Pro-oxidative diesel exhaust particle chemicals inhibit LPS-induced dendritic cell responses involved in T-helper differentiation

Ray Chun-Fai Chan, PhD,[a] Meiying Wang, MD,[a] Ning Li, MD, PhD,[a] Yoshiki Yanagawa, PhD,[b] Kazunori Onoé, PhD,[b] James J. Lee, PhD,[c] and Andre E. Nel, MD, PhD[a]

Los Angeles, Calif, Sapporo, Japan, and Scottsdale, Ariz

Background: Epidemiologic studies show that exposure to ambient particulate matter leads to asthma exacerbation. Diesel exhaust particles (DEPs), a model pollutant, act as an adjuvant for allergic sensitization. Increasing evidence shows that this effect could be mediated by an effect on dendritic cells (DCs).
Objective: Our aim was to elucidate the mechanism by which pro-oxidative DEP chemicals change DC function so that these antigen-presenting cells strengthen the immune response to an experimental allergen.
Methods: We exposed murine bone marrow–derived DCs and a homogeneous myeloid DC line, BC1, to DEPs and organic extracts made from these particles to determine how the induction of oxidative stress affects cellular maturation, cytokine production, and activation of antigen-specific T cells.
Results: DEP extracts induced oxidative stress in DCs. This change in redox equilibrium interfered in the ability of Toll-like receptor agonists to induce the expression of maturation receptors (eg, CD86, CD54, and I-A^d) and IL-12 production. This perturbation of DC function was accompanied by decreased IFN-γ and increased IL-10 induction in antigen-specific T cells. The molecular basis for the perturbation of DC function is the activation of a nuclear factor-erythroid 2 (NF-E2)–related factor 2–mediated signaling pathway that suppresses IL-12 production. NF-E2–related factor 2 deficiency abrogates the perturbation of DC function by DEPs.
Conclusion: These data provide the first report that pro-oxidative DEP chemicals can interfere in T_H1-promoting response pathways in a homogeneous DC population and provide a novel explanation for the adjuvant effect of DEPs on allergic inflammation.
Clinical implications: These data clarify the adjuvant effect of particulate air pollutants in allergic inflammatory disease.
(J Allergy Clin Immunol 2006;118:455-65.)

Key words: *Dendritic cells, T_H1/T_H2 cells, LPS, cell differentiation*

Exposure to particulate air pollutants is associated with a variety of adverse health effects, including exacerbation of allergic rhinitis and asthma.[1,2] Diesel exhaust particles (DEPs), a model particulate pollutant, exert adjuvant effects that promote allergic sensitization to common environmental allergens.[3] The DEP response is characterized by increased allergen-specific IgE production in parallel with decreased IFN-γ production in animal asthma models, as well as in the exacerbation of allergic rhinitis in human nasal challenge studies.[4] A mechanistic understanding of the basis of this adjuvant effect could help to clarify how particulate matter (PM) might lead to an increase in asthma prevalence.[5]

It is increasingly being recognized that the generation of reactive oxygen species (ROS) and oxidative stress play an important role in PM-induced allergic inflammation.[5] In addition to the contribution of the particles themselves, redox-cycling organic chemical compounds, such as polycyclic aromatic hydrocarbons and quinones, generate oxidative stress in target cells.[1] PM-induced oxidative stress is a hierarchical event, in which protective cellular responses at lower levels yield to proinflammatory effects at higher levels of oxidative stress.[1,6] The ultimate response is dependent on the type of intracellular activation pathway that is switched on by oxidative stress.[6] Thus lower levels of oxidative stress leads to the release of nuclear factor-erythroid 2 (NF-E2)–related factor 2 (Nrf2) to the nucleus, where this transcription factor initiates the expression of protective phase II enzymes, such as heme oxygenase 1 (HO-1) and catalase.[1,6] These enzymes exert antioxidant, detoxification, and anti-inflammatory effects. Higher levels of oxidative stress activates the nuclear factor (NF) κB and mitogen-activated protein kinase signaling cascades that are responsible for transcriptional activation of cytokine, chemokine, and adhesion molecule genes.[1,6] These proinflammatory pathways can be suppressed by phase II enzymes, suggesting a dynamic equilibrium between protective and proinflammatory cascades.[6]

Although DEPs affect macrophages and epithelial cells, most of these target cells are not directly involved in antigen-specific immune responses.[2] An alternative

From [a]the Division of Clinical Immunology and Allergy, Department of Medicine, University of California, Los Angeles; [b]the Division of Immunobiology, Institute for Genetic Medicine, Hokkaido University, Sapporo; and [c]the Division of Pulmonary Medicine, Mayo Clinic Scottsdale.

Supported by US Public Health Service Grants, RO-1 ES10553, RO1 ES12053, RO1 ES013432, and U19A1070453 (UCLA Asthma and Allergic Disease Research Center) and the US Environmental Protection Agency STAR grant (RD-83241301) to the Southern California Particle Center. This manuscript has not been subjected to the US Environmental Protection Agency's peer and policy review.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication February 16, 2006; revised June 6, 2006; accepted for publication June 8, 2006.

Reprint requests: Andre E. Nel, MD, PhD, Division of Clinical Immunology and Allergy, Department of Medicine, University of California, Los Angeles, CA 90095. E-mail: anel@mednet.ucla.edu.

