Phase II Enzymes Induction Blocks the Enhanced IgE Production in B Cells by Diesel Exhaust Particles[1]

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Oxidant pollutants such as diesel exhaust particles (DEPs) can initiate and exacerbate airway allergic responses through enhanced IgE production. These effects are especially pronounced in individuals in whom phase II antioxidant enzyme responses are impaired. We confirmed that DEPs and DEP extracts (DEPX) can act directly on B lymphocytes and showed that DEPX could enhance IgH ε germline transcription in a B cell line and in PBMCs. We therefore studied the regulation in B cells of NAD(P)H: quinone oxidoreductase (NQO1) as a typical model phase II enzyme and its role in modulating DEPX-enhanced IgE responses. DEPX increased NQO1 mRNA expression in a dose-dependent manner. NQO1 protein induction by DEPX was confirmed by Western blot. DEPs induced activity of the antioxidant response element located in the NQO1 gene promoter. Induction of both NQO1 mRNA and protein expression could be blocked by coculture with an antioxidant and partly repressed by inhibitors of PI3K and p38 MAPK, but not by inhibitors of MAPK/ERK kinase (MEK/ERK) or protein kinase C. The ability of DEPX to enhance IgE production was blocked by the induction of phase II enzymes, including NQO1 in B cells by the chemical sulforaphane. These findings suggest that a natural protective mechanism in B cells from oxidant pollutants such as diesel particles is the expression of phase II enzymes through induction of antioxidant response elements and support the approach of overexpression of these enzymes as a potential future chemopreventative strategy. ***The Journal of Immunology*****, 2006, 177: 3477–3483.**

There is accumulating epidemiological and experimental evidence that exposure to ambient particles is associated with the occurrence of allergic diseases (1–4). Diesel exhaust particles (DEPs)[3] are a major component of suspended atmospheric pollutants and have been shown to initiate and exacerbate airway allergic responses (5). DEPs and their constituent chemicals can target multiple cells including epithelial cells, macrophages, mast cells, and lymphocytes and induce the production of cytokines, chemokines, and other inflammatory mediators (6–8). An important consequence of this inflammation, observed both in human and animal models, is the increased production of IgE Ab, the hallmark of allergic disease (9–11). In addition to this adjuvant effect, DEPs can also induce or augment IgE production by acting directly on B cells (11, 12). The mechanisms by which DEPs mediate their action are still not fully understood, but it is clear that generation of oxidative stress and reactive oxygen species (ROS) is an important component (13).

Ng et al. (14) have shown that in monocytes low levels of exposure to chemical extracts of DEP (DEPX) result in the formation of ROS and lead to the activation of antioxidant response elements (AREs). In eukaryotes, ARE is thought to be important in mediating chemical-induced activation of antioxidant genes, a defense mechanism against oxidative stress (15, 16). Activation of these genes can occur by the translocation of the basic leucine zipper transcription factor NF E2 p45-related factor-2 (Nrf2) into the nucleus where it binds the AP1-NFE2 motif (GCTGAGTCATGAT GAGTCA) in the ARE (17, 18). Among the genes induced are those for phase II drug-metabolizing enzymes that protect cells against the toxic effects of xenobiotics and oxidatively labile compounds by conjugating them and their byproducts into small hydrophilic moieties, thereby rendering them into less toxic forms (19). The importance of these phase II enzymes in regulating inflammatory responses to pollutants is illustrated by studies showing that individuals with nonfunctioning variants of phase II enzyme genes are highly susceptible to the proallergenic and proallergic effects of DEPs and ozone (20, 21). Thus, in individuals lacking the ability to make GST μ1 (GSTM1), upon challenge with DEPs their allergen-specific IgE responses are 50-fold higher than in individuals whose GSTM1 is present (20).

It is likely that the severity of responses to pollutants is critically dependent on the magnitude of phase II enzyme responses. We believe that a possible future therapeutic strategy to counteract the effects of diesel and other pollutants is through the induction of phase II enzyme genes to high levels. Therefore, it is important to understand how these enzymes are regulated by pollutants in cells that govern allergic responses. Although genes of phase II enzymes are ubiquitously expressed, their induction by oxidant chemicals has been primarily studied in hepatocytes. In this study we examine IgE production in B cells and its association with phase II responses induced by chemical extracts of DEPs. We confirm the ability of DEPX to directly target B cells to enhance IgE production and show that this may be achieved by increased ε germline transcription. We have chosen a typical phase II enzyme, NAD(P)H:quinone oxidoreductase (NQO1), as a model member of the family. We show that DEPX can up-regulate NQO1 expression by induction of ARE, identify a putative pathway, and demonstrate that potent induction of the Nrf2 transcription factor will increase

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[3] Abbreviations used in this paper: DEP, diesel exhaust particle; DEPX, DEP extract; ARE, antioxidant response element; Nrf2, NF E2 p45-related factor-2; NAC, *N*-acetylcysteine; NQO1, NAD(P)H:quinone oxidoreductase 1; ROS, reactive oxygen species; GSTM1, GST μ1; Q-PCR, quantitative PCR; εGT, ε germline transcript.

