

Letter to the Editor

A combination of dexamethasone and anti-IL-17A treatment can alleviate diesel exhaust particle-induced steroid insensitive asthma*To the Editor:*

A recent comprehensive and systematic review of worldwide traffic emissions and health science by a special panel convened by the Health Effects Institute found sufficient evidence that exposure to traffic-related air pollution (TRAP) causes asthma exacerbation in children.¹ Diesel exhaust particles (DEPs) represent the major component of TRAP particulate matter and the main contributor to TRAP-related asthma exacerbations in children. We have previously shown that in children with allergic asthma, TRAP exposure is associated with earlier sensitization, and increased asthma prevalence and severity.^{2,3} Asthma severity, defined as more frequent weekly symptoms, was associated with increased IL-17A but not IL-4, IL-5, or IL-13 blood levels.² Indeed, although asthma has long been described as a disease resulting from an abnormal T_H2 immune response to environmental allergens, accumulating evidence suggests a role for T_H17 cells, especially in severe asthma.⁴ A recent study demonstrated that dual-positive T_H2/T_H17 cells and IL-17A were present at a higher frequency in the bronchoalveolar lavage fluid (BALF) of patients with severe asthma.⁵ Furthermore, the study found that these T_H2/T_H17 cells were resistant to dexamethasone-induced cell death. We recently reported that DEP coexposure augmented allergen-induced airway hyperresponsiveness (AHR), eosinophilia, and T_H2 and T_H17 cytokines levels, and resulted in increased numbers of T_H2/T_H17 cells in the BALF.² Collectively, these data suggest that a subgroup of patients with asthma with high DEP exposure and mixed T_H2/T_H17 responses may benefit from anti-IL-17A therapy alone or in combination with steroids. Although inhibition of IL-17 receptor A did not result in significant improvement among subjects with moderate to severe asthma in a recent randomized controlled trial,⁶ targeted anti-IL-17 therapy in a subset of patients with severe asthma with high DEP exposure may be beneficial alone or in combination with steroids.

A significant proportion of patients with asthma remain symptomatic despite treatment with high-dose inhaled corticosteroids. This group has been defined as having severe asthma, refractory asthma, or treatment-resistant asthma. This group of patients comprises about 15% of patients with asthma but accounts for more than 50% of health care utilization related to this disease.⁷ Although it is evident that DEP exposure results in increased asthma severity in children and in mouse models,^{2,3} the impact of DEP exposure on steroid responsiveness in the context of asthma is largely unknown. Hence, our goal was to directly examine the impact of DEP exposure on the steroid sensitivity of allergen-induced asthma. Specifically, we exposed BALB/c mice intratracheally to saline, DEP (100 μ g), house dust mite (HDM) extract (Greer; 10 μ g), or HDM + DEP 9 times over a 3-week period as previously described.^{2,3} Once asthma was established, mice were treated with once-daily dexamethasone (2 mg/kg) or a vehicle control as outlined in Fig 1, A. This dose of dexamethasone was effective and resulted in weight loss, decreased spleen size, and decreased lung cells in all animals (see Fig E1, A and B, in this article's Online Repository at www.jacionline.org).

As we have previously shown,² coexposure to DEP significantly increased HDM-induced AHR compared with exposure to HDM alone (Fig 1, B). Dexamethasone treatment abrogated HDM-induced AHR. Interestingly, 1 mouse in this group did not respond. This mouse was treated with a 25% lower dexamethasone dose because of its small size, suggesting that perhaps there is a threshold dose that is required for optimal responsiveness. In contrast, 4 days of systemic steroid treatment only partially reduced AHR in mice coexposed to HDM + DEP (Fig 1, B). Dexamethasone abrogated cell recruitment, including eosinophils into the airways in HDM-exposed mice (Fig 1, C). HDM-exposed mice did not exhibit a significant neutrophilic recruitment into the airways, in contrast to DEP-exposed mice, which had a strong neutrophilic response. However, the neutrophilia remained unchanged following treatment with dexamethasone (Fig 1, C). Mice exposed to HDM + DEP mice had a mixed eosinophilic and neutrophilic response as we previously reported.² Dexamethasone treatment of coexposed mice resulted in a partial decrease in BALF eosinophil levels and no change in neutrophil levels, resulting in a shift toward predominant neutrophilic BALF inflammation following dexamethasone treatment (Fig 1, C).

