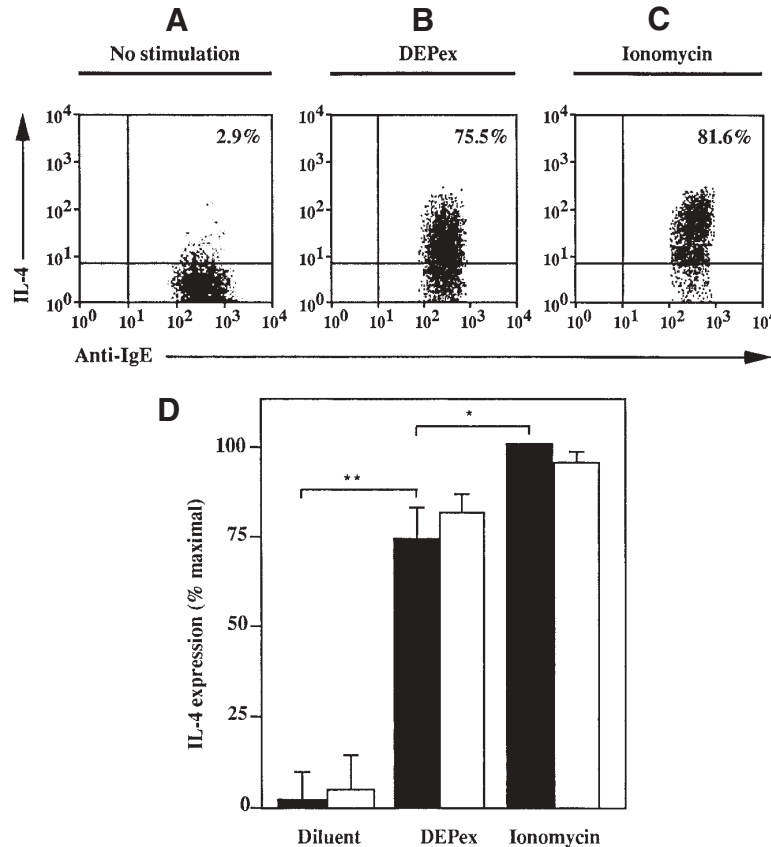


**FIG 1.** DEPex induces basophil IL-4 expression. PBMCs were incubated in the absence (A) or presence (B) of DEPex (1  $\mu$ g/mL) or ionomycin (1  $\mu$ mol/L) (C) for 4 hours; fixed; stained with antibodies to IgE, lineage markers, and IL-4; and analyzed by means of flow cytometry. After gating on IgE<sup>high</sup>, lineage<sup>low</sup> basophils, a cytokine versus IgE dot plot was generated. Statistical markers were placed on the basis of isotype-matched control staining. The percentages given in each panel (A, B, and C) represent the percentages of basophils producing IL-4. D, Means  $\pm$  SD of 5 allergic donors (filled column) and 4 nonallergic donors (open column) are shown. Results are expressed as the percentage of maximal IL-4 production. The mean maximal IL-4 expression (100%) was 82%. Statistically significant differences are noted: \* $P$  < .05 and \*\* $P$  < .01.

## Statistical analysis

Data are expressed as the means  $\pm$  SD. The paired  $t$  test was performed to compare intrasubject differences of cytokine expression or histamine release. A  $P$  value of less than .05 was considered significant.

## RESULTS

### DEPex induces basophil IL-4 production

When PBMCs from allergic patients were exposed to DEPex (1  $\mu$ g/mL), the majority of basophils expressed IL-4 (range, 68.5%-80.7%; mean  $\pm$  SD, 73.9%  $\pm$  5%;  $n$  = 5; Fig 1). This IL-4 expression was similar to the level seen after ionomycin activation. In the absence of activation, basophils expressed minimal IL-4. Similar results were observed with PBMCs from nonallergic subjects (Fig 1, D). Thus DEPex induces IL-4 expression in human basophils to a degree similar to that resulting from ionophore stimulation.

