Vehicular exhaust particles promote allergic airway inflammation through an aryl hydrocarbon receptor–notch signaling cascade

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Background: Traffic-related particulate matter (PM) has been linked to a heightened incidence of asthma and allergic diseases. However, the molecular mechanisms by which PM exposure promotes allergic diseases remain elusive.

Objective: We sought to determine the expression, function, and regulation of pathways involved in promotion of allergic airway inflammation by PM.

Methods: We used gene expression transcriptional profiling, *in vitro* culture assays, and *in vivo* murine models of allergic airway inflammation.

Results: We identified components of the Notch pathway, most notably Jagged 1 (Jag1), as targets of PM induction in human monocytes and murine dendritic cells. PM, especially ultrafine particles, upregulated T_H cytokine levels, IgE production, and allergic airway inflammation in mice in a Jag1- and Notch-dependent manner, especially in the context of the proasthmatic IL-4 receptor allele *Il4raR576*. PM-induced Jag1 expression was mediated by the aryl hydrocarbon receptor (AhR), which bound to and activated AhR response elements in the *Jag1* promoter. Pharmacologic antagonism of AhR or its lineage-specific deletion in $CD11c^+$ cells abrogated the augmentation of airway inflammation by PM.

Conclusion: PM activates an AhR-Jag1-Notch cascade to promote allergic airway inflammation in concert with proasthmatic alleles. (J Allergy Clin Immunol 2015;136:441-53.)

Key words: *Traffic-related particulate matter, diesel exhaust particles, ultrafine particles, asthma, allergic airway inflammation, Jagged 1, Notch, aryl hydrocarbon receptor, airway hyperresponsiveness*

Asthma is a major health problem worldwide, the prevalence of which has reached unprecedented levels in recent years.[1] Its increase to the status of a modern epidemic has been driven by the interaction of susceptibility genes in affected subjects with emerging environmental factors unleashed by the industrial revolution and lifestyle changes.[2] The latter include changes in microbial exposure integrated under the rubric of the hygiene hypothesis, changes in diet and activity, and, significantly, exposure to airborne particles and pollutants in carbon footprint–heavy economies.[3] A large body of research has linked asthma development with urbanization and exposure to pro-oxidative, traffic-related air pollutants, most notably particulate matter (PM) and ozone, by virtue of their initiation of airway and systemic inflammation and their proallergic adjuvant function.[4-7]

The acute increase in the incidence of atopy and asthma in urban areas has been related in part to fine particles (FP; ≤2.5 μm in diameter) and ultrafine particles (UFP; ≤0.18 μm in diameter) of PM emitted by vehicular traffic, particularly that associated with diesel engines.[8-11] These particles have been reported to promote T_H2 and T_H17 cell responses and upregulate IgE production in the exposed host.[6,12,13] They differentially affect the lung function of asthmatic patients, suggesting that genetic and epigenetic predispositions to atopy cooperatively promote the airway inflammatory responses seen after diesel exhaust particles (DEP) exposure.[14]

Ambient PM exposure acts as an adjuvant that primes the immune response to common environmental allergens.[15] Intranasal instillation of ambient UFP derived from vehicular emissions enables a *de novo* immune response in the absence of added adjuvants.[16] Recently, it was demonstrated that simple inhalation of ambient UFP could effectively boost the secondary immune response to an experimental allergen, indicating that vehicular traffic exposure might exacerbate allergic inflammation in already sensitized subjects.[12] Neither the precise molecular mechanisms by which PM exposure promotes allergic sensitization nor the identity of the PM subcomponents involved are clear. However, PM-induced oxidative stress can induce T_H skewing of the immune response through affecting the antigen-presenting function of dendritic cells (DCs).[17-21] This could involve antigen uptake, antigen presentation, DC costimulatory activity, and cytokine production.