0091-6749/$32.00

doi:10.1016/j.jaci.2006.06.006

Abbreviations used
APC: Antigen-presenting cell
BMDC: Bone-marrow derived dendritic cell
DC: Dendritic cell
DEP: Diesel exhaust particle
DEP^{ex}: Organic diesel exhaust particle extract
ERK: Extracellular signal–regulated kinase
FITC: Fluorescein isothiocyanate
HO-1: Heme oxygenase 1
MBB: Monobromobimane
NAC: N-acetylcysteine
NF-E2: Nuclear factor-erythroid 2
NF-κB: Nuclear factor κB
Nrf2: NF-E2–related factor 2
OVA: Ovalbumin
PM: Particulate matter
ROS: Reactive oxygen species
SFN: Sulforaphane
TLR: Toll-like receptor
WT: Wild-type

explanation for the adjuvant effects of DEPs is the perturbation of antigen-presenting cell (APC) function. In this regard it has been demonstrated that DEPs can suppress IL-12 production in monocyte-derived dendritic cells (DCs), whereas the study of mononuclear cells from atopic patients has shown that organic DEP extracts synergize with purified allergen in inducing cytokine production.[7] Most data suggest that the generation of oxidative stress at the APC level favors T_H2 skewing of the immune response while suppressing T_H1 differentiation.[8]

We were interested to explore the role of oxidative stress in the adjuvant effects of DEPs, with particular emphasis on the effect of pro-oxidative DEP chemicals on DC function. Our data demonstrate that organic DEP extracts interfere in LPS-induced IL-12 production in DCs and IFN-γ production in T cells but promote IL-10 production. An Nrf2-dependent oxidative stress pathway that suppresses NF-κB activation is a key component in the modulation of DC function. Decreased T_H1 differentiation is a possible explanation for the adjuvant effect of DEPs in allergic disease.

METHODS

Animals

DO11.10, BALB/c, and *Nrf2*$^{-/-}$ mice were used as described in this article's supplementary Methods text in the Online Repository at www.jacionline.org. The UCLA Animal Research Committee approved all animal experiments.

Reagents and suppliers

Reagents and suppliers are listed in this article's supplementary Methods text in the Online Repository at www.jacionline.org.

Preparation of organic DEP extracts

DEPs were a gift from Dr Masaru Sagai (National Institute of Environment Studies, Tsukuba, Iabaraki, Japan). These particles and extracts were prepared as described in this article's supplementary Methods text in the Online Repository at www.jacionline.org.

Generation of bone marrow–derived DCs and cell culture

Generation of bone marrow–derived DCs (BMDCs) and maintenance of BC1 cell culture are described in this article's supplementary Methods text in the Online Repository at www.jacionline.org.

Flow cytometric analysis

Cells were stained and analyzed as described in this article's supplementary Methods text in the Online Repository at www.jacionline.org.

Measurement of intracellular thiol levels

Intracellular thiol levels were measured by means of flow cytometry with monobromobimane (MBB), as described in this article's supplementary Methods text in the Online Repository at www.jacionline.org.

Western blotting

Western blotting to determine HO-1 expression and degradation of IκB proteins is described in this article's supplementary Methods text in the Online Repository at www.jacionline.org.

Electrophoretic mobility shift assays

$CD11c^+$ cells were purified from day 7 BMDCs from wild-type (WT) and *Nrf2*$^{-/-}$ mice by using CD11c MicroBeads. $CD11c^+$ BMDCs and BC1 cells were stimulated with 5 ng/mL LPS for 30 minutes before the preparation of nuclear extracts with NE-PER nuclear and cytoplasmic extraction reagents (Pierce, Rockford, Ill) according to the manufacturer's instructions. Electrophoretic mobility shift assays to determine the presence of Rel proteins in the nucleus was performed as described in this article's supplementary Methods text in the Online Repository at www.jacionline.org.

Cytokine assays

BC1 cells or BMDCs (2×10^5 cells in 500 μL of culture medium) were stimulated with DEPs (10 μg/mL), LPS (5 ng/mL), or DEPs plus LPS in 24-well culture plates (Falcon; BD Biosciences, San Jose, Calif). Supernatants were harvested after 24 hours and stored at −20°C until murine IL-12p40, murine IL-12p70, murine IL-4, and IL-10 levels were measured by means of ELISA.

Assessment of DO11.10 T-cell activation by BC1 cells

BC1 cells were plated at 1×10^6 cells per well in 12-well plates and then treated with 100 ng/mL ovalbumin peptide ($OVA_{323-339}$; Bachem) along with DEPs, LPS, or LPS plus DEPs in complete Iscove's Modified Dulbecco's Medium (IMDM) for 24 hours. BC1 cells were irradiated at 30 Gy with a ^{137}Cs irradiator (Nordion, Ottawa, Ontario, Canada). $CD4^+$ T cells, 2×10^5, from DO11.10 mice, purified by using mouse CD4 MicroBeads (Miltenyi Biotec, Auburn, Calif), were cocultured with 2×10^4 irradiated BC1 cells per well (96-well) for 48 hours. The supernatants were harvested for murine IFN-γ, IL-10, and murine IL-4 measurement.

Statistical analysis

Data were analyzed for statistical significance by using the Student *t* test. A *P* value of less than .05 was regarded as statistically significant.

