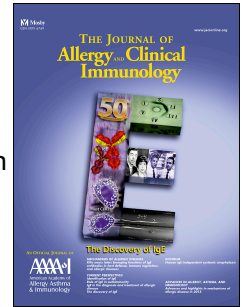


# Accepted Manuscript

Pre-Pregnancy Exposure to Diesel Exhaust Predisposes Offspring to Asthma through IL-1 $\beta$  and IL-17A

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**Pre-Pregnancy Exposure to Diesel Exhaust Predisposes Offspring to Asthma through IL-1 $\beta$  and IL-17A**

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## Capsule Summary

Using a mouse model we provided evidence that pre-pregnancy exposure to diesel exhaust predisposes offspring to asthma. We identified immunological mechanisms of this predisposition to facilitate future development of asthma prevention strategies.

## Key Words

Pre-pregnancy exposure; diesel exhaust particles; asthma; mouse model; interleukin 1 $\beta$ ; interleukin 17A

## Abbreviations

AHR, airway hyperresponsiveness  
BAL, bronchoalveolar lavage  
DEP, diesel exhaust particles  
ELISA, enzyme-linked immunosorbent assay  
IL, interleukin  
i.n., intranasal  
i.p., intraperitoneal  
OVA, ovalbumin  
PAH, polycyclic aromatic hydrocarbons  
PBS, phosphate-buffered saline  
PCR, polymerase chain reaction  
PM, particulate matter  
PMD, pre-mating day

48 PND, postnatal day

49 Th, T helper

50 TNF, tumor necrosis factor

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**To the Editor:**

Prenatal environmental exposures, including exposure to diesel exhaust are increasingly recognized as important risk factors for asthma<sup>1, 2, E1-E4</sup>. In the human population, prenatal exposure to diesel exhaust has been measured by several different means including levels of polycyclic aromatic hydrocarbons (PAH)-DNA adducts in the cord blood, concentrations of fine particles (PM<sub>2.5</sub>) in the environment of a pregnant mother (personal monitors) and proximity of maternal residence to a major road<sup>1, 2, E1, E2</sup>. All measures of the exposure were positively associated with childhood asthma. What remains unclear is whether predisposition to asthma in children can be attributed solely to gestational exposures or is a collective result of exposures occurring during and before pregnancy. The evidence provided by association studies in humans spurred causative studies in mice. Using mouse models, we and others showed that gestational exposure to diesel exhaust particles (DEP) caused development of asthma predisposition in offspring<sup>3, 4</sup>. As is true of studies on the human population, effects of pre-pregnancy exposure in mice are unknown.

To address this gap in knowledge we developed a mouse model (see Fig E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). In this model, C57BL/6 female mice were intranasally exposed to DEP (50 µg) or PBS on days -29, -26, -23, -20, -17, -14 before mating with unexposed males (see this article's Methods section in the Online Repository at [www.jacionline.org](http://www.jacionline.org)). There were no exposures during pregnancy. Pups were immunized and challenged with ovalbumin (OVA) or treated with PBS. The immunization was intentionally suboptimal (occurring during the neonatal period) to reveal the priming effect of maternal exposure. The immunization included a single intraperitoneal injection of a low dose (5 µg)

94 ovalbumin (OVA) with Imject Alum (1 mg) on postnatal day 5 (PND 5). The intranasal  
95 challenge with OVA (50 µg) or PBS was done on PND 23, 24 and 25. Mice were analyzed on  
96 PND 28. The following groups of pups were studied: pups exposed to DEP prenatally and  
97 challenged with PBS after birth (DEP-PBS pups) or OVA after birth (DEP-OVA pups), pups  
98 exposed to PBS prenatally and challenged with PBS after birth (PBS-PBS pups), or OVA after  
99 birth (PBS-OVA pups). Pre-pregnancy exposure predisposed offspring to asthma. Two types of  
100 effects were observed: the effects of DEP alone and the effects of combined exposure to DEP  
101 and OVA. The first type of effects was revealed by comparison of DEP-PBS pups with PBS-PBS  
102 pups. The DEP-PBS pups had increased airway resistance to methacholine (Fig 1, A).  
103 Interestingly, this enhancement of airway hyperresponsiveness (AHR) was accompanied by only  
104 marginal increases of inflammation parameters. In fact, lung histology did not reveal any  
105 inflammatory infiltrates (Fig 1, B). The bronchoalveolar lavage (BAL) fluid showed a marginal  
106 increase of eosinophils and neutrophils (Fig 1, C). Pulmonary IL-5, IL-13 and IFN $\gamma$  were at the  
107 normal level (Fig 1, D). The effects of combined exposure were uncovered by comparison of  
108 DEP-OVA pups with PBS-OVA and DEP-PBS pups. The asthma in DEP-OVA pups was fully  
109 manifested. Compared to PBS-OVA pups, DEP-OVA pups had enhanced AHR (Fig 1, A), larger  
110 peribronchial cellular infiltrates (Fig 1, B) and increased number of eosinophils and lymphocytes  
111 in the BAL fluid (Fig 1, C). The AHR in DEP-OVA pups was also higher than that in DEP-PBS  
112 pups (Fig 1, A). Finally, DEP-OVA pups had increased levels of pulmonary IL-5 and IL-13 (Fig  
113 1, D). IFN $\gamma$  was not affected (Fig 1, D). Comparison with our previously-described model<sup>3</sup>  
114 revealed that pre-pregnancy and pregnancy exposures affected similar traits of asthma. The  
115 models differ with regard to the magnitude of some of these traits, including BAL eosinophils  
116 and peribronchial infiltrates. In the context of postnatal exposure to OVA, pre-pregnancy and