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production of phase II enzymes in B cells and thereby block DEP-enhanced IgE production.

Materials and Methods

Reagents

RPMI 1640 was obtained from Invitrogen Life Technologies. pGL3 luciferase reporter vectors and the Dual luciferase reporter assay system were purchased from Promega. SB203580 was obtained from A.G. Scientific, and LY294002, calphostin C, and PD98059 were from Calbiochem. *N*-acetylcysteine (NAC) was purchased from Sigma-Aldrich. Goat anti-NQO1 polyclonal Ab and HRP-labeled donkey anti-goat IgG were purchased from Abcam. Mouse anti-β-actin mAb was obtained from Santa Cruz Biotechnology. A plasmid midi kit was purchased from Qiagen. IsoPrep was obtained from Robbins Scientific. DL-Sulforaphane (1-isothiocyanato-4-(*R*)-(methylsulfinyl)-butane; $CH_3S(CH_2)_4NCS$) was obtained from LKT Laboratories and diluted in PBS for addition to cell cultures. DEPs were a generous gift from Dr. M. Sagai (National Institute for Environmental Studies, Tokyo, Japan). These particles were generated by a light duty, four-cylinder diesel engine (4JB1 type; Isuzu Motors) using standard diesel fuel and methanol extracts prepared as previously described (11, 22).

PBMC isolation and B cell purification

PBMCs were prepared by Ficoll-Hypaque density centrifugation using IsoPrep. Briefly, whole blood was diluted by an equal volume of 0.9% NaCl. The diluted blood was carefully layered over IsoPrep and centrifuged at 1900 rpm for 20 min. The lymphocytes at the sample and IsoPrep interface were carefully transferred to a new tube and diluted with medium. After centrifugation at 1100 rpm for 10 min, the cell pellets were resuspended with RPMI 1640, and B cell isolation was performed as previously described (11). Briefly, T cells were depleted by two cycles of rosette formation with sheep RBCs treated with 2-aminoethylisothiouronium bromide (Sigma-Aldrich). Two cycles of adherence to plastic dishes in the presence of serum were performed to remove monocytes/macrophages. The resulting cell populations contained <1% contaminating T cells ($CD3^+$) and >98.5% B cells ($CD19^+CD20^+$) as determined by cytofluorographic analysis. The Human Subject Protection Committee of the University of California (Los Angeles, CA) approved all studies, and all subjects gave informed consent.

Cell line determination

PCR-restriction fragment length polymorphism was performed on four human lymphocyte cell lines (DG75, Ramos 2G6, BL2, and CL-01) to determine wild-type, mutant-type, or heterozygous NQO1 polymorphism status (*NQO1* C609T mutation). Briefly, the target PCR product of 266 bp was amplified by the sense primer 5′-GAATTGGTTGACTTACCTC-3′ and the antisense primer 5′-TTTCTCCTCATCCTGTACCT-3′. The PCR products (20 μl) were digested with 5 U of *Hin*fI restriction endonuclease at 37°C for 4 h (Fermentas Life Sciences) in a total volume of 25 μl, and the products were separated by electrophoresis on an ethidium bromide-stained 2.5% agarose gel. Mutation resulted in smaller fragments of 151 and 78 bp as compared with the wild-type allele (229 bp).

Cell culture, DEPX stimulation, and treatment

PBMCs, purified B cells, and B cell line DG75 were maintained in RPMI 1640 containing 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The DG75 cell line was chosen because it contained the wild-type (functional) form of the *NQO1* gene. Other B cell lines (Ramos 2G6, BL2, and CL-01) were observed to contain variant forms of *NQO1*, resulting in the nonproduction of fully functional NQO1. To examine the induction of NQO1 gene expression by DEPX, total 1×10^6 PBMCs or 3×10^6 DG75 cells were placed in 24-well plates or in 6-well plates overnight and stimulated by 2.5, 5.0, 10.0, or 20.0 μg of DEPX for 6 h for mRNA analysis or 16 h for protein assay. To investigate the effect of antioxidant NAC and kinase inhibitors on the induction of NQO1 by DEPX, the cells were preincubated with the appropriate concentration of NAC and kinase inhibitors of PD98059, LY294002, SB203580, and calphostin C for 2 h before stimulation with 20 μg of DEPX. After 6 or 16 h of incubation, the cells were collected for real-time quantitative PCR and Western blot assay. Controls were treated with DMSO at a final concentration of 0.1%. To test the effect of sulforaphane on IgE production, PBMCs were cultured at 1×10^6/ml and B cells at 0.5×10^6/ml. B cells (200 μl) were introduced into each well, and quadruplicates were used for each point in each experiment. Cultures were kept at 37°C and 5% CO_2 for 14 days. Cells were cultured in the presence of 200 U/ml rIL-4 and anti-CD40 (0.1 μg/ml) with or without DEPX (100 ng/ml). Sulforaphane at 0–30 μM was added at the start of culture.