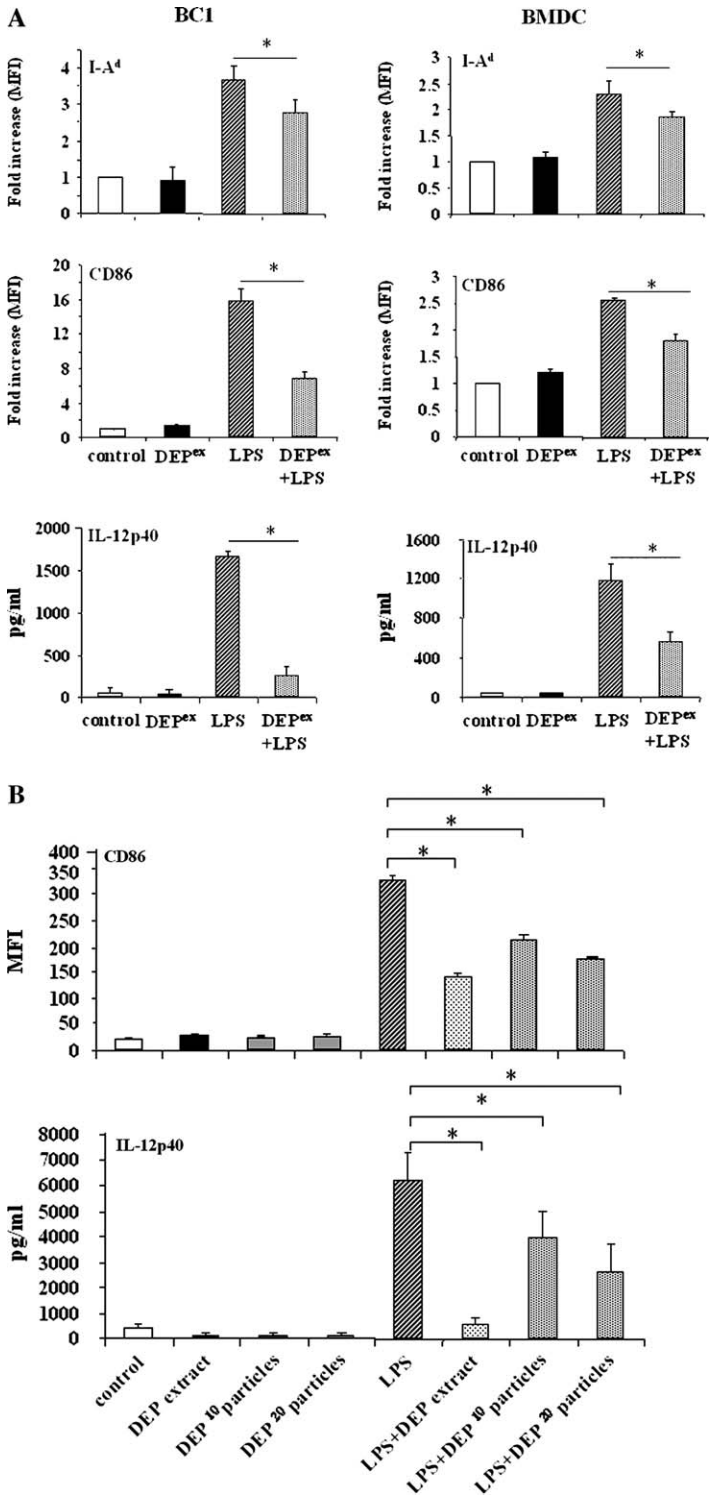

FIG 1. Intact DEPs and their extract suppress LPS-induced CD86 and I-A^d expression and IL-12 production in DCs. **A**, Flow cytometric analyses of I-A^d or CD86 expression on BC1 cells were assessed by using FACScan *(left panel)*. Similar analysis was performed for BMDCs from BALB/c mice *(right panel)*. CD86 and I-A^d expression was determined in CD11c$^+$ cells: ELISA of IL-12p40 in culture media *(lower panel)*. **B**, CD86 expression and IL-12 production by BC1 cells exposed to intact particles. Results are presented as means ± SD from 3 independent experiments (*$P < .01$).

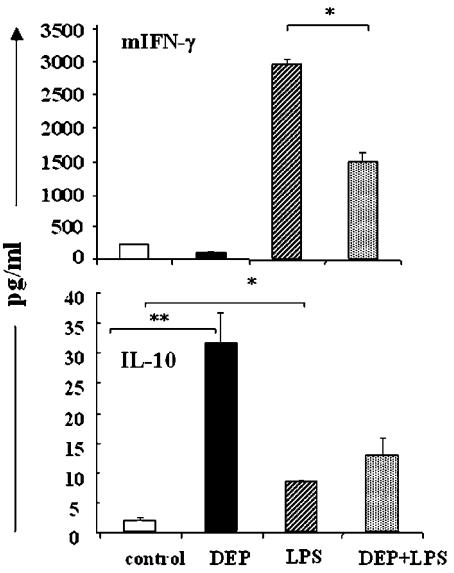

FIG 2. DEP chemicals suppress IFN-γ and promote IL-10 secretion in DO11.10 T cells coincubated with antigen-presenting BC1 cells. IFN-γ and IL-10 levels were assessed by means of ELISA after 48 hours' incubation. Results are presented as means ± SD from 3 independent experiments (**$P < .001$; *$P < .01$).

RESULTS

Organic DEP extracts inhibit the maturation of BMDCs and a DC line during LPS stimulation

Unstimulated BC1 cells behave phenotypically and functionally as immature myeloid DCs.[9] Exposure of this homogeneous DC line to an organic DEP extract (DEP^{ex}), which includes a number of well-characterized pro-oxidative chemicals,[1,10] did not change the expression of the MHC class II molecule I-A^d or the costimulatory molecule CD86 (Fig 1, *A*). There was also no effect on the expression of the adhesion molecule CD54 (see Fig E1 in the Online Repository at www.jacionline.org). Stimulation with LPS increased the expression of the above maturation markers through a pathway that involves the Toll-like receptor (TLR) 4 (Fig 1, *A*, and Fig E1, *A*, in the Online Repository at www.jacionline.org). Simultaneous introduction of DEP^{ex} significantly suppressed the expression of I-A^d, CD86, and CD54 (Fig 1, *A*, and Fig E1 and Table E1 in the Online Repository at www.jacionline.org). These changes were dependent on the DEP^{ex} dose (see Table E2 in the Online Repository at www.jacionline.org). No significant BC1 toxicity occurred with extract doses up to 20 μg/mL.

Similar effects were seen in BMDCs. Treatment with DEP^{ex} induced a significant decrease in I-A^d and CD86 expression in CD11c$^+$ gated cells (Fig 1, *A*, *right panel*). Taken together, these data indicate that organic DEP chemicals interfere in the expression of several DC maturation markers.

DEP^{ex} inhibits IL-12 secretion in LPS-exposed DCs

IL-12 is important for T_H1 skewing of the immune response by LPS.[11] Supernatants from LPS-treated BC1 and BMDC cultures were used to measure IL-12p40 levels. Although DEP^{ex} failed to exert an effect on IL-12p40 production, it could suppress the LPS-induced production of this cytokine in BC1 cells and BMDCs (Fig 1, *A*, *lower panels*). The response inhibition was almost linear in the DEP^{ex} dose range of 2.5 to 20 μg/mL (see Fig E2 in the Online Repository at www.jacionline.org). Similar effects were seen in measuring IL-12p70 levels (see Fig E3 in the Online Repository at www.jacionline.org). These data demonstrate that in addition to interfering in the expression of DC maturation markers, pro-oxidative DEP chemicals inhibit IL-12 production.

