

Enhanced nasal cytokine production in human beings after in vivo challenge with diesel exhaust particles

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Background: Diesel exhaust particles (DEPs) have been implicated in the worldwide increased incidence of allergic airway disorders over the past century. They can enhance in vivo IgE production in the human upper respiratory mucosa.

Objective: The study was carried out to determine whether DEPs can alter the production of cytokines by cells residing in the nasal mucosa.

Methods: Eighteen hours after intranasal challenge with saline solution or DEPs, we studied the levels of messenger RNA for cytokines in nasal lavage cells and the number of subjects in whom cytokine mRNA could be detected.

Results: Before challenge, most subjects' nasal lavage cells had detectable levels of only interferon- γ , IL-2, and IL-13 mRNA. After challenge, the cells produced readily detectable mRNA for IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, and interferon- γ . In addition, the levels of all cytokine mRNA increased. Enhanced IL-4 protein was also present in the postchallenge lavage fluid. Although the cells in nasal lavage before and after challenge do not necessarily represent the same cells either in number or type, the broad increase in cytokine production was not simply the result of an increase in T cells recovered in the lavage fluid.

Conclusion: An increase in nasal cytokine expression after exposure to DEPs can be predicted to contribute to enhanced local IgE production and thus play a role in the increased incidence of respiratory allergic disease. (*J Allergy Clin Immunol* 1996;98:114-23.)

Key words: Human IgE, pollution, cytokines, mucosal immunity, allergy

A variety of factors may be involved in the increased incidence of airway allergic disease in recent years. Changes in allergen exposure and

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Abbreviations used

DEPs:	Diesel exhaust particles
IFN:	Interferon
PAH-DEP:	Polyaromatic hydrocarbons in diesel exhaust particles
PAHs:	Polyaromatic hydrocarbons
PBMCs:	Peripheral blood mononuclear cells
PCR:	Polymerase chain reaction
RT-PCR:	Reverse transcription-polymerase chain reaction

increased allergen loads have been suggested as possible explanations, but although localized epidemics of allergen exposure have been recorded, it is unlikely that new or higher concentrations of allergens are responsible for the global increases.¹ Epidemiologic links between atmospheric pollutants and the increase in the incidence and morbidity of allergic airway disease are now well established.¹⁻⁵ Major components of inhaled pollution in

industrialized cities are diesel exhaust particles (DEPs) and their associated polyaromatic hydrocarbons (PAHs). We have previously directly addressed the effects of DEPs and PAHs on the human allergic responses IgE both in vitro and in vivo.^{6,7} We showed that PAH-DEP could enhance IgE production in vitro and preferentially upregulate production of certain messenger RNAs coding for distinct isoforms of the ϵ chain.⁶ We also showed that DEPs could induce a heightened IgE response in the human upper airway after intranasal challenge. The action of DEPs was surprisingly isotype-specific. Thus although IgE and IgG₄ levels were increased by nasal challenge with DEPs, the levels of IgA, IgM, and total IgG and IgG₃ remained unchanged. This in vivo increase in IgE production was associated with a markedly increased number of IgE- but not IgA-secreting cells and altered production of ϵ mRNA isoforms. DEPs and PAHs were therefore capable of altering IgE production both qualitatively and quantitatively in vivo and in vitro, respectively.

In previous studies, we focused on direct effects on B cells. However, DEPs and their chemical constituents may be expected to interact with and affect T cells, as well as monocytes or macrophages and even epithelial cells.⁶⁻⁹ We have now examined the changes in local cytokine production that accompany the enhanced local IgE response. We have compared cells from different sources to determine the role of the local environment in the observed changes. We show that in vivo nasal challenge with DEPs increased cytokine production from cells residing in the nasal mucosa, whereas in vitro stimulation of cells recovered from nasal lavage fluid with DEP or stimulation of peripheral blood mononuclear cells (PBMCs) with PAH-DEP did not increase cytokine mRNA production. These results show that DEPs affect non-B cells in a fashion that is likely to contribute to enhanced nasal IgE production and imply that such cells in the local tissues may be critical for the outcome of exposure to DEPs.

METHODS

Subjects

Fourteen healthy, nonsmoking volunteers (eight men and six women), ranging in age from 23 to 48 years, were recruited for this study. Eight had no history of asthma, allergic rhinitis, or any allergic or chronic respiratory illness. Six had a history of seasonal allergies but were free of symptoms and did not take any medication 3 days before or at any time during the study. All atopic subjects were outside their allergy season. None of the

volunteers had previously had any known extensive or extraordinary exposure to pollutants. All studies were approved by the Human Subject Protection Committee of the University of California at Los Angeles.

