

The Organic Component of Diesel Exhaust Particles and Phenanthrene, a Major Polyaromatic Hydrocarbon Constituent, Enhances IgE Production by IgE-Secreting EBV-Transformed Human B Cells *in Vitro*

ALBERT TSIEN,* DAVID DIAZ-SANCHEZ,* JOHNNY MA,* AND ANDREW SAXON*†‡¹

*Hart and Louise Lyon Laboratory, Division of Clinical Immunology and Allergy, †The Molecular Biology Institute, and ‡The Jonsson Comprehensive Cancer Center, University of California Los Angeles School of Medicine, Los Angeles, California 90024-1680

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Suspended airborne particulate matter such as diesel exhaust particles (DEP) have been implicated in the increased incidence of respiratory allergic diseases that has occurred over the past century. Studies *in vitro* and *in vivo* have shown that DEP may enhance allergic antibody (IgE) expression. DEP contain a wide spectrum of polycyclic aromatic hydrocarbons (PAH) that have been reported to have direct effects on the immune system, including the modulation of IgE production using various human and murine cell populations. We investigated the effects of the organic extract of DEP (PAH-DEP) and particularly, phenanthrene, a major component of DEP, *in vitro* on IgE production by 2C4/F3, a human Epstein–Barr virus transformed isotype switched, IgE producing B cell line. Phenanthrene consistently enhanced 2C4/F3 IgE production two- to threefold. This *in vitro* enhancement was associated with an increased expression of total IgE mRNA. Furthermore, the pattern of mRNA's coding for distinct isoforms of the epsilon chain was altered by both DEP-PAH and phenanthrene. While phenanthrene increased the level of productive epsilon transcripts, it did not increase epsilon germ line transcription. These effects were not due to an alteration of the cell cycle. Unstimulated 2C4/F3 cells contained detectable mRNA for IL6, IL10, TNF- α , and interestingly IL4; however, addition of PAH-DEP or phenanthrene did not significantly alter the level of these cytokines and thus did not appear to account for our findings. Thus, we have used our *in vitro* model to dissect the mechanism of DEP-PAH on IgE production in postswitch IgE producing cells and shown that phenanthrene, an important component in DEP and other pollutants, can act in a similar manner. © 1997 Academic Press

Epidemiological studies demonstrate that increased air pollution may play an important role in the expression of the allergic response (Takafuji *et al.*, 1989a; Corbo *et al.*, 1993; Bascom *et al.*, 1991; Emanuel, 1988). An important contributor of the airborne pollution are diesel exhaust particles (DEP) and the various associated polycyclic aromatic hydrocarbons (PAH) (Rykowski and Brochu, 1986; Spangler and Samet, 1991; Wallace, 1991; Ryan and Lambert, 1991). During the industrialization of Japan, a 500-fold increase in the number of diesel cars and a greater than 1000-fold increase in the amount of polyaromatic hydrocarbons associated with diesel exhaust in the air paralleled the increase in allergic airway diseases (Kaneko *et al.*, 1980). Evidence for this linkage has come from a variety of studies. For example, Miyamoto *et al.* found that specific allergic antibody (IgE) production to antigen was enhanced in mice inhaling or inoculated with DEP along with antigen (Muranaka *et al.*, 1986; Takafuji *et al.*, 1987, 1989b). Recently, we demonstrated that polyaromatic hydrocarbons extracted from diesel exhaust particles (PAH-DEP) enhanced human IgE production *in vitro* from peripheral blood mononuclear cells costimulated with IL-4 plus CD40 mAb (Takenaka *et al.*, 1995). Additionally, we have shown in humans that *in vivo* intranasal challenge using DEP induced enhanced production of IgE at the nasal mucosa (Diaz-Sanchez *et al.*, 1994a).

DEP is composed of a heterogeneous collection of carbonaceous soot particles and a wide variety of organic compounds composed of unburned fuel hydrocarbons plus pyrolysis products including PAH (Cooke and Dennis, 1985). The variability between preparations of DEP and the mixture of PAH contained therein make it difficult to standardize DEP or their extracts for long-term study or to ensure reproducibility between investigators. In this study we attempted to resolve these difficulties by establishing an appropriate model that can be used to study the mechanisms of PAH-DEP enhancement of IgE production.

Phenanthrene is a major organic constituent of DEP, com-

¹ To whom correspondence should be addressed at Division of Clinical Immunology and Allergy, 52-175 Center for the Health Sciences, UCLA School of Medicine, Los Angeles, CA 90024-1680.

prising 6% of the total organic extract), while phenanthrenes as a family (including phenanthrene, methylphenanthrenes and C2-, C3-, and C4-phenanthrenes) constitute 52% of the total PAHs (Barfknecht *et al.*, 1981). In this study we established an *in vitro* model for the PAH-DEP direct effects on B cells, using a continuously growing IgE producing EBV transformed B cell line. We used this model to show that phenanthrene, as well as PAH-DEP, could significantly increase IgE mRNA and protein production in cells that have undergone isotype switch to IgE. Additionally, we used this model to dissect the mechanisms involved in this IgE enhancement.

