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Diesel Exhaust Particle-Treated Human Bronchial Epithelial Cells Upregulate Jagged-1 and OX40L in Myeloid Dendritic Cells via Thymic Stromal Lymphopoietin

Bertram Bleck,* Doris B. Tse,* † Terry Gordon,‡ Mohammad R. Ahsan,* and Joan Reibman* ‡

Ambient particulate matter, including diesel exhaust particles (DEP), promotes the development of allergic disorders. DEP increase oxidative stress and influence human bronchial epithelial cell (HBEC)–dendritic cell interactions via cytokines, including thymic stromal lymphopoietin (TSLP). Upregulation of TSLP results in Th2 responses. Using primary culture HBEC and human myeloid dendritic cell (mDC) cocultures, we show in this study that DEP upregulation of Th2 responses occurred via HBEC-dependent mechanisms that resulted from oxidative stress. Moreover, DEP-treated HBEC and ambient particulate matter-treated HBEC upregulated OX40L and the Notch ligand Jagged-1 mRNA and expression on mDC. Upregulation of OX40L as well as Jagged-1 on mDC required HBEC and did not occur in the presence of N-acetylcysteine. Furthermore, OX40L and Jagged-1 upregulation was inhibited when HBEC expression of TSLP was silenced. Thus, DEP treatment of HBEC targeted two distinct pathways in mDC that were downstream of TSLP expression. Upregulation of OX40L and Jagged-1 by mDC resulted in mDC-driven Th2 responses. These studies expand our understanding of the mechanism by which ambient pollutants alter mucosal immunity and promote disorders such as asthma. The Journal of Immunology, 2010, 185: 000–000.

Air pollution is associated with an increase in allergic asthma (1). Ambient particulate matter (PM) and diesel exhaust particles (DEP), the largest single source of airborne PM from vehicular traffic, have been documented in both animal and human studies to participate in allergic immune responses (1). Observational human studies, including a recent prospective birth cohort study of >2000 children, show an increased risk of atopic diseases and allergic sensitization in relation to exposure to ambient PM (2–6). DEP are adjuvants for allergic inflammation, and exposure to DEP in the context of an allergen increases IgE production in human and animal studies (7, 8). Exposure to DEP results in an increase in inflammatory markers in airways and peripheral blood of healthy and asthmatic individuals (9–11). These findings reinforce the need to understand mechanisms by which ambient PM and DEP promote airway immune responses toward an allergic phenotype.

Human bronchial epithelial cells (HBEC) are the first targets for most inhaled pollutants. DEP generate oxidative stress in airway as well as other cells, and this process can promote immune responses (12, 13). Dendritic cells (DC) are abundant in the airway (14), and we have suggested that DEP modify allergic sensitization by the effect of HBEC on local DC. We have demonstrated that HBEC treated with ambient PM or DEP release chemokines (CCL20) associated with the recruitment of immature DC (15). Furthermore, DEP-treated HBEC upregulate cytokines (GM-CSF and thymic stromal lymphopoietin [TSLP]) associated with the maturation and Th2 polarization of DC (16–19). TSLP is an IL-7–like cytokine that regulates Th2 cell differentiation via its effect on DC (20). We have shown that TSLP is produced by DEP-treated HBEC and that its production results from oxidative stress (19). A recent animal study supports the role of DC in DEP-induced adaptive immunity (21). We now expand upon these studies and suggest that DEP treatment of HBEC results in TSLP and reactive oxygen intermediates that upregulate OX40L and selective Notch pathways, two DC signals that promote a Th2 response. These studies further our understanding of mechanisms by which DEP promote airway immune responses toward those associated with asthma and allergy.

