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Review Article

The effects of early low dose exposures to the Environmental Estrogen Bisphenol A on the Development of Childhood Asthma

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Abbreviations: BALF: Broncho Alveolar Lavage Fluid; BBzP, di-n-butyl phthalate and butylbenzyl phthalate; BPA: Bisphenol A; BW: Body Weight; ER: Estrogen Receptor; IP: Intraperitoneal; FEV1: Forced Expiratory Volume in the first second of expiration; Intraperitoneal; MBzP, Monobenzyl phthalate; NHANES: National Health and Nutrition Examination Survey; OVA: Ovalbumin; PN: Post Natal; PND: Post Natal Day

SUMMARY

Exposure to environmental chemicals is a potential cause for the rapid increase in the prevalence of allergic asthma over the last few decades. The production of the environmental estrogen bisphenol A, the monomer of polycarbonate plastics, has increased rapidly over the last 50 years, such that bisphenol A is one of the most highly produced chemicals. It is detectable in the urine of the vast majority of the human population. While the relationship between the increase of bisphenol A in our environment and the prevalence of asthma does not prove a cause and effect relationship, it provides a strong rationale for experiments that have tested the hypothesis. Because of its small molecular size and hydrophobicity, bisphenol A is easily transferred from the mother to the fetus, via the placenta and in breast milk.

We have reviewed all the publications available on medline on the human epidemiological studies of the early bisphenol A exposure on the development of allergic asthma and experimental studies using mouse model of the effects of early bisphenol A exposure on the development of asthma. There are eight human epidemiological studies and five mouse model studies currently published.

The human studies suggest that bisphenol A exposure in early life enhances the likelihood of developing asthma on at least one of the study groups. The effects of early bisphenol A exposure were observed as an enhanced development of asthma before adolescent in the animal model.

INTRODUCTION

Allergic diseases, including asthma are often considered to be related to our genes and/or environment. However, changes of the genetic code in a population typically take a long period of time, suggesting that the recent, rapid increase in the prevalence of allergic diseases is more likely to be due to the changes in our environment, which may alter gene expression (gene/environment interaction [1]). Further, during the last few decades, campaigns to promote smoking cessation in the US and elsewhere have reduced exposure to direct and second hand smoke in pregnant women and their children. The concentrations of the six other common air pollutants (ozone, carbon monoxide, nitrogen dioxide, particulate matter, sulfur dioxide and lead) have also decreased by 60% during these decades (EPA, http://www.epa.gov/airtrends/aqtrends.html), yet the overall prevalence of asthma has increased during this period. Another important consideration is that the increasing prevalence of allergic rhinitis and asthma is seen predominantly in women after adolescent, while prevalence of allergic rhinitis and asthma in boys and young men decrease during and after adolescent. These observations coupled with an increase in the incidence of new or recurrent asthma in women who took hormone replacement therapy after

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menopause [2], suggest an effects of female hormones on the development of allergic diseases, including asthma. Other reports show the human and mouse fetuses are T helper type (Th) 2 dominant [3,4] and endogenous (e.g. estradiol, E_2) and exogenous (environmental) estrogens induce Th2 development [5-7].

Based on these clinical observations and the concomitant high production and ubiquitous exposure of humans to BPA, we chose to focus our studies of asthma development on environmental estrogens, and particularly on BPA, as a model for assessing the effects of exposure to estrogenic chemicals on the development of allergic diseases. We review here evidence that early life exposures to BPA may enhance the development of allergic asthma (Figure 1).