Nasal washes and DEP provocation

Nasal washes and provocation challenges were performed as previously described.⁷ Briefly, 5 ml of normal saline solution was delivered into each nostril of each subject, and after 10 seconds, the wash fluid was collected. The initial wash was discarded. The subjects then performed four subsequent nasal washes at 10-minute intervals, and each wash was collected in a separate tube. The tubes were centrifuged at 350 g for 10 minutes at 4° C, and the aqueous supernatants were separated from the cell pellets. Ten minutes after the last wash, 0 or 0.15 mg of DEPs suspended in 200 μ l of saline solution was sprayed into each nostril. The doses of DEPs were chosen according to previous reports⁷ in which we had titrated the amount of DEPs administered to volunteers and examined the amount of immunoglobulins present in nasal washes. Previous reports have shown that persons are exposed to a similar dose (0.2 mg) in a period of a day in Los Angeles¹⁰; additionally, sitting behind a bus will expose a person to a similar amount, but in one dose, and therefore simulate the bolus effect seen in this study.

DEPs were obtained from light-duty diesel passenger cars and processed as previously described.^{7,11} Nasal washes were performed immediately before and 18 hours after challenge. The period of 18 hours was chosen because maximal expression for cytokines was observed to occur between 18 and 24 hours (data not shown), as previously reported.¹²⁻¹⁴

Immunoglobulin, albumin, and IL-4 determination

The levels of IgE and albumin in the nasal washes were measured by ELISA, as previously described.^{7,15,16} IL-4 protein was measured after lavage samples were concentrated with Centricon concentrators (Amicon, Beverly, Mass.). A commercial ELISA kit system (Endogen, Cambridge, Mass.) was used according to the manufacturer's instructions. The detection limit for the IL-4 ELISA was 1 pg/ml. For all ELISA strategies, samples were run in duplicate, and optical density values that differed from the mean by more than 10% were re-assayed.

Preparation of cells

PBMCs were prepared by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density centrifugation with Lymphoprep (1077 kg/m³; Nycomed Pharma, Oslo, Norway). Cells were cultured in RPMI-1640 medium (M.A. Bio-products, Walkersville, Md.) supplemented with 10% fetal calf serum (HyClone, Laboratories, Logan, Utah), 2 mmol/L glutamine, 50 U/ml penicillin, and 50 μ g/ml streptomycin and placed in 96-well round-bottom micro-

TABLE I. Primers and probes for cytokine RT-PCR amplification

Sequence (5' to 3')		
IL-2	5':	ACTCACCAGGATGCTCACAT
IL-2	3':	AGGTAATCCATCTGTTTCTAGA
IL-2	Probe:	CTGGAGGAAGTGCTAAATTTAGCT
IL-4	5':	CCTCTGTTCTTCCTGCTAGCATGTGCC
IL-4	3':	CAACGTAATCTGGTTGGCTTCCTTCAC
IL-4	Probe:	AGAGCAGAAGACTCTGTGCACCGAG
IL-5	5':	ATGAGGATGCTTCTGCATTTG
IL-5	3':	ACTTTCTATTATCCACTCGGTGTTTATTAC
IL-5	Probe:	GCCAATGAGACTCTGAGGATTCCTG
IL-6	5':	ATGTAGCCGCCCCACACAGA
IL-6	3':	CATCCATCTTTTTCAGCCAT
IL-6	Probe:	CTGAGAAAGGAGACATGTAACAAGA
IL-10	5':	ATGCCCCAAGCTGAGAACCAAGACCCA
IL-10	3':	TCTCAAGGGGCTGGGTCTAGCTATCCCA
IL-10	Probe:	CAGGTGAAGAATGCCTTTAATAAGCTCCAACAGA AAGGCATCTACAAAGCCATGAGTGACTTTGACATG
IL-13	5':	AGGGAGCTCATTGAGGAGCTGGTC
IL-13	3':	GAGCAGGTCTCTTACAAACTGGGC
IL-13	Probe:	CAGAGGATGCTGAGCGGATTCTGCCCG
INF- γ	5':	AGTTATATCTTGGCTTTTCA
INF- γ	3':	ACCGAATAATTAGTCAGCTT
INF- γ	Probe:	ATTTGGCTCTGCATTATTTTCTGT
β -Actin	5':	GTGGGGCGCCCCAGGCACCA
β -Actin	3':	CTCCTTAATGTCACGCACGATTTT
β -Actin	Probe:	CTGCTGACCGAGGCCCGCTGAACCCC
CD38	5':	CTGGACCTGGGAAAACGCATC
CD38	3':	GTAATGAGCATCATCTCGATC
CD38	Probe:	GCCGACACACAAGCTCTGTTGAGGA