Materials and Methods

Reagents

DMEM, MEM, penicillin-streptomycin, FBS, trypsin-EDTA solution, and PBS were purchased from Invitrogen (Carlsbad, CA). Bronchial epithelial cell growth medium and bronchial epithelial cell basal medium were purchased from Lonza (Walkersville, MD). Picoll was obtained from Amersham Biosciences (Piscataway, NJ). A magnetic cell separator and magnetic beads were from Miltenyi Biotec (Auburn, CA). GM-CSF, IL-2, IL-4, IL-1β, IL-6, TNF-α, and IFN-γ were from PeproTech (Princeton, NJ). PMA, ionomycin, mitomycin C, and PGE2 were from EMD Chemicals (Gibbstown, NJ). Fluorescent reagents for FACS analyses were obtained from BD Immunocytometry Systems (San Jose, CA), BD Pharmingen (San Diego, CA), Beckman Coulter/Immunotech (Brea, CA), or R&D Systems (Minneapolis, MN). N-acetylcysteine (NAC) was from MP Biomedicals (Solon, OH).

DEP were derived from a 1.6-L Volkswagen diesel engine (40 kW) running under standard city driving cycle conditions according to U.S. test protocol FTP 72 (U.S. Environmental Protection Agency, 1992) and were...
a gift of D. L. Costa (U.S. Environmental Protection Agency, Durham, NC). DEP were diluted in cell culture medium, vortexed (5 times, 10 s), sonicated (1 min), and added to cells in the defined concentrations. Because DEP sediment to the bottom of cell culture dishes, DEP concentrations were based on the available surface area (μg/cm²). Endotoxin activity in DEP (100 μg/ml), a concentration that was 10-fold higher than that used in most of the experiments, was below the lower limit of detection (0.01 endotoxin unit/ml; PyroGene recombinant factor C assay; Lonza).

PM suspensions were collected using a high-volume three-stage impactor (ChemVol model 2400; Rupprecht & Patashnick, Albany, NY) and PM were collected using a cell viability dye (Allamar Blue; Invitrogen, Carlsbad, CA) for 900 min. The coarse and fine PM fractions were collected on polycarbonate filters (GC-55 and Non-Woven, respectively; McMaster-Carr, Robbinsville, NJ), whereas the ultrafine PM fractions were collected on polycarbonate final filters (GC-55 and Non-Woven, respectively; McMaster-Carr, Robbinsville, NJ). All sampling substrates were precleaned using sterile solutions prior to exposure and extracted after exposure in sterile and pyrogen-free water using sonication. Samples were obtained from Midtown Manhattan (Hunter College, New York, NY) and the South Bronx, New York, NY.

Cells
HBEC included primary HBEC (pHBEC) or 16HBEC14+ cells (16HBEC) as defined for each study. Primary HBEC were purchased from Lonza and cultured as described (19). Primary HBEC were provided by Dr. D. Gneuven (University of Vermont, Burlington, VT) and cultured as described in BMM supplemented with 10% FBS (22).

Immature myeloid DC (mDC) were directly isolated from PBMC according to expression of the mDC marker CD1c (CD1c) using the CD1c DC isolation kit (Miltenyi Biotecn). mDC were cultured in DMEM supplemented with FBS (10%, v/v), 2-ME (5 mM), and IL-4 (10 ng/ml). mDC were routinely checked for immaturity by assessing expression of maturation markers (CD80, CD83) by FACS, as well as their inability to induce robust T cell proliferation in an allogeneic MLR (data not shown). For coculture experiments, immature mDC (1 × 10⁵ cells/well) were cultured with confluent pHBEC or 16HBEC (48 h) and cells were subsequently trypsinized (0.5 g/l, 5 min) for analysis. mDC were discriminated from HBEC by MACS according to their expression of CD11c and CD32c. CD4⁺CD45RA⁺CD4RO T cells were isolated from PBMC using the CD4 MultiSort kit in combination with a depletion of CD45RO⁺ cells using CD45RO magnetic beads (Miltenyi Biotecn) according to the manufacturer’s instructions.