Intact particles inhibit CD86 expression and IL-12 production in LPS-exposed DCs

The effect of intact DEPs was tested on the responses shown in Fig 1, *A*. DEPs were sonicated to break up the large aggregates that form during storage. These particle suspensions were added to BC1 cultures to achieve final concentrations in the range 10 to 20 μg/mL. This is roughly equivalent to 4 to 8 μg/mL DEP^{ex}. Intact particles were taken up in BC1 cells and suppressed LPS-induced CD86 and IL-12 production in a dose-dependent fashion (Fig 1, *B*). The intact particles were not as effective as DEP^{ex}.

DC exposure to the DEP extract affects their ability to induce cytokine production in antigen-specific T cells

BC1 cells were treated with OVA peptide ($OVA_{323-339}$) before irradiation and coincubation with CD4$^+$ T cells from DO11.10 mice to further assess the effect of DC perturbation on T-cell activation. These T cells express a transgenic T-cell receptor that recognizes $OVA_{323-339}$ in the context of the BALB/c MHC class II (I-A^d).[12] Under basal conditions, the cocultured T cells produce a small quantity of IFN-γ in the presence of antigen-pulsed BC1 cells (Fig 2, *upper panel*). Prior treatment with LPS leads to increased expression of costimulatory receptors and IL-12 production (as shown in Fig 1). This allows these APCs to enhance IFN-γ production in cocultured T cells (Fig 2, *upper panel*). Exposure of BC1 cells to DEP^{ex} plus LPS induced a statistically significant decrease in IFN-γ production compared with that seen in cells treated with LPS only (Fig 2, *upper panel*). Interestingly, exposure of the BC1 cells to DEP^{ex} alone, before antigen pulsing, induced a statistically significant increase in IL-10 production in the cocultured T cells (Fig 2, *lower panel*). The same treatment did not elicit effects on IL-4 and IL-13 production (not shown). The increase in IL-10 production was not dependent on LPS. Please notice that DEP^{ex} alone did not stimulate IL-10 production in DCs (see Fig E3 in the Online Repository at www.jacionline.org), which rules out the possibility that the IL-10 in Fig 2 is of DC origin.

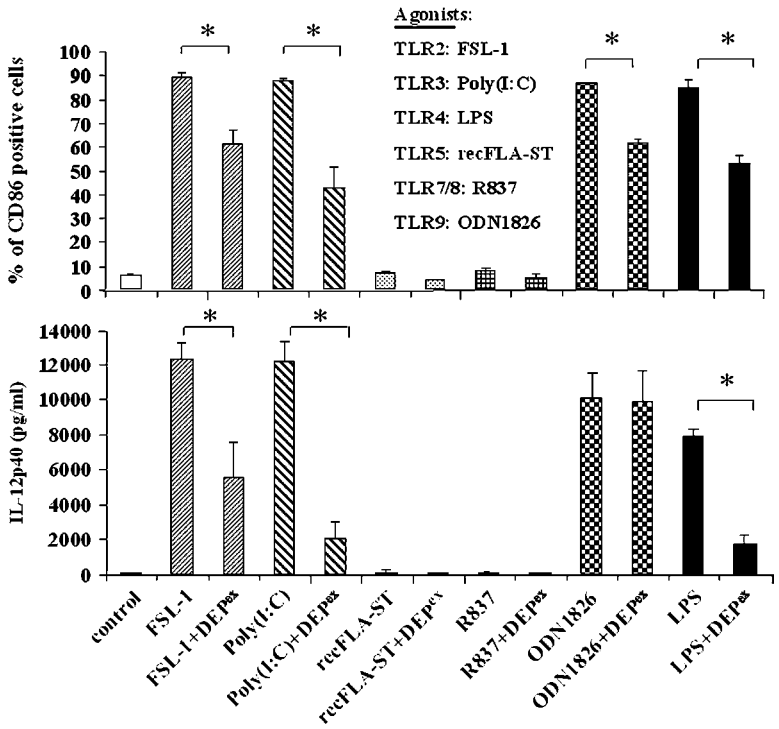

FIG 3. A, DEP^{ex} inhibits TLR-induced CD86 in BC1 cells *(upper panel)*. Cells were treated for 24 hours with 10 μg/mL DEP in the presence or absence of TLR agonists, as described in the Methods section. DEP^{ex} inhibits TLR-induced IL-12p40 production in BC1 cells *(lower panel)*. Results are presented as means ± SD from 3 independent experiments (*$P < .05$). *FSL-1*, Synthetic diacylated lipoprotein; *Poly(I:C)*, polyinosine-polycytidylic acid, *recFLA-ST*, recombinant flagellin from *Salmonella typhimurium*; *R837*, imiquimod; *ODN1826*, synthetic oligonucleotide.

Using a BMDC/DO11.10 coculture system, we could demonstrate that DEP exposure in this DC population also induces IL-10 secretion in the antigen-specific T cells (not shown). These data indicate that DC exposure to DEP^{ex} modifies their function, with the ability to change the types of cytokines being produced in cocultured T cells. The combination of a decrease in IFN-γ production plus increased IL-10 could favor T_H2 differentiation and might explain the adjuvant effect of the particles.