Next, we examined the T-cell populations present in the lungs of mice with asthma following dexamethasone treatment. As mentioned above, dexamethasone decreased the total number of lung cells regardless of exposure (Fig E1, B). Exposure to HDM induced a modest increase in lung IL13⁺ T_H2 cells, which was completely abrogated following dexamethasone treatment (Fig E1, C). In contrast, HDM + DEP exposure resulted in a marked increase in lung IL13⁺CD4⁺ T_H2 cells, which was only partially abrogated by dexamethasone (Fig E1, C). Dexamethasone treatment decreased lung T-cell levels, but the decrease in T_H17 cells did not reach significance while the T_H2 cells were markedly decreased following dexamethasone treatment (Fig 1, D).

Although T_H17 cells and dual-positive T_H2/T_H17 cells are thought to be steroid resistant,^{4,8} 4 days of dexamethasone treatment did significantly decrease IL-13/IL-17A double producers in HDM + DEP coexposed mice (Fig 1, D). One potential explanation is that the treatment regimen we used was high and may have had a profound systemic impact. Indeed, dexamethasone-treated mice lost about 5% to 10% of their body weight and we observed a dramatic decrease in their spleen size and weight (Fig E1, A). Thus, the dexamethasone treatment regimen that we used appears to be more immunosuppressive than "comparable doses" of corticosteroid used in humans, as previously suggested.⁹ Even if dexamethasone did not directly impact T_H17 cell survival, it may affect the generation of cytokines important in their induction or maintenance. Indeed, dexamethasone decreased not only lung messenger RNA levels of IL4 but also T_H17 -related cytokine levels including IL-1 β , IL-6, and IL-17A in mice coexposed to HDM + DEP (see Fig E2 in this article's Online Repository at www.jacionline.org). Nevertheless, dexamethasone treatment only partially reduced the number of T_H17 and IL-13/IL-17A double-producing cells in the lungs of mice coexposed to HDM + DEP. Because we previously demonstrated a role for IL-17A in asthma exacerbations induced by HDM + DEP,² we investigated the impact of anti-IL-17A treatment alone or in combination with dexamethasone on AHR induced by HDM + DEP. Briefly, we delivered anti-IL-17A