MATERIALS AND METHODS

Reagents. Diesel exhaust particles were obtained by collecting the exhaust from a light duty diesel passenger car onto a cyclone impactor equipped with a dilution tunnel constant volume sampler system as described (Muranaka *et al.*, 1986). The DEP were collected on high capacity glass fiber filters. The particulate DEP consists of large chain aggregates of DEP in which the individual particles are $<0.1 \mu\text{m}$ in diameter. The DEP containing filters were stored in glass containers under nitrogen gas to preserve all the PAHs. To prepare PAH-DEP, a high capacity filter was extracted in dichloromethane (Aldrich Chemical Co., Milwaukee, WI) as this was shown to be highly efficient in recovering total PAH-DEP (Yamane *et al.*, 1988). We extracted 134 mg of PAH-DEP from a filter with 330 mg of total DEP. The resulting extract was adjusted to a stock concentration of 2 mg of organic material/ml in dichloromethane and stored at 4°C. Working concentrations of PAH were dissolved serially in 10-fold dilutions (2000 to 0.2 ng/ml) 2% acetone (Fisher Scientific, New Jersey) plus 10% fetal calf serum-RPMI 1640 (cRPMI, a culture media solution). The concentrations of DMSO in the test samples were no higher than 0.1%. An additional preparation of PAH-DEP was generously provided by Dr. Hiroshi Takenaka (Department of the Department of Otorhinolaryngology, Kyoto Prefectural University of Medicine, Kyoto, Japan).

Phenanthrene was purchased from Aldrich Chemical Co. and dissolved in acetone at 500 $\mu\text{g/ml}$. This concentrate was then diluted serially with cRPMI for addition to cell cultures. Cycloheximide (Sigma Chemical Co. St. Louis, MO) was added to cell cultures at a final concentration of 100 $\mu\text{g/ml}$ to stop protein synthesis so that the amount of preformed or surface IgE could be measured.

Cell preparation and culture conditions. The 2C4/F3 cell line, an EBV transformed IgE producing human B cell line (Thyphronitis *et al.*, 1991) was a kind gift from Dr. Fred Finkelman (Uniformed Services School of Medicine, Bethesda, MD). It was maintained in complete RPMI 1640. The 2C4/F3 cells (5×10^4 cells/ml) were cultured at 10^4 cells in 0.2 ml complete RPMI 1640 per well in 96-well round-bottomed microtiter culture plates (Nunc, Roskilde, Denmark). These conditions had previously been established as optimal for IgE production. Cultures were maintained with a selected concentration of an aromatic hydrocarbon or PAH-DEP at 37°C in 5% CO₂. Supernatants were harvested on Day 3 for IgE measurement after preliminary experiments showed that optimal enhancement of IgE was observed at 3 or later. Preformed IgE in 2C4/F3 was accounted for by parallel cultures treated with cycloheximide. Since cycloheximide inhibits *de novo* protein synthesis, IgE found in the cycloheximide controls was considered background and was subtracted from the controls and the test samples.

IgE and cytokine measurement. IgE in the supernatants of cultured cells was measured by isotype specific ELISA as previously described (Macy *et al.*, 1988; Zhang *et al.*, 1991). Briefly, microtiter plates (Nunc, Roskilde, Denmark) for IgE assays were coated with monoclonal antibodies

to human IgE mAb CIA-E-7.12 and CIA-E-4.15 at 2 $\mu\text{g/ml}$ overnight. After washing, 100 μl of samples and standards were added for overnight incubation. After further washing the detection antibody, alkaline phosphatase labeled anti-IgE was added at 1/3000 (Kirkegaard and Perry Labs Inc., Gaithersburg, MD). Absorbance at 405 nm was read with an ELISA reader (Bio-Tek Instruments, Inc., Burlington, CA). Standard for IgE was the PS IgE myeloma protein. The sensitivity of the IgE assay was 0.5 ng/ml. All samples were run in quadruplicate. Color development was measured on a Bio-Tek Instruments Model EL310 autoreader. Cytokine mRNAs were measured using the same primers and conditions as previously described (Diaz-Sanchez *et al.*, 1996). The PCR used a supermix reaction mixture that contained (per sample) 1 U Perfect Match (Stratagene Ltd., La Jolla, CA), 50 mM KCl, 50 mM Tris-HCl (pH 9.0), 2.5 mM MgCl₂, 0.1% (w/v) gelatin, 0.2 mM dNTP, 2.0 U *Taq* polymerase (Promega, Madison, WI), and 25 μM of the oligonucleotide primers. Aliquots were then amplified by PCR on a preheated (94°C) Perkin Elmer thermal cycler (Perkin Elmer Cetus, Emeryville, CA) for 35 cycles. Each cycle consisted of denaturation at 94°C for 1 min, followed by 2 min of annealing/extension at 55°C for IL-2 and IFN- γ or 65°C for all other cytokines, β -actin, and CD3 δ . An aliquot (1/20th) of the PCR product was then electrophoresed on 1.5% agarose gels and visualized by ethidium bromide staining.

