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A Mouse Model Links Asthma Susceptibility to Prenatal Exposure to Diesel Exhaust

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Abstract

Background—Most asthma begins in the first years of life. This early onset cannot be merely attributed to genetic factors, because the prevalence of asthma is increasing. Epidemiological studies have indicated roles for prenatal and early childhood exposures, including exposure to diesel exhaust. However, little is known about the mechanisms. This is largely due to paucity of animal models.

Objective—We aimed to develop a mouse model of asthma susceptibility through prenatal exposure to diesel exhaust.

Methods—Pregnant C57BL/6 female mice were given repeated intranasal applications of diesel exhaust particles (DEP) or phosphate-buffered saline (PBS). Offspring underwent suboptimal immunization and challenge with ovalbumin (OVA) or received PBS. Pups were examined for features of asthma; lung and liver tissues were analyzed for transcription of DEP-regulated genes.

Results—Offspring of mice exposed to DEP were hypersensitive to OVA, indicated by airway inflammation and hyperresponsiveness, increased serum levels of OVA-specific IgE, and increased levels of pulmonary and systemic T-helper (Th)2 and Th17 cytokines. These cytokines were primarily produced by natural killer (NK) cells. Antibody-mediated depletion of NK cells prevented airway inflammation. Asthma susceptibility was associated with increased transcription of genes known to be specifically regulated by the aryl hydrocarbon receptor (AhR) and oxidative

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stress. Features of asthma were either marginal or absent in OVA-treated pups of PBS-exposed mice.

Conclusion—We created a mouse model that linked maternal exposure to DEP with asthma susceptibility in offspring. Development of asthma was dependent on NK cells and associated with increased transcription from AhR- and oxidative stress-regulated genes.

Keywords

prenatal exposure; diesel exhaust particles; asthma; mouse model; natural killer cells; aryl hydrocarbon receptor; interleukin 5; interleukin 13; interleukin 17

Introduction

Over the last several decades, the prevalence of asthma has continuously increased. In the last 10 y, the prevalence of asthma in the United States has increased from 7.3% (20.3 million persons in 2001) to 8.4% (25.7 million persons in 2010)[1]. This recent rise in prevalence implicates industrialization and urbanization-generated environmental exposures in disease pathogenesis. Prenatal and early childhood exposures are likely to have the highest impact as they occur in periods of intense developmental programming and thereby have the potential to induce long-term memory in cells, systems and organs. The role of early programming is underscored by the fact that asthma symptoms, in most cases, start in the first years of childhood. The argument for prenatal/maternal influences is provided by the observation on strong association of childhood asthma with maternal asthma[2–4]. Among children less than 5 y old, the risk of asthma is more than 3-fold greater for those with mothers with asthma than fathers with asthma.[2] One of the plausible explanations is that offspring predisposition to asthma is shaped prenatally, by an altered intrauterine environment. This alteration of the intrauterine environment is imposed by maternal disease and/or disease-triggering maternal exposures. In support of the latter hypothesis, there are many epidemiological studies showing an association between various *in utero* exposures and asthma susceptibility[5–12]. Among prenatal insults with linkage to asthma, solid and consistent epidemiological evidence has been provided for exposure to traffic-related pollution, including diesel exhaust[5–8]. Mothers who lived near highways during pregnancy are more likely to have children with asthma[5]. Prenatal exposure to polycyclic aromatic hydrocarbons (PAH), which are diesel exhaust-derived toxins, is associated with increased risk of allergic sensitization and early childhood wheeze[6,8].

Although epidemiological data supports the hypothesis on prenatal origins of asthma, the mechanistic understanding is still very poor. There are many obstacles to conducting mechanistic studies during pregnancy and infancy. Intentional exposures of pregnant women are unethical. Studies of infants have been limited by scant size of biological samples and ethical concerns. Last but not least, only few animal models are available. In regard to prenatal diesel exhaust exposures, the positive link to asthma has been established by three earlier models[13–15]. We have created a complementary mouse model and provided new mechanistic insights.

Methods

Mouse model

Time-mated C57BL/6 female mice were anaesthetized with isoflurane and given intranasal applications of diesel exhaust particles (DEP, 50 μg, National Institute of Standards and Technology, Gaithersburg, MD; SRM 2975)[15–18] in 50 μl PBS (15 mice) or 50 μl of PBS alone (9 mice) on gestation days (GD) 3, 6, 9, 12, 15, and 18. The volume was delivered through 2 sequential injections of 25 μl, 15 min apart, each into a different nostril.

On postnatal day (PND) 5, pups of 10 DEP-exposed mice and 5 PBS-exposed mice (5–8 pups from each mother) were given intraperitoneal injections of 50 μl of the immunizing mixture, which contained OVA (5 μg) and Imject Alum (0.5 mg aluminum hydroxide and 0.5 mg magnesium hydroxide; Thermo Scientific, Rockford, IL) in PBS. Pups from another 5 DEP-exposed mice and 4 PBS-exposed mice were given injections of 50 μl PBS. On PNDs 20, 21, and 22, OVA-immunized offspring of three DEP-exposed mice were given injections of either the anti-NK1.1 antibody or mouse IgG2a isotype (control) (NK cell depletion experiment; see detailed method in the Online Repository). On PNDs 23, 24, and 25, all pups underwent pulmonary challenges. OVA-immunized pups were given intranasal applications of 50 μg of OVA in 15 μl of PBS and PBS-injected pups were given intranasal applications of 15 μl of PBS without OVA, all under isoflurane anesthesia. On PND 27 (22 days after immunization and 2 days after final pulmonary challenge), blood samples were collected (by tail nick) and serum was isolated. FlexiVent studies were conducted on PND 28 (3 days after final pulmonary challenge). A separate set of mice was used to obtain bronchoalveolar lavage fluid (BALF) and lung and liver tissues[19,20]. For each measured parameter, offspring of 3–7 mice per group were analyzed. All experiments were approved by the institutional animal care and use committee at National Jewish Health. Other methods can be found in the Online Repository.