Cell RNA extraction and reverse transcription reaction

RNA was extracted from cells by the routine TRIzol method. RNase-free DNase (1 μl) was applied to eliminate genomic DNA contamination in a total volume of 10 μl containing 4 μg of total RNA. cDNAs were synthesized using DNase-treated total RNA (4 μg), 1× first stand buffer (diluted from 5× first strand buffer), 0.01M DTT, 0.5 mM each dNTP, 1 μM oligo(dT), 40 U of RNase inhibitor, and 200 U of Moloney murine leukemia virus reverse transcriptase (Invitrogen Life Sciences) in a 25-μl reaction mixture at 42°C for 2 h.

Real-time quantitative PCR assay

Real-time quantitative PCR (Q-PCR) was performed using the QuantiTect SYBR Green PCR kit (Qiagen) on an iCycler iQ real-time PCR detection system (Bio-Rad) according to manufacturer's instruction. The primers for phase II enzymes used in Q-PCR are listed in Table I. The program was conducted under the following conditions. After the initial activation step at 95°C for 15 min, there were 40 cycles of denaturation at 94°C for 15 s and annealing at 60°C for 1 min followed by melting curve analysis consisting of one cycle at 95°C for 1 min, one cycle at 55°C for 1 min, and 80 cycles starting at 55°C with 0.5°C gradient accretion. Data were analyzed by iCycler iQ optical system software (version 3.0a). The average of three independent measurements was used to calculate the relative gene expression levels compared with *GAPDH* expression levels.

Western blot assay for NQO1 protein

The cell pellets were lysed with 150 μl of radioimmune precipitation assay lysis buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 150 mM NaCl, 50 mM Tris-HCl (pH 7.5), 1 mM PMSF, 4 mM EDTA, 10 mM NaF, and 2 mM Na_3VO_4). Forty micrograms of total proteins was loaded for electrophoresis on 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membrane. The membrane was incubated with 1/2000 anti-NQO1 Ab for 1 h. After three washes with TBS, the membrane was incubated with 1/5000 anti-goat IgG for 1 h. An ECL (Amersham Biosciences) Western blot kit was used to detect the signals. The membrane was stripped by stripping buffer (2% SDS, 100 mM 2-ME, and 50 mM Tris (pH 6.8)) and reblocked by 5% nonfat milk for 3 h. The same membrane was incubated with 1/2000 anti-β-actin Ab for 1 h. After three washes, the membrane was incubated with 1/2500 secondary Ab. β-Actin was used as internal control of the NQO1 protein assay.

NQO1-ARE construction of plasmid

The *NQO1* fragment of 362 bp containing ARE (AAATCGCAGTCA CAGTGACTCAGCAGAATCTGAGCCTAGG) located in *NQO1* promoter (from −477 to −438 upstream of the transcription start site) was amplified by the sense primer 5′-GGCGGCTAGCCCATTACCTGCCTT GAGGAG-3′ with the *Nhe*I site and anti-sense primer 5′-GGCGCTC GAGCAAAAATCTGGCTGGGGGTG-3′ with the *Xho*I site. The PCR product and the pGL3 luciferases reporter vectors were digested with the restriction endonuclease enzymes *Nhe*I and *Xho*I (New England Biolabs) at 37°C for 2 h. After purification of the digested PCR product and the pGL3 vector, the PCR product was cloned into *Nhe*I and *Xho*I sites of the pGL3 vectors by using T4 DNA ligase at 16°C overnight. The positive plasmid with NQO1-ARE was extracted and purified by a Qiagen plasmid midi kit.

Table I. *Primer sequence of quantitative PCR*

Gene	Primer Sequence
NQO1	Forward: 5′-CTTACGCTGCCATGTATGAC-3′ Reverse: 5′-GAGTGTGCCCAATGCTATAT-3′
GSTM1	Forward: 5′-CAGTCAGCCGCATCTTCTTT-3′ Reverse: 5′-CGCCCAATACGACCAAATC-3′
GSTP1	Forward: 5′-CCAAGTTCCAGGACGGAGA-3′ Reverse: 5′-CTGGTCCTTCCCATAGAGC-3′
GAPDH	Forward: 5′-CAGTCAGCCGCATCTTCTTT-3′ Reverse: 5′-CGCCCAATACGACCAAATC-3′

Cell transfection and luciferase reporter assay

DG75 cells grew in RPMI 1640 and reached 1×10^6/ml concentration. The cells were centrifuged at 1100 rpm for 5 min and resuspended in the medium with 20% FBS. DG75 cells (5×10^6) in 400 μl of medium were cotransfected with 10 μg of the reporter plasmid containing NQO1-ARE and 50 ng of the control plasmid (*Renilla*) using an electroporator (Bio-Rad) with voltage set at 225 V and capacitance set at 975 μF. Cells were cultured in RPMI 1640 containing 10% FBS and were rested for 24 h before the addition of various stimuli. After treatment, the transfected cells were collected and lysed with the luciferase lysis buffer according to the manufacturer's instruction. The Dual luciferase reporter assay kit and a Monolight 2010 luminometer (Analytical Luminescence Laboratory) were used to measure the luciferase activity.