PBMCs were cultured with DEPex over a wide concentration range to further characterize the effect of DEPex on basophil IL-4 expression (0-2  $\mu$ g/mL). Fig 2 shows that DEPex increased IL-4 expression in basophils in a con-

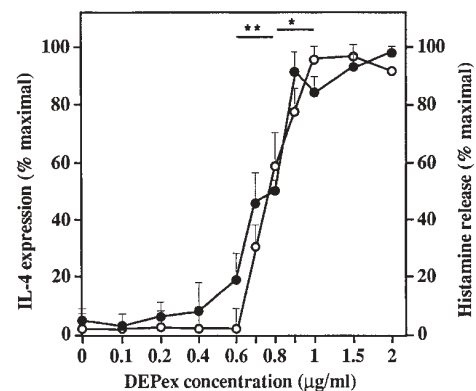


**FIG 2.** Concentration dependence of DEPex activity. PBMCs and enriched basophils were incubated with DEPex (0-2  $\mu$ g/mL) to evaluate IL-4 expression (open circles) by means of flow cytometry and degranulation by means of histamine assay (filled circles). Means  $\pm$  SD of 6 and 5 independent experiments are shown for IL-4 expression and histamine release, respectively. Results are expressed as the percentages of maximal basophil IL-4 expression and histamine release. The mean maximal IL-4 expression and histamine release were 75.1% and 27 mmol/L, respectively. Statistically significant differences are noted: \* $P$  < .05 and \*\* $P$  < .01.

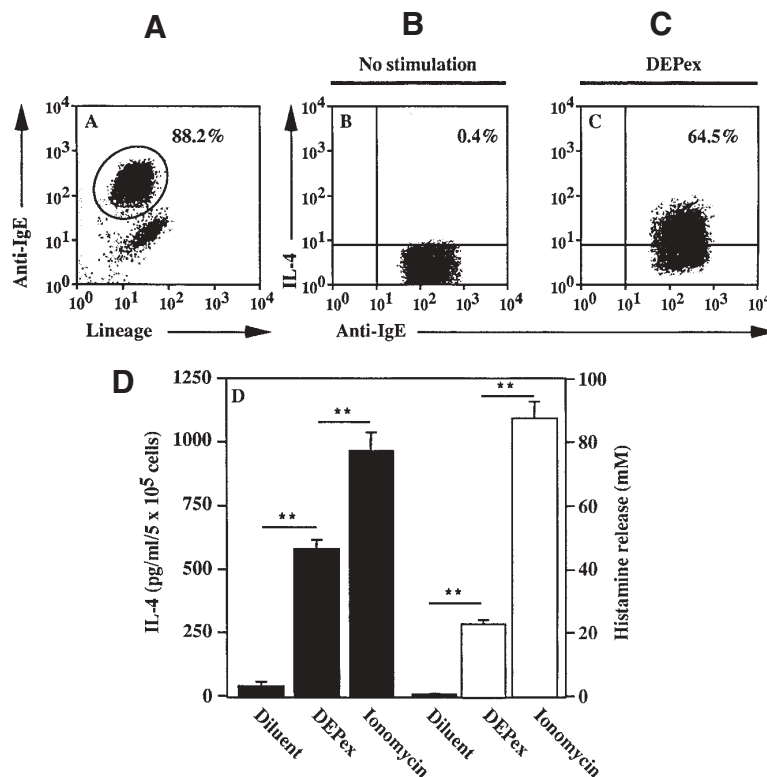


**FIG 3.** Basophils are a target of DEPex. Enriched basophils (**A**) were incubated with or without DEPex (1  $\mu$ g/mL; **B** and **C**) for 4 hours. Purity and frequency of IL-4-producing basophils were determined by means of flow cytometry. The percentage of basophil purity is given in **A** (circle), and percentages in **B** and **C** represent the percentages of basophils producing IL-4. Similar data were obtained in 4 independent experiments. Basophil IL-4 (filled column) and histamine (open column) releases into the supernatant were analyzed by means of ELISA and histamine assay, respectively (mean  $\pm$  SD,  $n = 4$ , **D**). Each experiment was performed in duplicate. Statistically significant differences are noted: \*\* $P < .01$ .

centration-dependent manner. A significant increase was observed when DEPex was present at 0.8  $\mu$ g/mL. The maximum response was observed at 1  $\mu$ g/mL.