Treatment of HBEC with DEP, carbon, fine ambient PM, in the presence or absence of NAC, did not induce cell death compared with untreated HBEC using a cell viability dye (Allamar Blue; Invitrogen, Carlsbad, CA) (data not shown). mDC were recovered, pelleted, and reacted with a mixture consisting of V500-anti–CD11c, FITC-anti–Jagged-1, PE-anti–Ox40L, and PE-Cy5 anti–CD83 (30 min, 0°C), washed (cold PBS), and fixed (PBS containing 1% formaldehyde). Cytofluorometric analyses were performed on a FACSArray (BD Biosciences, San Jose, CA). Forward and 90° angle scattered 488-nm laser light intensities were used to exclude cellular debris. Fluorescence intensities were detected using 405 nm excitation for V450, 488-nm excitation for FITC, and 562-nm excitation for PE and PE-Cy5 through appropriate dichroic band-pass filters (450/50 nm for V450, 530/30 nm for FITC, 585/15 nm for PE, and 660/20 nm for PE-Cy5). Spillover detected by inappropriate channels was corrected by electronic compensation. Expression of surface molecules was determined as mean FITC, PE, or PE-Cy5 fluorescence intensities on the V450-gated mDC population.

Data analysis
Data are presented as mean ± SE. Significance was determined by a Student t test for comparing two variables or one-way ANOVA for multiple variable comparisons, with p value of <0.05 considered significant.

Results
DEP-treated HBEC promote DC-dependent Th2 polarization via reactive oxygen species

We have recently shown that DEP-treated HBEC (pHBEC and 16HBEC) upregulate TSLP and support maturation of immature mDC toward a phenotype that promotes Th2 polarization of naive T cells (19). We have previously shown that DEP, but not carbon, upregulate reactive oxygen species (ROS) in HBEC using 5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate, an oxidation-sensitive fluorescent probe, as a measure (19). Because DEP increase oxidative stress in our studies as well as in those others (19, 24), we examined whether the Th2 polarization induced by mDC exposed to DEP-treated HBEC was dependent on the generation of ROS. Immature mDC were treated as described, isolated, and used as stimulator cells in an MLR and CD4⁺ T cell polarization measured by the ratio of IL-5/IFN-γ in cell supernatants. As shown in Fig. 1A, CD4⁺ T cells exposed to mDC that had been cocultured with resting pHBEC released low levels of both IL-5 and IFN-γ (IL-5/IFN-γ ratio of 0.07 ± 0.02 [mean ± SE]; n = 3). CD4⁺ T cell supernatants from an MLR with mDC cocultured with DEP-treated pHBEC had an increase in the IL-5/IFN-γ ratio (0.29 ± 0.13, p < 0.05, DEP-treated versus resting pHBEC). In contrast, CD4⁺ T cell supernatants from an MLR with mDC that had been cocultured with DEP-treated pHBEC in the presence of NAC had a reduced IL-5/IFN-γ ratio (0.06 ± 0.02, p < 0.05 versus DEP-treated pHBEC). No increase in the IL-5/IFN-γ ratio was noted for CD4⁺ T cells exposed to mDC cultured with carbon-treated pHBEC (0.06 ± 0.02, p < 0.05 versus resting pHBEC). As ex-
We have previously demonstrated that mDC exposed to DEP-treated HBEC upregulate OX40L mRNA in mDC. Immature mDC were cultured alone or with pHBEc in the absence or presence of DEP (3 μg/cm²). mDC were isolated and OX40L mRNA was assayed (Fig. 2A). OX40L mRNA expression was increased in mDC cultured with resting pHBEc compared with mDC alone (11.3 ± 2.5 x 10³ versus 0.1 ± 0.1 x 10³, respectively, p < 0.05, n = 3). Exposure of immature mDC to DEP-treated pHBEc further upregulated OX40L mRNA above resting levels (26.9 ± 10.0 x 10³, p < 0.05 versus resting pHBEc, n = 3). No significant effect was seen with carbon. The increase was similar to that seen after treatment with MF2 (20.7 ± 7.6 x 10³, p < 0.05 versus resting pHBEc, n = 3), whereas the presence of MF1 during coculture did not increase OX40L mRNA (0.9 ± 0.5 x 10³, p < 0.05 versus resting pHBEc, n = 3). Findings were similar for mDC exposed to 16HBEC (Fig. 2B).