IgH ε germline transcription

The human B lymphoma cell line Ramos 2G6 was maintained in RPMI 1640 medium. PBMCs were isolated from healthy volunteers as described in the paragraph *PBMC isolation and B cell purification* above in this section. Total 2×10^6 Ramos 2G6 cells per well in 6-well plates and 5×10^6 PBMCs per well in 24-well plates were stimulated by various concentrations of DEPX extracts in the presence of IL4 (10 ng/ml) and anti-CD40 (5 μg/ml) for 3 days. The cells were collected for *IgH* ε germline transcription assay. The primer pairs 5′-AGCTGTCCAGGAACCCGACAGG GAG-3′ and 5′-GTTGATAGTCCCTGGGGTGTA-3′ were used to amply the ε germline transcripts (εGTs). *GAPDH* was used as an internal control.

IgE determination

IgE in the cultured PBMCs and purified B cells was measured by isotype-specific ELISA as previously described (23, 24). Briefly, microtiter plates were coated with mAbs to human IgE mAb, CIA-E-7.12 and CIA-E-4.15, at 2 μg/ml overnight. After washing, 100 μl of samples and standards were added for overnight incubation. After further washing of the detection Ab, alkaline phosphatase-labeled goat anti-IgE was added at 1/3000 (Kirkegaard & Perry Laboratories). Absorbance at 405 nm was read with an ELISA reader (Bio-Tek Instruments). Standard for IgE was the IgE myeloma protein PS. The sensitivity of the IgE assay was 0.2 ng/ml. All samples were run in quadruplicate.

Results

Induction of NQO1 by DEPX in PBMCs and B lymphocytes

DEPX increased mRNA gene expression for *NQO1* in both PBLs and the B lymphocyte cell line DG75. When PBMCs were cocultured with DEPX, a distinct dose-response relationship was observed by Q-PCR (Fig. 1). NQO1 expression was increased 12.6- and 16.3-fold over baseline in PBMCs from two individuals after stimulation with 20 μg/ml DEP. A similar dose-response induction

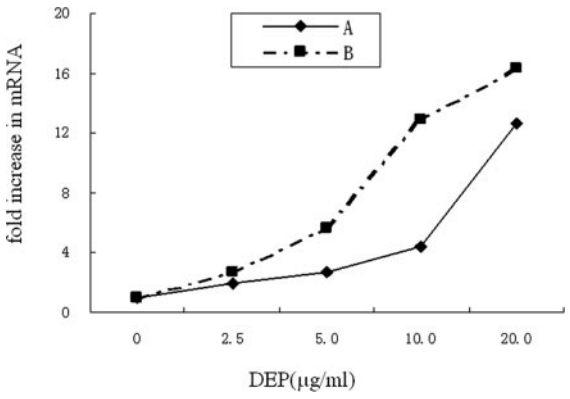

FIGURE 1. *NQO1* mRNA expression induced by DEPX in PBMCs. PBMCs were isolated from fresh blood donated by two volunteers, subjects A and B. The 1×10^6 cells were placed in 24-well plates and stimulated with various concentrations of DEPX from 2.5 to 20.0 μg/ml. The dose-response relationship between NQO1 mRNA expression and DEPX was observed. PBMC isolation and Q-PCR were performed as described in *Materials and Methods*.

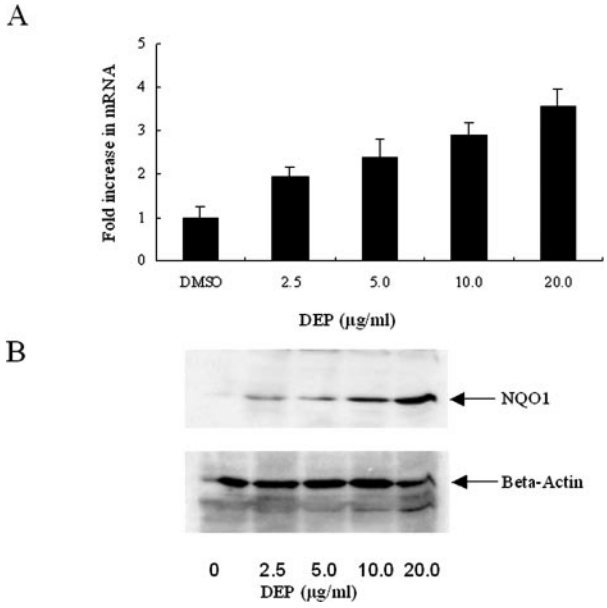

FIGURE 2. Induction of NQO1 gene expression by DEPX in lymphocytes. The DG75 cells were stimulated with concentrations of DEPX from 2.5 to 20.0 μg/ml. *A*, Q-PCR analyses showing dose-dependent induction of *NQO1* gene transcription by DEPX after 6 h of stimulation. The values were obtained from two individual experiments. *B*, Western blot showing the dose-dependent induction of NQO1 protein in DG75 cells treated with DEPX for 16 h. β-Actin was used as internal control. Q-PCR and Western blot were performed as described in *Materials and Methods*.

in both mRNA and protein levels was observed in DG75 cells (Fig. 2, *A* and *B*).