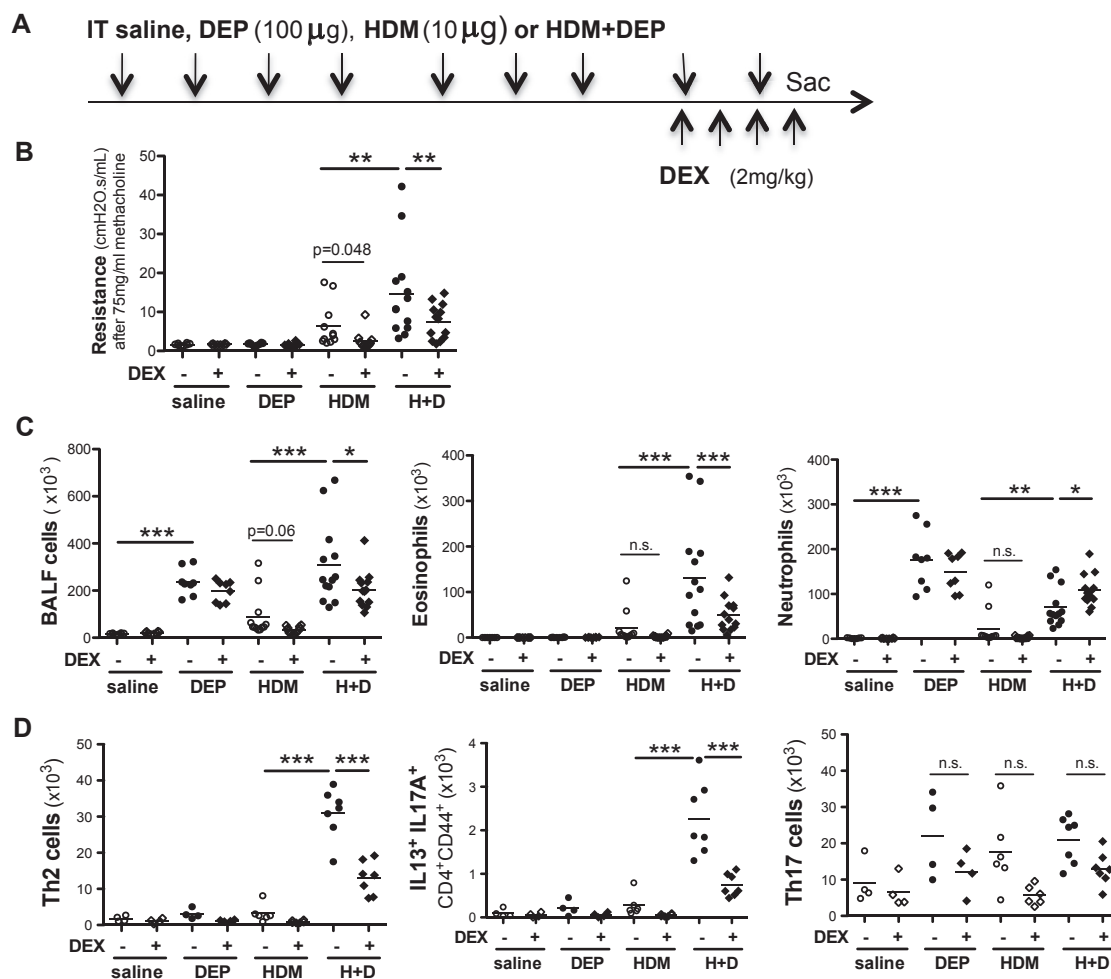


FIG 1. Impact of steroid treatments on HDM + DEP-induced asthma exacerbations. **A**, Protocol (it, intratracheal; ip, intraperitoneal). **B**, Airway resistance to methacholine challenges was assessed using FlexiVent ($n = 8-13$ mice/group from 2 separate experiments). **C**, Total BALF cell numbers and BALF eosinophil and neutrophil counts. **D**, Lung Th2 and Th17 cells were identified by their expression of CD3, CD4, CD44, and IL-13 or IL-17A, respectively, following acquisition on a FACS Canto III (Becton Dickinson, Mountain View, Calif) and analyzed with FlowJo software (Tree Star, Ashland, Ore). $n = 4-7$ mice/group; *n.s.*, Not significant. * $P < .05$, ** $P < .01$, *** $P < .001$, 1-way ANOVA with Bonferroni multiple comparison test.

(200 µg) or an IgG₁ control antibody intratracheally as depicted in Fig 2, A. In contrast to our previous published work where prophylactic treatment with similar doses of anti-IL-17A significantly alleviated HDM + DEP-induced AHR exacerbations,² blocking IL-17A once disease is established failed to significantly decrease AHR (Fig 2, B). Dexamethasone treatment alone had only a modest impact on AHR (Fig 2, B). Anti-IL-17A treatment, in combination with dexamethasone, significantly reduced AHR compared with either treatment alone (Fig 2, B), supporting the notion that IL-17A contributes to steroid resistance. Dexamethasone treatment decreased BALF eosinophilia and promoted BALF neutrophilia as expected (Fig 2, C). Similar to our previous observation,² anti-IL-17A treatment did not alter BALF neutrophil levels (Fig 2, C), most likely because DEP can directly stimulate epithelial cells to secrete neutrophil chemokines.

Although the combination treatment with dexamethasone and anti-IL-17A was effective, the combination did not fully abrogate AHR in mice exposed to HDM + DEP; thus, we increased the total dose and duration of dexamethasone treatment as indicated

in Fig 2, D. Increasing the magnitude of the dexamethasone treatment significantly but incompletely reduced AHR compared with the case of untreated mice exposed to HDM + DEP (Fig 2, E). Adding anti-IL-17A treatment to the enhanced dexamethasone regimen resulted in complete abrogation of AHR in all mice (Fig 2, E). Although these results suggest that increasing steroid dosage and/or duration of treatment can reduce DEP-induced asthma exacerbations, hard-to-treat patients respond poorly to high doses of steroids and more aggressive steroid doses would be associated with unacceptable side effects. Thus, patients with allergic asthma who are exposed to high levels of DEP may benefit from combination treatment with inhaled corticosteroids and anti-IL-17A.

Recently, a randomized double-blind placebo-controlled study of an anti-IL-17 receptor mAb (brodalumab) demonstrated positive results only in a subgroup of patients with moderate to severe asthma,⁶ underscoring the need to identify specific asthma endotypes that would benefit from blocking the IL-17 pathway and/or DEP-associated neutrophilia. Our data suggest that