DEP extract interferes in the effect of several TLR agonists

A number of TLRs, other than TLR4, elicit T_H1 skewing of the immune response through IL-12 production and DC maturation. To determine whether DEP^{ex} could affect other TLR responses, BC1 cells were treated with a range of TLR agonists. In addition to inhibiting the LPS effect (TLR4), crude DEP^{ex} suppressed CD86 expression in response to TLR2, TLR3, and TLR9 agonists (Fig 3, *upper panel*). No effect could be demonstrated during treatment with TLR5, TLR7, and TLR8 agonists (Fig 3, *upper panel*). Similar effects were seen on IL-12 production, except that TLR9 did not interfere in IL-12 production (Fig 3, *lower panel*). The lack of interference in the IL-12 response during oligonucleotide treatment is unclear. These data indicate that pro-oxidative DEP chemicals interfere in the effects of several TLRs.[11]

DEPs interfere in LPS-induced NF-κB activation

On engagement of the TLR4 pathway, LPS activates the NF-κB cascade, which is involved in transcriptional activation of the genes that encode for maturation receptors and IL-12 (see Table E3 in the Online Repository at www.jacionline.org). To determine whether DEP^{ex} perturbs NF-κB activation, we looked at IκB degradation and the release of Rel proteins to the nucleus. IκB degradation is an important regulatory step in the release of Rel proteins to the nucleus.[13] IκBα degradation started within 15 minutes of LPS addition and was followed by an autoregulatory increase 60 minutes later (Fig 4, *upper panel*). In contrast, IκBβ degradation starts at 15 minutes and is sustained for a longer duration (Fig 4).[14] Addition of DEP^{ex} to LPS-treated cells interfered in IκBβ degradation in a dose-dependent fashion. In contrast, there was a lesser effect on IκBα. It should be noted that DEP^{ex} alone has no effect on IκBβ degradation (see Fig E4 in the Online Repository at www.jacionline.org). In the accompanying electrophoretic mobility shift assay, we demonstrated that DEP^{ex} is also capable of interfering in the formation of p50/p65

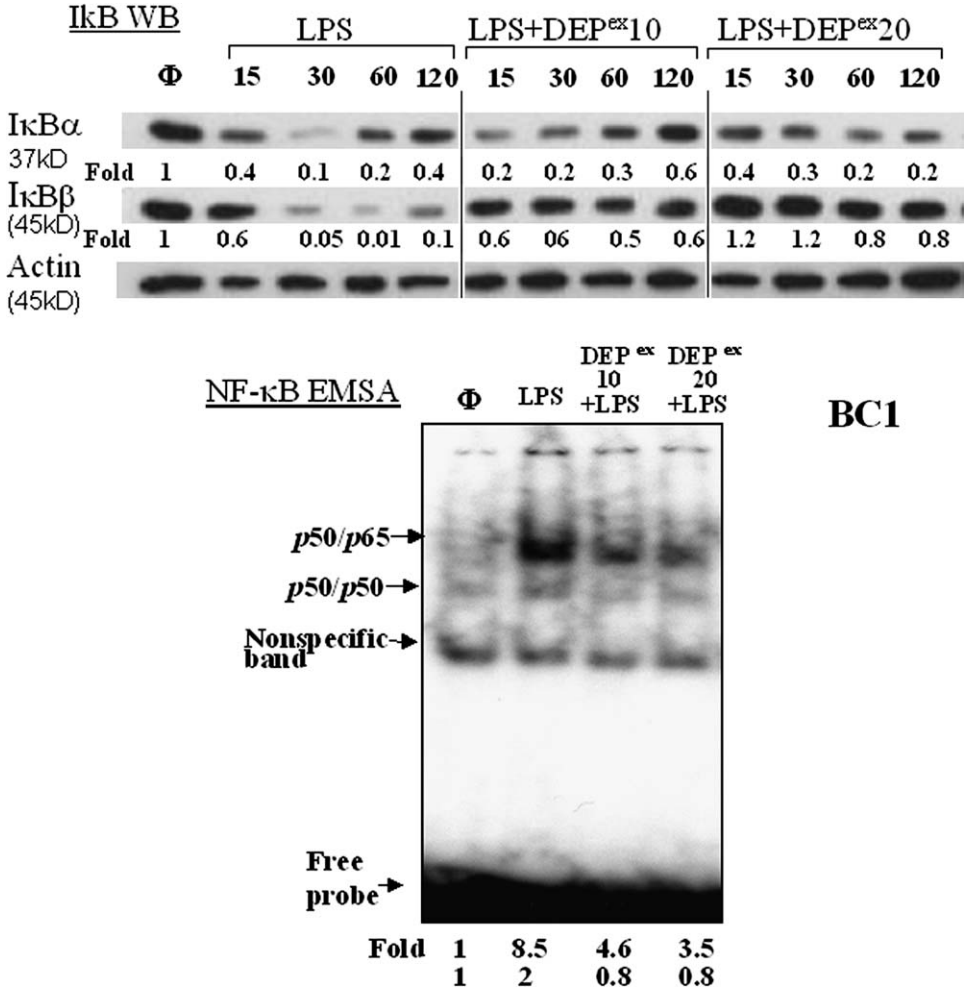

FIG 4. DEP chemicals inhibit LPS-induced NF-κB activation in BC1 cells. Western blotting showing the inhibitory effect of DEPs on LPS-induced IκBα and IκBβ degradation is shown *(upper panel)*. Results of an electrophoretic mobility shift assay demonstrating the nuclear localization of Rel proteins are shown *(lower left panel)*.

and p50/p50 shift complexes in BC1 nuclei (Fig 4, lower right panel). The supershift experiment that was conducted to identify these shift complexes is shown in the Fig E4 in the Online Repository at www.jacionline.org.

Effects of the DEP extract are mediated by pro-oxidative DEP chemicals that engage the Nrf2 pathway

We have previously shown that redox-cycling DEP chemicals are capable of ROS generation and the induction of oxidative stress.[1,10] To determine whether oxidative stress is involved in the perturbation of DC function, we used a fluorescent dye, MBB, to follow changes in the intracellular thiol content in DCs. This demonstrated a dose-dependent decrease in MBB fluorescence (see Fig E5 in the Online Repository at www.jacionline.org). Much of this decrease is due to glutathione depletion, which is indicative of the induction of oxidative stress.