BPA was first synthesized by a Russian chemist Alexander Dianin in 1891, and tests were conducted in the 1930s to determine whether BPA could be used for hormone replacement therapy [8,9]. However BPA proved less effective than other synthetic estrogens. In 1952, manufacturing processes for polymerizing BPA monomers to form polycarbonate plastic were developed and the production of BPA has increased exponentially since then (Figure 2, modified from [10]). In addition to polycarbonate plastic containers, BPA is widely used as a coating of metal cans, baby bottles, toys and receipt papers, and to a lesser extent in other household plastics. Thus, BPA is

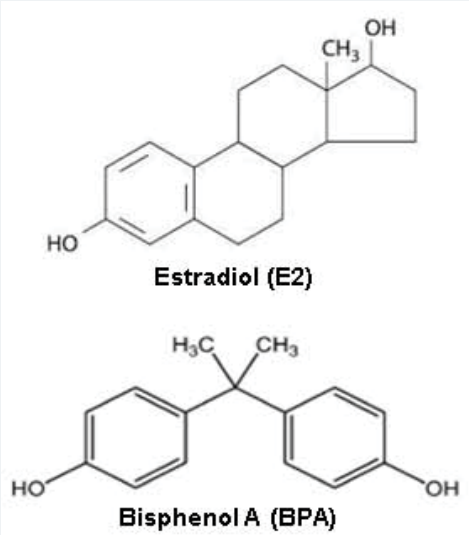

Figure 1: The structures of endogenous estrogens and BPA.

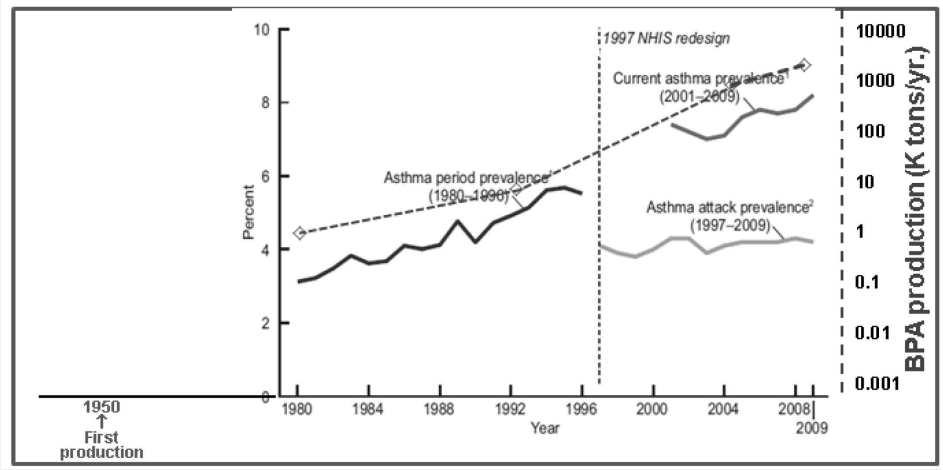

Figure 2: Temporal relationships between the increases of asthma prevalence and BPA production.

now ubiquitous, not only in our environment, but also in human tissues and secretions [11]. Its small molecular weight (228.29 g/mol) and hydrophobicity also allow BPA to be rapidly transferred to fetus through placenta and also into the breast milk [12], providing pathways into the next generation.

BPA exposure and metabolism in humans

We identified eight English language manuscripts concerning the relationship between human exposure to BPA and the subsequent development of asthma. These exposures are mainly via consumption of food and drinks [13], and also non-oral exposures such as dermal exposure is reported [14,15]. BPA is absorbed through gastrointestinal tract and enters the hepatic portal vein to the liver, and then to the systemic circulation. Most of the absorbed BPA is conjugated to glucuronide by predominantly by UDP-glucuronosyltransferases in the intestinal epithelium and in liver. This makes BPA more hydrophilic and allows it to be excreted into urine [16]. However, the biologically active form is predominantly residual unconjugated BPA, which can bind to estrogen receptors (ERs) in various tissues and alters biological processes [17]. Thus, it is the small amount of unconjugated BPA in the blood and tissues, termed the "internal dosage"-as assessed from blood measures-that is likely to have the most important effects in human toxicology [18]. However, BPA concentrations/excretion into the urine "exposure assessment from urinary measures of BPA" is most commonly used in epidemiological studies.