Effect of DEPX on ARE located in NQO1 promoter

The ARE is known to regulate the expression and coordinated induction of many detoxifying enzyme genes in responses to antioxidants and xenobiotics. DEPX could induce ARE activity in the *NQO1* gene promoter. We cotransfected a reporter gene construct containing the ARE located in the NQO1 promoter and a control reporter gene, *Renilla*, into the DG75 B cell line. Stimulation of the cells with DEPX for 16 h resulted in a dose-dependent increase of ARE activity in these cells after 36 h of transfection (Fig. 3). A 5.07-fold increase was observed in NQO1 ARE activity induced by 20 μg/ml DEPX compared with control using DMSO.

Induction of NQO1 gene expression by DEPX on DG75 cell is inhibited by pretreatment with NAC

ROS generated by DEP was shown to be involved in multiple DEP-induced kinase activation. The antioxidant NAC is a thiol-reducing agent that can antagonize cellular ROS (25) and block DEP-induced enhanced IgE production in vivo in mice (26). We therefore examined the regulatory role of the cellular redox state in DEP-induced NQO1 gene expression. DG75 B cells were pretreated with 20 μM NAC or control for 2 h and then stimulated with 20 μg/ml DEPX. We observed that whereas ARE activity was partially blocked by NAC (Fig. 4*A*), induction of mRNA and protein by DEPX was greatly suppressed by NAC (Fig. 4, *B* and *C*).

DEP and its chemicals have been reported to activate several different signaling pathways. We examined the role of different pathways in NQO1 induction by DEPX. DG75 cells were pretreated with different specific inhibitors (SB203580 for p38 MAPK, LY294002 for PI3K, calphostin C for protein kinase C, and PD98059 for MAPK/ERK kinase (MEK/ERK) for 2 h before

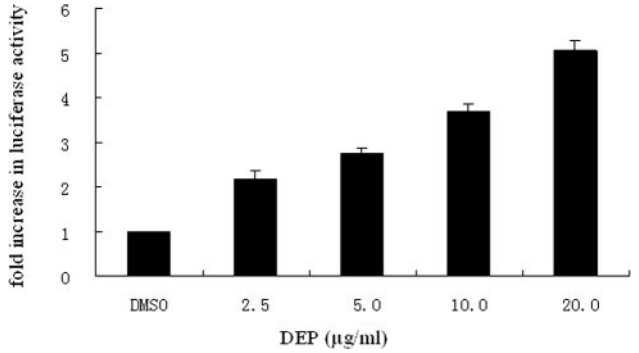

FIGURE 3. Effect of DEPX on ARE activity mediated *NQO1* gene expression in DG75 by dual-reporter gene assay. The 5×10^6 DG75 cells in 400 μl of medium transiently transfected with 5-μg reporter constructs containing the *NQO1* gene *ARE* and 50 ng of control reporter expressing *Renilla* luciferase. Thirty-six hours after transfection, the cells were incubated with concentrations of DEPX from 2.5 to 20.0 μg/ml for 16 h. The cells were incubated with DMSO as a parallel control. Dural luciferase reporter assay was performed on a Monolight 2010 luminometer. The values represented the mean $\pm$ SE of two independent experiments with each experiment done in triplicate.

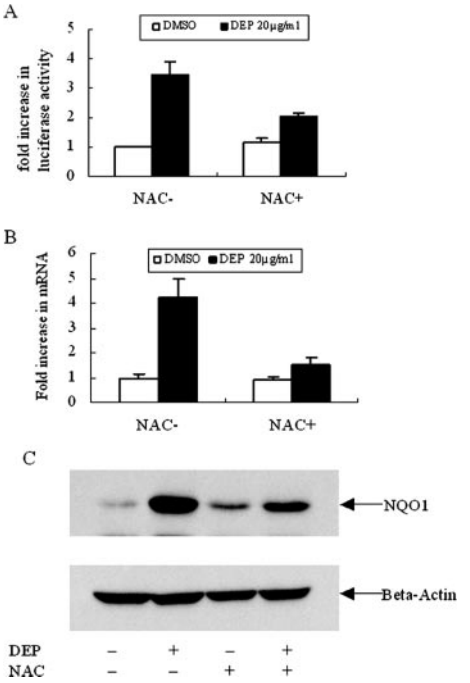

FIGURE 4. Effect of NAC on induction of NQO1 gene expression by DEPX in the lymphocyte cell line DG75. *A*, The DG75 cells were cotransfected with luciferase reporter construct containing an *ARE* located in the NQO1 promoter and a control reporter expressing *Renilla* luciferase. The cells were seeded in a 12-well plate after 36 h with transfection. Pretreatment with 20 μM NAC for 2 h, and then the cells were stimulated with 20 μg/ml DEPX for 16 h. The Dual luciferase reporter assay was performed as described in *Materials and Methods*. *B* and *C*, The cells (1×10^6/ml) were seeded in 6-well plates overnight and pretreated with 20 μM NAC for 2 h. After that, the cells were treated with 20 μg/ml DEPX for 6 or 16 h. The total RNA and cDNA were prepared for NQO1 gene transcriptional expression (*B*). The cells were lysed and analyzed for NQO1 protein by Western blot assay (*C*).