titer plates (Nunc, Roskilde, Denmark). PBMCs were cultured at 1×10^6 /ml. Then 200 μ l was introduced into each well, and five replicates were used for each point in each experiment. All cultures were placed in a Napco incubator (Precision Scientific, Inc., Chicago, Ill.) and kept at 37°C and in an atmosphere of 5% CO₂. Cells were stimulated for 18 hours in vitro with 5 ng/ml PAH-DEP, which had previously been established to be the optimal amount for induction of IgE in vitro⁶ or with 0.3 mg of DEPs in solution.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA from the cells recovered from nasal washes or from cell cultures (1 to 5×10^6 cells/sample) was isolated and then reverse-transcribed with Moloney murine leukemia virus reverse-transcriptase (Bethesda Research Laboratories, Gaithersburg, Md.) as previously described.¹⁷ To lower nonspecific background bands, all reactions were performed in laminar flow hoods with pipettes dedicated for polymerase chain reaction (PCR) use. The PCR strategies were

based on those reported by Yamamura et al.,¹⁸ which allowed comparison of cytokine mRNA in different samples. This PCR strategy had been demonstrated to be quantitative throughout a broad range of complementary DNA concentrations by the use of cytokine cDNA-containing plasmids. The PCR used a supermix reaction mixture that contained per sample: 1 U Perfect Match (Stratagene Ltd., La Jolla, Calif.), 50 mmol/L KCl, 50 mmol/L Tris-HCl (pH 9.0), 2.5 mmol/L MgCl₂, 0.1% (wt/vol) gelatin, 0.2 mmol/L deoxyribonucleoside triphosphate, 2.0 U Taq polymerase (Promega, Madison, Wis.), and 25 pmol of the oligonucleotide primers. Aliquots were then amplified by PCR on a preheated (94°C) Perkin Elmer thermal cycler (Perkin Elmer Cetus, Emeryville, Calif.) for 35 cycles. Each cycle consisted of denaturation at 94°C for 1 minute, followed by 2 minutes of annealing/extension at 55°C for IL-2 and interferon (IFN)- γ or 65°C for all other cytokines, β -actin, and CD38. The primers used are shown in Table I. An aliquot (one twentieth) of the PCR product was then electrophoresed on 1.5% agarose gels and visualized by ethidium bromide staining. A distinct band

corresponded to the positive identification of production of cytokine for a subject. A distinct band was one that was clearly seen on an ethidium bromide-stained agarose gel to be greater than the negative control. Southern blots were used in instances in which the presence or absence of a distinct band could not clearly be distinguished or was debatable.

To confirm that the bands observed after PCR, although the appropriate size, were indeed the PCR products expected, a representative sample of PCR results was confirmed by transfer of the electrophoresed PCR products to nylon membranes (Hybond-N membrane; Amersham Corp., Arlington Heights, Ill.), and hybridization with phosphorous 32-radiolabeled oligonucleotide probes complementary to internal sequences of the PCR products as previously reported.^{17,18} The probes used are shown in Table I. The validity and exactness of this PCR strategy and these primers had already been established and published by Yamamura et al.,¹⁸ and the primers were obtained directly from this group. The appearance of multiple bands for some of the PCR products (e.g., IL-4) is normal and has been previously demonstrated in Southern blots.¹⁸

RESULTS

DEPs enhance nasal IgE levels

The IgE levels in the nasal washes of 14 subjects, which were performed immediately before or 4 days after nasal challenge with DEPs, were measured to confirm that DEPs enhanced IgE production as previously reported. Fig. 1 shows that IgE levels rose after challenge, so that by day 4 they were significantly higher than prechallenge levels. No IgE enhancement was observed after saline challenge. The cDNAs from these challenges were used for the measurement of cytokine mRNAs.

Cytokine mRNA production in unstimulated and DEP-stimulated nasal lavage cells and PBMCs

Table II shows the number of subjects in whom it was possible to detect mRNA for the appropriate cytokine and also shows that in contrast to PBMCs (which generally express little if any detectable cytokine mRNA in the resting or unstimulated state), unstimulated cells recovered from nasal mucosa do make detectable amounts of mRNA for several cytokines. The amounts of cDNA were normalized to yield equivalent PCR products of β -actin, a "housekeeping gene" constitutively expressed by cells and a direct measure of the amount of cells from which RNA was extracted. Before challenge, detectable mRNA for IFN- γ was present in all 14 samples, whereas IL-10