To confirm that HBEC were required for the DEP effect, immature mDC were exposed to DEP (6 h, 48 h) in the absence of HBEC and OX40L mRNA was assayed (Fig. 2C). OX40L mRNA was minimal in immature mDC and was not significantly increased after DEP (3 μg/cm²) or carbon exposure (data not shown for 6 h; data yielded similar results). OX40L mRNA was significantly increased after exposure to recombinant TSLP (15 ng/ml) and MF2 but not after MF1.

We next examined whether OX40L upregulation by DEP-treated HBEC was a result of HBEC-derived TSLP and was ROS-dependent. As shown in Fig. 3, anti-TSLP, but not control IgG, reduced OX40L mRNA expression in mDC exposed to DEP-treated 16HBEC. To confirm that the increase in mDC OX40L mRNA expression was due to epithelial cell-derived TSLP, 16HBEC were transfected with an siTSLP duplex pool to silence TSLP expression as described (19). OX40L mRNA expression was significantly reduced in mDC exposed to DEP-treated TSLP-silenced 16HBEC compared with those that were exposed to DEP-treated 16HBEC or DEP-treated 16HBEC with siCONTROL (Fig. 3). OX40L mRNA expression was also significantly reduced in mDC that were exposed to DEP-treated 16HBEC in the presence of NAC. These data suggested that OX40L mRNA expression in mDC was due to TSLP derived from DEP-treated HBEC and that this process was associated with the generation of ROS.

Selective mDC Notch ligand mRNA is upregulated by DEP-treated HBEC via TSLP and ROS

Th2 polarization of T cells requires multiple IL-4-dependent and –independent signals (26). IL-4–independent signals include those derived via distinct Notch ligands expressed by DC with Notch receptors on T cells (27). We therefore examined whether exposure of mDC to DEP-treated pHBEc upregulated Jagged-1, Jagged-2, and Dll4 mRNA. Jagged-1 was significantly upregulated in mDC that had been cocultured with DEP-treated pHBEc compared with mDC cocultured with resting pHBEc (3.3 ± 0.4 x 10³ versus 1.5 ± 0.4 x 10³, p < 0.05, n = 3) (Fig. 4A). MF2, but neither carbon nor MF1, upregulated Jagged-1 mRNA. Dll4 mRNA was upregulated by MF1, but no increase was seen with either DEP-treated 16HBEC or MF2 (Fig. 4A). This finding was similar for mDC exposed to 16HBEC (Fig. 4B) in which DEP increased Jagged-1 mRNA. Jagged-2 mRNA in mDC was not increased by DEP-treated HBEC (data not shown).

To confirm that DEP upregulation of Jagged-1 was associated with mDC exposure to HBEC, immature mDC were treated with DEP (3 μg/cm²), recombinant TSLP, or appropriate stimuli in the absence of HBEC and Notch ligand mRNA measured by RT-PCR. Resting mDC expressed low-level Jagged-1, which was not increased by DEP at 6 h (data not shown) or 48 h (Fig. 4C). TSLP
and MF2, but not MF1, significantly increased Jagged-1 mRNA in mDC. Dll4 mRNA was increased in mDC treated with MF1, but not DEP or TSLP (Fig. 4C). Jagged-2 expression was not significantly upregulated by any of these stimuli (data not shown).