Results

A mouse model of asthma susceptibility through prenatal exposure to DEP

To develop our mouse model of prenatally-induced asthma susceptibility, we selected the most valuable elements from existing models (protocols by Fedulov et al.[13], Auten et al.[14], and Reiprich et al.[15]; see Table EII in the Online Repository). Similar to Auten et al.[14], we used C57BL/6 mice. This approach allows an immediate use of genetically-modified mice that are typically on the C57BL/6 background. We also incorporated the principle of repeated maternal exposure, similar to Auten et al.[14] and Reiprich et al.[15], because human exposure to diesel exhaust is chronic. For maternal challenges, we used DEP, because their pro-asthma activity was proven by all three prenatal exposure protocols and by other studies in adult mice and humans[13–15, 21–31]. The inflammatory activity of DEP is attributed to components such as PAH, quinones, sulfuric acid, and metal oxides[32].

In our model, 50 μg of DEP was repeatedly applied to pregnant C57BL/6 mice via the intranasal route (Fig 1). Control mice received PBS. The DEP dose of 50 μg is commonly used in mouse models, including those by Auten at al. and Fedulov et al [13, 14, and 16]. We considered using exposure to titanium dioxide or carbon black particles because they were

used in some studies as "control" particles. [13, 33] We chose not to because these particles have specific biologic effects including the effects on fetal development[13, 34, and 35]. From the model of Fedulov et al., we took the idea of suboptimal immunization of offspring[13]. The rationale was that standard immunization protocols produce vigorous inflammation, so effects of maternal DEP exposure may become masked. The selected protocol of suboptimal sensitization included a single injection of a low dose of OVA during the neonatal period, when antigen exposure elicits a weak immune response or even immune tolerance[36, 37]. Intranasal challenge with OVA or PBS was done on PND 23, 24 and 25. Mice were analyzed 72h after the final challenge.

Prenatal exposure to DEP reduces body weight

Exposures had no effect on litter size (5–8 pups from mothers in each group). At the age of 4 weeks, mice exposed prenatally to DEP and then challenged with PBS after birth (DEP-PBS mice) or OVA after birth (DEP-OVA mice) had significantly reduced body weight (12.49±0.34 and 12.27±0.29 g, respectively) compared to mice exposed to PBS prenatally and after birth (PBS-PBS mice) (13.74±0.37 g; $P<.01$ for both comparisons), but not to mice exposed to PBS prenatally and OVA after birth (PBS-OVA mice) (12.82±0.46 g; $P>.05$ for both comparisons). There was no significant difference in the body weight between DEP-OVA and DEP-PBS mice, and, between PBS-OVA and PBS-PBS mice. Thus, postnatal exposure to OVA did not have significant effect on the body weight.

In utero exposure to DEP facilitates induction of airway inflammation

DEP-OVA pups developed peribronchial inflammatory infiltrates (Fig 2, A and B). BALF from these mice had increased numbers of cells, including eosinophils, neutrophils, and lymphocytes, compared to BALF from pups of other groups (Fig 2, C–G). Airway inflammation was absent in PBS-PBS, PBS-OVA, and DEP-PBS mice.

Prenatal exposure to DEP facilitates development of airway hyperreactivity

Airway response to methacholine is generally weak in pups/young mice, compared with adult mice. Further, baseline airway resistance in pups is high. The baseline airway resistance values for PBS-PBS, PBS-OVA, DEP-PBS, and DEP-OVA mice were high and comparable (2.22±0.22, 2.15±0.14, 2.11±0.17, and 2.02±0.15 cm H_2O.s/ml, respectively; $P>.05$ for all comparisons). Nonetheless, DEP-OVA mice showed augmented airway resistance following exposure to methacholine (Fig 2, H). In addition, a less-pronounced but significant increase in airway resistance was observed in PBS-OVA mice. Airway hyperresponsiveness can occur in the absence of inflammation[38]. This hyperresponsiveness may be due to activation of airway cells such as mast cells. PBS-OVA mice had some OVA-specific IgE (discussed below; Fig 4), which might activate resident mast cells upon OVA challenge.

Prenatal exposure to DEP primes pulmonary T-helper (Th)2 and Th17-type responses

Lung tissues from DEP-OVA mice had higher levels of mRNAs encoding the Th2 cytokines IL4, IL5, and IL13; the Th17 cytokine IL17; and the pro-inflammatory cytokines IL6 and TNFα, compared with PBS-PBS and PBS-OVA mice (Fig 3, A and Fig E1, A–G in the

Online Repository). Levels of *Il4*, *Il5*, *Il13*, *Il17*, and *Tnfα* transcripts corresponded with levels of proteins in BALF (Fig 3, B). DEP-OVA mice had reduced expression of the Th1 cytokine IFNγ at the protein level (Fig 3, B). PBS-OVA mice did not have any significant increases in cytokine production, compared with PBS-PBS mice.

Prenatal exposure to DEP increases production of allergen-specific IgE

OVA-specific IgE was detected in serum of all mice immunized with OVA, regardless of prenatal exposure (Fig 4). Thus, our OVA exposure protocol is sufficient to induce OVA-specific IgE, but insufficient to produce pulmonary inflammation in the absence of the prenatal stimulus. Sera from DEP-OVA mice had significantly higher concentrations of the OVA-specific IgE than sera from all other groups of mice.

In utero exposure to DEP promotes production of cytokines by NK cells

The production of IL5, IL13 and IL17 was higher in splenocytes from DEP-OVA mice compared to mice from other studied groups (Fig 5, A–C). In DEP-OVA mice, the majority (>60%) of cytokine-positive cells were NK cells (Fig 5, D–F). In these pups, CD4+ T cells accounted for only 0.9%–1.5% of cytokine-positive cells (Fig 6, D–F). The percentages of NK cells expressing IL5, IL13 and IL17 were highest in DEP-OVA mice and equal to 11%, 16% and 9%, respectively (Fig 6, G–I). DEP-OVA mice had also highest percentage of NK cells expressing the activation marker CD69 (27%; Fig E2 in the Online Repository). Frequencies of NK cells in spleens of mice from studied treatment groups were similar (PBS-PBS mice, 2.65%±0.33%; PBS-OVA mice, 2.55%±0.42%; DEP-OVA mice, 2.49% ±0.39%).