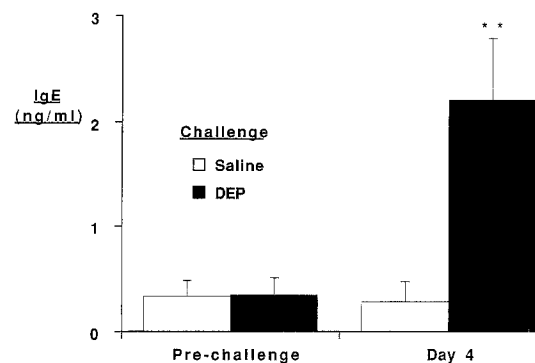


FIG. 1. An IgE response induced by in vivo DEP challenge. Subjects were challenged intranasally with saline solution or a total of 0.3 mg of DEPs. Each bar represents the mean IgE level of 14 subjects, determined immediately before or 4 days after challenge. IgE levels were determined by ELISA. Error bars represent 1 standard deviation. ** $p < 0.01$, paired t test between pre- and postchallenge levels.

mRNA was detected in none of the samples. Eight subjects' samples had IL-2 mRNA, three had IL-4 and IL-5 mRNA, and four had detectable IL-6 mRNA. IL-13 mRNA was present in 11 subjects' lavage fluid. Unstimulated PBMCs purified from the same 14 subjects before in vivo challenge with DEPs showed expression of IL-2 mRNA in four, whereas minimal IL-13 and IL-4 mRNA were detected in three subjects. No other cytokine expression was observed in the unstimulated PBMCs.

Cells were recovered from nasal lavages performed 18 hours after in vivo exposure to 0.3 mg of DEPs, and cytokine mRNAs were quantitated. Again, cDNA samples were normalized to β -actin before RT-PCR analysis. Challenge with DEPs in vivo increased the number of subjects in whom production of mRNA could be detected for all cytokines except IFN- γ , which was already present in all 14 samples before challenge. As shown in Table II, mRNA for the cytokines assayed could be detected in nearly all 14 subjects at this time.

We sought to determine whether the action of DEPs on cytokine production could be mimicked in vitro. We examined the effect of DEPs on mRNA cytokine production by PBMCs and by cells recovered from nasal lavage. The mRNA from cells stimulated in vitro with PAH-DEP or with DEPs in solution was extracted and used in RT-PCR to determine cytokine mRNA levels after normalization for β -actin. As shown in Table II, after in vitro stimulation with either particulate DEPs or PAH-DEP, PBMCs from only a minority of the subjects produced detectable levels of IL-2,

TABLE II. Cytokine mRNA production in unstimulated and DEP-stimulated nasal lavage cells and PBMCs

Cytokine	Nasal lavage cells		PBMCs		
	Unstimulated (14)*	DEP (14)*	Unstimulated (14)*	+DEP (11)*	+PAHs in DEPs (11)*
IL-2	8	12	4	2	4
IL-4	3	13	3	3	1
IL-5	2	14	0	0	0
IL-6	4	14	0	1	0
IL-10	0	12	0	0	0
IL-13	11	14	3	0	2
IFN- γ	14	14	0	0	0

RT-PCR was performed on mRNA from cells recovered from nasal lavages 18 hours after in vivo challenge with 0.3 mg of DEPs or from PBMCs from the same subjects stimulated for 18 hours in vitro with 0.3 mg/ml DEPs or from PBMCs stimulated for 18 hours in vitro with 5 ng/ml PAHs in DEPs. Complementary DNA samples were adjusted to produce similar intensity bands for β -actin after PCR amplification and visualization on ethidium bromide-stained agarose gels. These samples were then used to determine the presence of mRNA for the different cytokines. If a distinct band was observed on Southern blot analysis of amplified PCR products, the sample was deemed positive.

*Numbers in parentheses are numbers of subjects studied.

IL-4, IL-6, and IL-13 mRNA. In these samples no levels of IL-5, IL-10, or IFN- γ mRNA could be measured in these PBMCs. Although the results of only one dose of PAH-DEP are shown, similar results were observed through a range of doses from 0.5 to 500 ng/ml (data not shown). Although data for only one concentration of particulate DEPs is shown, the experiment was performed with a range of concentrations from 0.1 ng/ml to 1 mg/ml. At the top concentration, the DEPs had some toxic effects; for all other concentrations, the effects were similar to that shown for 0.3 mg/ml (data not shown). This lack of enhanced cytokine mRNA expression was also observed when PBMCs were obtained from blood drawn from subjects 18 hours after challenge with DEPs and when nasal lavage cells were recovered before stimulation and then stimulated ex vivo with DEPs (data not shown). The lack of stimulation was assessed by observation that both the number of subjects and the intensity or levels of the mRNA for the cytokines measured were unchanged.

In vivo challenge with DEPs increases cytokine mRNA levels

In vivo challenge with DEPs not only increased the number of subjects in whom the different cytokine mRNAs could be detected but also increased the expression of mRNA for each of the cytokines. Fig. 2 shows that after normalization for β -actin, following DEP challenge, the levels of all cytokines measured were greater than those detected in prechallenge cells. Results for two average responsive subjects are shown in Fig. 2; the

same result was observed for all 14 subjects. Indeed, the increase seen in those other subjects was often far greater than that shown here. Challenge with saline solution did not result in any change in cytokine mRNA levels as compared with prechallenge expression (data not shown).