exposure to 20 μg/ml DEP. The Dual reporter luciferase assay showed that inhibition of p38 MAPK and PI3K partially blocked NQO1-ARE induction by DEPX (Fig. 5*A*) as well as the induction of NQO1 mRNA and protein. (Fig. 5, *B* and *C*). In contrast, inhibition of protein kinase C failed to block DEPX induction of NQO1-ARE activity and mRNA and protein expression. Inhibition of MEK/ERK partly suppressed ARE activity but had no effect on NQO1 mRNA and protein expression.

IgH ε *germline transcription enhanced by DEPX*

IgH ε germline transcription is required for IgE class switch recombination. Class switch recombination is essential for IgE production. The mechanism of IgE production by DEPX is still not clear. In this study, we examined whether the production of the *IgH* ε germline transcripts was affected by DEPX. Ramos 2G6 cells and PBMCs were stimulated with DEPX at various doses in the presence of IL-4 and anti-CD 40. The spontaneous IgH ε germline transcription occurred in Ramos 2G6 cells and PBMCs. The *εGTs* could be detected in the cells without IL-4 and anti-CD40, but the level was relatively low. The result of Q-PCR showed the DEPX enhanced εGT production in both the B cell line and PBMCs in a dose-response manner (Fig. 6).

Induction of phase II enzymes can block DEPX-enhanced IgE production

DEPX can act directly on B cells to increase IgE production. If production of phase II enzymes such as NQO1 is indeed a cytoprotective mechanism, we reasoned that high-level induction of these enzymes should ablate the effect of DEPX. Therefore, we stimulated primary B cells or PBMC with IL-4, anti-CD40, and DEPX in the presence or absence of sulforaphane, a potent phase II inducer. As expected, after 14 days the cells cocultured with DEP-extract had IgE levels 200–600% higher than those cultured with IL-4/anti-CD40 alone. Fig. 7 demonstrates that the addition of sulforaphane to B cells resulted in a dose-dependent inhibition of DEP-induced IgE potentiation. At concentrations of sulforaphane of 6 μM and higher, IgE levels from cells stimulated without DEPX and those with DEPX were indistinguishable. The addition of sulforaphane in the B cell line Ramos 2G6 and in PBMCs resulted in increased gene expression of the sentinel phase II enzyme *NQO1*, and the induction of mRNA levels of the other enzymes such as *GSTM1* and *GSTP1* was also observed (Table II). No cytotoxicity was observed at any of the concentrations used (data not shown). Similar results were observed with PBMCs (data not shown).

Discussion

DEPs and their chemicals can directly activate B cells to enhance production of the allergic Ab IgE (20, 23, 27). Elevated IgE levels are thought to be important contributors of the heightened allergic airway response induced by DEPs. In this study we show that B cells will also make a cytoprotective response to diesel extracts and that if this response can be sufficiently elevated it can block the IgE potentiation effect. These results support the view that chemoprevention of the proinflammatory and proallergic effects of oxidant pollutants may be possible by enhancing production of phase II metabolizing enzymes.

DEPs are model particulate pollutants. Both human and animal models have demonstrated that they can exacerbate allergic airway disease (5–7). In addition, they can also promote de novo allergic sensitization to a neoallergen. In both of these primary and secondary allergic responses IgE is thought to play a critical role. DEP can stimulate IgE production both directly by interacting with an allergen to enhance isotype switching to IgE and indirectly by promoting a Th2 cytokine milieu that favors IgE. Both pathways have been demonstrated in vivo in humans and mice (12, 28–30).