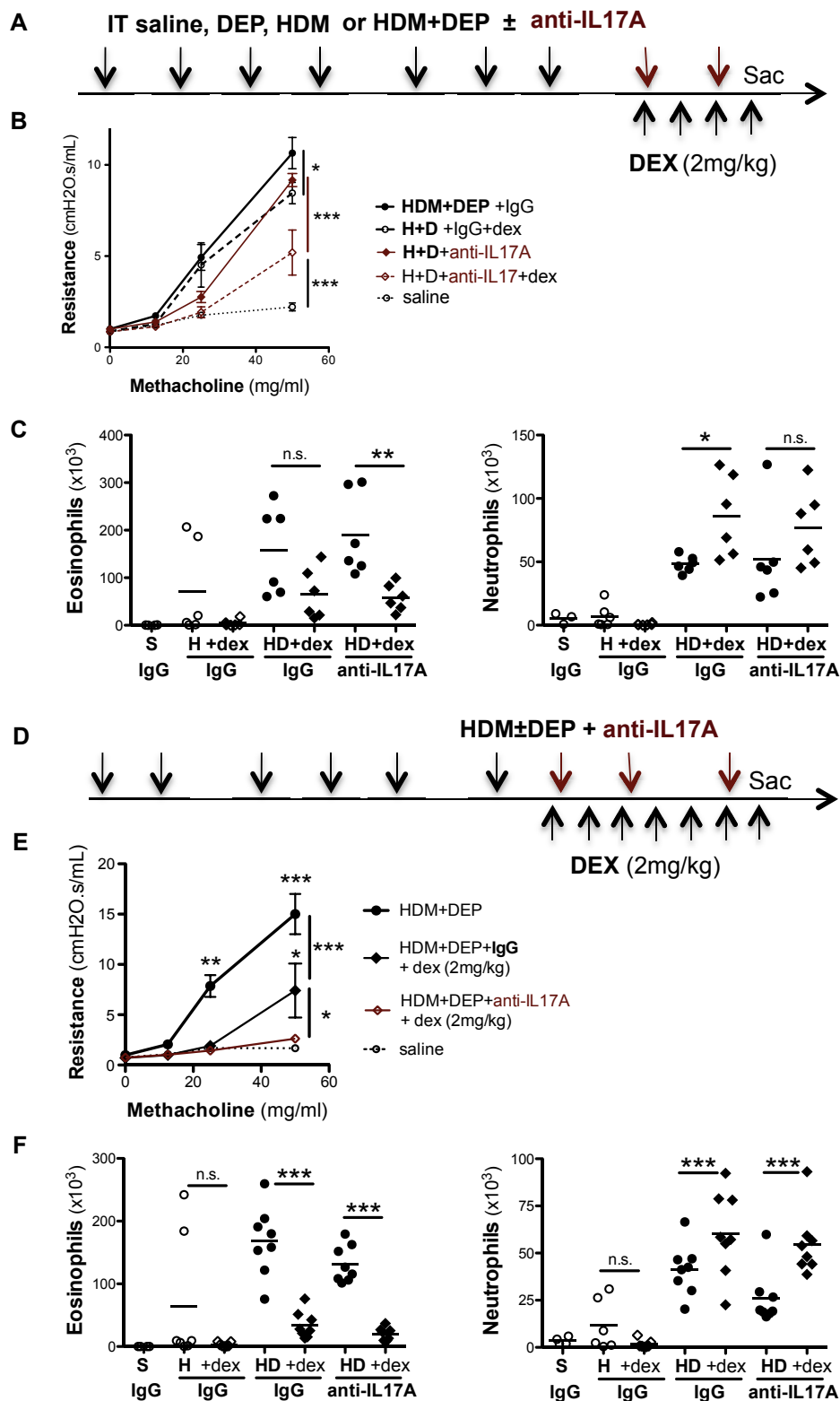


FIG 2. Combination treatment abrogates HDM + DEP-induced AHR. **A**, Protocol: 4 days of treatment. **B**, Airway resistance following treatment with dexamethasone (2 mg/kg) and/or anti-IL-17A (200 μ g) or an IgG₁ control antibody ($n = 3-6$ mice/group). **C**, BALF eosinophil and neutrophil counts. **D**, Protocol: 1 week of treatment. Airway resistance (**E**) and BALF eosinophil and neutrophil counts (**F**). *H*, HDM; *HD*, HDM + DEP; *n.s.*, not significant; *S*, saline. $n = 4-8$ mice/group; 2-way ANOVA, $*P < .05$, $**P < .01$, $***P < .001$.

patients with exposure to high levels of TRAP/DEP characterized by elevated levels of serum IL-17A and/or increased numbers of T_H2/T_H17 double producers may represent this treatment-responding endotype.