To determine whether the increased mRNA expression for cytokines was due solely to increased T cell presence, the amounts of cDNA of all samples collected were normalized to yield equivalent CD3 δ PCR products, and cytokine mRNA levels were then determined. CD3 δ is constitutively expressed by T cells and T cells only and thus is a direct measure of the amount of T cells from which RNA was extracted. On exposure to DEPs, there was a modest increase in the expression of CD3 δ mRNA, implying that the number of T cells present had increased (Fig. 2). However, Fig. 3 shows that even when adjusted to equivalent levels of T cells, after challenge with DEPs, the levels of cytokine mRNA expression were enhanced in cells recovered from nasal washes. This increase in cytokine mRNA production was apparent in cells from all the subjects and for all cytokines studied.

In vivo challenge with DEPs increases IL-4 protein levels

To confirm that the effect of DEPs on cytokine mRNA production was translated at the protein level, we used an ELISA system to measure IL-4 protein levels before and after in vivo DEP challenge. Fig. 4 shows that challenge with DEPs enhanced IL-4 protein levels in the lavage fluid.

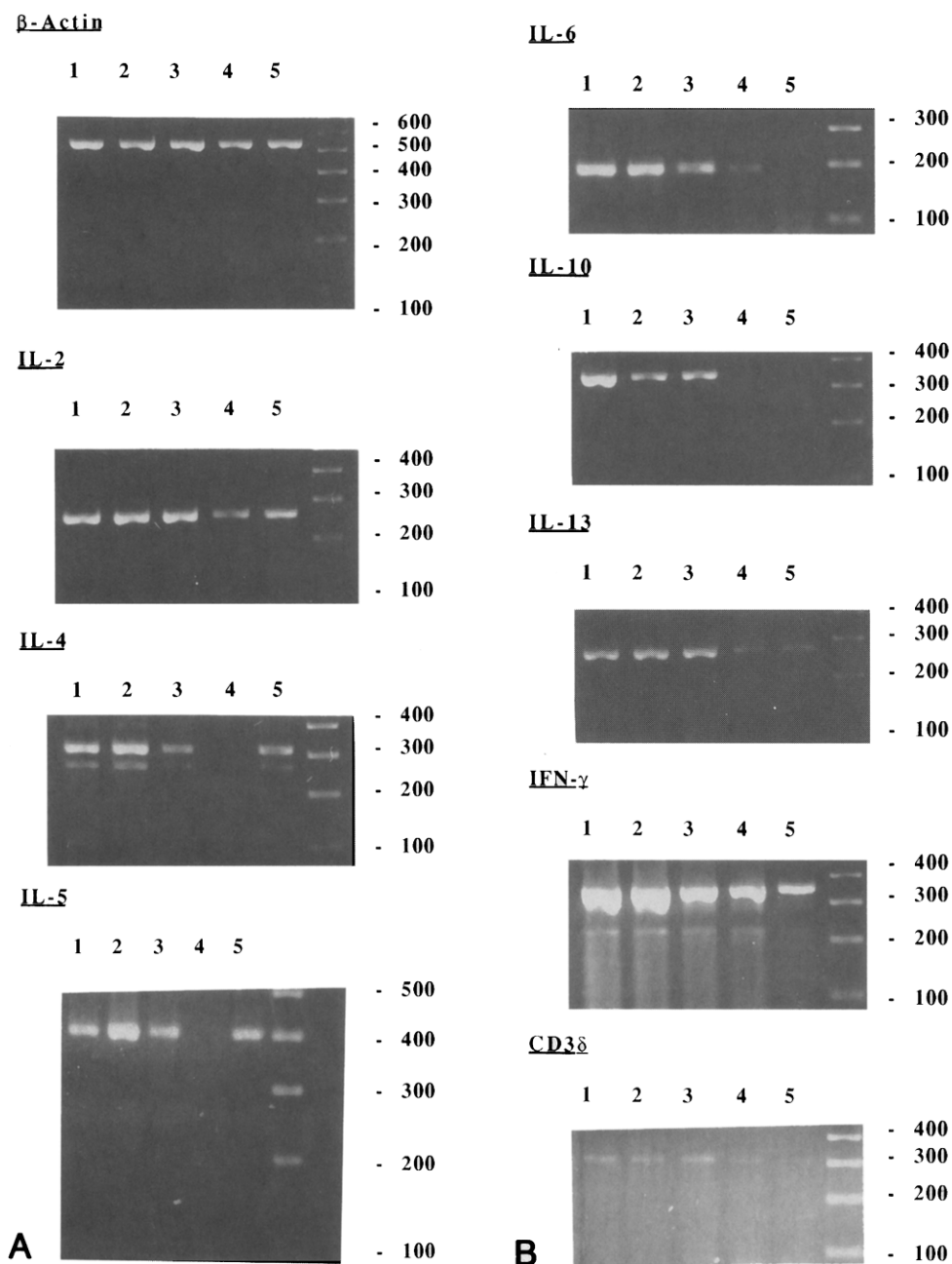


FIG. 2. In vivo challenge with DEPs increases cytokine mRNA expression. Cells from nasal lavages performed before and 18 hours after in vivo exposure to 0.3 mg of DEPs were used to obtain cDNA and amplified with β -actin mRNA-specific primers. Complementary DNA samples were adjusted to produce similar intensity bands on an ethidium bromide-stained agarose gel for β -actin after PCR amplification, and then these samples were used in PCR amplification with mRNA-specific primers for: IL-2, IL-4, and IL-5 (**A**) and for IL-6, IL-10, IL-13, IFN- γ , and CD38 (**B**). The amplified cDNA was separated on an agarose gel. Lane 1 shows the results from PBMCs stimulated with phorbol myristate acetate and ionomycin, a regimen known to express all cytokine mRNA, which served as a positive control. Amplified cDNAs from cells recovered after challenge from two representative subjects (lanes 2 and 3) were compared with those obtained before challenge (lanes 4 and 5). Although results for only two average subjects are shown, similar results were observed in all 14 subjects.

