Inhalation of diesel exhaust and allergen alters human bronchial epithelium DNA methylation

Rachel L. Clifford, PhD,[a]* Meaghan J. Jones, PhD,[a] Julia L. MacIsaac, PhD,[a] Lisa M. McEwen, BSc,[a] Sarah J. Goodman, BSc,[a] Sara Mostafavi, PhD,[a,b,c] Michael S. Kobor, PhD,[a,c,d]‡ and Chris Carlsten, MD[e]‡

Vancouver, British Columbia, and Toronto, Ontario, Canada

Background: Allergic disease affects 30% to 40% of the world's population, and its development is determined by the interplay between environmental and inherited factors. Air pollution, primarily consisting of diesel exhaust emissions, has increased at a similar rate to allergic disease. Exposure to diesel exhaust may play a role in the development and progression of allergic disease, in particular allergic respiratory disease. One potential mechanism underlying the connection between air pollution and increased allergic disease incidence is DNA methylation, an epigenetic process with the capacity to integrate gene-environment interactions.
Objective: We sought to investigate the effect of allergen and diesel exhaust exposure on bronchial epithelial DNA methylation.
Methods: We performed a randomized crossover-controlled exposure study to allergen and diesel exhaust in humans, and measured single-site (CpG) resolution global DNA methylation in bronchial epithelial cells.
Results: Exposure to allergen alone, diesel exhaust alone, or allergen and diesel exhaust together (coexposure) led to significant changes in 7 CpG sites at 48 hours. However, when the same lung was exposed to allergen and diesel exhaust but separated by approximately 4 weeks, significant changes in more than 500 sites were observed. Furthermore, sites of differential methylation differed depending on which exposure was experienced first. Functional analysis of differentially methylated CpG sites found genes involved in transcription factor activity, protein metabolism, cell adhesion, and vascular development, among others.
Conclusions: These findings suggest that specific exposures can prime the lung for changes in DNA methylation induced by a subsequent insult. (J Allergy Clin Immunol 2017;139:112-21.)

Key words: *Allergen, particulate matter, diesel exhaust, air pollution, epigenetics, DNA methylation*

Allergic disease is increasing worldwide with approximately 30% to 40% of the world's population now affected.[1] The development of allergic disease is determined by an interaction between genetic and environmental factors.[2] Air pollution is one of the major environmental factors impacting the development and exacerbation of allergic diseases, in particular respiratory diseases including asthma and allergic rhinitis.[3]

Diesel exhaust (DE) contributes the majority of particulate matter present in urban air pollution,[4] and diesel exhaust particles (DEPs) reach the alveolar/gas exchange regions.[5] DE increases bronchial inflammation and airways resistance in healthy subjects, whereas short-term exposure to diesel traffic reduces airway function in patients with asthma.[6] Exposure to DE plays a major role in the development and progression of allergies.[4,5] For example, high DEP exposure is associated with more frequent asthma symptoms in children with allergic asthma.[7] Interestingly, coexposure to allergen and DEPs increases allergen specific IgE, asthma severity, airway inflammation, and airway responsiveness in humans or in mouse models.[7-9]

The molecular mechanisms responsible for the synergy between DE and allergen remain unclear. As mutations occur too slowly to explain the recent rapid increase in allergy-associated disease, epigenetics is a potential mechanism by which gene-environment interactions may rapidly influence disease incidence and progression.[10] DNA methylation was among the first epigenetic mechanisms to be identified[11] and is the most extensively studied in relationship to disease[12] and human populations.[13] It is the potentially reversible addition of a methyl group to DNA cytosine residues, primarily where a cytosine is followed directly by a guanine (CpG sites).[14] Consistent with epigenetics serving as a potential mediator between environment and genome, differential DNA methylation of a number of genes involved in multiple cellular processes including immune responses,[15-17] nitric oxide synthesis,[18,19] and DNA binding[16] has been associated with air pollution exposure. We have previously shown that controlled exposure to DE alters the methylation of CpG sites in circulating mononuclear cells.[20]

From [a]the Centre for Molecular Medicine and Therapeutics, Child and Family Research Institute, University of British Columbia, Vancouver; [b]the Department of Statistics, University of British Columbia, Vancouver; [c]the Canadian Institute for Advanced Research, Toronto, Ontario; [d]Human Early Learning Partnership, School of Population and Public Health, University of British Columbia, Vancouver; and [d]the Air Pollution Exposure Laboratory, Chan-Yeung Centre for Occupational and Environmental Lung Disease, Division of Respiratory Medicine, Department of Medicine, University of British Columbia, Vancouver.

*Rachel L. Clifford, PhD, is currently affiliated with the Department of Respiratory Medicine and Nottingham Respiratory Research Unit, University of Nottingham, Nottingham, United Kingdom.

‡These authors contributed equally to this work as senior authors.

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Corresponding author: Chris Carlsten, MD, 2775 Laurel St, 7th Fl, Vancouver, British Columbia, Canada V5Z 1M9. E-mail: carlsten@mail.ubc.ca.