Populations of enriched basophils were tested (Fig 3) to help establish the basophil as the cell directly responsive to DEPex. The frequency of IL-4<sup>+</sup> basophils ranged from 20.3% to 75.5% (mean  $\pm$  SD, 51.1%  $\pm$  24%) after DEPex exposure (Fig 3, C), whereas virtually no cells were positive when cultured with diluent alone. This increase in the number of IL-4<sup>+</sup> basophils was also reflected in IL-4 protein production (Fig 3, D). Exposure of enriched basophil populations to DEPex (1  $\mu$ g/mL) resulted in markedly elevated levels of secreted IL-4 (576  $\pm$  40.4 pg/mL) comparable with those seen after ionomycin stimulation (965.6  $\pm$  77.5 pg/mL) and significantly higher than those observed with the diluent controls (34.6  $\pm$  19.1 pg/mL,  $P < .01$ ).

#### DEPex induces basophils to degranulate

In parallel experiments enriched basophils were cultured with a broad range of DEPex concentrations (0-2  $\mu$ g/mL) for 20 minutes to investigate the effect of DEPex on histamine release. Fig 2 shows that histamine is secreted in a concentration-dependent manner.

Exposure of enriched basophils to ionomycin (1  $\mu$ mol/L) induced a high release of histamine (87.2  $\pm$  6.8 mmol/L) that was significantly higher than those observed after either DEPex activation (1  $\mu$ g/mL; 22.6  $\pm$  1.5 mmol/L;  $P < .01$ ) or with the diluent controls (0.5  $\pm$  0.4 mmol/L;  $P < .01$ ; Fig 3, D). Finally, DEPex induced a similar release of histamine in allergic and nonallergic subjects (data not shown).

#### DEPex does not synergize with allergen to increase basophil IL-4 expression and histamine release

Basophil IL-4 expression and histamine release were analyzed after allergen exposure, DEPex exposure, or both to investigate possible synergy between DEPex and allergen-IgE basophil activation. As expected, allergen activation induced 5% to 20% of basophils to produce IL-4 and to release histamine (5.5  $\pm$  2.6 mmol/L, Fig 4). After culture with both DEPex and allergen, basophil IL-4 frequency and histamine release also increased (77.5%  $\pm$  8.8% and 24.8  $\pm$  3.3 mmol/L, respectively) but was no higher than that seen after stimulation with DEPex alone (73.5%  $\pm$  5.2% and 22.6  $\pm$  1.5 mmol/L, respectively). In

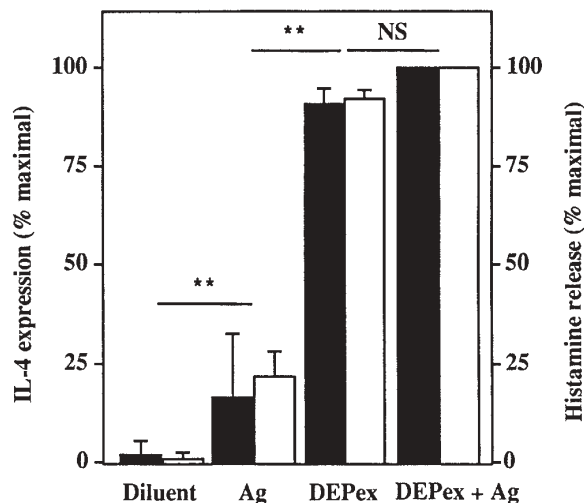


**FIG 4.** DEPex does not synergize with antigen to increase basophil IL-4 production and histamine release. PBMCs from allergic subjects were incubated with media, antigen, DEPex (1  $\mu$ g/mL), or antigen plus DEPex. IL-4 production was analyzed by means of flow cytometry (filled column) and histamine release by means of histamine assay (open column). The mean  $\pm$  SD of 4 independent experiments is shown, and results are expressed as the percentage of maximal IL-4 expression. The mean maximal IL-4 expression and histamine release were 77.5% and 24.8 mmol/L, respectively. Statistically significant differences are noted: \*\* $P$  < .01. NS, Not significant; Ag, antigen.