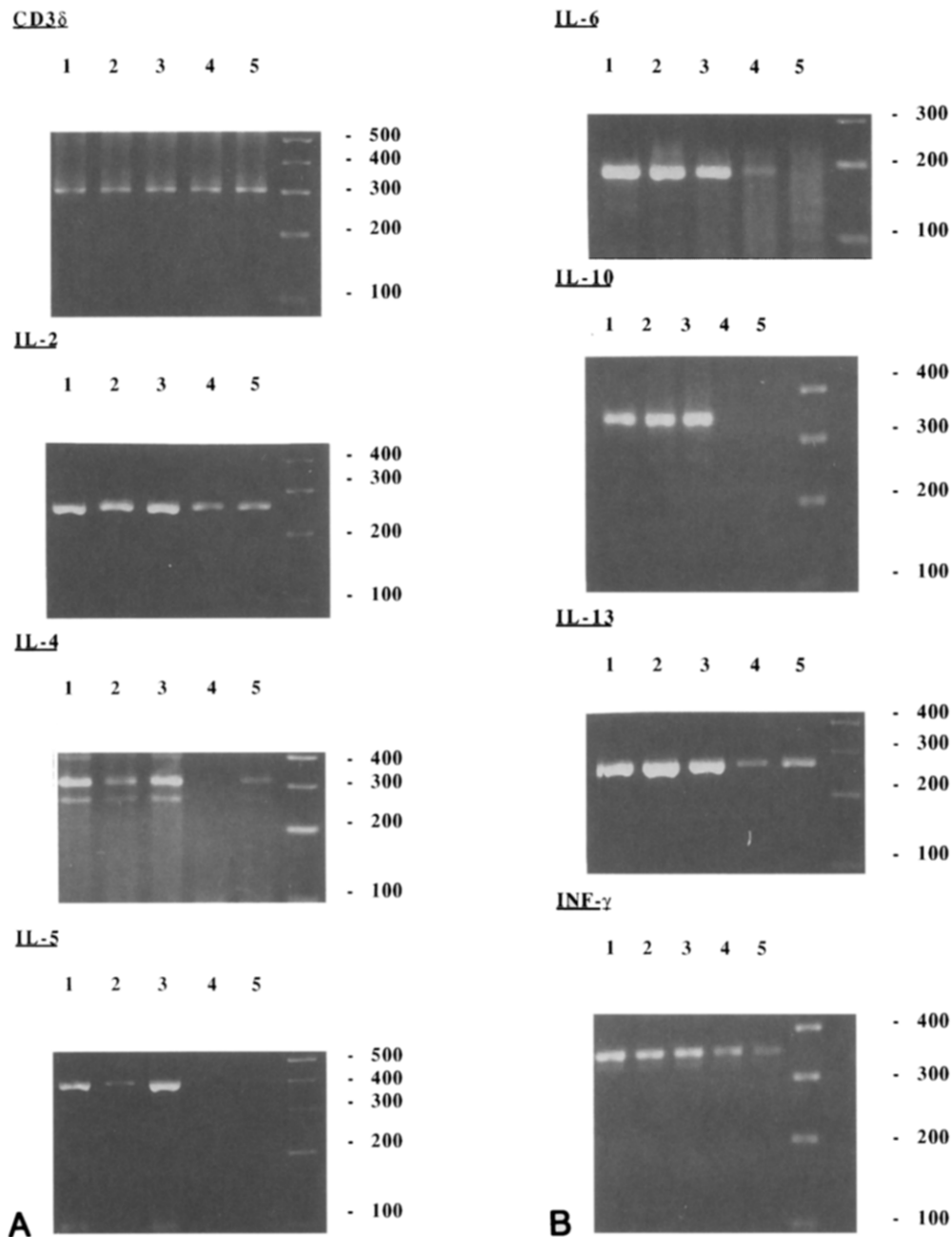


FIG. 3. Increased cytokine mRNA expression by DEPs is not due to increased T cell numbers alone. Cells from nasal lavages performed before and 18 hours after in vivo exposure to 0.3 mg of DEPs were used to obtain cDNA and amplified with CD3 δ mRNA-specific primers. Complementary DNA samples were adjusted to produce similar intensity bands on an ethidium bromide-stained agarose gel for CD3 δ after PCR amplification, and then these samples were used in PCR amplification with mRNA-specific primers for IL-2, IL-4, and IL-5 (**A**) and for IL-6, IL-10, IL-13, and IFN- γ (**B**). The amplified cDNA was separated on an agarose gel. Lane 1 shows the results from PBMCs stimulated with phorbol myristate acetate and ionomycin, a regimen known to express all cytokine mRNA, which served as a positive control. Amplified cDNAs from cells recovered after challenge from two representative subjects (lanes 2 and 3) were compared with those obtained before challenge (lanes 4 and 5). Although results for only two subjects are shown, similar results were observed in all 14 subjects.

One representative cytokine was studied, because previous reports had shown that the levels of most cytokines in nasal lavage samples are too low to be detected in an unstimulated state.¹⁹ As expected, no IL-4 could be detected in lavage fluids collected before challenge in any of the subjects studied. In contrast, in all but one of the nasal washes obtained 18 hours after challenge with DEPs, IL-4 could be detected at levels ranging from 1.54 to 4.96 pg/ml.

DISCUSSION

We have previously shown that DEPs can enhance human IgE responses in vivo and that this increase is due to the expansion of the local population of IgE-secreting cells.