To determine whether the increase in Jagged-1 induced by DEP-treated 16HBEC resulted from epithelial cell-derived TSLP and was ROS-dependent, immature mDC were exposed to DEP-treated 16HBEC in the presence of anti-TSLP or TSLP-silenced 16HBEC (Fig. 5). Anti-TSLP reduced Jagged-1 mRNA compared with mDC exposed to DEP-treated 16HBEC (1.3 ± 0.5 × 10^3 versus 5.2 ± 1.7 × 10^3, respectively, \( p < 0.05, n = 3 \)). mDC exposed to TSLP-silenced 16HBEC also had a significant reduction in Jagged-1 mRNA compared with mDC exposed to DEP-treated 16HBEC (1.0 ± 1.0 × 10^3 versus 4.9 ± 2.3 × 10^3, respectively, \( p < 0.05, n = 3 \)). mDC cocultured with DEP-treated 16HBEC and NAC also had a reduction in Jagged-1 mRNA (1.3 ± 0.2 × 10^3).

OX40L and Jagged-1 mRNA in response to HBEC treated with urban ambient PM

DEP are derived from a single source engine. To examine whether ambient PM, which is comprised of DEP as well as particles with other chemical components, had a similar effect, we used fine ambient PM from two urban sources: Midtown Manhattan (Hunter College) and the South Bronx. mDC were exposed to resting or ambient PM-treated HBEC, and mRNA for OX40L, Jagged-1, and Dll4 was measured as described for cells exposed to DEP. Hunter College and South Bronx PM were used at 3 mg/cm^2. As shown in Fig. 6, mDC mRNA for OX40L was upregulated after exposure to resting HBEC, and it further increased in the presence of DEP-, Hunter College-, and South Bronx-treated HBEC, but not carbon-treated HBEC. mDC mRNA for Jagged-1 but not DLL4 was upregulated after exposure of mDC to ambient PM-treated HBEC. There was no significant difference in the effect of DEP, Hunter College PM, and South Bronx PM when used at the same concentration.

DEP-treated HBEC upregulated Jagged-1 and OX40L expression on the surface of mDC

To confirm that mRNA upregulation was associated with surface expression of OX40L and Jagged-1, we performed FACS analyses...
as described and monitored mean fluorescence intensity (MFI). As shown in Fig. 7A, OX40L expression was increased in mDC exposed to 16HBEC (1.74 ± 0.29-fold increase compared with resting mDC alone, n = 3, p < 0.05). Consistent with mRNA data, exposure of mDC to DEP-treated 16HBEC resulted in a further increase in OX40L expression (3.02 ± 0.67-fold increase compared
with mDC exposed to resting 16HBEC, \( n = 3, p < 0.05 \). An increase in Jagged-1 expression was seen when mDC were cocultured with 16HBEC (1.59 ± 0.03-fold increase compared with mDC alone) with a further increase in Jagged-1 in mDC exposed to DEP-treated 16HBEC (2.26 ± 0.16-fold compared with mDC and resting 16HBEC, \( n = 3, p < 0.05 \)). Upregulation of OX40L and Jagged-1 was inhibited in the presence of NAC. Neither OX40L nor Jagged-1 was upregulated in mDC exposed to carbon-treated 16HBEC.

Fig. 7B is one of three experiments illustrating the heterogeneity in the mDC responses revealed by FACS. A small increase in the frequency of cells expressing OX40L (1.9 ± 1.4%) and Jagged-1 (3.3 ± 2.2%) alone, or coexpressing OX40L and Jagged-1 (4.1 ± 1.1%), was seen in mDC exposed to untreated 16HBEC (detected as the difference between mDC cultured with untreated 16HBEC and mDC cultured alone [mean ± SE, \( n = 3 \)). Similar heterogeneity in the mDC response to DEP-treated 16HBEC (detected as the difference between mDC cultured with DEP-treated 16HBEC and mDC cultured alone) was also seen in the increase of OX40L+ cells (7.5 ± 5.4%), Jagged-1+ cells (6.0 ± 3.6%), and OX40L+Jagged-1+ cells (5.0 ± 2.0%). Interestingly, only a minority of mDC upregulated their expression of OX40L or Jagged-1 in response to untreated 16HBEC (9.3 ± 2.4%) or DEP-treated 16HBEC (18.5 ± 3.0%), an increase that was associated with a similar rise in the number of CD11c+ cells that upregulated their expression of the activation marker CD83 (data not shown).

