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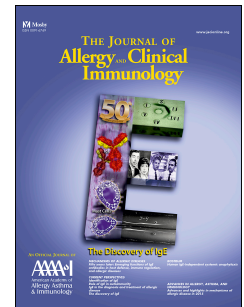
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Are We What Our Mothers Made Us? Lessons from Epigenetics

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Some questions are as old as mankind. Ralph Waldo Emerson, from whose writings the title of this piece is borrowed, was not the first poet to muse over the relationship between mothers and their children. Biologists have been doing the same for a long time, and in recent years the literature on the prenatal origins of disease has been piling up, fueled by novel models and powerful technologies. In our field, more and more evidence suggests that the trajectory to asthma and allergic diseases begins *in utero*, when exposure to the maternal environment influences the child's responses to exposures encountered after birth (1), possibly through an epigenetic calibration of set points that control later responsiveness. Indeed, maternal smoking in pregnancy, prenatal maternal stress, asthma and obesity are all associated with asthma risk in the child [reviewed in (2)]. Interestingly all of these conditions, while distinct, have been reported to modify the child's epigenome, which may act as a gatekeeper of the asthma trajectory (3).

Because asthma and allergy are rapidly on the rise primarily in the Western world, maternal exposures that are frequent in and typically associated with Western lifestyles are most actively interrogated for their impact on the child's risk for asthma. Hence the attention given to phthalate exposure. Phthalates, which are commonly added to plastics to increase their flexibility, transparency, and durability, are everywhere. Exposure derives primarily from the diet, particularly from food and beverage packaging containing PVC, but also from the inhalation of dust from vinyl flooring, carpet backing, and products such as fragrances, dryer sheets, air fresheners, and sunscreens (4).

However, reports of associations between phthalates and asthma have been inconsistent and little is known about potential underlying mechanisms. A study from Germany now reports a significant association between maternal urinary concentrations of the phthalate metabolite mono-n-butyl phthalate (MnBP) during pregnancy and increased risk of asthma in children

enrolled in LINA (Lifestyle and environmental factors and their Influence on Newborns Allergy risk), a prospective mother-child cohort enrolled in Leipzig (5). It is noteworthy that among the 10 phthalate metabolites that were measured, only MnBP was found to be associated with increased asthma risk in children. Such a selective effect may explain why previous studies (which measured different metabolites under different exposure conditions) produced inconsistent results.

The main question, of course, is which mechanisms link phthalate exposure to increased asthma risk. Jahreis and her colleagues sought to answer this question by turning to a “trans-generational” ovalbumin (OVA)-driven mouse model of asthma (Figure 1). Through their drinking water, Balb/c dams were exposed to concentrations of butyl benzyl phthalate (BBP, of which MnBP is a metabolite) chosen to yield urine MnBP levels comparable to those detected in highly exposed LINA mothers. Exposure occurred during pregnancy or perinatally, that is, during both pregnancy and breastfeeding. Grown-up offspring were then sensitized with OVA without further BBP exposure. A third group of mice were exposed to BBP as adults, starting one week before OVA sensitization. The results were impressive. While exposure of adult mice to BBP had no effect on experimental asthma, prenatal and perinatal BBP exposure significantly enhanced lung inflammation and airway responsiveness.

Maternal BBP exposure also led to genome-wide DNA hypermethylation in splenic CD4⁺ T cells isolated from both mothers and offspring, and the asthma-inducing effects of phthalate exposure were reduced when one-week old pups from BBP-exposed dams were treated with the DNA methyl-transferase inhibitor 5-aza-2'-deoxycytidine (Aza). Therefore, the authors argue that the asthma-promoting effects of BBP were mediated by epigenetic mechanisms. The hypothesis is

plausible even though the effects of Aza and its toxicity are so broad that the interpretation of these results is not entirely straightforward. Combined analysis of DNA methylation and gene expression highlighted pathways that might be mechanistically linked to the effects of phthalates. CD4 T cell hypermethylation was accompanied by reduced expression of genes involved in Th2 cell differentiation, including *Zfp101*, a gene that acts as a repressor of GATA3-mediated Th2 cell development. The authors suggest that *Zfp101* suppression might contribute to the increased allergic response observed in exposed mice.

Exposure to BBP during pregnancy and breastfeeding also modestly enhanced eosinophilic airway inflammation and airway hyperresponsiveness (albeit not OVA-specific IgE levels) in the F2 generation, and again treatment of F2 pups with Aza reduced these effects. However, the Th2 differentiation-related genes that were hypermethylated and suppressed in the F1 generation were not differentially expressed in F2 pups. Moreover, the effects on F2 pups were seen when BBP was administered during both pregnancy and breastfeeding. This implies that the impact of phthalate exposure on the F2 generation, and the patterns of effect transmission across generations, need to be further clarified. Indeed, if maternal BBP exposure increased metabolite levels in breast milk [as reported in humans (6)], then F1 pups were exposed not only prenatally but also directly.

Speaking about trans-generational effects, a broader caveat is in order. The authors present their mouse model as an epigenetic trans-generational one, but trans-generational inheritance refers to the germline-mediated inheritance of epigenetic information between generations (with ensuing phenotypic variation) *in the absence of direct environmental influences* (7). Many exposures are known to promote epigenetic alterations that influence somatic cells and the

disease status of exposed individuals (F0 generation). In pregnant females, direct environmental exposure may also cause epigenetic modifications in the next two generations (F1 and F2) through the fetus and its germline, but for the initial exposure to cause a true trans-generational phenotype, its effects must persist to subsequent generations (F3 and beyond) (7). In our case, exposure of pregnant mice (F0 generation) to BBP also exposed the fetuses (F1 generation) and the germ cells present in those developing fetuses. These exposed germ cells then became the F2 generation. To definitively conclude that the effects of phthalates described by Jahreis and her colleagues are trans-generational, the F3 generation should be examined, especially because the immune and epigenetic profiles observed in F2 mice were quite different from those seen in F1 pups, and moreover, as mentioned above, it remains unclear whether F1 mice were or were not directly exposed.

This caveat aside, the results presented by Jahreis and her colleagues are intriguing and warrant further investigation in areas so far under-explored. For instance, it would be interesting to know whether paternal exposure plays a role in the effects of phthalates. Indeed, pancreatic β -cell dysfunction in female rat offspring was shown to be programmed by chronic high-fat diet in their fathers, not their mothers (8). It would also be critical to assess the effects of phthalates not just on adaptive immune cells but also on other lung components (the epithelium and smooth muscle first and foremost) – after all, allergen-driven Th2 inflammation is not all there is to asthma. Moreover, it would be important to know whether genetic variants modify the association between phthalate exposure and asthma, as recently reported for *SOD2* (9), and to what extent post-translational histone modifications [especially histone H3 Lys27 trimethylation, a major emerging player in trans-generational epigenetic inheritance in animal models (10)] are involved in these processes. And finally, perhaps the most intriguing question of them all: are the effects of phthalate exposure completely recapitulated by the modifications induced in the

host's cells, immune or otherwise, or are they also mediated through a reconfiguration of the maternal microbiome, which in turn reprograms the microbiome and immune development of the child during early life? Obviously, it is too early to tell. But the work of Jahreis and her colleagues sets the stage for exciting discoveries about the pathways that control the prenatal origins of allergic diseases.

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Legend to Figure 1.

Impact of prenatal, perinatal, and adult exposure to phthalates in an ovalbumin-dependent experimental asthma model. AHR: airway hyperresponsiveness, BBP: butyl benzyl phthalate.