⁷ Our results showed that DEPs in vivo and PAH-DEP in vitro⁶ can affect B-cell IgE production both quantitatively and qualitatively. In this report we extend these findings to provide a further mechanism by demonstrating that DEP can act in vivo to potentiate cytokine production at the human upper respiratory mucosal surface. An increase in localized cytokine expression is likely to result in enhanced local IgE production and an increase in incidence of symptomatic respiratory allergic disease. Because the enhanced IgE and IgG₄ production we observed appears to result from DEPs expanding a preexisting population of IgE-committed memory B cells,⁷ it is noteworthy that many of the cytokines induced by DEPs—such as IL-4, IL-6, and IL-13—are B-cell proliferation factors.^{20, 21}

In this study we have used PCR methods to measure cytokine mRNA levels in both cells from nasal washes and PBMCs because cytokine protein levels from nasal mucosa cells are normally below detection levels of most assay systems.²² Thus other studies have failed to detect any IL-4 protein in nasal wash samples from nonchallenged subjects,¹⁹ a result also observed in this study. Other studies have circumvented the difficulty in measuring protein by studying cytokine mRNA levels (e.g., by using in situ hybridization).¹³ However, because there is good evidence for important posttranscriptional control of cytokine production, we confirmed our PCR-based mRNA findings at the protein level for the sentinel cytokine, IL-4.

Unstimulated PBMCs express little or no mRNA for most cytokines. In contrast, cells recovered from nasal lavages from untreated subjects showed expression of some mRNA for IL-2, IL-13, and IFN- γ —and to a lesser extent—IL-4, IL-5, and IL-6. It is likely that constant exposure to inhaled

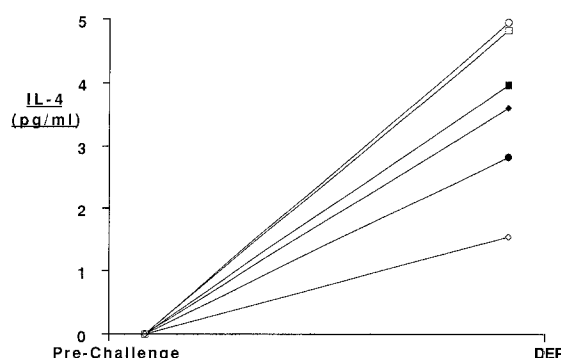


FIG. 4. In vivo challenge with DEPs induces IL-4 protein production. IL-4 protein was measured in nasal washes performed before and 18 hours after in vivo challenge with 0.3 mg of DEPs. Results from six subjects are shown.