Cell cycle analysis. 2C4/F3 cells incubated with various concentrations of phenanthrene were centrifuged in cRPMI and resuspended in PBS buffer at $5 \times 10^5/\text{ml}$. Cells were then stained with propidium iodide solution (0.05 mg/ml in 0.1% sodium citrate) and DNA content analyzed by rapid flow cytofluorometric analysis as described by Krishan (1975), utilizing the fact that cells in different stages of the cell cycle contain different amounts of the dye-DNA complex.

Measurement of epsilon mRNA isoforms by reverse transcription-PCR. Alternative splicing of the single human functional epsilon heavy chain gene results in at least five different epsilon mRNA isoforms (Zhang *et al.*, 1992, 1994). We have previously developed modified polymerase chain reaction techniques to compare the relative amounts of different isoforms of epsilon mRNA produced (Diaz-Sanchez *et al.*, 1994a). Briefly total cellular RNA from the cells recovered from 2C4/F3 cultures was isolated and then reverse transcribed as previously described (Zhang *et al.*, 1992).

Two different PCR reactions were performed on the resultant cDNA. The PCR products were quantitated as previously described (Diaz-Sanchez *et al.*, 1994a). The first PCR, termed "PCR1," amplified the three epsilon mRNA isoforms CH4-M1'-M2, CH4-M2', and CH4-M2" (Zhang *et al.*, 1994) using the primers

A: 5'-GGCCATCGATAGGCAGCGAGCCCCTCACAGACCG-3'

and

B: 5'-GGCCGTCGACGACGGGGATGCCAGGCAGTTGGATC-3'.

To amplify the mRNA for housekeeping gene β -actin in PCR1, the following primers were used:

5'-GTGGGGCGCCCCAGGCACCA-3'

and

5'-ACCTGGCCATCAGGCAGCTC-3'.

The second PCR, termed "PCR2," was used to amplify two other ϵ mRNA splices, the CH4-S form (the classic secreted variant) and the alternative spliced CH4'-CH5 form. Primers

C: 5'-TCATGCGGTCCACGACCAAGA-3'

and

D: 5'-GTCATTGCAACAGTGGACAG-3'

were used to amplify the epsilon isoforms in PCR2, while β -actin mRNA amplified with primers 5'-CGCGAGAAGATGACCCAGATC-3' and 5'-ATCACGATGCCAGTGGTACGG-3'.

For quantitation, aliquots of 20 ml of each internally labeled PCR product were run on an 8% nondenaturing polyacrylamide gel, dried, and then exposed to Kodak XAR-5 film. The autoradiograph was scanned on an Apple One 24-bit scanner at 300 dpi and converted into a TIFF uncompressed format and the resultant pattern analyzed using the Scan Analysis program from Biosoft (Cambridge, UK) which delineates peaks and integrates the area under each peak.

Quantitation of germline and productive epsilon mRNA transcripts. Reverse transcription using 2C4/F3 cells and kinase labeling of downstream primer was performed as previously reported (Diaz-Sanchez *et al.*, 1994a). PCR quantitation was then performed to amplify epsilon germline or productive transcripts along with β -actin transcripts. The cocktail mixture for each sample contained 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 1.5 mM $MgCl_2$, 1 mM dNTP, 2.0 U *Taq* polymerase (Promega), and 50 pM each of primers. Aliquots were then amplified by PCR on a preheated (94°C) Perkin Elmer thermal cycler for 25 cycles. Each cycle consisted of denaturation at 94°C for 1 min, followed by 1 min at 60°C and then 2 min at 72°C. The primers used for germline transcripts were

E: 5'-AGGCTCCACTGCCCGGCACAGAAAT-3'

and

F: 5'-GGACAAGTCCACGTCCATGA-3'.

The primers for productive transcripts were primer F as above and

G: 5'-TCGACTTCTGGGGCCAAGGG-3'.

Beta-actin was amplified in this PCR by using the primers 5'-TCACCAACTGGGACGACATGGAG-3' and 5'-TCCTTAATGTCACGCATGATTC-3'. The quantitation of the PCR products and the validation of this PCR strategy was performed using the method previously reported (Diaz-Sanchez *et al.*, 1994a) with minor modifications.