117 pregnancy DEP increased eosinophils by 3.2 and 5.6 fold and peribronchial infiltrates by 2.1 and  
118 3.5 fold, respectively. To further explore the effects of pre-pregnancy DEP, offspring lungs were  
119 examined for mRNA encoding DEP-inducible cytokines<sup>5, E5-E7</sup>, including IL-1 $\alpha$ , IL-1 $\beta$ , IL-17A,  
120 IL-17F, IL-23 and TNF $\alpha$ . DEP alone induced transcripts for IL-1 $\beta$ , IL-17A and IL-23 as  
121 revealed by comparison of DEP-PBS and PBS-PBS groups (Fig 1, E). We also analyzed the  
122 combined effect of DEP and OVA. Compared to PBS-OVA pups, DEP-OVA pups had higher  
123 levels of pulmonary transcripts for IL-1 $\beta$  and IL-17A (Fig 1, E). Furthermore, DEP-OVA pups  
124 had more of these transcripts than DEP-PBS pups. Thus, effects of DEP and OVA on these two  
125 cytokine transcripts were additive. DEP-OVA and PBS-OVA pups were not different with  
126 respect to levels of transcripts for IL-1 $\alpha$ , IL-17F, IL-23 and TNF $\alpha$ . Since IL-1 $\beta$  and IL-17A  
127 transcripts produced the strongest differentiating signal between the DEP groups and their  
128 controls, we decided to focus on these two cytokines in further experiments. DEP-OVA pups had  
129 increased concentration of IL-1 $\beta$  and IL-17A proteins in the BAL fluid as compared to all three  
130 other groups of pups (Fig 1, F). Interestingly, the BAL concentration of IL-1 $\beta$  and IL-17A  
131 proteins was same in DEP-PBS and PBS-PBS pups. Thus, DEP primed IL-1 $\beta$  and IL-17A  
132 pathways through induction of cytokine transcripts; OVA potentiated the DEP effects on  
133 transcripts and upregulated the corresponding proteins. IL-1 $\beta$  transcripts were also increased in  
134 lungs of pregnant (gestation day 10.5) DEP-exposed mothers (Fig 1, G). This was associated  
135 with increased levels of the aryl hydrocarbon receptor (Ahr) pathway transcripts including Ahr,  
136 Ahrr, Cyp1a1 and Cyp1a2 (Fig 1, H). The transcriptional factor Ahr regulates IL-1 $\beta$  production  
137 and is the key signaling target of DEP<sup>E5, E8</sup>. These results suggest that Ahr signaling persists  
138 after cessation of DEP exposure into the pregnancy and may contribute to the elevation of the IL-  
139 1 $\beta$  transcript. The two groups of mothers did not show any difference in the level of IL-17A

transcript. One possible explanation is that, at the studied time point, the IL-17A gene/transcript is subjected to a negative feedback mechanism that negates the action of Ahr.

We examined the importance of IL-1 $\beta$  and IL-17A in offspring asthma through the use of depleting antibodies. DEP-OVA pups were used for experiments. Depleting antibodies and control immunoglobulins were injected one day before immunization and again one day before airway challenge with OVA. Compared to control IgG-injected pups, anti-IL-1 $\beta$ -injected pups had reduced AHR (Fig 2, A), decreased peribronchial inflammation (histology; Fig 2, B) as well as numbers of eosinophils, neutrophils and lymphocytes in the BAL fluid (Fig 2, C). Neutrophils and areas of peribronchial infiltrates were brought to baseline, meaning that for these parameters, there was no statistical difference between the IL-1 $\beta$ -depleted DEP-OVA group and the PBS-PBS group. Consistently with reduced inflammation, depletion of IL-1 $\beta$  led to reduction in IL-1 $\beta$ , IL-5, IL-13 and IL-17A transcripts (Fig 2, D). Depletion of IL-17A completely abrogated BAL neutrophilia (Fig 2, G). In this regard, the IL-17A-depleted DEP-OVA group was statistically indistinguishable from the PBS-PBS group. More importantly, compared to control IgG-injected pups, anti-IL-17A-injected pups had reduced AHR (Fig 2, E). Consistently with previous publications<sup>5,6</sup>, IL-17A depletion had no effect on eosinophils, lymphocytes, macrophages and peribronchial inflammation (Fig 2, F and G). In agreement with this, IL-17A depletion did not affect pulmonary IL-1 $\beta$ , IL-5 and IL-13 (Fig 2, H). Interestingly, IL-17A depletion led to marked reduction of the IL-17A transcript (Fig 2, H). The data point to existence of an additional mode of IL-17A regulation i.e. a positive feedback loop that does not involve IL-1 $\beta$ .