addition, no synergistic effect on IL-4 expression was observed when basophils were costimulated with antigen plus suboptimal doses of DEPex (range, 0.1–0.8  $\mu$ g/mL), which were unable, by themselves, to induce IL-4 production. Similarly, no significant difference was noted in IL-4 protein secretion after stimulation with DEPex alone or allergen plus DEPex (data not shown).

### Kinetics of IL-4 production

Previously, we reported that basophil activation by specific allergen led to early and transient IL-4 expression in allergic subjects only.<sup>23</sup> PBMCs from allergic subjects were cultured with DEPex for 2, 4, 8, or 20 hours to analyze the kinetics of DEPex-induced IL-4 expression. In contrast to allergen-induced IL-4 expression, which was transient, DEP-driven IL-4 expression peaked at 2 hours and was sustained throughout the 20-hour culture ( $n = 3$ , Fig 5). Similar time kinetics were noted with cells from nonallergic subjects (data not shown). Neither antigen nor DEPex altered basophil survival when compared with that observed in the absence of activation (data not shown). Little if any IL-4 was expressed in nonbasophil cells, as assessed by gating on the IgE<sup>low</sup>, lin<sup>high</sup> population (data not shown).

### Effect of DEPex on basophils is abrogated by NAC

Because reactive oxygen species have been implicated in DEP-induced cytokine expression in other cell types, we examined the effect of the thiol-reducing agent NAC

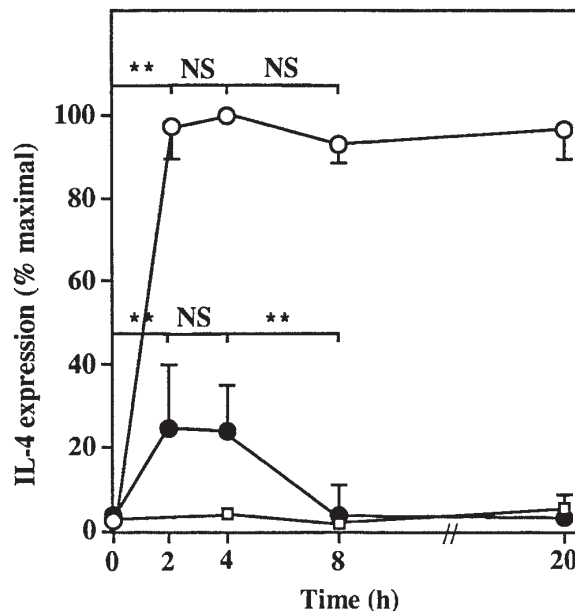


**FIG 5.** Kinetics of cytokine expression by means of DEPex-stimulated basophils. PBMCs from allergic subjects were stimulated in vitro with media (open squares), antigen (closed circles), or DEPex (open circles) for 0 to 20 hours at 37°C; fixed; stained for IgE, lineage, and IL-4; and analyzed by means of flow cytometry. Means  $\pm$  SD of 3 independent experiments with 3 donors are shown. Results are expressed as the percentage of maximal IL-4 expression. The mean maximal IL-4 expression was 77%. Statistically significant differences are noted: \*\* $P$  < .01. NS, Not significant; Ag, antigen.

on basophil IL-4 expression. PBMCs from 5 subjects were preincubated with or without NAC before DEPex exposure. DEPex alone induced  $65\% \pm 11\%$  of basophils to express IL-4. However, when the cells were preincubated with NAC (10 mmol/L), only 1.8% of basophils stained positively for IL-4 ( $1.8\% \pm 1.5\%$ ,  $P$  < .01, Fig 6). This inhibitory effect of NAC preincubation occurred in a dose-dependent fashion (data not shown). In contrast, neither ionomycin- nor allergen-driven basophil IL-4 expression was modified by NAC preincubation.