An independent verification was performed using a RNAase protection assay to confirm that the PCR quantitation was accurate. A labeled RNA probe was synthesized using T_3 RNA polymerase (MAXIscrip Kit, Ambion, Austin, TX). This probe was complementary to a portion of the germline epsilon mRNA which spanned the I ϵ , CH ϵ 1, and CH ϵ 2 exons. The probe was gel purified and hybridized to the same samples containing epsilon germline and productive transcripts used in the PCR validation. The mixture was treated with ribonuclease (RPAII Kit, Ambion, Austin, TX) to degrade single-stranded, nonhybridized RNA, separated on a 5% polyacrylamide gel, and visualized by autoradiography. The intensity of the protected germline and productive epsilon bands were compared side by side using an Apple One 24-bit scanner and the resultant patterns were analyzed using the Scan Analysis program from Biosoft (Cambridge, UK). A representative experiment using premixed germline and productive mRNA at different ratios showed the following outcome: a RT-PCR gave germline:productive ratios of 1:1, 1:10, and 10:1, while, using the same RNA material, the RNAase protection assay showed a germline:productive ratio of 1:0.67, 1:5.8, and 19.6:1. Thus, the PCR strategy provided a valid quantitative measurement for germline and productive epsilon transcripts.

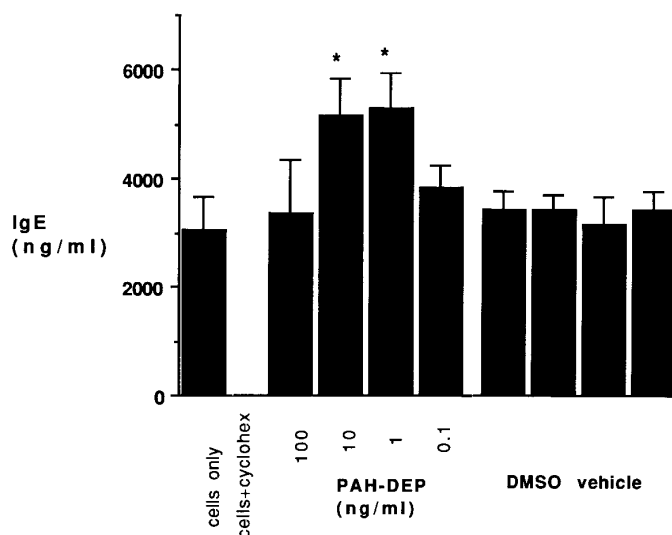


FIG. 1. Enhancing effect of PAH-DEP on IgE production by 2C4/F3 cells. 2C4/F3 (10^4 cells/0.2 ml) were cultured with 100, 10, 1, or 0.1 ng/ml of PAH-DEP at 37°C for 3 days. * $p < 0.05$ compared to cells plus DMSO vehicle alone, Mann-Whitney *U* test. Supernatants were harvested on Day 3 for IgE measurement. Values represent the mean IgE plus 1 standard deviation of four replicate cultures.

RESULTS

PAH-DEP enhances IgE production by 2C4/F3, a human IgE secreting EBV transformed B line. The 2C4/F3 cells were incubated with concentrations of PAH-DEP ranging from 0.1 to 100 ng/ml. Compared to controls, cells cultured with PAH-DEP at 1 to 10 ng/ml for 3 days doubled their IgE production ($p < 0.05$) (Fig. 1). This level of enhancement of IgE production by PAH-DEP was seen at this 1–10 ng/ml dose range in four other experiments. At doses lower than 1 ng/ml or equal to or greater than 100 ng/ml, PAH-DEP did not statistically increase IgE production compared to that of the vehicle controls. Time course experiments ranging over 5 days showed that IgE production by 2C4/F3 was optimally enhanced by PAH-DEP following 3 days of incubation (data not shown). Controls included 2C4/F3 cells maintained with a diluent vehicle and cells incubated with cycloheximide 50 μ g/ml to account for any IgE present at the initiation of culture.

Phenanthrene, an aromatic hydrocarbon component of diesel exhaust, enhances IgE production by 2C4/F3 cells. Cells were incubated with varying concentrations of phenanthrene for 3 days. Controls were cells maintained with a diluent vehicle as well as cells incubated with cycloheximide. The 2C4/F3 cells were incubated with between 0.001 and 100 ng/ml of phenanthrene (Fig. 2). Compared to medium or vehicle controls, concentrations of phenanthrene at 10 and 1 ng/ml produced significantly more IgE in three of four experiments (Mann-Whitney *U* test, $p < 0.05$). This