**DEP-treated HBEC upregulated Th2 polarization by mDC Jagged-1 and OX40L**

Having demonstrated the upregulation of two pathways associated with CD4+ Th2 polarization by DC, we next examined whether these pathways were required for CD4+ Th2 polarization by mDC exposed to DEP-treated 16HBEC. Immature mDC were exposed to DEP-treated 16HBEC in the absence or presence of anti-OX40L or anti-Jagged-1. mDC were isolated and Jagged-1 mRNA was measured by RT-PCR. Data are expressed as relative mRNA expression of Jagged-1 compared with GAPDH (2−ΔΔCt) (mean ± SE, \( n = 3 \). \( sp < 0.05 \).

**FIGURE 6.** Ambient PM-treated 16HBEC upregulate the expression of mDC OX40L and Jagged-1, but not Dll4 in mDC. Immature mDC were cultured (48 h) with DEP, Hunter College, or South Bronx fine ambient PM (3 \( \mu g/cm^2 \)) in the presence of untreated or treated 16HBEC and mRNA for OX40L (A), Jagged-1 (B), or Dll4 (C) measured by RT-PCR. Data are presented as a representative experiment performed in triplicate and are expressed as relative mRNA expression of OX40L, Jagged-1, or Dll4 compared with GAPDH (2−ΔΔCt) (mean ± SE, in triplicate). \( sp < 0.05 \).

**FIGURE 7.** B is one of three experiments illustrating the heterogeneity in the mDC responses revealed by FACS. A small increase in the frequency of cells expressing OX40L (1.9 ± 1.4%) and Jagged-1 (3.3 ± 2.2%) alone, or coexpressing OX40L and Jagged-1 (4.1 ± 1.1%), was seen in mDC exposed to untreated 16HBEC (detected as the difference between mDC cultured with untreated 16HBEC and mDC cultured alone [mean ± SE, \( n = 3 \)). Similar heterogeneity in the mDC response to DEP-treated 16HBEC (detected as the difference between mDC cultured with DEP-treated 16HBEC and mDC cultured alone) was also seen in the increase of OX40L+ cells (7.5 ± 5.4%), Jagged-1+ cells (6.0 ± 3.6%), and OX40L+Jagged-1+ cells (5.0 ± 2.0%). Interestingly, only a minority of mDC upregulated their expression of OX40L or Jagged-1 in response to untreated 16HBEC (9.3 ± 2.4%) or DEP-treated 16HBEC (18.5 ± 3.0%), an increase that was associated with a similar rise in the number of CD11c+ cells that upregulated their expression of the activation marker CD83 (data not shown).

**Discussion**

Increasing epidemiologic data suggest that ambient PM and, in particular, DEP promote the development of allergic disorders (1). To understand the impact of these pollutants on human health and to gain insights into mechanisms leading to potential interventions, it is important to understand how these pollutants affect...
adaptive immune responses at mucosal sites. DC direct T cells to respond as T regulatory, Th1, Th2, or Th17 cells, and the instruction depends on the signals that DC receive as immature cells (28, 29). We have suggested that DEP perturb the interaction between airway epithelial cells and DC, resulting in DC that promote Th2 polarization (19, 30). We have previously shown that DEP promote a Th2 response by stimulating HBEC to produce GM-CSF and TSLP and upregulate mature mDC that support T cell proliferation and Th2 skewing (19, 30). Our present data expand our previous observations, and we now demonstrate that DEP-treated HBEC induce mDC that promote Th2 polarization via TSLP and ROS regulation of two distinct pathways involving OX40L and specific Notch receptors.