### DISCUSSION

In human subjects studies have demonstrated that DEPs enhance allergen-specific IgE production, which is followed by an increase of chemokine production and an alteration of cytokine production toward a T<sub>H</sub>2-like profile.<sup>4,9</sup> A key feature in those experiments was the early production (4–6 hours) of IL-4 from local mucosa (CD117<sup>+</sup>) mast cells.<sup>9,16</sup> IL-4, in addition to providing a necessary signal for IgE isotype switching, is required for the differentiation of naive T cells in T<sub>H</sub>2 cells.<sup>29</sup>

Basophils are a major source of early IL-4 production in allergic patients and may be involved in the initiation of allergic inflammation.<sup>23</sup> In the present study we thus sought to determine whether DEPex has a direct effect on human basophils or synergizes with allergen in stimulating basophil IL-4 production. For these in vitro experi-

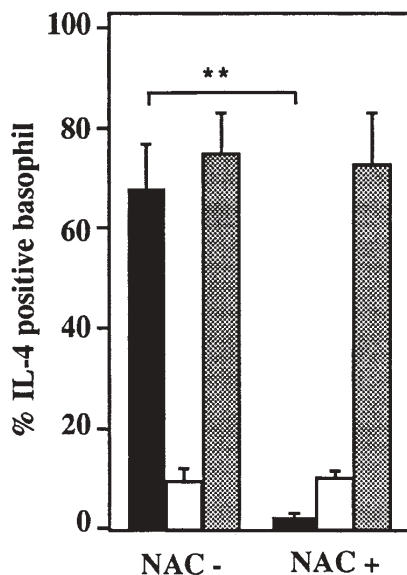


**FIG 6.** NAC abrogates IL-4 expression on DEPex-activated basophils. PBMCs were preincubated with media or NAC (10 mmol/L) for 1 hour at 37°C and then activated with DEPex (1000 ng/mL, filled column), antigen (open column), or ionomycin (shaded column) for 4 hours. IL-4 production was analyzed by means of flow cytometry, and the frequency of IL-4<sup>+</sup> basophils was determined. Means  $\pm$  SD of 5 independent experiments are shown, and results are expressed as the percentage of basophil IL-4 expression. Statistically significant differences are noted: \*\* $P < .01$ .

ments, we prepared and used DEPex rather than the DEPs themselves because use of particulate matter in vitro cultures is technically problematic.

Our data demonstrate that DEPex markedly induces basophil IL-4 production from both allergic and nonallergic subjects. This effect of DEPex occurs in a dose-dependent manner (Fig 1 and 2). Similar frequencies of IL-4<sup>+</sup> cells were observed among the IgE<sup>high</sup>, lineage<sup>low</sup> cells when both PBMCs and enriched basophil populations were stimulated with DEPex, suggesting that DEPex acts directly on basophils (Fig 3). Furthermore, gating on nonbasophil cells showed little if any IL-4 immunostaining, verifying the basophil as the source of the DEPex-induced IL-4.

To address IL-4 secretion, which is not examined by means of flow cytometry, we analyzed IL-4 production with purified basophils by using ELISA. Results of IL-4 secretion were consistent with the flow data, demonstrating marked release of IL-4 into the supernatant of DEPex-activated basophils (Fig 3). These data reinforce the biologic relevance of DEPex stimulation on basophil cytokine production.

In this study we have demonstrated that DEPex is capable of inducing basophil cytokine production in an allergen-independent fashion. The levels achieved were far greater than with allergen and were in the range observed for ionomycin stimulation. This occurred equally with cells from allergic and nonallergic donors. We tested basophils from nonatopic, as well as allergic,

subjects for synergy between DEPex and allergen because basophils from each subject group have differing activation requirements.<sup>23</sup> Synergy was not observed in basophils from either donor source (Fig 4).