Cells respond to the generation of oxidative stress by initiating new responses. One of the most sensitive oxidative stress responses is the expression of protective phase II enzymes. These enzymes are responsible for ROS scavenging effects, detoxification of chemicals, and exerting anti-inflammatory effects.[15] An example is HO-1, which constitutes a sensitive oxidative stress marker.[16] It is noteworthy that DEP^{ex} induced a dose-dependent increase in HO-1 expression in BC1 cells, and this effect could be inhibited by the thiol antioxidant N-acetylcysteine (NAC; see Fig E5 in the Online Repository at www.jacionline.org). In addition to its radical scavenging effects, NAC interacts directly with electrophilic DEP chemicals, preventing their participation in ROS generation.[6] This effect was used to show that NAC could neutralize the inhibitory effects of DEP^{ex} on CD86 expression and IL-12 production (not shown).

We have recently demonstrated that pro-oxidative DEP chemicals induce a hierarchical oxidative stress response, in which Nrf2-mediated phase II enzymes regulate pro-inflammatory responses.[6,17] The expression of HO-1 and other phase II enzymes is regulated by the bZIP

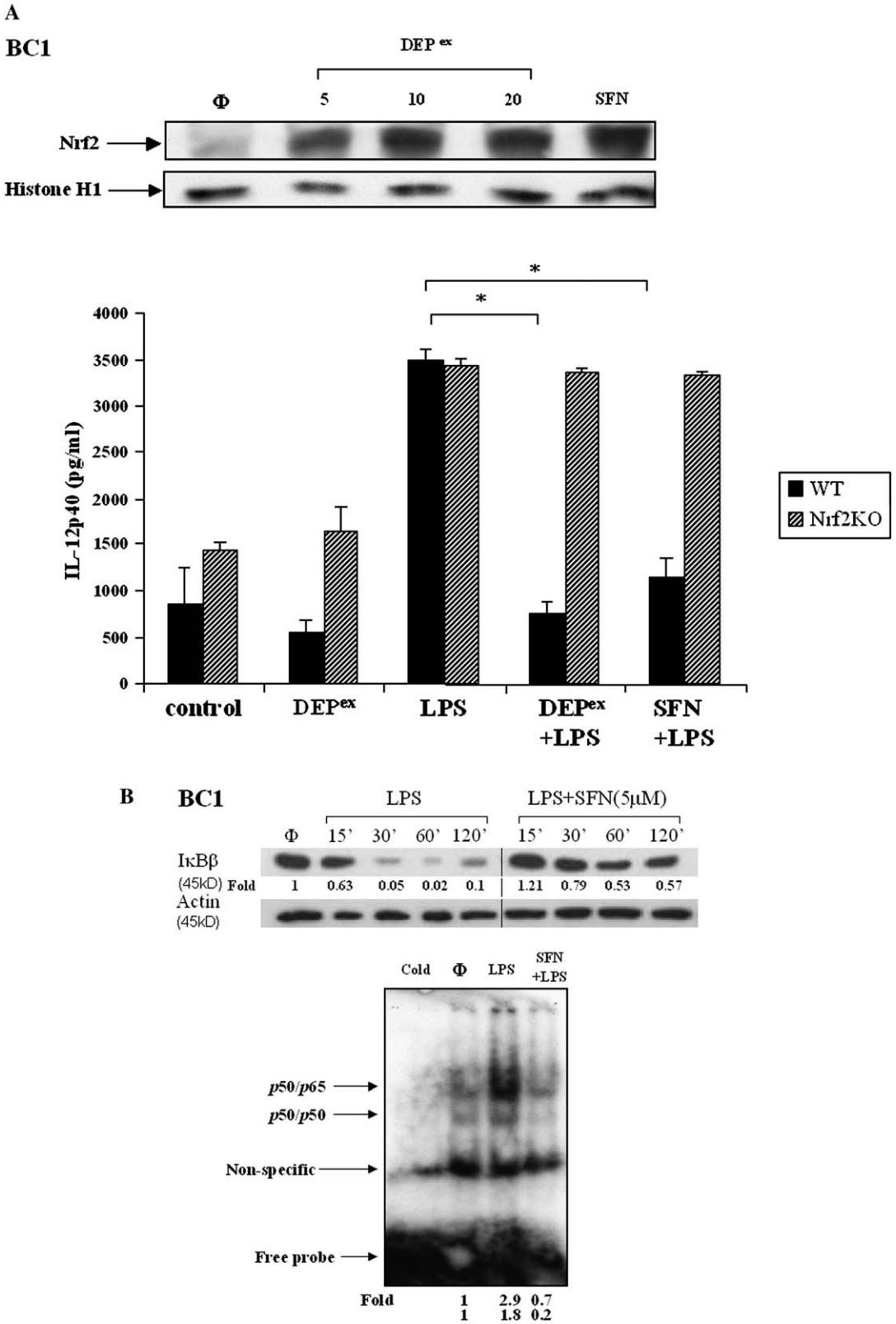

FIG 5. A, Immunoblot showing Nrf2 nuclear translocation 1 hour after the introduction of the stimulus is shown *(top panel)*. IL-12p40 levels in the supernatant from WT and Nrf2-deficient BMDCs are shown *(lower panel)*. **B**, Inhibition of LPS-induced IκBβ degradation *(upper panel)* and NF-κB nuclear translocation *(lower panel)* by SFN.

transcription factor Nrf2, which is released to the nucleus by electrophilic DEP chemicals.[18] We now confirm that the same is true in BC1 cells and that the nuclear translocation of Nrf2 is dependent on the DEP^{ex} dose (Fig 5, *A*, *upper panel*). To determine whether this transcription factor is required for the suppression of IL-12 production, we used BMDCs from Nrf2-deficient mice to see whether the response differs from that seen in $Nrf2^{+/+}$ littermate control animals. This provided a clear demonstration that IL-12 production could not be suppressed in Nrf2-deficient cells (Fig 5, *A*, *lower panel*). In contrast, response inhibition in WT BMDCs was intact (Fig 5, *A*). This inhibitory effect was dependent on the dose of DEP^{ex} (see Fig E2 in the Online Repository at www.jacionline.org). Nrf2 deficiency also negated the ability of DEP^{ex} to interfere in LPS-induced CD86 expression (not shown).