The role of oxidative stress in DEP-stimulated airway inflammation has been well described (1, 24, 31, 32). We have demonstrated the production of ROS in HBEC in response to DEP, and the addition of NAC inhibits DEP induced TSLP expression in HBEC (19). We now show that NAC inhibited Th2 skewing of mDC exposed to DEP-treated HBEC. Furthermore, we demonstrated that upregulation of both mDC OX40L and Jagged-1 mRNA by DEP-treated HBEC was inhibited in the presence of NAC. We demonstrated this effect using both primary and transformed epithelial cells to confirm the transformed cell model for subsequent studies. These findings suggest that ROS generation is associated with epithelial cell-derived TSLP, resulting in OX40L and Jagged-1 signaling in mDC and subsequent Th2 skewing.

Our previous studies suggest the importance of soluble mediators derived from HBEC after DEP treatment in DC maturation and Th2 polarization, and a recent murine study supports the role of DC–epithelial cell interactions in DEP-induced Th2 skewing (21, 30). We have previously shown that DEP upregulate TSLP in HBEC and that this expression results in Th2 polarization of mDC by DEP-treated HBEC (19). TSLP, predominantly derived from epithelial cells, has been considered a “master switch” of the mucosal immune response (33, 34). Human and murine mDC express high levels of the TSLP receptors (TSLPR/CRLF2 and IL-7Ra), and via STAT3 and STAT5 signaling, their engagement results in mDC proliferation and the production of inflammatory Th2-promoting cytokines (25, 34–36). TSLP-stimulated DC activate naive T cells and upregulate Th2 memory cells (37, 38). Interestingly, TSLP only conditions the lung for a Th2 response, and lung-specific TSLP transgene expression requires antigenic stimulation for the full inflammatory response (39). In humans, bronchial epithelial cell expression of TSLP is associated with the...
severity of asthma (33, 40, 41). Our new data expand upon our previous observations and suggest that DEP promote a Th2 response via epithelial cell-derived TSLP that results in mDC expression of Th2-promoting signals.

We now show OX40L upregulation in immature mDC exposed to DEP-treated HBEC and the importance of TSLP and ROS in this process. OX40L (CD252, TNFSF4, gp34), a member of the TNF superfamily, is selectively expressed by TSLP-activated DC (42) and binds OX40 expressed on activated naive CD4+ and CD8+ T cells (43). Engagement of OX40 results in activation of signaling pathways that preferentially lead to generation of inflammatory Th2 CD4+ T cells (42–44). OX40L regulates memory as well as naïve T cells (43, 45–47) and blocks the generation of peripheral T regulatory cells and regulatory activity of naturally occurring CD4+ CD25+Foxp3+ T regulatory cells (43, 48). Thus, the OX40 pathway promotes Th2 diseases by multiple mechanisms. Animal models support the importance of the OX40 pathway in asthma since OX40L−/− mice have reduced Th2 responses and allergen sensitization (49, 50), and anti-OX40L decreases inflammation and CD4+ effector and memory T cells in animal models of TSLP-driven asthma (51). Allergen-specific Foxp3+ T regulatory cells and airway tolerance are also reduced by OX40L expression (52). Our studies now suggest that OX40L, via TSLP, may also be involved in the DEP promotion of allergic diseases.