Effects of BPA exposure on allergic diseases-human epidemiological studies

There are eight epidemiological studies of the effects of BPA exposure on the development of allergic asthma published accessible in Medline: Vaidya et al. [19] Spanier et al. [20,21], Donohue et al. [22], Kim et al. [23], Whyatt et al. [24], Gascon et al. [25] and Wang et al. [26] (Table 1). Each of these eight human epidemiological studies identified at least one indicator of an association between BPA exposure and asthma, which was significantly increased with prenatal or self (direct) BPA exposure of children.

Vaidya et al. performed a secondary analysis of urinary BPA data from the National Health and Nutrition Examination Survey (NHANES) 2005-2006 (http://www.cdc.gov/nchs/nhanes.htm). Using the data from 10,348 subjects older than 6 years, they examined the urinary BPA concentrations/excretion (BPA/Cr) ratio from 2,548 (24.6% of the total subjects: 1,270 males and 1,278 females). They then related the

Table 1: Human epidemiological data of early BPA exposure on the development of allergic asthma.

Author year	Recruitment N	Recruitment year	BPA measure	Outcome wheezing	asthma diagnosis	
Vaidya 2012	10,348	2005-2006	6yr-	6yr-	6yr-	asthma in females allergen specific IgE antibodies asthma episode in past 12mo in females
Spanier 2012	365	2003-2006	G16, G26w	every 6mo to 3yr		wheeze at age 6mo w uBPA at 16 w ~wheeze at age yr w uBPA
Donohue 2013	568	1998-2006	3rd Trimester 3, 5, 7yr	5, 6, 7yr	5-12yr	↓wheeze at age 5yr w prenatal uBPA wheeze at age 5&6yr w uBPA at age 3 yr wheeze at age 7yr w uBPA at age 7yr
Spanier 2014	208	2003-2006	G16, G26w	every 6mo to 5yr	4, 5yr	↓FEV1 at age 4yr w maternal uBPA wheezing w 16w uBPA ~FEV1 or wheeze w child uBPA
Kim 2014	127		7-8yr	9-10, 11-12yr	9-12yr	wheeze at 11-12yr w uBPA at 7-8yr in girls asthma at 11-12yr w uBPA at 7-8yr in girls
Whyatt 2014	292	1998-2006	3, 5, 7yr	5, 6, 7, 9, 11yr	5yr	asthma, persistent wheeze, exsecise-induced wheeze among prenatal MBzP only
Gascon 2015	462	2004-008	G12, G32w	6mo, 14mo, 4yr, 7yr	7yr	wheeze at any age chest infection at any age bronchitis
Wang 2016	453		3, 6yr	3, 6yr	3,6yr	asthma at 3, 6yr

urinary BPA excretion to the blood concentration of total IgE, specific IgE antibodies against 19 allergens, and other urinary environmental estrogens (Benzophenone-3, 4-tert-Octylphenol, Triclosan, Methyl paraben, Ethyl paraben, Propyl paraben and Butyl paraben). The diagnosis of asthma was based on the history of wheezing, the number of eosinophil in peripheral blood, total IgE concentration and history of other atopic diseases. Urinary BPA concentration had a significant positive association with increased concentrations of IgE antibodies to various specific allergens and with the history of asthma episodes in the previous 12 months among women, but not with those in men.

Spanier et al. collected samples of urine from 365 women at 16±2 weeks of pregnancies, in their birth cohort study from 2003 to 2006 in Cincinnati, Ohio, and measured the urinary BPA concentration and BPA/creatinine ratios in samples from women older than 18 years and paired these with their children. They followed the mother-infant pairs for five years and reported the follow-up data collected from phone interviews when each child was 6, 18 and 30 months old and home visits at child's age of 12, 24 and 36 month, and 3 and 5 years of age [20,21]. They used a questionnaire from National Health and Nutrition Examination Survey (NHANES) to identify those with wheezing, "Has [child's name] had wheezing or whistling in his/her chest in the last 6 months?" They also conducted a trajectory analysis to identify distinct groups of wheeze trajectories and phenotypes. This study identified a significant correlation between maternal urinary BPA concentration at 16 (but not 26) weeks of pregnancy with child's wheezing by postnatal age 6 months. Spanier et al. continued this study using phone interviews and tests of lung function on 4 and 5 year olds. They identified a significant association between maternal urinary BPA concentrations at 16 and 26 weeks of pregnancy with decreased forced expiratory volume in the first second of expiration (FEV1) of 4 years old children, but not with FEV1 at 5 years.