particulates results in the presence of a few activated cells in the nasal mucosa. In vivo exposure to DEPs resulted in a non- T_{H1} or non- T_{H2} increase in cytokine mRNA expression in cells recovered after nasal lavage. There was enhanced production of mRNA for a broad array of the cytokines. IgE commitment appears to result from the preferential activation of T_{H2} -type T cells,^{23, 24} cells that produce IL-4, IL-5, IL-6, and IL-13 but not IFN- γ .^{25, 26} DEPs alone appeared to induce or activate either T_{H0} -type cells that have an unrestricted pattern of cytokine production²⁷ or both T_{H1} -like and T_{H2} -like cells simultaneously. That DEPs alone do not show a T_{H1} or T_{H2} pattern is predictable because the stimulus provided is likely nonspecific and augments programs that cells have already initiated.⁶ This and the cytokines produced would be compatible with our findings that DEPs or PAH-DEP augment cells already committed to IgE production.

Studies by others have demonstrated IL-4 and IL-5, but not IFN- γ , mRNA expression in cells from allergen-induced late-phase cutaneous and bronchial reactions.^{28, 29} Furthermore, allergen challenge activated T_{H2} cells in the airway mucosa³⁰ and in bronchoalveolar lavage fluid of patients with allergic asthma.³¹ Allergen exposure, with its stimulation of early IL-4 release, along with DEPs' potent stimulation of T_{H0} -like cells, may well lead to a heightened T_{H2} -type cytokine response because initial IL-4 exposure will drive T_{H0} cells toward T_{H2} -like differentiation. Alternatively, it is possible that DEP-induced increases in T_{H2} -type cytokines are simply functionally far greater than the concomitant stimulation of T_{H1} -like cytokines and thereby would favor increased IgE production.

Inhalation of DEPs results in qualitative and quantitative effects on IgE production in human beings and rodents.^{7, 11, 32} Alternate 3' RNA splicing in human beings results in generation of multiple ϵ mRNA coding for distinct IgE isoforms from the single active ϵ gene.³³⁻³⁵ The ϵ mRNA isoform pattern is not fixed in vivo because DEPs can preferentially select against one of the forms. Recently, we have shown that in vitro, DEPs and IL-10 have similar effects on both IgE levels and ϵ isoform patterns⁶ (personal observations). Thus increased expression of IL-10 by DEPs in vivo may be responsible for the change in ϵ isoform pattern observed with DEPs.

The mechanism by which DEPs cause increased cytokine production remains to be elucidated. It is interesting that no cytokine enhancement was observed when PBMCs were stimulated with DEPs or PAH-DEP in vitro. It is possible that there is suppression of cytokine production by some factor produced by the PBMCs or that a "mucosal factor" that was produced in vivo contributed to the enhancement of cytokine synthesis in nasal lavage cells. However, it should be noted that nasal lavage cells obtained before challenge could not be stimulated in vitro. Additionally, preliminary experiments have shown that PBMCs cultured in the presence of nasal washes after exposure to DEPs do not enhance cytokine production (data not shown). Subjects were challenged with DEPs, the cells were spun out of the nasal washes, and the supernatant was concentrated and added to PBMC cultures at different concentrations. No enhancement was observed in mRNA production for any of the cytokines studied. This argues against the presence of a mucosal factor. If such a factor is present, it may interact only with resident mucosal cells and not with PBMCs. An important caveat to our results is that it is not possible to assume that the cells recovered in nasal secretions reflect the cells resident within the mucosa. Thus it may be that the cytokine-producing cells recovered are interacting with resident mucosal cells, cells that are not necessarily recovered during the lavage procedure.

It is important to recognize that the cells in nasal lavage before and after challenge do not necessarily represent the same cells, either in number or type. Therefore many of the actions of DEPs may be due to cellular infiltration or proliferation, or more unlikely, given the short time frame, differentiation. It is possible that the action of DEPs could be partly due to recruitment of T cells because inhalation of DEPs caused an increase in

T cell numbers, as measured by an increase in CD3 δ transcripts. However, this T cell increase cannot wholly explain the effects of DEPs because when the samples were normalized for CD3 δ , an increase in cytokine mRNA production was still observed. It is just as likely that the increased cytokine expression is partly due to some other cell types in the nasal mucosa, such as monocytes or macrophages or mast cells, the latter of which are known to produce IL-4.³⁶ Furthermore, the majority of cells recovered from nasal lavages are epithelial cells, cells now reported to produce IL-6 and IL-10,^{37, 38} which are proinflammatory cytokines. Current studies are directed at finding the source of the cytokines and determining how they affect the local upper airway allergic response.

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