Depletion of NK cells prevents development of airway inflammation

To study the role of NK cells in our model, OVA-immunized offspring of DEP-exposed mice were given injections of the NK cell-depleting antibody anti-NK1.1[39–41] (or the isotype control IgG) 72 hrs, 48 hrs, or 24 hrs before the first intranasal OVA challenge. The anti-NK1.1 antibody efficiently depleted NK cells. In spleens of mice injected with the anti-NK1.1 antibody, only 0.11%±0.03% of cells were NK cells (NKp46+CD3−), whereas in spleens of mice injected with the isotype control IgG, 2.53%±0.34% of cells were NKp46+CD3− ($P<.01$). NK cell depletion significantly reduced airway inflammation (Fig 6, A–G).

Prenatal exposure to DEP affects expression of AhR signature transcripts

From toxicological standpoint, PAH are important components of DEP[42, 43]. PAH cross the placenta[44]. Prenatal exposure to PAH increases the risk of allergic sensitization and wheezing in children [6–8]. PAH activate the transcriptional factor AhR[45]. Activated AhR regulates several components of immune response, including the production of IL17[46–48], which we found to be increased in DEP-OVA mice. We therefore investigated AhR-dependent transcription in mice exposed to DEP in utero. We measured levels of AhR signature transcripts, encoding: the AhR repressor (*AhRR*); cytochrome P450, family 1, subfamily A, polypeptide 1 (*Cyp1a1*); and cytochrome P450, family 1, subfamily B, polypeptide 1 (*Cyp1b1*)[45, 49]. We analyzed liver in addition to the lung because liver is an

important target for AhR ligands[50]. Levels of all 3 transcripts were increased in lungs of 4-week old DEP-PBS and DEP-OVA pups (Fig 7A and Fig E3, A–C in the Online Repository). These pups also demonstrated augmented levels of *AhRR* and *Cyp1b1* in livers (Fig 7, B and Fig E3, D and F in the Online Repository). Interestingly, PBS-OVA pups had increased levels of *Cyp1a1* mRNA in lung, compared with PBS-PBS mice (Fig 7, A and Fig E3, B in the Online Repository). This may be due to activation of the *Cyp1a1* promoter by non-AhR transcription factors in response to OVA exposure. In summary, prenatal exposure to DEP was associated with increased transcription from AhR-regulated genes in the lung and the liver. Interestingly, this result was obtained using 4 week old offspring, thus roughly 4 weeks after final maternal exposure to DEP.

Prenatal exposure to DEP results in upregulation of oxidative stress signature transcripts

PAH are metabolized via AhR-inducible CYP1A1 and CYP1B1, which leads to generation of reactive oxygen species (ROS)[51]. ROS are also produced upon exposure to DEP-derived quinones or allergens [52]. We measured levels of the ROS-sensitive transcripts heme oxygenase 1 (*Hmox1*) mRNA and nuclear factor (erythroid-derived 2)-like 2 (*Nrf2*) mRNA[52]. We observed moderate upregulation of *Hmox1* mRNA in lungs and livers of DEP-OVA pups, and, to a lesser extent, in lungs but not livers of PBS-OVA pups, compared with PBS-PBS mice (Fig 8 and Fig E4 in the Online Repository).

Discussion

We developed a new mouse model of maternal transmission of asthma susceptibility. In this model, suboptimal OVA immunization and challenge of pups born to DEP-exposed mice resulted in airway hyperresponsiveness and eosinophilic inflammation, with increased expression of Th2- and Th17-type and inflammatory cytokines. Features of asthma were either marginal or absent in OVA-treated pups of vehicle-exposed mice. Prenatal exposure to DEP therefore lowers the threshold for induction of asthma in response to allergens. The Th2- and Th17-type cytokines were mainly produced by NK cells; depletion of these cells blocked development of airway eosinophilic inflammation. Prenatal exposure to DEP upregulated expression of genes known to be controlled by AhR and oxidative stress; the upregulation persisted 1 month after birth, even though mice were no longer exposed to DEP.

We chose to expose prenatal mice to DEP because the link between DEP and asthma is well established[5–8, 21–31, 53–58]. Epidemiological studies associated exposure to diesel exhaust with development of new asthma and exacerbation of pre-existing asthma[5–8, 53–58]. The causality was determined in controlled exposure studies in humans and adult mice[21–31]. DEP exposure increased production of IL17, Th2-type cytokines, and specific IgEs; increased eosinophilic inflammation in the upper and lower respiratory tract; and increased vascular permeability and airway resistance[21–31, 59]. Although there is some understanding of how postnatal exposure to diesel exhaust triggers asthma, the mechanisms underlying asthma induction following in utero exposure have not been characterized. Through development of this mouse model, we hope to provide a tool for mechanistic studies.

Our model incorporated the most valuable elements of earlier models. Table EII in the Online Repository contains experimental details of these models. The field was pioneered by Fedulov et al.[13]. In their model, pregnant BALB/c mice were given a single intranasal dose of DEP on GD 14. Pups were immunized through a single intraperitoneal injection of low-dose OVA in alum during the neonatal period and challenged with aerosolized OVA when they were 2 weeks old. Asthma developed only in DEP-exposed pups. We followed this immunization strategy. The model by Auten et al. used C57BL/6 mice and combined repeated prenatal exposure to diesel exhaust (inhalation) or DEP (intranasal application) with chronic postnatal ozone exposure[14]. Prenatal exposures increased ozone-induced airway hyperreactivity.