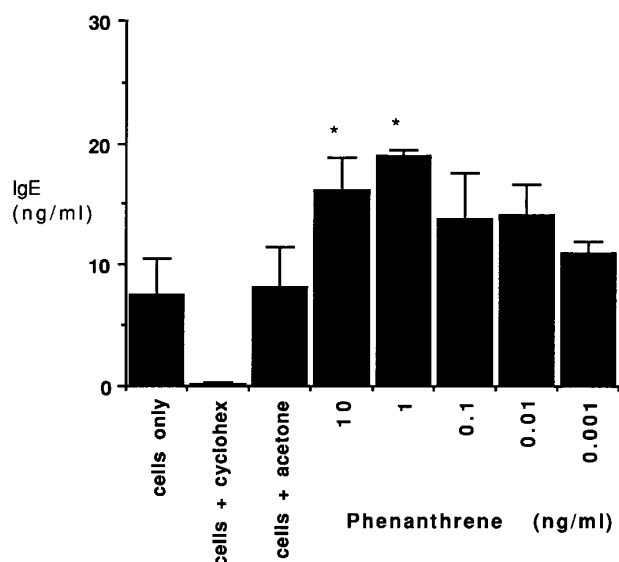


FIG. 2. Enhancing effect of phenanthrene on 2C4/F3 IgE production. 2C4/F3 (10^4 cells/0.2 ml) were incubated with 10.0 to 0.001 ng/ml of phenanthrene at 37°C for 3 days. * $p < 0.05$ compared to cells cultured with acetone vehicle alone, Mann-Whitney U test. Supernatants were harvested on Day 3 for IgE measurement. Values represent the mean IgE and 1 standard deviation of four replicate cultures.

enhancement demonstrated a dose-dependent response similar to that found in PAH-DEP experiments. We then tested the ability of phenanthrene to augment IL-4 plus anti-CD40 driven IgE production from B cells purified from freshly collected peripheral blood lymphocytes in a manner analogous to that previously observed with PAH-DEP (Takenaka *et al.*, 1995). Phenanthrene (Fig. 3) at 2 ng/ml enhanced IgE 30% over the standard 12-day culture.

Neither cell proliferation or survival could fully account for the increased IgE production induced by phenanthrene at this concentration, since the number of viable 2C4/F3 cells present after 3-day cultures (as determined by trypan blue exclusion) was not different from that seen with the vehicle control treatment. Cell cycle analysis was performed in order to determine if the augmented IgE response was due to accumulation of cells in a certain phase of the cycle. As shown in Table 1, the proportion of 2C4/F3 in the phases of cell cycle was unchanged.

PAH-DEP and phenanthrene alter the epsilon mRNA isoform pattern but not cytokine profile in 2C4/F3 cells. Figure 4 shows that PAH-DEP not only quantitatively altered IgE production but also qualitatively altered the IgE response by changing the relative ratios of the different epsilon splice variants produced by 2C4/F3 cells. PAH-DEP at 5 ng/ml resulted in a 6.3-fold increase in the level of total epsilon mRNA compared to control cultures along with the preferential production of the CH4-M2' splice variant which was increased 18.7-fold. The relative amounts of three other epsilon

splice variants (CH4-M1'-M2; CH4-M2'' and CH4-S) increased by between 4.3- and 7.1-fold. However, the CH4'-CH5 splice variant increased only 1.3-fold. Similar though less dramatic results were observed at concentrations of PAH-DEP from 1 to 50 ng/ml.

Phenanthrene at 5 ng/ml led to a similar preferential enhancement of mRNA for the CH4-M2' splice variant and a relative decrease in the CH4'-CH5 splice variant in 3-day cultures. Thus, while the total epsilon mRNA increased 4.9-fold, the level of mRNA for CH4-M2' rose 14.3-fold and CH4'-CH5 was 0.9-fold compared to control.

Cytokines strongly influence the IgE response. Accordingly, 2C4/F3 cell expression of mRNA for a variety of cytokines was assessed. Messenger RNA for IL6, IL10, TNF- α , and IL4 could be detected in unstimulated cells. As expected, no IL2, IL5, or IFN- γ mRNA could be detected. When PAH-DEP was added at 0.1 to 100 ng/ml, there was no change in the level of expression of any of the cytokines compared with that seen in the 2C4/F3 controls after correction for β -actin. Similarly phenanthrene (0.01–100 ng/ml) failed to alter mRNA expression for any of the tested cytokines (data not shown).

Phenanthrene enhances epsilon productive transcript levels in 2C4/F3 cells. To confirm whether the increased production of IgE protein observed was due to the increased transcription of the epsilon gene, germline and productive epsilon transcript levels were directly measured by quantitative PCR as described under Materials and Methods. This was necessary as the PCRs employed for determination of epsilon isoforms only indirectly measured total epsilon