Here we demonstrate for the first time that pre-pregnancy exposure to diesel exhaust causes asthma predisposition in offspring. The observed transgenerational effects of diesel exhaust were twofold. Diesel exhaust had its own, allergen-independent effect that manifested in increased AHR. The effect on inflammation was minimal. Diesel exhaust also made offspring hypersensitive to allergens. Offspring of DEP-exposed mothers vigorously responded to the allergen treatment that caused only minimal response in controls. The combined treatment with diesel exhaust and the allergen led to fully manifested asthma with increased levels of IL-5 and IL-13, eosinophilic inflammation and the highest level of AHR. The transgenerational transmission of predisposition to hypersensitivity to allergens and asthma critically relied on IL-1 $\beta$  and IL-17A. Depletion of IL-1 $\beta$  in allergen-treated pups of DEP-exposed mothers abolished all features of asthma. IL-1 $\beta$  induced AHR through IL-17A. Depletion of IL-17A reduced AHR but had no effect on IL-5, IL-13, eosinophils and peribronchial inflammation.

Our work indicates that the effects of diesel exhaust are long lasting and transmittable to the next generation. We previously described the next generation effects of the exposure occurring during pregnancy<sup>3</sup>. Here we demonstrate that transgenerational transmission occurs even if the exposure occurs weeks before pregnancy. Thus, harmful transmittable signals can persist in a mother for weeks and affect the fitness of future offspring. These persistent signals may include DEPs themselves, DEP-derived compounds (e.g. polycyclic aromatic hydrocarbons that activate Ahr) and the Ahr pathway and its targets.

DEP exerted effects in isolation and in combination with an allergen. DEP alone augmented AHR. In addition, DEP increased levels of IL-1 $\beta$  and IL-17A transcripts. Interestingly, levels of

186 respective proteins were not changed. Consistently with lack of effect on cytokine proteins, DEP  
187 alone did not trigger any histologically-detectable inflammation; the effects on BAL counts were  
188 very subtle. The significance of increased levels of IL-1 $\beta$  and IL-17A transcripts was revealed  
189 upon exposure to the allergen. In this context, priming by DEP led to increased production of IL-  
190 1 $\beta$  and IL-17A proteins. Primed cytokine pathways were the source of allergen hypersensitivity  
191 and airway pathology. Our results are consistent with previous reports that supported the link  
192 between immune cell priming/memory and the accumulation of untranslated cytokine mRNAs<sup>E9-</sup>  
193 <sup>E11</sup>. Upon cell re-stimulation, the increased transcript levels allowed for a rapid and high-level  
194 induction of cytokine proteins, accounting for enhanced capacities of memory T cells to trigger  
195 immune responses.

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197 IL-1 $\beta$  is a master regulatory cytokine that has capacity to initiate and enhance a number of  
198 immune responses, including the allergic response. Normally, mucosal administration of OVA  
199 does not lead to airway inflammation but instead causes immune tolerance to this antigen. Co-  
200 administration of IL-1 $\beta$  breaches this tolerance, induces type 2 and type 3 immunity and  
201 eosinophilic inflammation of airways<sup>7, E12</sup>. These strong enhancer activities of IL-1 $\beta$  were also  
202 observed in our model. Pups born to vehicle-exposed mothers produce very little IL-1 $\beta$  and were  
203 hypo-responsive to OVA. Pups born to DEP-exposed mothers responded to OVA and this  
204 response was dependent on upregulation of endogenous IL-1 $\beta$ . IL-1 $\beta$  acted as an upstream  
205 regulator of type 2 (IL-5, IL-13, eosinophils) and type 3 (IL-17A, neutrophils) responses.

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IL-1 mediates pro-asthma effects of some but not all environmental pollutants. IL-1 $\beta$  is required for toluene diisocyanate-induced asthma<sup>E13</sup>. Conversely, IL-1R signaling opposes the development of nitrogen dioxide-mediated asthma<sup>E14</sup>.

Priming of the IL-1 $\beta$  pathway may be responsible for inborn predisposition to human asthma. Elevated IL-1 $\beta$  mRNA at birth and the increased IL-1 $\beta$  protein in the first four months of life are predictive for early ( $\leq 2$  years) onset childhood wheeze<sup>8, E15</sup>.

IL-17A and IL-17A-producing cells are increased in childhood asthma and positively correlate with asthma severity<sup>5, 9, E16</sup>. In children with asthma, IL-17A associates with exposure to DEP<sup>5</sup>. Furthermore, there are associations between childhood asthma and polymorphisms in the IL-17A gene<sup>E17</sup>.