These results demonstrating that DEPex is able to efficiently initiate IgE-independent basophil cytokine production indicate the presence of mechanisms by which DEP exposure may polarize the adaptive immune response toward a more T<sub>H</sub>2-like outcome. This would account for the ability of DEPs to drive sensitization to a neoantigen, as observed on nasal coadministration with keyhole limpet hemocyanin.<sup>22</sup>

Synergy of antigen and DEPs on cytokine production has been observed after in vivo nasal or bronchial challenges. In such studies the cell types involved in cytokine release were either not clearly defined<sup>9,10,30</sup> or their surface phenotypes were consistent with mast cells.<sup>16</sup> These in vivo results may be representative of the complex cellular milieu, where DEPs could interact with multiple cell types. Finally, in vivo challenges were performed with DEPs instead of organic extracts and used a total dose of 0.3 mg of DEPs, which is difficult to compare with the concentrations used for the in vitro experiments. Overall, divergent experimental conditions might explain the absence of the synergistic effect of organic components of DEPs and allergen on in vitro basophil IL-4 production.

The kinetics of DEPex-induced basophil IL-4 production provides additional evidence for a cytokine production pathway distinct from that induced through FcεRI by allergen. Specific allergen activation led to a rapid basophil IL-4 production that peaked at 2 hours and then quickly declined.<sup>23</sup> In contrast, DEPs rapidly induced IL-4 at 2 hours and was then sustained at peak levels out to 20 hours (Fig 5). DEPex stimulation of an early and persistent basophil IL-4 response may thus be relevant in maintaining and exacerbating the allergic inflammatory response.

In addition to the effects of DEPex on cytokine production, our results demonstrate that DEPex strongly induced basophils to degranulate (Figs 2 and 3). A similar effect of DEPex on mast cells has been previously described.<sup>31</sup> Taken together, these data suggest an additional role of DEPex on allergic response by enhancing the immediate-phase response. However, in contrast to the IgE-dependent histamine release from DEPex-activated mast cells,<sup>31</sup> our results demonstrate an IgE-independent effect of DEPex on basophil degranulation, suggesting the presence of different mechanisms of cell activation.

Organic chemical compounds contained in DEPs generate reactive oxygen species in both macrophages and epithelial cells,<sup>27,32</sup> with the resulting transcription of cytokine genes.<sup>33,34</sup> DEPex contains bifunctional inducers (polycyclic aromatic and halogenated hydrocarbons) that lead to the production of reactive oxygen species through the induction of cytochrome P450A1<sup>35</sup> and monofunctional inducers (quinones or derivatives) that can directly drive oxidative stress.<sup>36</sup> However, involvement of such metabolic pathways in other cell types remains speculative. In the current study we showed that preincubation of PBMCs with NAC, a thiol-reducing

agent, completely abrogated DEPex-induced cytokine production in basophils (Fig 6). These data indicate that DEP-mediated reactive oxygen species generation is involved in peripheral blood basophil induction of cytokine gene transcription. Furthermore, the dose-response effect of DEPex on basophil IL-4 expression (Fig 2) suggests that reactive oxygen species are also generated in a dose-dependent manner.

In summary, we have shown that basophils are a target for DEP effects through exposure to the chemical constituents in DEPs. We demonstrated that DEPex stimulated basophil IL-4 production and histamine release, regardless of the allergic status of the subjects. DEPex was able to induce allergen-independent IL-4 expression and histamine release. The effect of DEPex on cytokine production was mediated through oxidant stress because it could be completely blocked with NAC. In vivo, induction of tissue basophils by chemicals found in DEPs may participate in the critical early appearance of IL-4 that leads to the enhanced  $T_H2$ -like responsiveness characteristic of the DEP adjuvant effect.

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