Ambient air pollution impairs regulatory T-cell function in asthma

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Background: Asthma is the most frequent chronic disease in children, and children are at high risk for adverse health consequences associated with ambient air pollution (AAP) exposure. Regulatory T (Treg) cells are suppressors of immune responses involved in asthma pathogenesis. Treg-cell impairment is associated with increased DNA methylation of Forkhead box transcription factor 3 (Foxp3), a key transcription factor in Treg-cell activity. Because AAP exposure can induce epigenetic changes, we hypothesized that Treg-cell function would be impaired by AAP, allowing amplification of an inflammatory response.

Objectives: To assess whether exposure to AAP led to hypermethylation of the Foxp3 gene, causing impaired Treg-cell suppression and worsened asthma symptom scores.

Methods: Children with and without asthma from Fresno, Calif (high pollution, Fresno Asthma Group [FA], n = 71, and Fresno Non Asthmatic Group, n = 30, respectively), and from Stanford, Calif (low pollution, Stanford Asthma Group, n = 40, and Stanford Non Asthmatic Group, n = 40), were enrolled in a cross-sectional study. Peripheral blood Treg cells were used in functional and epigenetic studies. Asthma outcomes were assessed by Global Initiative in Asthma score.

Results: Fresno Asthma Group Treg-cell suppression was impaired and FA Treg-cell chemotaxis were reduced compared with other groups ($P \leq .05$). Treg-cell dysfunction was associated with more pronounced decreases in asthma Global Initiative in Asthma score in FA versus the Stanford Asthma Group. Foxp3 was decreased in FA compared with the Fresno Non Asthmatic Group ($P \leq .05$). FA also contained significantly higher levels of methylation at the Foxp3 locus ($P \leq .05$).

Conclusion: Increased exposure to AAP is associated with hypermethylation of the Foxp3 locus, impairing Treg-cell function and increasing asthma morbidity. AAP could play a role in mediating epigenetic changes in Treg cells, which may worsen asthma by an immune mechanism. (J Allergy Clin Immunol 2010;126:845-52.)

Key words: *Ambient air pollution, asthma, immune system, regulatory T cell, Treg, epigenetics*

Asthma, which is characterized by reversible airway obstruction and inflammation, is the most frequent chronic disease in children. Children with asthma have an increased risk of exacerbations when exposed to higher concentrations of ambient air pollutants,[1-3] and some recent studies suggest that ambient air pollutants, especially those from traffic sources, may increase the risk for new onset of asthma.[4,5] Many children with asthma are atopic—that is, they are allergically sensitized to aeroallergens—on the basis of symptoms and/or results of allergen challenge or detection of allergen-specific IgE.[6,7] Ambient air pollutants have been shown to enhance allergic responses through IgE-mediated pathways. *In vitro* studies by Diaz-Sanchez and colleagues[8-12] have shown that diesel exhaust particles (DEP) enhance IgE production that is mediated through increases in isotype-specific IgE mRNA. These responses can be reproduced with phenanthrene, a polycyclic aromatic hydrocarbon (PAH).[13] In mice, increases in IgE after exposure to DEP are associated with increased IL-4, a signature cytokine for $CD4^+$ T_H2-type responses that promote allergies and asthma, in part by increasing allergen-specific IgE production.[14] In addition, controlled nasal exposure of atopic human beings to DEP enhances responses to allergens.[15]

There is growing evidence that regulatory T (Treg) cells play an essential role in inhibiting the proximal pathways of allergic sensitization and IgE production in response to allergen exposure.[16] Treg numbers are reduced in the bronchoalveolar lavage fluid of subjects with asthma,[7,17] and this is accompanied by reduced circulating numbers of Treg cells[18] and impaired chemotaxis of Treg cells to lung epithelial cells.[19] Consequently, impaired function and decreased presence of Treg cells in the lung can lead to worsening asthma pathology.

Forkhead box transcription factor 3 (Foxp3) is important in Treg-cell development and function. Methylation of cytosine-phosphate guanine residues in transcriptional regulatory regions of the Foxp3 gene represses the Foxp3 expression and ultimately Treg-cell function.[20] In contrast, complete demethylation of these transcriptional regulatory regions is associated with and appears to be required for stable Foxp3 expression in Treg cells.[20-25] Thus, it is plausible that environmentally induced increases in methylation at the Foxp3 locus could lead to

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Abbreviations used

CARB: California Air Resources Board
$CD4^+$ T cell: Effector T-cell ($CD4^+CD25^{lo}CD127^{hi}$)
DEP: Diesel exhaust particles
FACES: Fresno Asthma Children's Environment Study
FA: Fresno asthma group
FNA: Fresno nonasthmatic group
Foxp3: Forkhead box transcription factor 3
GINA: Global Initiative for Asthma
HBEC: Human bronchial epithelial cells
PAH: Polycyclic aromatic hydrocarbon
$PM_{2.5}$: Particulate matter ≤2.5 μm with aerodynamic diameter
pp: Percent predicted
SA: Stanford asthma group
SNA: Stanford nonasthmatic group
Teff: Effector T
Treg: Regulatory T ($CD4^+CD25^{hi}CD127^{lo}$)

lower levels of Foxp3 expression and to decreases in Treg-cell function.