To further confirm that Nrf2 is responsible for inhibiting the DC responses, we used an electrophilic chemical, sulforaphane (SFN), that elicits phase II enzyme expression without inducing cellular toxicity.[19] SFN induced Nrf2 translocation to the nucleus (Fig 5, *A*, *upper panel*) and suppressed LPS-induced IL-12 production in WT, but not Nrf2-deficient, cells (Fig 5, *A*, *lower panel*). SFN also induced HO-1 expression and inhibited LPS-induced $I\text{-}A^d$ and CD54 expression (see Fig E6 in the Online Repository at www.jacionline.org).

To confirm a possible connection between Nrf2 and the NF-κB cascade, we looked at the effect of SFN on LPS-induced NF-κB activation (Fig 5, *B*). This demonstrated that SFN treatment interferes in IκBβ degradation, as well as the release of Rel proteins to the nucleus (Fig 5, *B*). Similar effects were seen in BMDCs (see Fig E7 in the Online Repository at www.jacionline.org). Also, DEPs interfered in extracellular signal–regulated kinase (ERK) activation in DCs (see Fig E8 in the Online Repository at www.jacionline.org). Taken together, these data demonstrate the importance of the Nrf2-dependent pathway in modulating DC function under conditions of oxidative stress.

DISCUSSION

We assessed the effect of an organic DEP extract on DC maturation and antigen-specific T-cell activation. Several reports indicate that DCs are important APCs in asthma.[20] The present study demonstrates that DEPs inhibit TLR-induced DC maturation, IL-12 secretion, and IFN-γ secretion in antigen-specific T cells. The modulation of DC function is triggered by pro-oxidative DEP chemicals, which are responsible for Nrf2-mediated induction of antioxidant and anti-inflammatory pathways. This communication provides new information about the role of DEPs as an adjuvant in the immune system.

Lung-derived DCs in the mouse are mainly of myeloid origin and critical for the induction of T_H2 responses and eosinophilic airway inflammation in response to inhaled antigen.[21] DC activation and maturation are regulated by a variety of cytokines, costimulatory molecules, and bacterial products.[22] These events are accompanied by changes in DC morphology, phenotype, and ability to act as an APC.[23] From the perspective of asthma and allergic disease, IL-12 production is an important facet of DC function.[24] Rieder et al[25] found that DCs from atopic individuals produce less IL-12 than DCs from healthy individuals. This could favor T_H2 differentiation.[26] In this communication we show that DEPs inhibit DC maturation and IL-12 secretion, with a subsequent decrease in IFN-γ production in antigen-specific T cells (Figs 1 and 2). This is in agreement with the ability of DEPs to decrease IFN-γ production in LPS-treated animals, as well as in human subjects challenged with intranasal DEPs and ragweed.[4,27] Thus it is possible that interference in T_H1 immunity could favor an enhancement of the T_H2 responsiveness to a common environmental allergen (Fig 6). Another possibility is that DEPs might perturb an aspect of DC function that actively promotes T_H2 differentiation. This might explain the increase in the murine T_H2 cytokine IL-10 during DC coincubation with antigen-specific T cells (Fig 2). The molecular basis for the enhancement of IL-10 production is unknown.

Pro-oxidative DEP chemicals induce protective, as well as injurious, oxidative stress responses that are in dynamic equilibrium.[1,6] Thus Nrf2-induced phase II enzyme expression is capable of interfering in cellular activation and inflammation. We now demonstrate that the Nrf2 pathway is also capable of suppressing the pro-inflammatory effects of LPS in DCs (Fig 5). This notion was further confirmed by using SFN, a therapeutically useful inducer of phase II enzymes (Fig 5). SFN, similar to electrophilic DEP chemicals, induces Nrf2 release to the nucleus and suppresses LPS-induced IL-12 production (Fig 5).[19] Under basal conditions, Nrf2 is sequestered in the cytosol by the actin-binding protein Keap 1, which chaperones Nrf2 to a proteosomal degradation pathway.[17,18] In the presence of DEP chemicals or SFN, Nrf2 is released from Keap 1, allowing the transcription factor to accumulate in the nucleus. By using DCs from Nrf2-deficient animals, we demonstrate that Nrf2 is essential for suppressing IL-12 production and NF-κB activation by DEP^{ex} and SFN (Fig 5). All considered, the dynamic relationship between proinflammatory and anti-inflammatory effects in DCs demonstrates that the Nrf2 pathway dominates over the TLR pathways that promote IL-12 production and T_H1 differentiation.

It has previously been demonstrated that boosting of glutathione levels in the immune system favors T_H1 responses by interfering in IL-4 production.[28] Likewise, glutathione depletion has been reported to shift the immune response in the opposite direction, leading to T_H2 dominance.[8] A number of theories have been provided to explain these effects, including that oxidative stress modifies T-cell activation by perturbing APC activity, such as the expression of MHC gene products.[29] Another possibility to explain the decreased T_H1 differentiation is inhibition of IL-12 production, as discussed above (Figs 1 and 2).[7] A third possibility is that DEPs might promote T_H2 differentiation by changing DC characteristics that

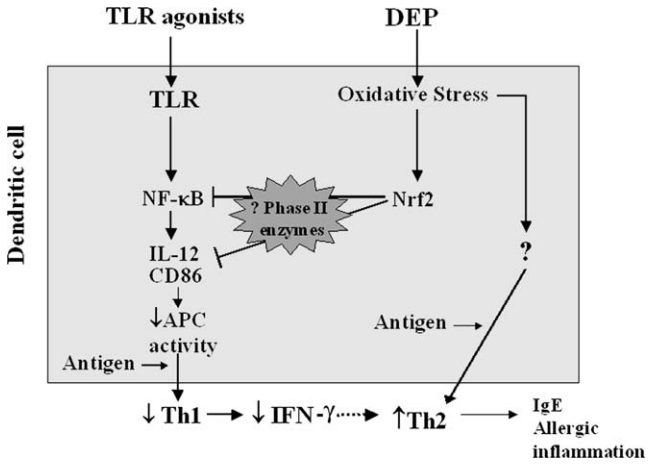

FIG 6. A model of the suggested signaling networks involved in T_H2 skewing by DEPs at the DC level.