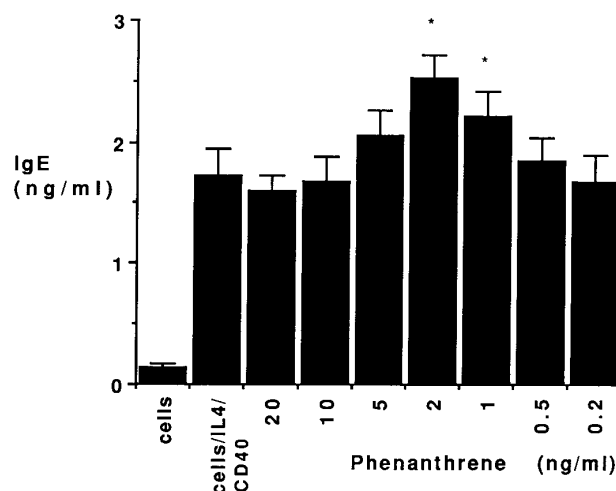


FIG. 3. Phenanthrene effect on IL-4 plus α CD40 driven IgE production by fresh B cells. B cells from purified from freshly collected PBL (10^5 /0.2 ml) were cultured for 12 days in the presence of 200 U/ml of IL4, plus 0.1 μ g/ml of α CD40 and different concentrations of phenanthrene (20, 2, or 0.2 ng/ml) added on Day 3. The mean IgE of four replicates cultures are shown. * $p < 0.05$ compared to cells cultured with IL4 plus α CD40 alone, Mann-Whitney U test.

TABLE 1
2C4/F3 Cell Cycle Analysis^a

Cell cycle phase	Phenanthrene (ng/ml)						
	Medium	Cyclohex	Acetone	5.0	0.5	0.05	0.005
G ₁	57.7	49.4	62.6	62.9	54.2	58.4	54.1
S	36.5	49.8	30.5	28.6	41.0	37.3	35.5
G ₂ + M	5.8	0.9	6.9	8.6	4.8	5.4	4.9

Note. The IgE-secreting cell line 2C4/F3 was cultured in the presence of different concentrations of phenanthrene or as a control acetone or cycloheximide. The percentage of cells in the different phases of cell cycle was determined by cytofluorographic analysis and is shown above.

^a Data are reported as percentage of total events.

mRNA and did not distinguish between germline and productive transcripts. β -Actin was used as an internal control.

As shown in Fig. 5 phenanthrene at concentrations of 0.05 to 5 ng/ml increased the levels of productive epsilon mRNA expression. This result is in agreement with the results for IgE protein production. In this experiment, at the maximal enhancing dose of phenanthrene for IgE protein (5 ng/ml), the total productive epsilon mRNA showed a greater than twofold increase. At higher concentrations (10 to 50 ng/ml) increased expression was still observed but mRNA levels were less than with the 5 ng/ml dose.

DISCUSSION

In this report, we demonstrated that the organic extract of DEP (PAH-DEP) as well as the specific polyaromatic hydrocarbon, phenanthrene, a major component of DEP, enhanced IgE

production by an IgE-producing B cell line. Previous studies have shown that PAH-DEP could enhance B cell IgE production from fresh PBLs *in vitro* (Takenaka *et al.*, 1995), while DEP enhanced IgE production from nasal mucosal cells *in vivo* (Diaz-Sanchez *et al.*, 1994a). The *in vitro* studies suggested that PAH-DEP acted on cells already committed to IgE production, since PAH-DEP could not substitute for IL-4 in inducing germ line epsilon transcription or for CD40 ligation in inducing isotype switching. In this study we extended those earlier findings by demonstrating that phenanthrene and PAH-DEP increased and altered IgE isoform mRNA levels and resulted in an enhanced IgE production from a cell line already committed to and producing IgE.

DEP contain a complex mixture of organic compounds with a major component being PAH (Barfknecht *et al.*, 1981). Extensive studies have characterized the nature of the PAHs present in diesel exhaust (Kittelson, 1984; Williams *et*