A model created by Reiprich et al. used BALB/c mice and incorporated repeated prenatal co-exposure to DEP and lipopolysaccharide (LPS), postnatal exposure to LPS, and standard (optimal) postnatal immunization and challenge with OVA[15]. Mice were intraperitoneally immunized with an optimal dose of OVA in alum at 6 and 8 weeks of age, and then given intranasal challenge with OVA 14–16 days and 21–23 days after the initial immunization. This OVA treatment induced asthma in control pups (offspring of mice given injections of vehicle). The DEP inhibited LPS-mediated protection against asthma. As discussed earlier, we incorporated the repeated DEP exposure principle from protocols by Auten at al. and Reiprich et al., and, as described by Auten at al., utilized C57BL/6 mice.

The link between prenatal diesel exhaust exposure and asthma was examined in 2 additional studies that reached different conclusions[60, 61]. In the model of Sharkhuu et al., pregnant mice repeatedly inhaled diesel exhaust[60]. Their pups were immunized (PND 42 and 43) and challenged (PND 54, 55 and 56) with intranasal OVA. The authors reported no asthma-promoting effects of prenatal exposure to diesel exhaust. In the model of Corson et al., pregnant mice repeatedly inhaled diesel exhaust and received intranasal applications of *Aspergillus fumigatus* extract [61]. Their pups then received several doses of *Aspergillus* extract, beginning at 9–10 weeks of age. In this model, the combined prenatal exposure to diesel exhaust and *Aspergillus* extract protected against airway eosinophilia. The discrepancies in outcomes between positive (Auten et al., Fedulov et al., and Reiprich et al.) and negative (Sharkhuu et al. and Corson et al.) studies could result from differences in methods used for prenatal exposure (instillation of the DEP suspension vs. inhalation of diesel exhaust) and postnatal exposure (allergen vs. ozone; differences in dose, route, timing, and type of postnatal allergen).

Pathogenesis of asthma involves Th2- and Th17-type cytokines. In our model, these cytokines were produced mainly by NK cells. This is in contrast to conventional mouse models of asthma, in which CD4+ T cells are primary source of Th2- and Th17-type cytokines. NK cells are believed to secrete IFNγ, TNFα, granzymes, and perforins[62], to kill cells infected with viruses and tumor cells[62]. Less is understood about IL17+/Th2-type cytokine+ NK cells, although studies have reported that these cells emerge during development of specific disorders[63–68]. Th2-type cytokine+ NK cells have been detected in patients with atopic asthma, atopic dermatitis, parasitic infections, and autoimmune diseases[63–66]. IL17+ NK cells have been observed in mouse models of toxoplasmosis and

hepatic ischemia/reperfusion injury[67, 68], but there are no reports of their association with asthma or allergy.

Our data strongly indicated that NK cells were responsible for development of airway inflammation in our model. Antibody-mediated depletion of these cells before allergen challenge prevented airway inflammation. Antibody-based strategies for depletion of NK cells were previously tested in conventional models of asthma. Using these approaches, four research groups showed that NK cells positively contribute to the initiation allergic inflammation, [69–72] whereas one group reported that NK cells suppress the resolution of pulmonary inflammation.[73, 74] The studies might have reached different conclusions because different experimental conditions or different phases of allergic response are associated with activation of distinct NK cell subsets. Alternatively, NK cells dynamically change phenotype during allergic response. A third possibility involves deletion of asthma-relevant non-NK cell subsets by injected antibodies.

Two antibodies are commonly used to deplete NK cells in vivo: anti-NK1.1 and anti-asialo-GM1 [39–41, 73]. We chose anti-NK1.1 because it is more specific for NK cells. One limitation of this approach is that anti-NK1.1 also depletes NK T cells. However, the role of NK T cells in airway inflammation in our model is probably not significant; in DEP-OVA mice, these cells accounted for only 8%–12% of cytokine-producing cells.

The AhR may provide a molecular link between maternal exposure to diesel exhaust and asthma in offspring. This receptor is activated by PAH—major components of DEP that pass through the placenta[44]. PAH-bound AhR activates transcription of many genes, including those that regulate the immune response[45–48]. Many studies have shown that AhR induces transcription of the *Il17* gene in T cells and type-3 innate lymphoid cells[46–48]. Accordingly, DEP were shown to stimulate IL17 production by splenocytes[75]. The AhR is expressed in NK cells,[76, 77] and it is likely that AhR regulates *Il17* also in NK cells.

The effect of the AhR on Th2-type cytokines is less studied. AhR deficiency impairs production of IL5 and IL13 by splenocytes and mast cells, respectively[78, 79]. Conversely, the AhR ligand 6-formylindolo[3,2-b]carbazole increases production of IL13 by mast cells[80]. DEP-derived PAH induce IL13 production by peripheral blood mononuclear cells[81]. AhR-deficient CD4+ T cells increase secretion of IL4 upon stimulation through CD3 and CD28[82]. In contrast, other studies have shown that PAH synergize with concanavalin A and antigen to stimulate IL4 production by T cells and basophils, respectively[83, 84].

The AhR also regulates the synthesis of inflammatory mediators including IL6 and TNFα[85, 86]. These cytokines are also induced by ROS; upregulation of transcripts that characteristic of the oxidative stress response (*Hmox1* mRNA), was observed in our DEP-OVA mice. Therefore, inflammatory responses in these mice might be the result of the combined effect of AhR and ROS signaling.

The AhR- and ROS-associated transcriptional response in DEP-OVA mice was systemic, because it was observed in two different organs i.e. lung and liver. Additional evidence for the systemic effects of DEP included the lower body weight of DEP-PBS and DEP-OVA mice, compared with PBS-PBS mice. One likely scenario is that in fetuses exposed to DEP,

activation of AhR and ROS pathways led to systemic priming of pro-inflammatory responses and systemic NK cell activation that ultimately facilitated postnatal induction of asthma upon lung-targeted allergen challenge.