In conclusion, our study reveals a causative relationship between pre-pregnancy exposure to DEP and development of asthma predisposition in offspring. The mechanism involves IL-1 $\beta$  and IL-17A. The study identifies molecular targets for development of approaches to prevent childhood asthma.

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## 236 **References**

237

- 238 1. Perzanowski MS, Chew GL, Divjan A, Jung KH, Ridder R, Tang D, et al. Early-life  
239 cockroach allergen and polycyclic aromatic hydrocarbon exposures predict cockroach  
240 sensitization among inner-city children. *J Allergy Clin Immunol* 2013;131:886-93.
- 241 2. Jedrychowski WA, Perera FP, Mauger U, Mrozek-Budzyn D, Mroz E, Klimaszewska-  
242 Rembiasz M, et al. Intrauterine exposure to polycyclic aromatic hydrocarbons, fine  
243 particulate matter and early wheeze. Prospective birth cohort study in 4-year olds. *Pediatr*  
244 *Allergy Immunol* 2010;21:e723-32.
- 245 3. Manners S, Alam R, Schwartz DA, Gorska MM. A mouse model links asthma  
246 susceptibility to prenatal exposure to diesel exhaust. *J Allergy Clin Immunol*  
247 2014;134:63-72.
- 248 4. Fedulov AV, Leme A, Yang Z, Dahl M, Lim R, Mariani TJ, et al. Pulmonary exposure to  
249 particles during pregnancy causes increased neonatal asthma susceptibility. *Am J Respir*  
250 *Cell Mol Biol* 2008;38:57-67.

5. Brandt EB, Kovacic MB, Lee GB, Gibson AM, Acciani TH, Le Cras TD, et al. Diesel exhaust particle induction of IL-17A contributes to severe asthma. *J Allergy Clin Immunol* 2013;132:1194-1204.
6. Wilson RH, Whitehead GS, Nakano H, Free ME, Kolls JK, Cook DN. Allergic sensitization through the airway primes Th17-dependent neutrophilia and airway hyperresponsiveness. *Am J Respir Crit Care Med* 2009;180:720-30.
7. Kobayashi T, Iijima K, Checkel JL, Kita H. IL-1 family cytokines drive Th2 and Th17 cells to innocuous airborne antigens. *Am J Respir Cell Mol Biol* 2013;49:989-98.
8. Herberth G, Offenberg K, Rolle-Kampczyk U, Bauer M, Otto W, Röder S, et al. Endogenous metabolites and inflammasome activity in early childhood and links to respiratory diseases. *J Allergy Clin Immunol* 2015;136:495-7.
9. Chien JW, Lin CY, Yang KD, Lin CH, Kao JK, Tsai YG. Increased IL-17A secreting CD4<sup>+</sup> T cells, serum IL-17 levels and exhaled nitric oxide are correlated with childhood asthma severity. *Clin Exp Allergy* 2013;43:1018-26.

**Figure legends**

**Figure 1:** Pre-pregnancy model. A-F, analysis of pups; G and H, analysis of mothers; A, Total lung resistance, 12-19 pups/group (group description in the main text), the table includes statistical analysis of the resistance data; B, Peribronchial inflammation, 8-15 pups/group; C, BAL cell counts, 11-17 pups/group; D, Cytokine proteins in lung homogenates, 9-12 pups/group; E, Cytokine transcripts in the lung, 8-15 pups/group; F, Cytokine proteins in the BAL fluid, 11-17 pups/group; G and H, Transcripts for cytokines (G) and components of the Ahr pathway (H) in maternal lungs (gestation day 10.5), 6-8 mothers/group; \*,  $P<0.05$ ; \*\*,  $P<0.01$ ; \*\*\*,  $P<0.001$ ; NS, not significant.

**Figure 2:** Cytokine depletion. Pups of DEP-exposed females were injected with antibodies against IL1 $\beta$  (A-D; 7-10 mice/group), IL-17A (E-H; 8-12 mice/group) or control IgGs before immunization and again before challenge with OVA. Pups were examined for total lung resistance to methacholine (A, E), peribronchial inflammation (B, F), BAL cell counts (C, G) and pulmonary cytokine transcripts (D, H); Anti-cytokine antibody vs. IgG control: \*,  $P<0.05$ ; \*\*,  $P<0.01$ ; \*\*\*,  $P<0.001$