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Abbreviations used
BEC: Bronchial epithelial cell
DE: Diesel exhaust
DEA: Diesel exhaust and allergen
DEP: Diesel exhaust particle
DES: Diesel exhaust and saline
FA: Filtered air
FAA: Filtered air and allergen
FAS: Filtered air and saline
TSS: Transcription start site

Similarly, exposure to ambient pollution is associated with increased DNA methylation and decreased expression of the forkhead box protein 3 and IFN-γ genes in regulatory and effector T cells, respectively, leading to impaired regulatory T-cell function and associated increased asthma morbidity.[15,21]

Studies of mechanisms underlying the interaction between allergy and DE exposure have been limited to animals or performed as observational studies (rather than controlled exposure studies), investigation of candidate targets rather than global alterations, or of easily accessible tissues such as blood[20] rather than sampling the primary site of exposure, the lung. Here, we tested the hypothesis that exposure to allergen and/or DE would alter DNA methylation in bronchial epithelial cells (BECs) by performing a randomized crossover-controlled exposure study to allergen and DE in humans. We collected BECs, the primary cell type exposed to inhalants, and assessed changes in global DNA methylation in response to exposure. We report that exposure to allergen, DE, or allergen and DE as a coexposure had modest effects on DNA methylation when assessed at 48 hours postexposure. However, exposure to both allergen and DE, with the exposures separated by 4 weeks, was strikingly different, significantly altering bronchial epithelial cell global DNA methylation. These results support the hypothesis that DNA methylation can serve as a molecular mechanism underlying the interaction between allergens and particulate air pollution on respiratory health.

METHODS

Study demographics

Seventeen white participants (see Tables I and II) were recruited to the Air Pollution Exposure Laboratory in Vancouver, British Columbia, Canada. Written consent was obtained from all subjects, and the protocols were approved by the institutional review board for human studies at the University of British Columbia. Participants were 20 to 46 years old (median = 27, SD = 7.8), all nonsmokers, and 47% had asthma (Table I). We excluded individuals with any of the following: (1) pregnancy/breast-feeding, (2) use of inhaled corticosteroids, (3) regular use of bronchodilator medication (ie, use of bronchodilators more than 3 times per week), (4) unstable asthma symptoms, (5) any use of vitamins A, C, E or other antioxidant supplements, (6) comorbid conditions judged by the investigators to increase risk of dropout, or (7) work in an industrial setting or other setting of significant inhaled exposures. Men (n = 7) and women (n = 10) were included. Sensitization to birch, timothy/Pacific grass, and house dust mite (*Dermatophagoides pteronyssinus* group 1) (Table II) was tested by skin prick test, using standardized extracts. Medical grade allergen extracts (Hollister-Stier, Spokane, Wash) in solutions were used. A wheal of 3 mm or more to at least one of those allergens was required for inclusion in the study. Subjects withheld long-acting β_2-agonists for 48 hours, short-acting β_2-agonists for 6 hours, long-acting antihistamines for 14 days, nonsteroidal anti-inflammatories and aspirin for 7 days, and short-acting antihistamines for 3 days before the skin test. A series of 10-fold dilutions of the test allergen were used to determine the lowest skin prick dose needed to elicit a 3-mm wheal, based on the strong correlation between the concentration of allergen leading to skin test positivity and that prompting airway responsiveness.[22] In those whose test allergen was birch, we required that they avoid apple, pear, sweet cherry, peach, plum, apricot, almond, celery, carrot, potato, hazelnut, mango, and chili pepper to minimize concerns for oral allergy syndrome.

TABLE I. Grouped participant demographics

Characteristic	Group I (n = 8)	Group II (n = 9)	*P* value
Age (y), mean ± SD	29.50 ± 2.771	27.33 ± 2.718	.5859 (*t* test)
Sex	Male: 3; Female: 5	Male: 4; Female: 5	1 (Fisher exact test)
Asthma status	Asthmatic: 3; Nonasthmatic: 6	Asthmatic: 5; Nonasthmatic: 4	.6372 (Fisher exact test)

TABLE II. Individual participant demographics

Subject	Age (y)	Sex	Asthmatic?	Allergen
DE-53	20	Female	Yes	*D pterinyssinus*
DE-58	31	Female	No	*D pterinyssinus*
DE-70	24	Female	Yes	*D pterinyssinus*
DE2-81	32	Male	No	Timothy/Pacific grass
DE2-90	34	Female	Yes	Timothy/Pacific grass
DE2-94	27	Male	No	*D pterinyssinus*
DE2-96	25	Female	No	*D pterinyssinus*
DE2-103	46	Male	No	Birch
DE2-105	27	Male	Yes	Timothy/Pacific grass
DE2-106	46	Female	Yes	*D pterinyssinus*
DE2-107	20	Female	No	Timothy/Pacific grass
DE2-109	31	Female	No	Birch
DE2-112	28	Male	Yes	Timothy/Pacific grass
DE2-114	23	Male	Yes	Timothy/Pacific grass
DE2-115	23	Female	Yes	*D pterinyssinus*
DE2-116	23	Male	No	*D pterinyssinus*

Exposure design and procedures

Subjects entered a crossover experiment[23-25] (Fig 1) using 2 conditions (DE [300 μg $PM_{2.5}/m^3$] or filtered air [FA]), the order of which was randomized and counterbalanced. DE exposure used a previously described system that excludes potential contamination with LPS,[26] except that in the present study we used a 2.5-kW constant load. The Air Pollution Exposure Laboratory was designed for the controlled inhalation of human subjects to aged and diluted DE to mimic "real-world" occupational and environmental conditions. Notably, the protocol allows for effective blinding to both the subject[27] and those analyzing all material and data.

One hour following each exposure to DE or FA, bronchoscopy was performed to deliver a saline-controlled segmental allergen challenge. A 5-mL solution of allergen extract in a concentration 10-fold lower than that minimal dose producing a positive wheal was instilled in a lower lobe bronchial segment, and 5 mL control (normal saline) was instilled in a contralateral lower lobe bronchial segment. Photographs were taken of the segments instilled, to guide subsequent bronchoscopy as needed. DE exposure preceded allergen instillation to avoid the possibility that starting with segmental allergen would lead to acute segmental bronchoconstriction and thus decreased deposition of diesel particulate matter within that segment. Forty-eight hours after allergen challenge, bronchial brushes of airway epithelial cells were obtained in the same segments. Side (right vs left) of