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Reduced mouse allergen is associated with epigenetic changes in regulatory genes, but not mouse sensitization, in asthmatic children

Rachel L. Miller[a,b,c,*,1], Hanjie Zhang[a], Jacqueline Jezioro[a], Mariangels De Planell Saguer[a], Stephanie Lovinsky-Desir[d], Xinhua Liu[e], Matthew Perzanowski[c], Adnan Divjan[c], Wanda Phipatanakul[f], Elizabeth C. Matsui[g]

[a] Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, Columbia University Medical Center, PH8E-101B, 630 W. 168th St., New York City, NY 10032, USA
[b] Division of Allergy, Immunology, and Rheumatology, Department of Pediatrics, Columbia University Medical Center, PH8E-101B, 630 W. 168th St., New York City, NY 10032, USA
[c] Department of Environmental Health Sciences, Columbia University, 722 W 168th St, 11th Floor, New York City, NY, 10032, USA
[d] Division of Pulmonary, Department of Pediatrics, Columbia University Medical Center, 3959 Broadway, CHC 7-701, New York City, NY 10032, USA
[e] Department of Biostatistics, Columbia University Medical Center, 722 W 168 St, 6 Floor, New York City, NY, 10032, USA
[f] Division of Pediatric Allergy/Immunology, Boston Children's Hospital, Harvard Medical School, 300 Longwood Ave, Boston, MA 02115, USA
[g] Division of Pediatric Allergy/Immunology, Johns Hopkins School of Medicine, CMSC 1102, 600 N. Wolfe Street, Baltimore, MD 21287, USA

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ABSTRACT

Chronic exposure to mouse allergen may contribute greatly to the inner-city asthma burden. We hypothesized that reducing mouse allergen exposure may modulate the immunopathology underlying symptomatic pediatric allergic asthma, and that this occurs through epigenetic regulation. To test this hypothesis, we studied a cohort of mouse sensitized, persistent asthmatic inner-city children undergoing mouse allergen-targeted integrated pest management (IPM) vs education in a randomized controlled intervention trial. We found that decreasing mouse allergen exposure, but not cockroach, was associated with reduced *FOXP3* buccal DNA promoter methylation, but this was unrelated to mouse specific IgE production. This finding suggests that the environmental epigenetic regulation of an immunomodulatory gene may occur following changing allergen exposures in some highly exposed cohorts. Given the clinical and public health importance of inner-city pediatric asthma and the potential impact of environmental interventions, further studies will be needed to corroborate changes in epigenetic regulation following changing exposures over time, and determine their impact on asthma morbidity in susceptible children.

1. Introduction

As many as 25–50% of inner-city children with asthma have evidence of allergic sensitization to mouse (Matsui et al., 2006; Ahluwalia et al., 2013). This trend suggests that chronic exposure to mouse allergen may contribute greatly to the inner-city asthma burden, particularly in the major metropolitan areas of the Northeastern United States. The multi-faceted environmental intervention by the Inner-City Asthma Study (ICAS) group, as well as others, has shown that successful reduction of indoor allergens can lead to long-lasting decreases in asthma symptoms, asthma exacerbations, missed school and disrupted sleep in children (Morgan et al., 2004; Pongracic et al., 2008; Johnson et al., 2009). Nonetheless, its ability to induce immune modulation is unknown and has implications for understanding the natural course of allergic asthma, identifying those at greater risk, and determining optimal treatment.

Environmental epigenetic regulation may induce immune modulation. This is supported by observations that measures of multiple environmental toxicants, including air pollution and allergens, are associated with altered inflammatory, allergic and regulatory gene methylation (Nadeau et al., 2010; Niedzwiecki et al., 2012; Salam et al., 2012). But far less studied are the impacts of potential changes in

Abbreviations: FOXP3, forkhead box P3 gene; Ig, immunoglobulin; ICAS, Inner-City Asthma Study; IPM, integrated pest management; IFN, interferon; IQR, interquartile range; MAAIT, Mouse Allergen and Asthma Intervention Trial; PASTURE, Protection Against Allergy: Study in Rural Environments

* Corresponding author at: Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, Columbia University Medical Center, PH8E-101B, 630 W. 168th St., New York City, NY 10032, USA.
E-mail address: rlm14@cumc.columbia.edu (R.L. Miller).
[1] Present address: Columbia University Medical Center, 630W. 168th St. PH8E-101B, NYC, NY, 10032, USA.