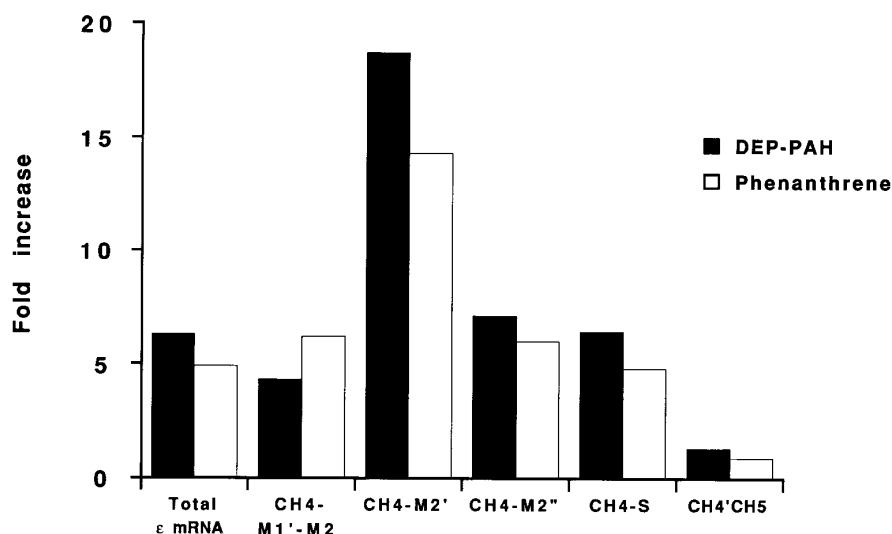


FIG. 4. DEP-PAH and phenanthrene alter the relative levels of the epsilon mRNA variants in 2C4/F3 cells. This figure shows the relative enhancement (as fold increase) for each of the epsilon mRNAs in 2C4/F3 cells in response to the addition of DEP-PAH (5 ng/ml) or phenanthrene (5 ng/ml). The levels are compared to the results obtained when cells were cultured with vehicle alone. The results shown are the mean and standard deviation of four experiments.

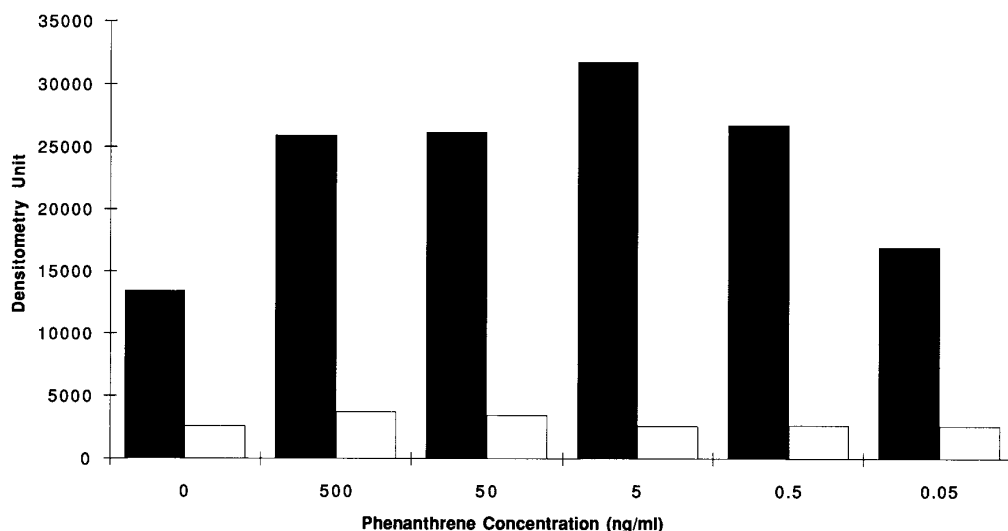


FIG. 5. Phenanthrene enhances the level of epsilon productive but not germline transcripts in IgE producing 2C4/F3 cells. Cells were stimulated with the indicated concentrations of phenanthrene for 3 days and harvested for RT-PCR analysis of epsilon mRNA levels. Autoradiographs of RT-PCR products were measured by densitometry. The shaded bars show the amount of epsilon productive transcript and the open bars show the amount of germline transcript produced.

al., 1986; Rykowski and Brochu, 1986; Andrews *et al.*, 1989). The majority of PAH found in DEP are relatively small polycyclic aromatic hydrocarbons containing up to four benzene rings although larger compounds are present. The most common PAHs in DEP are the phenanthrenes. PAH are highly hydrophobic and directly enter cells where they would be expected to bind to either the cytoplasmic Ah receptor or another PAH-binding/nuclear transporter protein (such as the 4S binding protein in mice) (Okey, 1990).

Once a bound aromatic hydrocarbon complex is translocated to the nucleus, it induces several enzyme systems and particularly the P450 cytochrome system. Activation of the P450 cytochrome system requires the induction and expression of the *cyp1A1* and *cyp1A2* genes (Reyes *et al.*, 1992). PAH with more than four rings have been shown to bind the Ah receptor and induce *cyp1A1* and *cyp1A2* gene expression. Previously, the role specificity of Ah activation in enhancing B cell IgE production was underscored using TCDD which functions solely through the Ah receptor (Takenaka *et al.*, 1995). Recently, radioligand binding studies have suggested that smaller tricyclic PAH such as phenanthrene do not bind the Ah receptor but nevertheless activate the *cyp1A2* gene (Chaloupka *et al.*, 1994). Taken together, these results suggest that more than one intracellular mechanism may be involved in upregulating B cell IgE production. Other mechanisms such as induction of oxidative stress induction may also be involved.

In this study, we present an important *in vitro* model to determine the direct effects of DEP and its polycyclic aromatic hydrocarbons on human IgE-secreting B cells. Previous studies have used purified B cells from PBL which are a hetero-

geneous population and which contain only a small proportion of B cells that are driven to IgE production. This current study provides a model that extends these findings by examining a homogenous population of cells that have already undergone isotype switching and are committed to production of IgE protein. This model is a valuable tool for mechanistic studies and by employing it, we have provided the first steps in elucidating the mechanisms by which DEP and DEP-PAH directly affect B cells and by extension allergic human diseases.