Figure 1

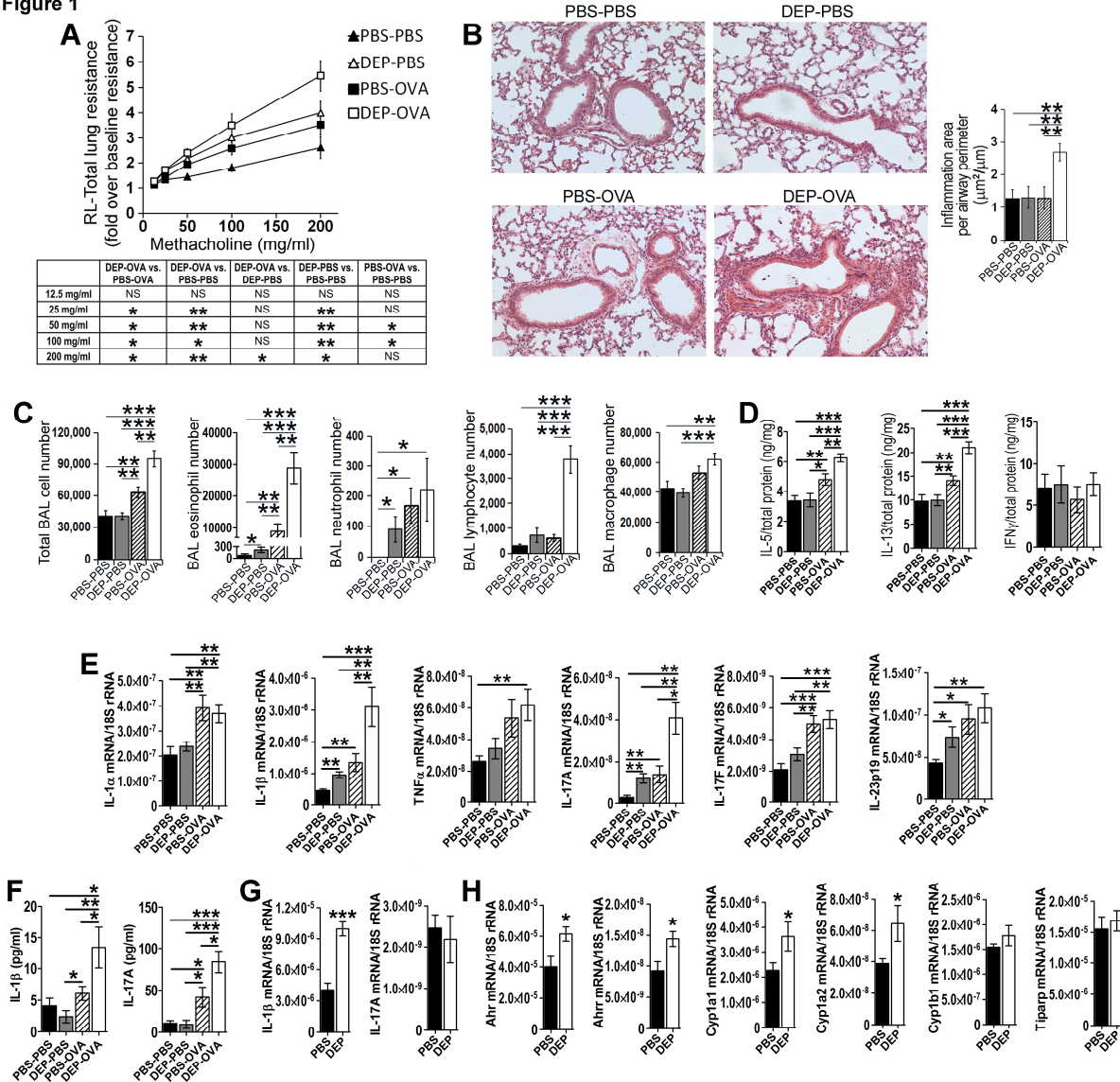
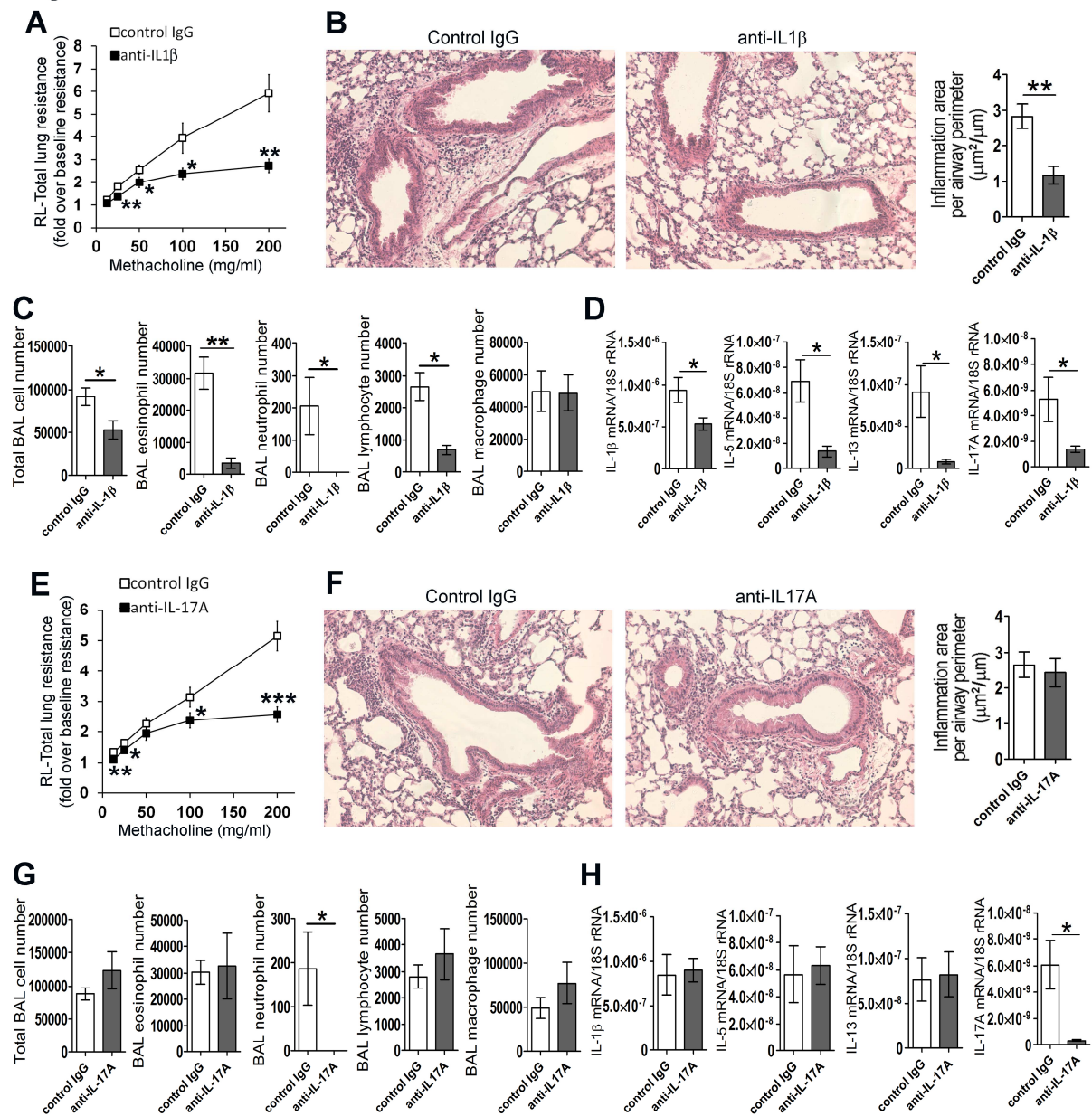