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exposures over time on changes in epigenetic marks, and whether these changes in epigenetic marks are clinically relevant. As an example, human rhinovirus infection changed global methylation in nasal epithelial cells to levels that varied by asthma diagnosis (McErlean et al., 2014). In the Normative Aging Study of elderly men, changes in particle numbers, levels of black carbon, and ozone over the preceding 4 weeks were associated with inflammatory gene specific changes in DNA methylation (Bind et al., 2014). However, comparable studies of changes in pediatric cohorts are scant. One exception is the Protection Against Allergy: Study in Rural Environments (PASTURE) study that observed significant differences in the DNA methylation of several genes assessed in cord blood and then repeated at age 4.5 years. The differences with aging during childhood also varied by whether there was prenatal exposure to a farm environment, and by whether the child was subsequently diagnosed with asthma (Michel et al., 2013).

To address this research gap, we measured prospectively and repeatedly promoter DNA methylation and expression of targeted asthma candidate genes associated with regulating allergy (T regulatory gene forkhead box P3 gene (*FOXP3*), and allergy suppressive gene interferon *(IFN)γ)*. Methylation of both genes previously was found to occur in association with ambient environmental exposures (Liu et al., 2008; Brand et al., 2012; Kohli et al., 2012; Niedzwiecki et al., 2012; Runyon et al., 2012; Tang et al., 2012) and with allergy and asthma (Liu et al., 2008; Brand et al., 2012; Runyon et al., 2012). We utilized pyrosequencing to capture and quantify small differences predicted to underlie potential changes in the exposure-outcome relationships (Murphy et al., 2012; Michel et al., 2013; Richmond et al., 2015; Clifford et al., 2017). This observational substudy combined measures from mouse sensitized, moderate to severe asthmatic inner-city children (n = 200; 6–17yr) undergoing mouse allergen-targeted integrated pest management (IPM) vs education in a randomized control intervention. We sampled buccal cells that comprise the aerodigestive track epithelium because they are accessed easily in children (Breton et al., 2011; Kuriakose et al., 2011; Lovinsky-Desir et al., 2014), and undergo molecular alterations following environmental exposures (Bhutani et al., 2008; Salam et al., 2012; Wan et al., 2014), and in association with airway inflammation (Breton et al., 2011; Salam et al., 2012). We postulated that reducing allergen exposure would modulate the immunopathology underlying persistent pediatric allergic asthma through epigenetic regulation. Specifically, we hypothesized that changes in mouse allergen exposure would be associated with changes in buccal cell methylation and expression, and that these would alter mouse-specific immunoglobulin (Ig)E.

2. Materials and methods

Children with persistent asthma and an exacerbation in the previous year underwent measurement of home settled bed dust and bed floor dust for mouse allergen (Mus m 1) and bed dust for cockroach (Bla g2) and dust mite allergen (Der f 1) by ELISA (Indoor Biotechnologies, Charlottesville, VA). Mouse-specific IgE (against mouse urine proteins, e72) was tested by Immunocap (ThermoFisher, Uppsala, Sweden) as described (Matsui et al., 2006; Sedaghat et al., 2016). Mouse exposed (determined by bed dust mouse allergen concentration of ≥0.4 μg/g or a bedroom floor dust mouse allergen concentration of ≥0.5 μg/g) and sensitized children were enrolled in a home-based Mouse Allergen and Asthma Intervention Trial (MAAIT; Fig. 1) (Sedaghat et al., 2016). The pest management education that was delivered as a control included information about setting mouse traps, sealing holes and cracks, and housekeeping practices. The IPM was delivered in treatments. The first treatment included targeted cleaning to remove allergen reservoirs, placement of traps, application of rodenticide, sealing holes and cracks, installation of allergen-proof mattress and pillow encasements (CleanBrands, LLC, Warwick, Rhode Island), and two portable air purifiers (Filtrete™ Room Air Purifier, 3M, St. Paul, Minnesota). This was followed by a visit 1–2 weeks later to reset traps and complete any work remaining from the primary visit. Each treatment was delivered by a licensed pest technician and by study staff.