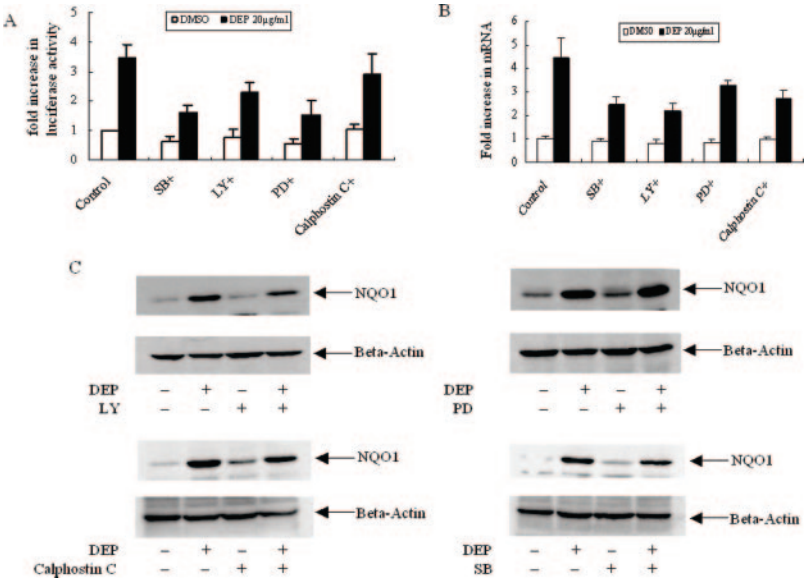

FIGURE 5. Effect of kinase inhibitor on induction of NQO1 gene expression by DEPX in DG75. The cells were pretreated with 10 μM LY294002 (LY), 20 μM PD98059 (PD), 20 μM SB203580 (SB), and 50 nM calphostin C before stimulation with 20 μg/ml DEPX (DEP), respectively. *A*, The DG75 cells were cotransfected with luciferase reporter construct containing an *ARE* located in *NQO1* promoter and a control reporter expressing *Renilla* luciferase. The cells were seeded in a 12-well plate overnight after 36 h with transfection. After pretreatment with the kinase inhibitor, the cells were stimulated with 20 μg/ml DEPX for 16 h. The Dual luciferase reporter assay was performed as described in *Materials and Methods*. *B* and *C*, The cells (1×10^6/ml) were seeded in 6-well plates overnight and pretreated with the inhibitors listed above. After that, the cells were treated with 20 μg/ml DEPX for 6 or 16 h. The cells were collected for Q-PCR assay (*B*) and Western blot assay (*C*).

Although the precise mechanisms by which particles such as DEPs mediate their effects are still not fully elaborated, it seems that the principal determinant is the ability to generate ROS and oxidative stress (31). The biological consequences of oxidative stress on the airways include activation of redox-sensitive transcription factors such as MAPK and NF–κB, which have been shown to regulate IgE isotype switching (32, 33). In this study we examined the effect of DEPX on the expression of εGTs (Fig. 6). We show that DEPX can directly enhance germline transcription in B cells, a necessary step required for the Sμ-Sε class recombination switch that is essential for IgE production. Although it is possible that other mechanisms may also be involved, it is likely that diesel exhaust can augment allergic responses by directly targeting B cells to increase production of ε germline transcripts.

DEP consists of a complex structure characterized by a carbon core absorbed with organic chemicals including quinones and polycyclic aromatic hydrocarbons. Polycyclic aromatic hydrocarbon metabolism induced by phase I drug-metabolizing enzymes produces electrophilic reactive metabolites, including ROS. Quinones can directly induce oxidative stress by redox cycling and are suspected of being responsible for the production of O_2^- and ·OH radicals (34). DEPX can induce gene expression of phase II drug metabolizing enzymes that can protect cells against the toxic effects of these oxidatively labile compounds (35).

Both population-based and experimental challenge studies have suggested that phase II enzymes could be involved in defense against pollution-induced allergic airway disease (20, 36). Individuals with polymorphisms in genes of key phase II enzymes that result in the absence or reduction of the protein are more susceptible to the adjuvant effects of oxidant pollutants (37, 38). For example, we have shown that IgE production in response to nasal challenge with DEPX plus allergen is >10-fold higher in GSTM1-null vs GSTM1-present subjects. In this study we have used NQO1 as a model phase II enzyme. Detoxification of quinones by NQO1 results in the formation of stable hydroquinones that can be readily conjugated and excreted.

In this study we show that the dose-dependent increase of NQO1 mRNA and protein expression in B lymphocytes is via

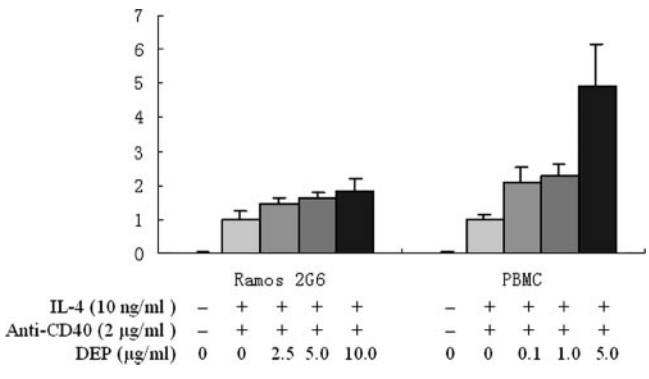

FIGURE 6. Enhancement of *IgH* ε germline transcription by DEPX. Total Ramos 2G6 cells (2×10^6/well) in 6-well plates and PBMCs (5×10^6/well) in 24-well plates were stimulated by various concentrations of DEPX in the presence of IL4 (10 ng/ml) and anti-CD40 (5 μg/ml) for 3 days. The cells were collected for ε germline transcription assay. The total RNA was extracted, and cDNA was synthesized for the Q-PCR as described in *Materials and Methods*. *GAPDH* was used as an internal control.