We also showed that DEP treatment of HBEC resulted in TSLP- and ROS-mediated upregulation of Jagged-1 on DC. Notch1 signaling is essential in T cell fate specification and instructs lymphoid progenitors to adopt T versus B cell fates (53). Recent studies also demonstrate that Notch signaling is required for both effector T cell and T regulatory cell differentiation (26, 53, 54). In DC, Dll1 promote DC-mediated induction of Th1 cell differentiation of naïve T cells (53, 55, 56), whereas Jagged-1 Notch receptor expression is associated with Th2 cell differentiation (26, 53, 57, 58) and is upregulated by Th2 cell-promoting stimuli (26, 27, 59, 60). Engagement of Jagged-1 promotes Th2 polarization in both naïve and memory T cells via an increase in the GATA3/T-bet ratio and subsequent STAT6 phosphorylation (27). IL-4 expression is downstream of Notch signaling (26, 61, 62); however, Th2 cell differentiation is induced via GATA3 even in situations in which IL-4 signaling is inhibited (58, 63). Thus, Notch signaling via Jagged supports Th2 cell differentiation in an IL-4–independent as well as IL-4–dependent manner (53). In humans, mDC constitutively express Jagged-1 in levels higher than any of the other Notch ligands (27). Inhibition of Notch signaling by a gamma-secretase inhibitor results in the inhibition of the asthma phenotype and downregulation of GATA3 (64). Our data suggest that HBEC treated with DEP selectively upregulated Jagged-1 in immature mDC and that Jagged-1 expression was associated with functional DC that supported Th2 differentiation of naïve T cells. Moreover, Jagged-1 upregulation was dependent on TSLP and the generation of ROS. Our finding that DEP-treated HBEC promoted DC that upregulated Jagged-1, but not Dll4, provides an additional mechanism by which DEP can promote Th2 responses and suggests the potential for an IL-4–independent process.

FACS analyses confirmed increased surface expression of both OX40L and Jagged-1 in mDC exposed to DEP-treated HBEC. FACS analyses also demonstrated that the mDC response to untreated and DEP-treated HBEC was heterogeneous, with mDC subsets expressing OX40L and Jagged-1 alone or expressing both ligands. These data also showed that only a minor percentage of mDC isolated from peripheral blood had the potential to respond to HBEC signals. These data suggest the possibility that a maturation threshold may be required for mDC–HBEC interaction and that there are subsets of mDC with the ability to respond.

DC have potential to directly access and phagocytize particles such as DEP, and they have been well shown to sample through epithelial cell tight junctions (65–68). DEP have also been reported to directly stimulate some cytokines (IL-1, TNF-α) in human monocyte-derived DC (69), and ambient PM obtained from Baltimore, MD has been shown to directly induce maturation of human monocyte-derived DC (70–72). Studies of isolated bone marrow-derived DC from murine models suggest a response of DC to carbon black particles or DEP and suggest that this is a complex process (73, 74). We and others did not identify direct activation of mDC by DEP or ambient PM in the absence of HBEC (19, 21, 30, 75, 76). In contrast, we demonstrated the importance of DEP-upregulated HBEC-derived signals, specifically TSLP, in the mDC expression of OX40L and Jagged-1. One explanation for the discrepancy in findings may be due to differences in the source of the particles and their chemical composition. We therefore suggest that the microenvironment provided by epithelial cell-derived cytokines is important for the Th2 polarization regulated by DC in response to DEP.

Our previous work and the initial experiments for this study were performed in human primary HBEC and were confirmed in 16HBEC, suggesting that for this area of study, the 16HBEC-transformed cell line functions in a manner that is similar to primary cells. DEP was used at a physiologically relevant concentration (3 μg/cm²), previously shown to induce cell activation without cell toxicity (30). Recent studies identify different origins of DC, and responses between monocyte-derived DC, plasmacytoid DC, and blood-derived DC may differ (77, 78). We used human blood-derived mDC for these studies, and the possibility exists that different responses may be identified for other DC. We identified Th2 skewing in the absence of allergen stimulation, and thus we suggest that DEP upregulation of TSLP and its downstream pathways may condition the lung for an enhanced allergen-regulated response.

In summary, our data, in combination with our previous studies, suggest that DEP-treated HBEC upregulate TSLP in a ROS-dependent manner, and that this upregulation results in functional mDC that support Th2 polarization. Th2 cell differentiation by mDC exposed to DEP-treated HBEC occurs via multiple pathways, including expression of OX40L and selective Notch ligands. These findings have implications for furthering our understanding of how pollutants may generate a permissive environment at the mucosal interface that supports the generation of a Th2 airway response.

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Disclosures
The authors have no financial conflicts of interest.

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