Donohue et al. recruited 568 mothers of African American or Dominican origin and their child pairs, who had lived in Northern Manhattan or South Bronx for at least 1 year, from 1998 to 2006. They analyzed their urinary BPA data from late in pregnancy, when the children were 3, 5 and 7 years old. Wheezing history was elicited for the previous 12 months and at 5, 6 and 7 years old, and one clinic visit between the ages of 5-12 years old. They identified an inverse association between maternal urinary BPA concentration during late pregnancy and child's wheezing at age 5 and 6 years. However, they also noted a significant correlation between the child's own urinary BPA concentration and wheezing at age 7 years. There was also a significant correlation between the child's urinary concentration of BPA and the prevalence of asthma at 3, 5 and 7 years of age [22].

Kim et al. collected 127 urine samples from 7-8 year old elementary school children in Seoul, Korea in 2005, who agreed to a baseline survey and a methacholine challenge test. They then identified their "wheezing outcomes" at age 9-10 and 11-12 years old. Children were considered to have asthma when they met either of the following criteria: 1) "wheezing or the use of asthma medication in the previous 12 months" or 2) the same history combined with an asthma diagnosis or a history of wheezing". They identified a significant correlation between urinary BPA concentration at 7-8 years old and wheezing and asthma at 11-12 years old among girls, but not in boys [27]. The Whyatt et al., group also analyzed the association of asthma and exposure combination of BPA, di-n-butyl phthalate and butylbenzyl phthalate (BBzP) among 229, inner-city women and their children at age 5-11 years [22]. They measured phthalates in spot urine collected from the mother during pregnancy at 33.9±3.1 week's gestation and BPA in child urine at 3, 5 and/or 7 years old. They found significant association between the children's urinary BPA concentration and respiratory outcomes, in those from women whose maternal prenatal urinary monobenzyl phthalate (MBzP) were above median, but not in those children from women whose values below the median.

In 2015, Gascon at al. reported a correlation between prenatal BPA exposure and respiratory tract infection and allergy, among subjects recruited in Catalonia, Spain, from 2004 to 2008 [25]. They analyzed the maternal urinary concentration of BPA at 12 and 32 weeks of gestation in 391 women and related these to symptoms of allergy, atopy and respiratory infections in the children at 6 and 14 months, and 4 and 7 years old. They found the relative risks of wheeze, chest infections and bronchiolitis, at any age, increased with each doubling in the concentration of the mother's prenatal urinary BPA [25].

Wang et al. [26] recruited 453 children age 3 and 6 years old from Taiwan and analyzed their own urinary BPA concentration, serum total IgE and diagnosis of asthma using the questionnaire from International Study of Asthma and Allergies in Childhood (ISAAC). They found an association between their urinary BPA concentration and asthma, as well as serum total IgE concentration.

One of the major differences between these studies was the timing/ages at which the subjects were tested for BPA exposure, particularly between pre and postnatal exposures. Interestingly, two recent reports from a consortium of European states showed a strong correlation between the mother's urinary BPA concentration and those of their child ($p=0.001$ and <0.001) at various postnatal ages [28,29]. This finding may suggest that a dietary lifestyle of the household is a major determinant of the extent of exposure to BPA for all of the family members.

Effects of BPA on the development of asthma-studies from mouse models

We identified six reports, two from our group [30,16] and one each from Bauer et al. [31], Petzold et al. [32], O'Brien et al. [33] and Nygaard et al. [34], concerning the effects of maternal BPA exposure on the development of asthma in mouse models (Table 2). Only mouse were used on the asthma model study for the effects of BPA, most likely because of the availability for lung function testing devices, whole body plethysmography and direct measurement of airway resistance.