There is evidence that AhR mediates allergic inflammation. AhR expression is increased in peripheral blood mononuclear cells from patients with allergic asthma[87]. The putative AhR ligand, 4-nonylphenol, enhances allergic inflammation in the lung[88]. The inflammation is reduced in mice carrying the AhR^d allele, which is defective in ligand binding. Mice that express a constitutively active form of AhR in keratinocytes spontaneously develop skin lesions that resemble atopic dermatitis[89]. Repeated intranasal administration of PAH aggravates allergic rhinitis in the guinea pig model[90]. PAH also increase production of allergen-specific IgE[91, 92]. We recognize that certain AhR ligands may be anti-inflammatory. Immunotoxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) promotes development of T-regulatory cells, and thereby suppresses allergic sensitization[93, 94]. It is now believed that TCDD-induced toxicity results from inappropriate activation of AhR, leading to deregulated physiologic functions[95].

In summary, we have developed a model of asthma susceptibility through prenatal exposure of mice to DEP. We provided mechanistic insight into this process, delineating the role of NK cells in asthma development, along with activation of AhR- and oxidative stress-regulated genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AhR	aryl hydrocarbon receptor
AhRR	aryl hydrocarbon receptor repressor
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
Cyp1a1	cytochrome P450, family 1, subfamily A, polypeptide 1
Cyp1b1	cytochrome P450, family 1, subfamily B, polypeptide 1
DEP	diesel exhaust particles
GD	gestation day
Hmox1	heme oxygenase 1

IL	interleukin
IN	intranasal
IP	intraperitoneal
LPS	lipopolysaccharide
NK cells	natural killer cells
Nrf2	nuclear factor (erythroid-derived 2)-like 2
OVA	ovalbumin
PAH	polycyclic aromatic hydrocarbons
PBS	phosphate-buffered saline
PND	postnatal day
Th	T helper
ROS	reactive oxygen species
TNF	tumor necrosis factor

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Key Messages

- Mice exposed in utero to diesel particulate matter have increased susceptibility to asthma.
- Asthma in these mice is mediated by NK cells and is associated with enhanced transcription of genes known to be specifically regulated by AhR and oxidative stress.

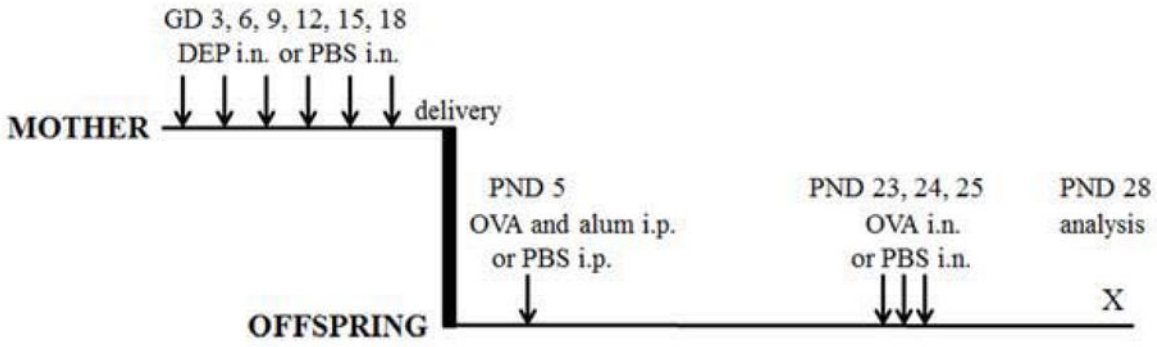

Figure 1. Experimental protocol

Timed pregnant C57BL/6 mice were given intranasal applications (IN) of either DEP or PBS on indicated gestation days (GD). Offspring were given intraperitoneal injections (IP) of PBS or a mixture of OVA and alum in PBS and then intranasal applications of OVA or PBS on indicated postnatal days (PND). These mice were then analyzed 3 days after the final IN application.