**Figure 2**

## Supplemental Materials

Pre-Pregnancy Exposure to Diesel Exhaust Predisposes Offspring to Asthma through IL-1 $\beta$  and IL-17A

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## Methods

### Mouse model

C57BL/6 females received six intranasal applications of DEP (National Institute of Standards and Technology, Gaithersburg, MD; SRM 2975) or PBS, three days apart (days -29, -26, -23, -20, -17 and -14 before mating). The intranasal instillations occurred under isoflurane anesthesia. For instillations, DEP was resuspended in PBS at 1 mg/ml and delivered at a dose of 50 µg (50 µl) per mouse. A volume of 50 µl was delivered through two sequential injections of 25 µl, 15 min apart, each injection to a different nostril. Two weeks after the final instillation females were mated with unexposed males. There were no exposures during pregnancy. Some females were sacrificed on gestation day 10.5 for organ recovery (day 0.5 denotes a day of the copulation plug detection). On postnatal day (PND) 5, pups were intraperitoneally injected with either 50 µl of PBS or 50 µl of the immunizing mixture containing OVA (5 µg) and Imject Alum (0.5 mg aluminum hydroxide and 0.5 mg magnesium hydroxide; Thermo Fisher Scientific, Waltham, MA) in PBS. On PND 23, 24 and 25 all pups underwent pulmonary challenges. OVA-immunized pups were intranasally administered with 50 µg of OVA in 15 µl of PBS. PBS-injected pups were intranasally challenged with 15 µl of PBS without OVA. Intranasal applications were performed under isoflurane anesthesia. On PND 28 mice were analyzed (airway resistance to methacholine, bronchoalveolar lavage (BAL), lung histology and pulmonary transcripts). All experiments were approved by the institutional animal care and use committee at National Jewish Health.

### **Cytokine depletion in vivo**

On PND 5, neonates of DEP-exposed females was intraperitoneally injected with 20 µg of either anti-IL-1β antibody (clone B122; BioLegend, San Diego, CA), anti-IL-17A antibody (clone 50104; R&D Systems, Minneapolis, MN), Armenian hamster IgG isotype control (control for anti-IL1β; BioLegend) or rat IgG2A isotype control (control for anti-IL17A; R&D Systems). On PND 6 pups were immunized with OVA in Imject Alum as described in the parent protocol. The antibody/control immunoglobulin administration was then repeated on PND 22 when reagents were delivered intravenously at a dose of 100 µg per mouse. All pups were intranasally challenged with OVA on PND 23, 24 and 25 as described in the parent protocol. Mice were sacrificed at 72 hours after the final challenge with OVA.