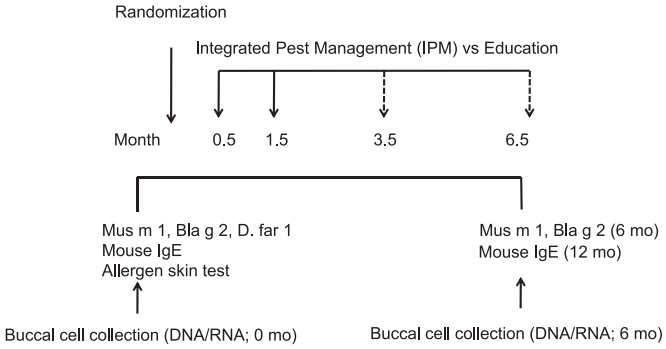

Fig. 1. Study algorithm. The IPM group received two IPM visits at 0.5 and 1.5 months (solid arrows). If there was evidence of mouse infestation at 3, 6, and 9 months, a subsequent IPM visit occurred at 3.5, 6.5 (see dashed arrows) and 9.5 months (not shown). The second mouse IgE level was measured at 12 month time-point, in contrast to the allergen (Mus m 1, Bla g 2) and epigenetic biomarkers, each measured at baseline and 6.0 months.

Extracted DNA underwent bisulfite conversion and pyrosequencing of the upstream enhancer (*FOXP3* only) and promoter (both genes) areas (Lovinsky-Desir et al., 2014). The *FOXP3* CpG loci within the gene promoter area were selected based on our previous associations with Treg function and asthma (Runyon et al., 2012) and their relatively large range in methylation levels across individuals (data not shown). The *IFN*γ CpG loci within the gene promoter were selected based on their conservation in mice (Niedzwiecki et al., 2012; Collison et al., 2013), specific roles in regulating gene expression (Gonsky et al., 2009; Brand et al., 2012; Kohli et al., 2012; Belsky et al., 2013), susceptibility to allergen and air pollution (Liu et al., 2008; Brand et al., 2012; Niedzwiecki et al., 2012), and previously implicated role in allergy and asthma (Kohli et al., 2012; Runyon et al., 2012).

Total RNA was extracted using Trizol (Molecular Research Center, Inc., Cincinnati, OH; Supplemental Tables 1A,B). qRT-PCR was carried out using the SuperScript First-Strand Synthesis System and the Applied Biosystems® 7500 Real-Time PCR Systems. The housekeeper gene cystatin A (CSTA) was selected based on its high and specific expression in the buccal mucosa (Magister and Kos, 2013). Mouse and cockroach allergen levels and buccal cells were collected at baseline and 6 months later. Dust mite allergen levels were collected at baseline only. Mouse IgE was assessed at baseline and 12 months later. The study was approved by the Institutional Review Boards of Columbia University Medical Center, Johns Hopkins University, and Harvard University.

The distribution of each variable was examined and an appropriate transformation was applied as necessary to meet parametric model assumptions. Analyses were conducted separately for each CpG gene site. Allergen levels and RNA values were natural log transformed. Values (eg, mouse allergen) below a limit of detection (LOD) were assigned a value at half the LOD. Methylation levels within the *FOXP3* enhancer highly correlated with each other (Spearman correlation coefficients 0.90–0.94) at each visit, and in subsequent analyses the average of the 4 values were used as a single variable.

The signed rank test was used to compare differences in allergen and mouse IgE levels between visits. The Kruskal-Wallis test was to used to detect bivariate associations between the methylation or gene expression levels and categorical variables including sex, site (Boston, Boston Children's Hospital, Harvard Medical School; Baltimore, Johns Hopkins University), race/ethnicity, insurance type, any reported exposure to second hand smoke, maternal allergy status and particulate matter less than 10 μm or not, at each visit. The spearman correlation coefficient was used to indicate bivariate association between quantitative variables, specifically the correlations among individual buccal biomarkers and between the arithmetic change in allergen and change in methylation. To examine concurrent associations between mouse allergen and