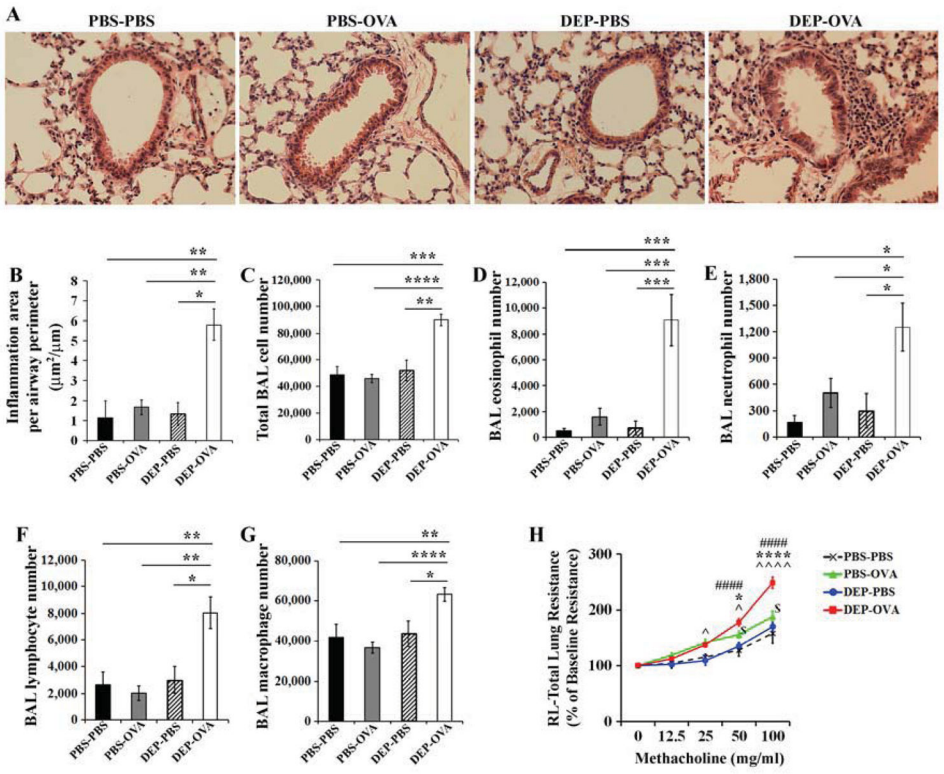

Figure 2. Airway inflammation and resistance

Experiments included PBS-PBS (prenatal and postnatal PBS), PBS-OVA (prenatal PBS, postnatal OVA), DEP-PBS (prenatal DEP, postnatal PBS), DEP-OVA (prenatal DEP, postnatal OVA) mice. A, B, peribronchial inflammation (histologic analysis), mean values (± standard error of the mean - SEM) from 3–6 mice/group; C–G, mean BAL cell counts (± SEM) from 10–21 mice/group; *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; H, total lung resistance, mean values (± SEM) from 9–18 mice/group; ####, DEP-OVA vs. PBS-PBS; */****, DEP-OVA vs. PBS-OVA; ^/^^^^, DEP-OVA vs. DEP-PBS; $, PBS-OVA vs. PBS-PBS.

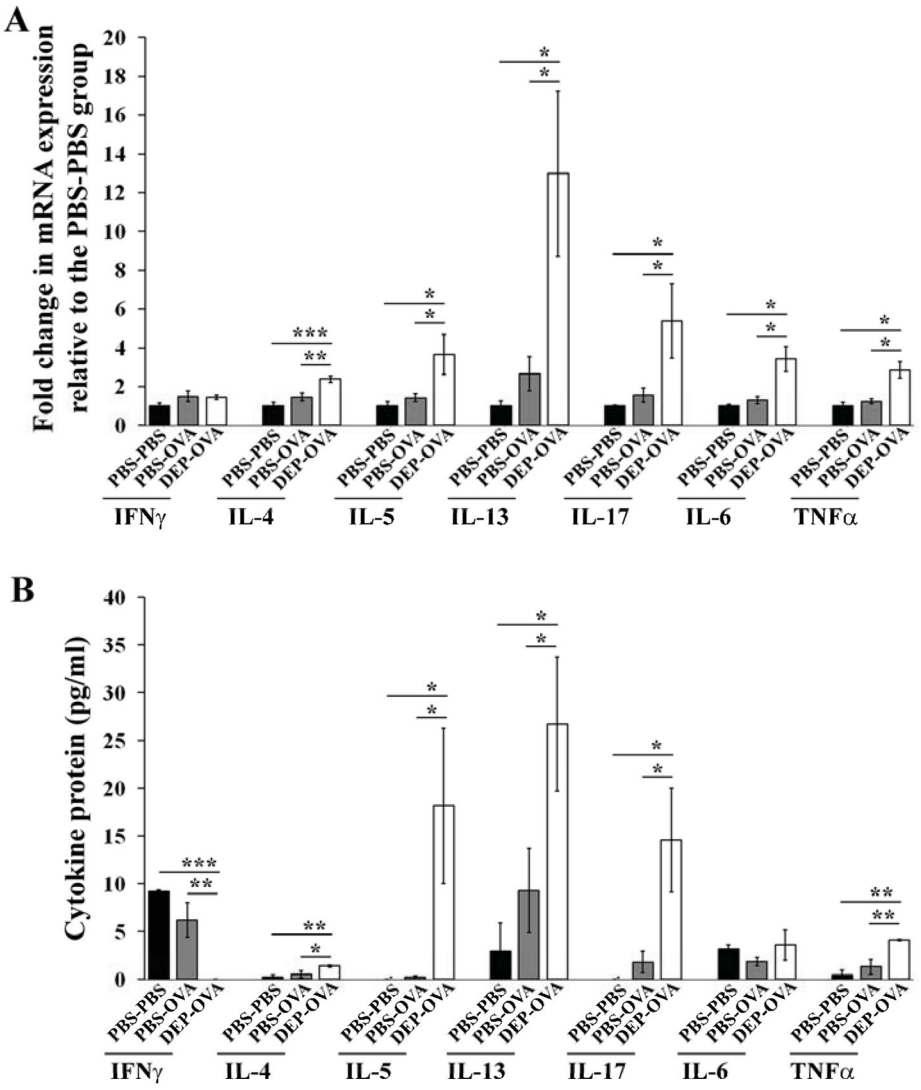

Figure 3. Expression of cytokines in lung

PBS-PBS, PBS-OVA, and DEP-OVA mice were analyzed for relative expression of cytokine transcripts in the lung tissue (A; mean values (± SEM) of 6–13 mice/group) and absolute level of cytokine proteins in BALF (B; mean values (± SEM) of 4–6 mice/group). Levels of each cytokine transcript were normalized to 18S rRNA and expressed as fold change in expression relative to the PBS-PBS group.