### **Measurement of airway hyperresponsiveness (AHR)**

Lung resistance was measured using the FlexiVent apparatus (Scireq, Montreal, CA) as described<sup>E18</sup>. Resistance values for each methacholine dose were expressed as a percent of baseline (saline inhalation) resistance. For statistical analysis, independent t tests were performed for each methacholine dose.

### **Histology and morphometric analysis**

Lungs were embedded in paraffin, sectioned and stained with hematoxylin and eosin. Images were acquired using the Nikon Eclipse TE 2000 inverted microscope, the Diagnostics Instruments camera model 4.2 and the Spot 5.0 software. The MetaMorph 7.0 software was used to measure areas of the peribronchial cellular infiltrate and a perimeter of the bronchial epithelial

basement membrane (proxy for airway perimeter). Results were expressed as an inflammation area per 1  $\mu\text{m}$  of airway perimeter.

## **Preparation of lung homogenates**

Lungs were homogenized at 100 mg/ml in the hypotonic buffer. The buffer contained 0.2x PBS and 1x Protease Inhibitor Cocktail (P8340, Sigma-Aldrich, St. Louis, MO). After two freeze-thaw cycles, debris were pelleted. 10xPBS was added to supernatants in order to equilibrate their osmolarity to 1xPBS. Concentration of total protein (Coomassie Plus Assay Kit; Thermo Fisher Scientific, Waltham, MA) and cytokines (ELISA) were measured in supernatants.

## **Cytokine ELISA**

Concentration of IL-1 $\beta$  and IL-17A in cell-free supernatants of the BAL fluid and IL-5, IL-13 and IFN $\gamma$  in lung homogenates was measured using ELISA kits (eBioscience, San Diego, CA and BD Biosciences, San Jose, CA). Cytokine concentration in lung homogenates was adjusted to total protein concentration.

## **Real time PCR**

RNA was extracted from Trizol lysates using RNeasy Plus Universal Mini Kit (Qiagen, Germantown, MD). cDNA was synthesized using ImProm-II reverse transcriptase and random primers (all from Promega, Madison, WI). cDNA was amplified and quantified using transcript-specific primers (Table E1), the Absolute qPCR SYBR Green mix (Thermo Scientific) and the ABI Prism 7000 Sequence Detection System (Life Technologies).

**Supplemental References**

- E1. Patel MM, Quinn JW, Jung KH, Hoepner L, Diaz D, Perzanowski M, et al. Traffic density and stationary sources of air pollution associated with wheeze, asthma, and immunoglobulin E from birth to age 5 years among New York City children. *Environ Res* 2011;111:1222-9.
- E2. Rosa MJ, Jung KH, Perzanowski MS, Kelvin EA, Darling KW, Camann DE, et al. Prenatal exposure to polycyclic aromatic hydrocarbons, environmental tobacco smoke and asthma. *Respir Med* 2011;105:869-76.
- E3. Burke H, Leonardi-Bee J, Hashim A, Pine-Abata H, Chen Y, Cook DG, et al. Prenatal and passive smoke exposure and incidence of asthma and wheeze: systematic review and meta-analysis. *Pediatrics* 2012;129:735-44.
- E4. Spanier AJ, Kahn RS, Kunselman AR, Hornung R, Xu Y, Calafat AM, et al. Prenatal exposure to bisphenol A and child wheeze from birth to 3 years of age. *Environ Health Perspect* 2012;120:916-20.
- E5. Provoost S, Maes T, Pauwels NS, Vanden Berghe T, Vandenabeele P, Lambrecht BN, et al. NLRP3/caspase-1-independent IL-1 $\beta$  production mediates diesel exhaust particle-induced pulmonary inflammation. *J Immunol* 2011;187:3331-7.
- E6. Ueng TH, Hung CC, Kuo ML, Chan PK, Hu SH, Yang PC, et al. Induction of fibroblast growth factor-9 and interleukin-1 $\alpha$  gene expression by motorcycle exhaust particulate extracts and benzo(a)pyrene in human lung adenocarcinoma cells. *Toxicol Sci* 2005;87:483-96.
- E7. Hartz AM, Bauer B, Block ML, Hong JS, Miller DS. Diesel exhaust particles induce oxidative stress, proinflammatory signaling, and P-glycoprotein up-regulation at the blood-brain barrier. *FASEB J* 2008;22:2723-33.