promote the development of T_H2 differentiation.[30] This could include the expression of OX40 and Notch ligands on the DC surface (Fig 6).[31,32] Although we found no direct evidence for an effect on IL-4 or IL-13 production, DC exposure to DEP^{ex} increased IL-10 production in cocultured T cells (Fig 2). IL-10 plays a role in the generation of T_H2 cells in the murine system[33] and has also been shown to enhance airway hyperresponsiveness independent of its immunomodulatory effects.[33,34] Our failure to find an increase in IL-4 and IL-13 production could relate to the use of DO11.10 $CD4^+$ T cells. It has been demonstrated that these transgenic T cells might alter their profile of T_H1 and T_H2 cytokines, depending on the dose of OVA peptide and the affinity of the T-cell receptor for the peptide-MHC complex.[35] Another possibility is that the induction of IL-10 production might reflect the activation of a regulatory T-cell phenotype that might be responsible for the downregulating T_H1 responses *in vivo*, thereby promoting allergic responses. However, there is no *in vivo* evidence that IL-10–producing regulatory T cells promote allergies. In fact, the induction of IL-10–producing regulatory T cells during allergen vaccination has been shown to be a possible mechanism by which such vaccination can induce clinical improvement of allergic disease.[36]

A key question is whether the DEP extract amount is relevant to the actual particle dose under real-life exposure conditions. This issue is also closely related to the question of how the particles gain access to the bronchial-associated lymphoid tissue. We have previously shown that calculation of the rate of PM2.5 deposition in the human tracheobronchial tree can be reconciled with the *in vitro* dose levels that result in hierarchical oxidative stress responses in macrophages and epithelial cells.[1] To make this comparison, it was necessary to convert the DEP^{ex} dose, calculated as mass per unit volume (in micrograms per milliliter), into particle mass per unitary surface area in the culture dish.[1] Our calculations show that 1 to 100 μg/mL of the extract is equivalent to an *in vitro* particle deposition dose of 0.2 to 20 $\mu g/cm^2$. According to the tenants of the hierarchical oxidative stress model, a dose of greater than 2 $\mu g/cm^2$ is sufficient to elicit pro-inflammatory effects in epithelial cells and macrophages. Pertaining to the *in vivo* side of the equation, we estimated that 24 hours of exposure to PM2.5 in a polluted urban environment could lead to a particle deposition rate of 2.3 $\mu g/cm^2$ at high-impact sites in the tracheobronchial region. This calculation is premised on the observation that inhaled particles are not symmetrically distributed throughout this region but tend to accumulate at so-called hotspots of deposition.[37] These hotspots tend to be located at airway bifurcation points and have been confirmed through the examination of human lung samples.[37-39] The same deposition sites might also constitute the preferred sites for particulate antigen deposition and presentation in the bronchial-associated lymphoid tissue. In this regard it has been demonstrated that the reactive lymphoid follicles that form in response to an inhaled experimental allergen tend to concentrate in the vicinity of primary bronchial branching points.[40] This could mean that the adjuvant and the allergen could be processed in overlapping regions in the lung. How exactly the allergen and the particles gain access to the participating APC at these sites is unclear but could involve DC dendrite extensions into the surface epithelium or particle uptake through the epithelial layer. Ultrafine particles (including DEPs) rapidly penetrate surface membranes in the lung.[41] Once in contact with DCs, small particles are taken up by specialized phagocytic and endocytic mechanisms. In fact, DCs prefer particulate antigen for immune response generation. We propose that the occasional uptake of PM and allergen into the same DC might boost the immune response by changing the APC characteristics of the cell. It is also possible that leaching of PM chemicals from the particle surface in the vicinity of the DC could lead to cellular uptake, with the ability to change the redox status of the cell. A further possibility is that bystander cells, such as macrophages and bronchial epithelial cells, could encounter the particles first and that the generation of

epithelial proinflammatory products might affect DC function; the extent to which this might contribute to the adjuvant effects of PM remains to be studied.[42]

LPS-induced DC maturation requires the activation of the NF-κB cascade.[43] The demonstration that DEP^{ex} could interfere in this cascade (Fig 4) is the first demonstration of a specific molecular pathway by which prooxidative DEP chemicals can disrupt T_H1 differentiation at the DC level. Moreover, this study is unique in using a homogeneous primary DC line to demonstrate this outcome. The exact cascade components or steps at which the Nrf2-mediated response products interfere in the TLR4 pathway are unknown. We do know that LPS-induced NF-κB activation can proceed through MyD88-dependent and MyD88-independent pathways.[11] In the MyD88-dependent pathway, the signal is propagated through the IL-1 receptor–associated kinase and the TNF receptor–associated factor 6, whereas the MyD88-independent pathway uses the Toll/IL-1 receptor (TIR) domain–containing adapter inducing IFN-β.[11] Whether one or more of these components could be targets for phase II enzymes will require further study. It has been demonstrated that phase II enzymes, such as HO-1, are capable of interfering in the NF-κB pathway.[44,45]

In summary, we have shown that DEPs contain prooxidative chemicals that induce a state of oxidative stress in DCs. This triggers an Nrf2-mediated cellular response pathway that interferes in proinflammatory signaling cascades that are responsible for IL-12 production (Fig 6). Decreased IL-12 production could be responsible for unopposed T_H2 activation and increased IgE production in response to common environmental allergens.

We thank Dr Owen N. Witte (University of California, Los Angeles, Calif) for providing DO11.10 mice. We also thank Hyon-Jeen Kim for expert advice.

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