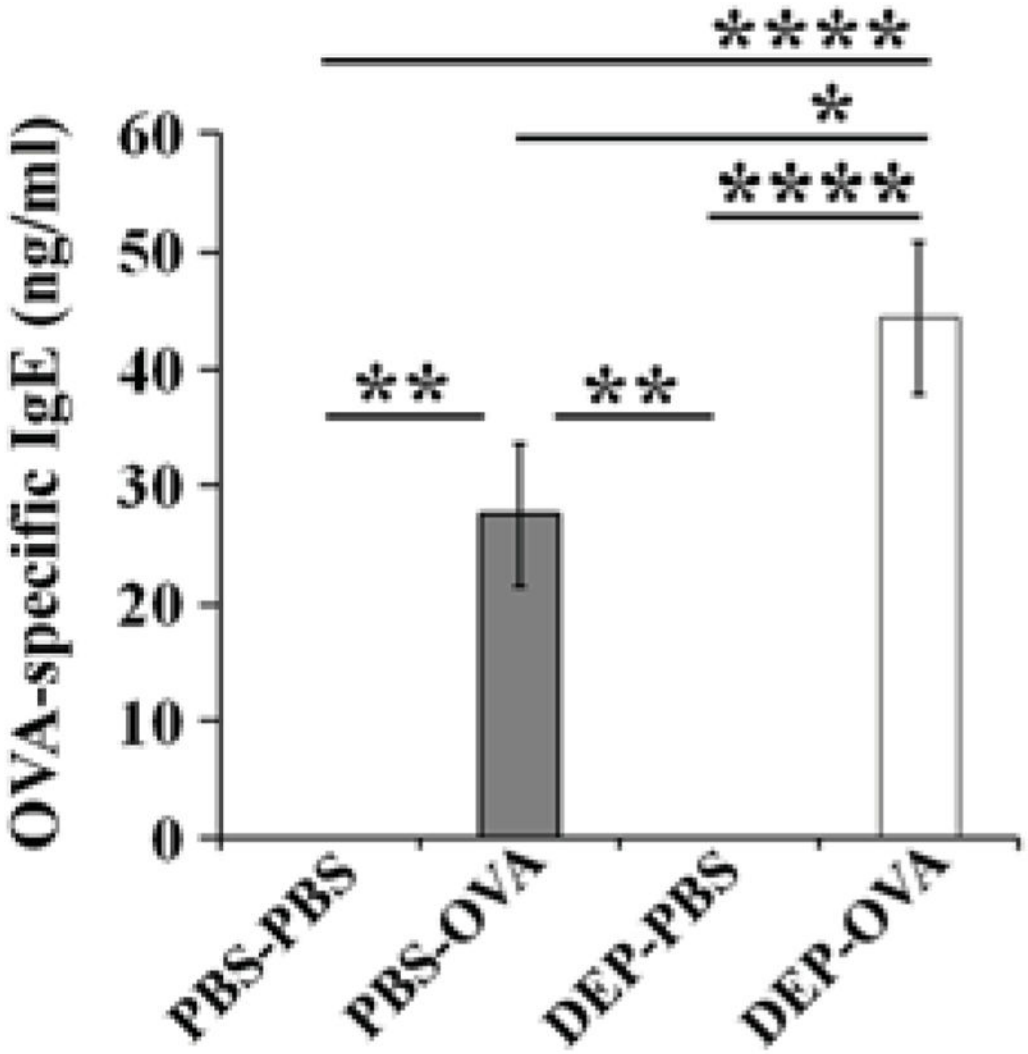

Figure 4. OVA-specific IgE in serum

Sera from PBS-PBS, PBS-OVA, DEP-PBS, and DEP-OVA mice were analyzed for concentration of OVA-specific IgE; mean values (± SEM) shown from 20–27 mice/group