- E8. Stevens EA, Mezrich JD, Bradfield CA. The aryl hydrocarbon receptor: a perspective on potential roles in the immune system. *Immunology* 2009;127:299-311
- E9. Walzer T, Marçais A, Saltel F, Bella C, Jurdic P, Marvel J. Cutting edge: immediate RANTES secretion by resting memory CD8 T cells following antigenic stimulation. *J Immunol* 2003;170:1615-9.
- E10. Marçais A, Tomkowiak M, Walzer T, Coupet CA, Ravel-Chapuis A, Marvel J. Maintenance of CCL5 mRNA stores by post-effector and memory CD8 T cells is dependent on transcription and is coupled to increased mRNA stability. *Eur J Immunol* 2006;36:2745-54.
- E11. Scheu S, Stetson DB, Reinhardt RL, Leber JH, Mohrs M, Locksley RM. Activation of the integrated stress response during T helper cell differentiation. *Nat Immunol* 2006;7:644-51.
- E12. Willart MA, Deswarte K, Pouliot P, Braun H, Beyaert R, Lambrecht BN, et al. Interleukin-1 $\alpha$  controls allergic sensitization to inhaled house dust mite via the epithelial release of GM-CSF and IL-33. *J Exp Med* 2012;209:1505-17.
- E13. Johnson VJ, Yucesoy B, Luster MI. Prevention of IL-1 signaling attenuates airway hyperresponsiveness and inflammation in a murine model of toluene diisocyanate-induced asthma. *J Allergy Clin Immunol* 2005;116:851-8.
- E14. Martin RA, Ather JL, Daggett R, Hoyt L, Alcorn JF, Suratt BT, et al. The endogenous Th17 response in NO<sub>2</sub>-promoted allergic airway disease is dispensable for airway hyperresponsiveness and distinct from Th17 adoptive transfer. *PLoS One* 2013;8:e74730.
- E15. Burgess SL, Lu M, Ma JZ, Naylor C, Donowitz JR, Kirkpatrick BD, et al. Inflammatory markers predict episodes of wheezing during the first year of life in Bangladesh. *Respir Med* 2016;110:53-7.

- E16. Kerzel S, Dehne J, Rogosch T, Schaub B, Maier RF, Zemlin M. Th17 cell frequency in peripheral blood from children with allergic asthma correlates with the level of asthma control. *J Pediatr* 2012;161:1172-4.
- E17. Schieck M, Michel S, Suttner K, Illig T, Zeilinger S, Franke A, et al. Genetic variation in TH17 pathway genes, childhood asthma, and total serum IgE levels. *J Allergy Clin Immunol* 2014;133:888-91.
- E18. Manners S, Alam R, Schwartz DA, Gorska MM. A mouse model links asthma susceptibility to prenatal exposure to diesel exhaust. *J Allergy Clin Immunol* 2014;134:63-72.

160 **Table E1:** Sequences of transcript-specific primers

| Transcript Name | Forward Primer          | Reverse Primer            |
|-----------------|-------------------------|---------------------------|
| Ahr             | AGGACCAGTGTAGAGCACAA    | GCTGTGACAACCAGCACAAA      |
| Ahrr            | GTGTACATACGCCGGTAGGA    | GGTGCCGTTTGGAAGGATTT      |
| Cyp1a1          | GACCTTCCGGCATTTCATCCT   | GCCATTCAGACTTGTATCTCTTGTG |
| Cyp1a2          | GATACACATCCTTTGTCCCCTTC | AAAGGGGTCTTTCCACTGCTT     |
| Cyp1b1          | AGGATGTGCCTGCCACTATT    | CACAACCTGGTCCAACTCAG      |
| IL-1 $\alpha$   | TCAGCAACGTCAAGCAACGG    | TGCTGATCTGGGTTGGATGGT     |
| IL-1 $\beta$    | GCTGAAAGCTCT CCACCTCA   | CGTTGCTTGGTTCTCCTTGT      |
| IL-5            | GAGGCTTCCTGTCCCTACTC    | CCCACGGACAGTTTGATTCTT     |
| IL-13           | GAGCAACATCACACAAGACCA   | GATGTTGGTCAGGGAATCCAG     |
| IL-17A          | AGACTACCTCAACCGTTCCAC   | CACTGAGCTTCCCAGATCACA     |
| IL-17F          | GAAGTGCACCCGTGAAACAG    | ATGGCAAGTCCCAACATCAAC     |
| IL-23 p19       | CCAGCGGGACATATGAATCTACT | GTCCTTGAGTCCTTGTGGGT      |
| Tiparp          | TCTCAGGAGCACTTGGAAGA    | GTAGACAGCCTTCGTAGTTGG     |
| TNF $\alpha$    | CTCTTCTCATTCTGCTTGTGG   | GATGATCTGAGTGTGAGGGTCT    |
| 18S rRNA        | CGGCGACGACCCATTCGAAC    | GAATCGAACCTGATTCCCCGT     |

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**Supplemental Figure Legends**

**Figure E1:** Pre-pregnancy model. C57BL/6 females were exposed intranasally (i.n.) to DEP or PBS on indicated pre-mating (PMD) days. Offspring were injected intraperitoneally (i.p.) with OVA in alum or PBS, and then, intranasally (i.n.) with OVA or PBS on indicated postnatal days (PND). Pups were analyzed three days after the final intranasal exposure.

Figure E1