A range of genetic, immunologic, and whole-animal approaches were used to elucidate molecular mechanisms by

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Abbreviations used
AhR: Aryl hydrocarbon receptor
BMDC: Bone marrow–derived dendritic cell
CB: Carbon black particles
DC: Dendritic cell
DEP: Diesel exhaust particles
DLL: Delta-like ligand
DMSO: Dimethyl sulfoxide
FICZ: 6-Formylindolo (3,2-b) carbazole
FP: Fine particles
GSI: Gamma-secretase inhibitor
Jag1: Jagged 1
Notch1c: Notch1 intracellular domain
OVA: Ovalbumin
PAH: Polycyclic aryl hydrocarbon
PAS: Periodic acid–Schiff
PM: Particulate matter
TLR4: Toll-like receptor
UFP: Ultrafine particles
WT: Wild-type

which PM exposure can program antigen-presenting cells to promote allergic diseases. Here we provide evidence for a critical role for an aryl hydrocarbon receptor (AhR)–Jagged 1 (Jag1)–Notch pathway in mediating the proinflammatory effects of PM in patients with allergic airway inflammation in interaction with proatopic *Il4ra* alleles.

METHODS

Mice

Il4raR576 mice were previously described.[22] The following mice were obtained from the Jax Labs (Bar Harbor, Me): BALB/c (wild-type [WT]), *Tlr4*$^{-/-}$ (B6.B10ScN-Tlr4$^{lps\text{-}del}$), *Ahr*$^{fl/fl}$ (Ahr$^{tm3.1Bra}$), *CD11c-cre* (B6.Cg-Tg[Itgax-cre]1-1Reiz/J), and *Nlrp3*$^{-/-}$ (B6.129S6-*Nlrp3*^{tm1Bhk}/J). *DO11.10Rag2*$^{-/-}$ mice were obtained from Taconic Farms (Hudson, NY). They were crossed with *Il4raR576* mice to generate *DO11.10Rag2*$^{-/-}$ *Il4raR576* mice. *Nrf2*$^{-/-}$ mice were a kind gift of Andre Nel.[23]

Particles

DEP were a kind gift of Dr Andre Nel and have been described previously.[24] Carbon black particles (CB; <500 nm) were purchased from Sigma-Aldrich (St Louis, Mo). Ambient FP (≤2.5 μm) and UFP (≤0.18 μm) were collected in an urban area of downtown Los Angeles by using a high-volume ultrafine particle sampler[25] operating at 400 L/min and loaded with a Zefluor filter (supported polytetrafluoroethylene, 3.0-μm pore, 8 × 10 inches; Pall Life Sciences, Port Washington, NY). For investigating the volatile and nonvolatile UFP fractions, we collected 2 UFP samples: N-UFP, which were collected during nighttime plus morning traffic hours (ie, rich in semivolatile organics partitioned to the PM phase), and Af-UFP, which were collected during the afternoon, when partitioning is minimal. The respective particles were suspended in an aqueous solution, with the hydrophilic components becoming part of the solution, whereas the solid nonsoluble UFP cores are left in suspension. The particle-loaded filters were soaked in ultrapure water, followed by 5 minutes of vortexing and 30 minutes of sonication, to transfer the particles into solution. The entire mixture was administered intranasally, as indicated below. Endotoxin levels were less than 0.1 ng/mL (data not shown).

Derivation of DCs

Bone marrow–derived dendritic cells (BMDCs) were obtained by culturing bone marrow aspirates for 7 days with GM-CSF at 20 ng/mL. The cells were either sham treated with PBS or treated with particles at 10 μg/mL in PBS for 18 hours before measurement of Notch pathway components by means of flow cytometry, real-time PCR, or both.