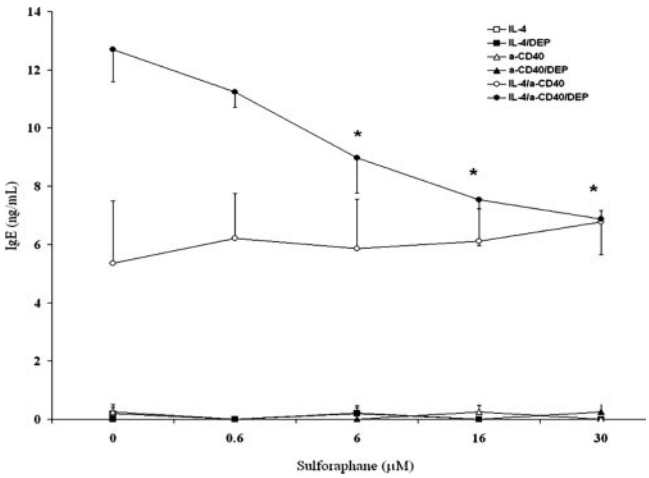

FIGURE 7. Inhibition of sulforaphane on DEPX-enhanced IgE production. Purified B cells were cultured in quadruplicate at 0.5×10^6/ml for 14 days in the presence of IL-4 (200 U/ml), CD-40 (0.1 μg/ml), DEPX (100 ng/ml), and different concentrations of sulforaphane from 0.6 to 30 μM. The medium was collected to measure the IgE. The value presented are mean ± SD of four experiments. *, $p < 0.05$ (statistical significance).

Table II. *mRNA expression of phase II enzymes induced by sulforaphane in B cell line and PBMC*

Gene	Sulforaphane Concentration (μM)	Relative to Medium Control in Ramos 2G6[a] (fold ± SD)	Relative to Medium Control in PBMC[b] (fold ± SD)
NQO1	5	5.24 ± 1.68	3.74 ± 2.40
	10	7.26 ± 1.91	3.56 ± 0.18
	15	10.13 ± 2.39	4.50 ± 0.81
	20	12.82 ± 2.82	5.03 ± 1.64
GSTM1	5	1.38 ± 0.37	2.00 ± 0.96
	10	1.51 ± 0.32	1.98 ± 1.30
	15	2.19 ± 0.58	3.00 ± 1.95
	20	1.78 ± 0.50	2.69 ± 1.27
GSTP1	5	1.95 ± 0.62	2.05 ± 1.33
	10	1.52 ± 0.42	1.99 ± 0.13
	15	1.39 ± 0.35	1.97 ± 0.48
	20	1.64 ± 0.38	1.42 ± 0.43

[a] The value was calculated from three independent experiments.
[b] The value was calculated from three donors.

ARE mediation and that p38 and PI3K, but not protein kinase C or the MEK/ERK pathways, are involved in this induction. The role of kinase pathways involved in NQO1 gene expression is controversial. However, our results agree with previous reports in IMR-32 human neuroblastoma cells showing that activation of the human *NQO1-ARE* by *tert*-butylhydroquinone is mediated by PI3K, not MEK/ERK (39). Jaiswal and coworkers (40, 41) identified ARE located in the *NQO1* promoter (−477 to −438) from the transcription start site. ARE is known to regulate the expression and coordinated induction of many detoxifying enzyme genes including *NQO1* (19, 42), *GST Ya subunit* (43), and *heme oxygenase-1* (44). The ARE is thus a transcriptional regulatory element that is widely distributed and protects against chemical-induced electrophile and oxidative toxicity through the expression of phase II conjugating enzymes. Several nuclear proteins have been shown to bind to the ARE either as homodimers or heterodimers. Analysis of ARE-nuclear protein complexes revealed a number of nuclear transcription factors including c-Jun, Jun-B, Jun-D, c-Fos, Fra1, Nrf1, Nrf2, and the small Maf protein had been shown to bind the human NQO1 gene *ARE* (16, 45, 46). There are several reports that NQO1 induction by *tert*-butylhydroquinone (a quinone and a phenolic antioxidant) is mediated through ARE by the Nrf2 transcription factor activation (47). NQO1 up-regulation induced by DEPX in human airway epithelial cells is most likely through the stress-sensitive Nrf2. DEPX can induce the translocation of Nrf2 to the nucleus and increase protein nuclear binding to the ARE, thereby leading to induction of antioxidant genes expression.

Xiao et al. (48) have suggested that in respiratory tract tissues and cells the biological response to oxidative stress is a hierarchical process in which the induction of the antioxidant effects of phase II enzymes is in competition with the pathways that generate allergic inflammation. In this work we have made use of the potent Nrf2 activator sulforaphane. Originally isolated from cruciferous vegetables, sulforaphane is the most powerful inducer of phase II enzymes identified to date (49–52). Preculture of B cells or PBMCs with sulforaphane significantly inhibited DEPX-induced IgE production. These results support our view that production of phase II enzymes is the primary defense mechanism of the body against the proallergenic effects of oxidant pollutants. Both in vivo and in vitro DEPX has been shown to target multiple cell types including airway epithelial cells and alveolar macrophages. Currently, studies are underway to determine whether phase II enzyme induction in these cells and in animal models will also ablate the effects of DEPX.

Disclosures

The authors have no financial conflict of interest.

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