To our knowledge, we were the first to report an effect of maternal BPA exposure on the development of asthma in their offspring. In our studies, we added 10 μg/ml of BPA to the drinking water of 8-10 weeks old female BALB/c mice (F0, about 2 mg BPA/KgBW/day), starting seven days before mating and continued throughout their pregnancy and nursing period. This protocol produced a similar range of BPA concentrations in the sera of the offspring (F1) to that described in human studies [18]. Some of the pups were sensitized to ovalbumin (OVA), using a "suboptimal" protocol, [35] in order to avoid overwhelming effects of OVA sensitization. The mice received a single intraperitoneal (i.p.) injection of 5 μg OVA on post natal day (PND) 4 and they were exposed to 3% OVA aerosol on PND 14-16. Airway hyperresponsiveness (AHR) was analyzed 48 hours after the last aerosol exposure. Serum OVA-specific IgE antibodies were assessed by ELISA, and eosinophilic airway inflammation were assessed by enumerating each cell type in the bronchoalveolar lavage fluid (BALF). Only the OVA sensitized pups from BPA exposed mothers (BPA/OVA) had significant increases in their serum IgE anti-OVA antibody concentration, and enhanced eosinophil numbers in BALF and AHR, relative to all three control groups (no BPA/no OVA, no BPA/OVA and BPA/no-OVA) [36]. Interestingly, these same indicators of allergic asthma were also observed in the F2 and F3 generation of females, despite the absence of additional exposures to BPA (non-published data).

We next examined the effect of prenatal and postnatal BPA exposure, separately [16]. Using the same exposure protocol as described above, we transferred half of the litters between BPA exposed and normal water control dams, within 48 hours of their birth, and used the same protocol to sensitize both groups, creating eight perinatal exposure/postnatal exposure/and sensitization groups. These experiments indicated that prenatal BPA exposure is necessary to produce the asthma phenotype in pups sensitized, using our "suboptimal" OVA protocol.

Table 2: Mouse model studies of early BPA exposure on the development of asthma phenotype.