T-cell/BMDCs cocultures

Splenic CD4^{+}DO11.10^{+}RAG2$^{-/-}$ T cells were purified by means of magnetic cell sorting negative selection (Miltenyi Biotec, Bergisch Gladbach, Germany). BMDCs were placed in aliquots at 2 × 10^{5} cells in 48-well plates and then either sham treated or treated with the respective PM species at 10 μg/mL per culture well overnight. For AhR inhibition experiments, BMDCs were treated with 30 nmol/L of the AhR antagonist CH223191 and washed after 16 hours. CD4^{+}DO11.10^{+}RAG2$^{-/-}$ T cells were added at 4 × 10^{5} cells per well in a final volume of 0.5 mL of 10% FBS/RPMI. Cultures were treated with the ovalbumin (OVA)$_{323\text{-}339}$ peptide at 1 μmol/L, as indicated. Notch pathway inhibitors included the gamma secretase inhibitor (GSI; Calbiochem, San Diego, Calif) added at 5 μmol/L and anti-murine Jag1 antibodies (BioLegend, San Diego, Calif) added at 2.5 μg/mL.

Allergic sensitization and challenge

Mice were sensitized to OVA by means of intraperitoneal injection of 100 μg of OVA in 100 μL of PBS and then boosted 2 weeks later with a second intraperitoneal injection of OVA in PBS. Control mice were sham sensitized and boosted with PBS alone. Starting on day 29, both OVA- and sham-sensitized mice were challenged with aerosolized OVA at 1% delivered through a Schuco 2000 nebulizer (Allied Health Care Products, St Louis, Mo) for 30 minutes daily for 3 days. Two hours before each OVA aerosol exposure, subgroups of mice were administered intranasally either PBS or PM (UFP, FP, or CB) at 10 μg/100 μL of PBS per instillation. Where indicated, GSI (0.3 mg/kg in dimethyl sulfoxide [DMSO] administered intranasally) or CH223191 (0.5 mg/kg in corn oil administered intraperitoneally) was introduced 4 hours before OVA challenge, with untreated mice receiving the DMSO vehicle, also intranasally. For anti-Jag1 antibody blocking, 150 μg of anti-Jag1 or isotype control antibodies in 100 μL of PBS buffer was administered daily for 3 consecutive days during OVA aerosol challenge. Mice were killed on day 32 after sensitization and analyzed. For dust mite–induced allergic airway inflammation, mice received 5 μg of lyophilized *Dermatophagoides pteronyssinus* extract in 100 μL of PBS intranasally for 3 days at the start of the protocol and then challenged with the same dose of *D pteronyssinus* extract on days 15 to 17 with or without PM. Where indicated, mice were treated with isotype control or anti-Jag1 antibodies or with DMSO (sham) or the AhR antagonist CH223191, as indicated, on the same days (days 15-17) of *D pteronyssinus* challenge. Mice were killed on day 18 and analyzed for measures of airway inflammation.

Lung histopathology staining

Paraffin-embedded lung sections were stained with hematoxylin and eosin, as previously described.[26] Lung inflammation was scored separately for cellular infiltration around the blood vessels and airways: 0, no infiltrates; 1, few inflammatory cells; 2, a ring of inflammatory cells 1 cell layer deep; 3, a ring of inflammatory cells 2 to 4 cells deep; and 4, a ring of inflammatory cells greater than 4 cells deep.[27] A composite score was determined by adding the inflammatory scores for both vessels and airways. The number and distribution of goblet cells was assessed by using periodic acid–Schiff (PAS) staining of mucin granules. Individual airways (bronchi/bronchioles) were scored for goblet cell hyperplasia according to the following scale: 0, no PAS-positive cells; 1, less than 5% PAS-positive cells; 2, 5% to 10% PAS-positive cells; 3, 10% to 25% PAS-positive cells; and 4, greater than 25% PAS-positive cells.[28]

Statistical analysis

The Student 2-tailed *t* test, 1- and 2-way ANOVA, and repeated-measures 2-way ANOVA with Bonferroni posttest analysis of groups were used to compare test groups, as appropriate. A *P* value of less than .05 was considered statistically significant.