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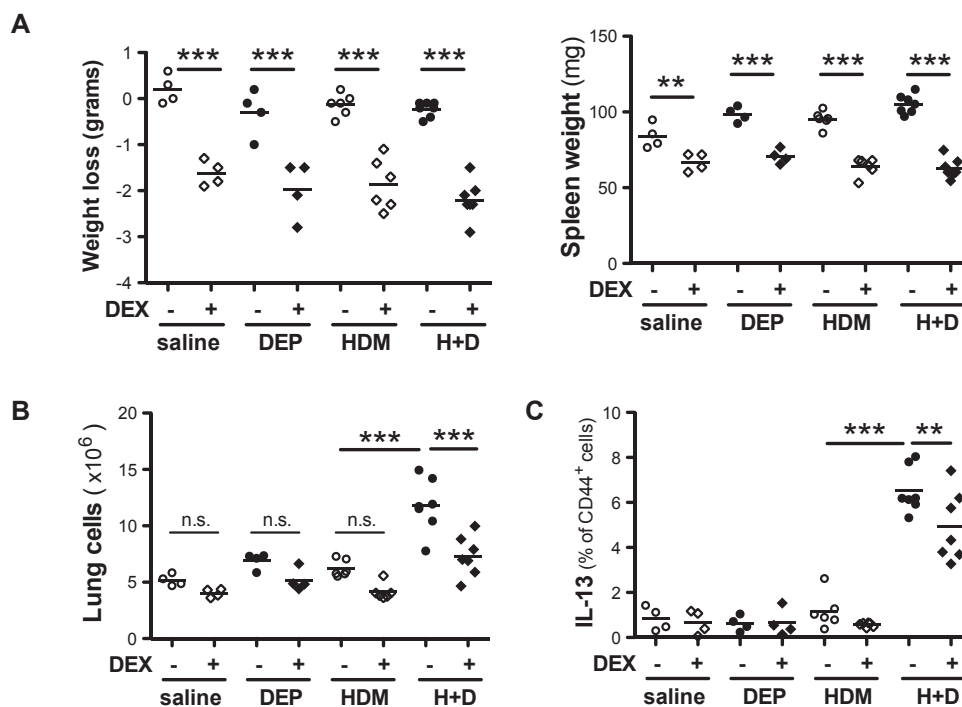


FIG E1. Immunosuppressive action of dexamethasone. **A**, Body weight loss and spleen weight loss. **B**, Total cells counts isolated from 2 right lung lobes following dexamethasone treatment. **C**, Percentage of IL-13⁺ cells among CD4⁺CD44⁺ lung effector T cells. *n.s.*, Not significant. ***P* < .01, ****P* < .001, 1-way ANOVA with Bonferroni multiple comparison test.

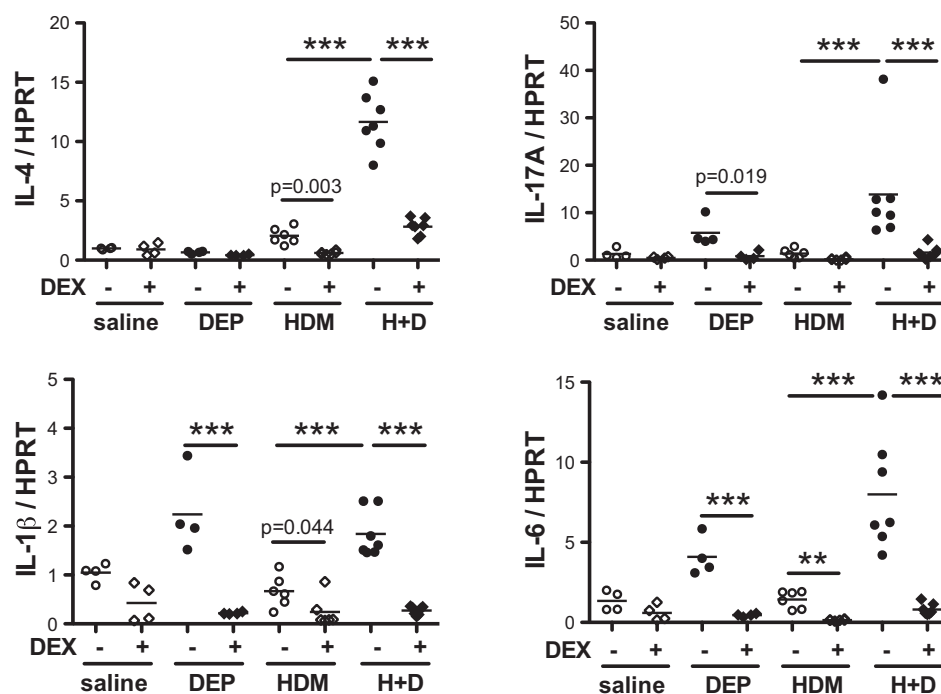


FIG E2. Dexamethasone decreased pro- T_H2 and T_H17 cytokines. IL-4, IL-17A, IL-1 β , and IL-6 mRNA levels were assessed by quantitative PCR. *P* values determined by *t* test or 1-way ANOVA with Bonferroni multiple comparison test (***P* < .01, ****P* < .001).