The results of this study suggest that the direct effects of PAH-DEPs and phenanthrene's on B cell IgE production were due to modulation of cells after isotype switch to IgE. PAH-DEP and phenanthrene increased IgE production by two- to three-fold in a cell line (2C4/F3) that had already switched ($p < 0.05$, Mann-Whitney *U* test). To dissect the possible mechanisms for the enhanced IgE production, the levels of epsilon germline and productive RNA transcripts, epsilon isoforms, and cytokine patterns of 2C4/F3 cells were studied. Neither phenanthrene nor DEP-PAH enhanced germline transcription or induced switching of the unrearranged epsilon allele in the 2C4/F3 cells. Thus, these compounds did not appear to act by providing a signal for epsilon germ line transcription nor did they provide the "second signal" involved in class switching observed with α CD40 or α CD58 mAb (Zhang *et al.*, 1991; Diaz-Sanchez *et al.*, 1994b). Yet, as shown by Fig. 5, the chemicals did enhance both productive IgE transcripts and production of IgE protein without altering cell survival or cycle. In this way, their action resembled that of IL-6 or IL-10 which can increase IgE production by stimulating B cell differentiation.

Figures 1, 2, and 3 show that IgE was produced in response to phenanthrene or DEP-PAH in a dose-response fashion. The doses at which these compounds were effective were narrow. PAH-DEP was effective at a concentration higher than 1 ng/ml, while phenanthrene was effective at greater than 0.1 ng/ml. However, at concentrations of PAH-DEP or phenanthrene greater than 10 ng/ml, the compounds were toxic in culture. Therefore, the IgE-enhancing effects were counterbalanced by the reduced cell viability engendered at the concentrations.

That DEP-PAH and phenanthrene altered B cell differentiation is supported by our data showing that these compounds altered the relative ratios of the various epsilon mRNA splice variants, a differentiation effect seen with IL-10 but not with IL-6 (Takenaka *et al.*, 1995; Diaz-Sanchez *et al.*, 1994a). IL-10, DEP-PAH, or phenanthrene favor the production of a splice variant that omits the transmembrane segment and encodes for a secreted protein (IgE grandé) that is 134 amino acids longer than the "classic" epsilon secreted (Lyczak *et al.*, 1996). DEP-PAH and phenanthrene also selected against production of the CH4'-CH5 splice variant which uses a novel splice different donor and acceptor site.

Nasal challenge with DEP increases a host of cytokine mRNAs expression in nasal lavage cells (Diaz-Sanchez *et al.*, 1994a). Thus a simple explanation for the observed changes IgE production by 2C4/F3 B cells could have been that the effects observed were secondary to an increase in their IL-10 production. However, this does not appear to be the case. Although unstimulated cultures of 2C4/F3 cells contained detectable mRNA for IL10, IL6, TNF- α , and even IL4, following stimulation with phenanthrene or DEP-PAH, the levels and the patterns of these cytokines were unchanged. The effects of DEP *in vivo* probably represent a combination of direct effects on postswitch B cells effects and effects on T cells, monocyte macrophages, and even mast cells that indirectly commit cells to IgE. Our studies in humans have definite parallels in *in vivo* murine studies where intranasal instillation of DEP has been shown to preferentially boost IgE antibody responses (Fujimaki *et al.*, 1995). This IgE enhancing effect has now also been shown for intraperitoneal administration of benzopyrene (Suzuki *et al.*, 1993), an important PAH in DEP as well as tobacco smoke (Guerin, 1992).

Epidemiological studies have linked increased incidence of asthma and allergy to airborne pollution (Takafuji *et al.*, 1989a; Corbo *et al.*, 1993; Turner, 1989; Ishizaki *et al.*, 1987). The argument in favor and against the role of pollutants in clinical allergic airways disease has been debated and discussed in many review articles (Rusznak *et al.*, 1994). There is no simple answer. Our studies on DEP are focused on a subtly different issue, whether the presence of DEP and similar organic respirable materials with the PAH they contain act as mucosal adjuvant to help initiate or enhance

IgE production to common inhaled allergens. If this is true, as our data suggest, then global pollution with respirable particles containing various PAHs are likely to be an important factor in increasing allergic respiratory disease by increasing allergic antibody responses in genetically susceptible individuals. In the present study, we have demonstrated that PAH-DEP and phenanthrene, a common organic component of DEP, enhance IgE production by human B cells. We have provided direct evidence that this occurs due to increased B cell differentiation. It is noteworthy that phenanthrenes, along with benzo[a]pyrene and a host of other PAH are found in tobacco smoke (Guerin, 1992) and tobacco smokers are known to have increased IgE levels and rates of atopy (Sherill *et al.*, 1994). Further studies will be needed to establish if these findings are related.

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