Epigenetic modulation by ambient air pollution may play a role in regulating the expression of genes important in the pathogenesis of inflammation in asthma.[26,27] Ambient air pollutants can affect DNA methylation and result in changes in chromatin structure.[28] Air pollutants also have been shown to initiate transformation of T_H1 to T_H2 cells,[26,27] leading to the proatopic cascade of T_H2 cytokines (eg, IL-4, IL-13) found in the bronchoalveolar lavage, sputum, and blood of patients with asthma.[26-28] Recent work has shown that transplacental exposure to airborne PAHs increases DNA methylation of 5' CpG islands of several genes.[13] Liu et al[14] demonstrated that altered methylation in genomic DNA correlated with changes in IgE levels. Baccarelli et al[27] reported changes in the status of overall DNA methylation among adults in association with exposure to black carbon, often an indicator of DEP from traffic sources.

Fresno is located at the southern end of California's Central Valley and is one of the fastest-growing areas of California.[29,30] During the years 2005 to 2007, the population of Fresno was exposed to annual average particulate matter ≤2.5 μm ($PM_{2.5}$) concentrations that exceeded the federal annual standard by over 40%.[1,29,31] The occurrence of asthma is also very high in Fresno. The 2005 lifetime prevalence of asthma in children 5 to 17 years in Fresno County was 34% (95% CI, 25-44) compared with 18% (95% CI, 17-19) for the state of California.[1,29]

Given these observations, we hypothesized that ambient air pollution exposure might worsen asthma in children by inducing epigenetic changes that would impair Treg-cell number and/or function. To test this, we examined the effect of ambient air pollutants on Treg-cell function in children with and without asthma and determined whether there were associations between methylation of relevant genes and any associations with health outcomes. Specifically, we hypothesized that (1) exposure to ambient air pollutants leads to impairment of Treg-cell function; (2) attenuation of Treg-cell function is associated with decreased lung function and increased asthma severity classification in children with asthma; and (3) hypermethylation of the Foxp3 gene, a gene important in Treg-cell development and maturation,[20] is associated with decreased gene product and suppression of Treg-cell function.

METHODS

All methods and procedures were approved by the University of California, Berkeley, and Stanford University Committees for the Protection of Human Subjects.

Subjects

We studied 4 groups. Children with and without asthma from Fresno, Calif (high pollution; FA, n = 71, and FNA, n = 30, respectively) and from Stanford, Calif (low pollution; SA, n = 40, and SNA, n = 40) were enrolled as a cross-sectional study. The first group of subjects (FA) consisted of children with asthma who have been followed for up to 8 years as part of the Fresno Asthmatic Children's Environment Study (FACES, R01 HL081521). Subjects were children whose residence was within 20 km of the California Air Resources Board (CARB) compliance monitoring site in Fresno. At baseline, subjects answered a detailed respiratory health and general history questionnaire and underwent prebronchodilator and postbronchodilator spirometry and skin prick testing with 14 aeroallergens antigens common in the Fresno area. The second group consisted of 30 additional subjects from Fresno without asthma (FNA). A third and a fourth group of children were recruited from the Stanford Lucile Packard Children's Hospital as follows: 40 children with asthma based on Global Initiative for Asthma (GINA) guidelines (http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.pdf; SA, third group) and 40 children without asthma (SNA, fourth group). The SA and SNA subjects had resided in the immediate vicinity of Palo Alto, Calif, for at least 8 years, and all their residences were >800 m from a major highway, which minimized exposure to traffic-related pollution.

Subjects from the second through fourth groups were frequency-matched by age and sex to the FA subjects. Subjects were excluded if they had been taking oral immunosuppressives within 5 days of the blood draw, had a chronic disease, and/or had acute infection. Subjects without asthma were defined by (1) normal pulmonary function tests, (2) lack of historical diagnosis of asthma, (3) total IgE (Immunocap, Phadia, Sweden) of <10 IU/mL, and (4) negative skin test results (for the same 14 aeroallergens tested in FA). For subjects with asthma, we used the updated GINA classification scheme for asthma severity (2007), which includes symptoms and lung function components and recognizes that treatment with medication can modify these components.

Exposure to air pollutants

Hourly, quality-assured, ambient air quality and meteorologic data collected at the First Street monitoring station in Fresno (for Fresno cohort) and in Redwood City, Calif (for Stanford cohort) were obtained from CARB. Subject-specific estimates of annual average PAH exposures were based on land use regression analyses that used 497 PAH measurements at the homes of a subset of the FACES cohort (n = 83). Details are presented in this article's Methods section in the Online Repository at www.jacionline.org.

Collection and processing of blood specimens

We obtained up to 25 mL whole blood from each subject, and assays were performed by using PBMCs and purified cell subsets. Treg cells ($CD4^+CD25^{hi}CD127^{lo}$) and effector T (Teff) cells ($CD4^+CD25^{lo}$ or ^{neg}) were purified by flow cytometry after staining of PBMCs with fluorochrome-conjugated mAbs. Treg and Teff cells were then used in standard ^3H thymidine proliferation assays, flow cytometry, quantitative PCR, and chemotaxis assays according to published methods.[18,19] Details are provided in the Online Repository in the Methods section.

Statistical analysis

Between-group means were compared with nonparametric Kruskal-Wallis ANOVA and pairwise posttest comparisons via the Dunn multiple comparison test (Graph Pad Prism Software 5.0; Prism Software, La Jolla, Calif). Linear regressions were fit with intercepts for comparisons between lung function and Treg-cell function and for the comparison between average exposure to PAHs and number of methylated CpG islands. All other regressions were fit