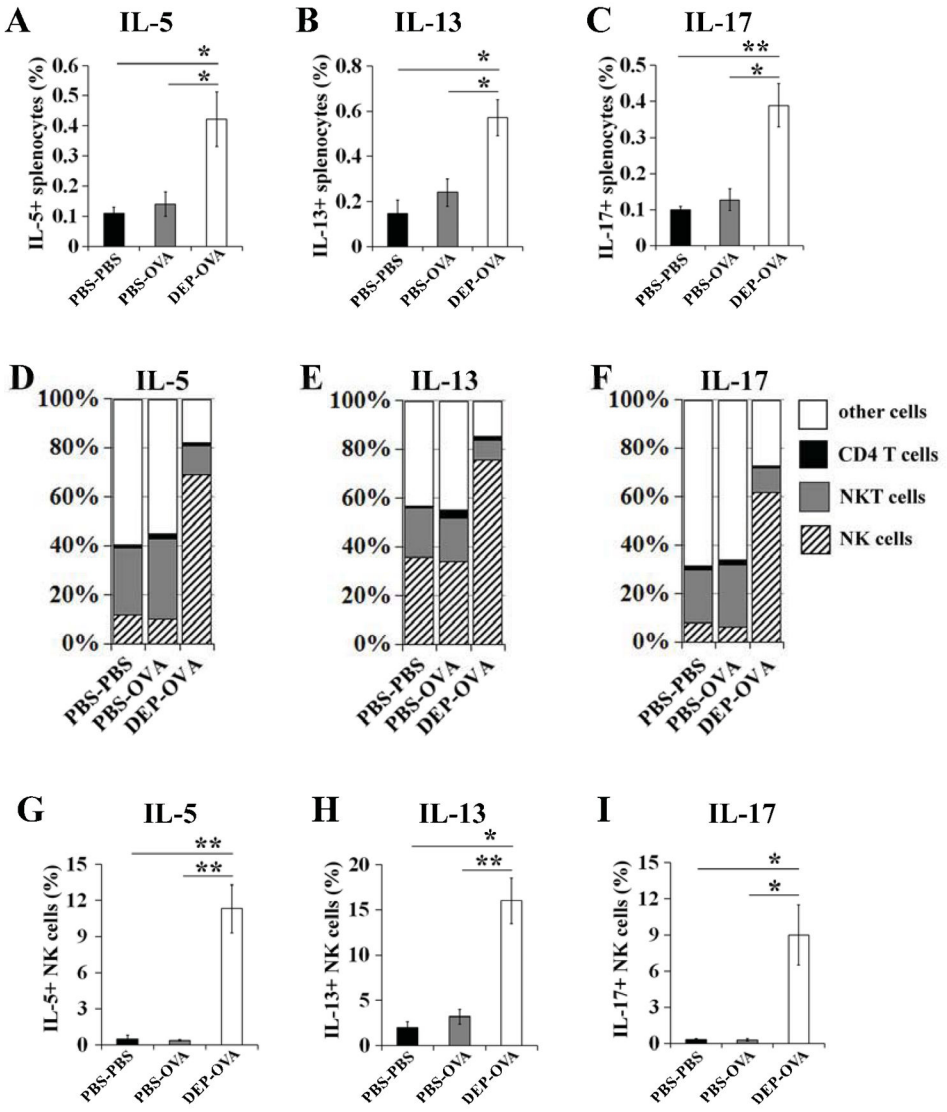

Figure 5. Cytokine expression in splenocyte populations

OVA-stimulated and immunostained splenocytes (CD3, CD4, NK1.1, NKp46 and a cytokine) from PBS-PBS, PBS-OVA, and DEP-OVA mice were analyzed by flow cytometry. CD4 T cells, NK and NKT cells were defined as CD3+CD4+NK1.1−NKp46−, CD3−NK1.1+NKp46+ and CD3+NK1.1+ cells, respectively. A–C, Cytokine+ splenocytes expressed as a percentage of all splenocytes. D–F, Percentages of cytokine+ cells attributable to individual cell populations. G–I, Cytokine+ NK cells expressed as a percentage of all NK cells; mean values (± SEM) shown from 6 mice/group

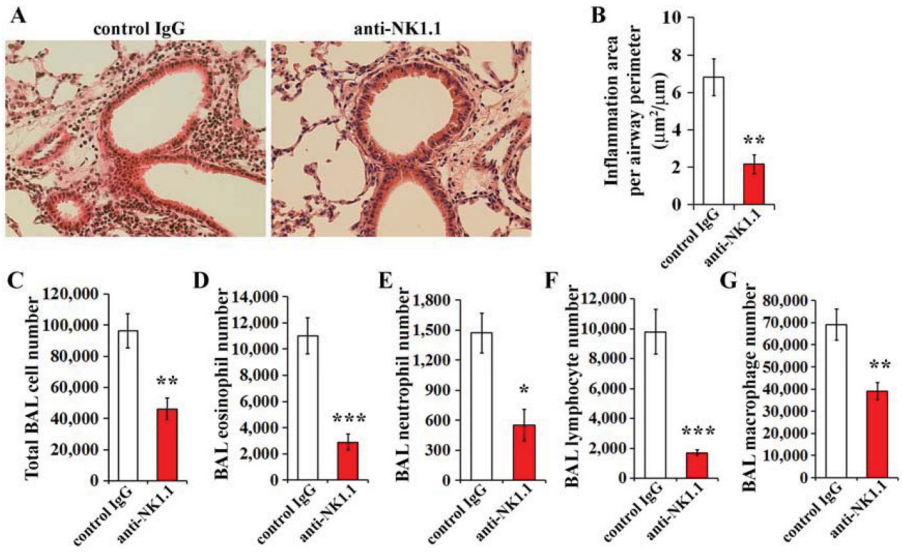

Figure 6. Effect of NK cell depletion

Pregnant mice were exposed to DEP and offspring were immunized and challenged with OVA (see Fig 1). Anti-NK1.1 or isotype control IgG were injected 72 hrs, 48 hrs, and 24 hrs before the first OVA challenge. A–B, Peribronchial inflammation (histologic analysis); C–G, BAL cell numbers; mean values (± SEM) shown from 8 mice/group

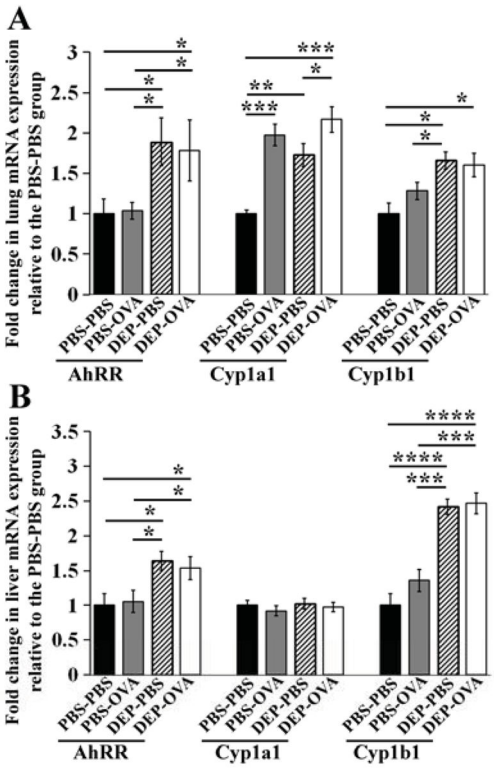

Figure 7. Expression of AhR signature transcripts

Lungs (A) and livers (B) from PBS-PBS, PBS-OVA, DEP-PBS, and DEP-OVA mice were analyzed for relative levels of *AhRR*, *Cyp1a1*, and *Cyp1b1* mRNA as in Fig 3; mean values (± SEM) shown from 6–13 mice/group

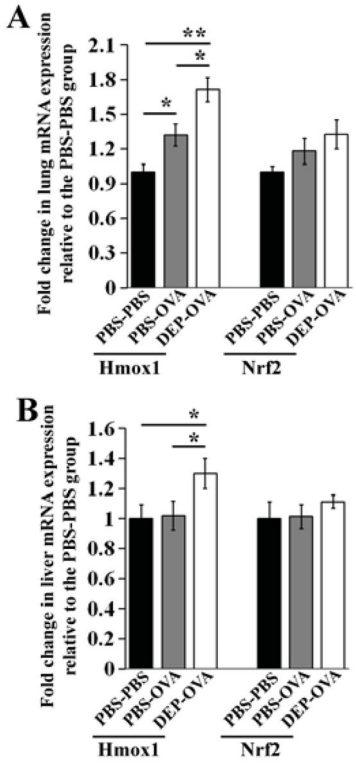

Figure 8. Levels of oxidative stress-regulated transcripts

Lungs (A) and livers (B) from PBS-PBS, PBS-OVA, and DEP-OVA mice were analyzed for relative levels of *Hmox1* and *Nrf2* mRNAs (as in Fig 3); mean values shown from 6–13 mice/group