Author	Strain	BPA exp		BPA dose		Allergen (OVA) sensitization				analyses	
Year		Star	end	µg/KgBW/d	route	Systemic		Airway		day	
		(PND)		(µg)		dose	age	dose	age	(PN)	
							(PN)		(PN)		
Midoro	BALB/c	-28	17	2,000	drinking	5 µg x 1 i.p.	4d	3% aerosol 10min	13-15d	17d	↑AHR in BPA/OVA ↑Eos in BALF in BPA/OVA ↑serum IgE anti-OVA in BPA/OVA
2010					water			x3d			
Nakajima	BALB/c	-28	21	2,000	drinking	5 µg x 1 i.p.	4d	3% aerosol 10min	18-20d	22d	↑AHR in BPA/BPPA/OVA and BPA/no BPA/OVA
2012		-28	0		water			x3d			↑Eos in BALF in BPA/BPA/OVA and BPA/no BPA/OVA
		0	21								
Bauer	C57BL/6	-21	21	0.5,5,50,500	gavage	100 µg x 2 i.t.	6w	1% aerosol 1hr	8w	8w	↓Nt in BALF in male BPA50/OVA woLPS
2012						w/wo 100 ng LPS		1d			~Eos in BALF in male/femle BPA/OVA
mucosal sensitization											↑lung histology in female high BPA/OVA wo LPS and female BPA/OVA w LPS ↑lung histology in male BPA/OVA wo LPS
Bauer	C57BL/6	-21	21	0.5,5,50,500	gavage	100 µg x 2 i.p.	6w	1% aerosol 1hr	7-11w	7-11w	↓Eos in BALF in female BPA0.5, 5, 50/OVA
2013								1d			~Eos in BALF in female BPA500/OVA
peritoneal sensitization											↓serum IgE anti-OVA in female BPA/OVA ~AHR in female BPA/OVA
Petzold	BALB/c	-28	21	1,000	drinking	20 µg x 2 i.p.	6&8w	20 µg/40 µL i.n.	8&9w	9 w	~AHR
2014								6d			~Eos in BALF ~serum IgE anti-OVA ~IL-4, IL-5, IL-13, IFNγfrom splenocytes
O'Brien	BALB/c	-35	21	0.008, 8, 8,400	chow	20 µg x 1 i.p.	12w	3% aerosol 20min	13w	13w	↑serum IgE anti-OVA in low BPA
2014								x2d			↑↑serum IgE anti-OVA in moderate and high BPA
											↑IL-13 from splenocyte from moderate & high BPA/OVA
											↑↑↑ IFNγ from splenocyte from BPA/OVA
											~IL-4, IL-5, TNFa from splenocytes from BPA/OVA
											↑total Leu in BALF in female high BPA/OVA
											↓total Leu in BALF in male low & high BPA/OVA
											↓Eos female high BPA/OVA
											~Mp, PMN, Ly in females BPA/OVA
											↓Mp, PMN, Eos in male low BPA/OVA
											↓PMN, Ly in male high BPA/OVA
											↓PMN in male moderate BPA/OVA
											↓IL-4, IL-13, TNFa in BALF from female low and high BPA/OVA
											~IL-4, IL-13, TNFa in BALF from male low and high BPA/OVA
											↓IL-17 in BALF from BPA/OVA
											↓CysLTs in BALF from high BPA/OVA
											~Eotaxin-1 in BALF from BPA/OVA
											↑RANTES in lung homogenate in low BPA/OVA
											no change in female lung histology
											↓inflammatory score in male high BPA/OVA
Nygaard	BAL B/c	0	21	1,400-4,500	drinking	10 µg x 2 i.p.	4&18d	10 µg i.n.	25d	30d	↑Eos in BALF in 100µg/ml
2015	OlaHsd			&14,000-44,000	wo adjuvant						↑trend serum IgE anti-OVA in 100µg/ml
											~serum IgG1, IgG2 anti-OVA
											non detectable IL-5, IL-10, IL-17, IFNγ in BALF
											~IL-4, IL-13, IL-10, IL-2, IL-17, IFNγ from splenocytes

Bauer et al. [31] and Petzold et al. [32], also examined the effects of maternal (prenatal) BPA exposure on their mouse pups, but did not evaluate the offspring for asthma manifestations until they were in their adolescent and adults. Bauer et al. gave 0.5, 5, 50 or 500 µg/Kg BW/day of BPA by gavage to C57BL/6 mice while Petzold gave 1,000 µg/Kg BW/day of BPA in their drinking water in BALB/c (F0) mice during their pregnancy and until the pups were 3 weeks old. They then (at 3 weeks) sensitized these pups with 100 µg of endotoxin-depleted OVA with or without

E. coli lipopolysaccharide (LPS) intratracheally (i.t.) three times, followed two weeks later by aerosol OVA challenge twice daily for three days (mucosal sensitization model), or with two injections of 100 µg OVA by i.p., when the adult offspring were 6-8 weeks old and then 1% OVA aerosol at 7-11 postnatal weeks of age (peritoneal sensitization model), and analyzed AHR, eosinophils in BALF and lung histology. Since BALB/c mice are sexually mature around 28-49 days (average 35 days) [37], these are considered adult asthma models. They found that adult female offspring demonstrated enhanced whole-lung inflammation, compared with vehicle treated controls in this mucosal sensitization model. No effect was seen in males. The $CD4^+$, $CD8^+$ and $CD4^+CD25^+FoxP3^+$ lymphocyte numbers were not significantly changed, compared to the vehicle controls in neither females nor males. Both groups did find a decrease in the number of eosinophil in BALF in the females that had received with 0.5, 5, 50 µg/Kg BW/day BPA exposures, but not in the males. The results of AHR measurements by invasive plethysmography were not different between the groups in the peritoneal sensitization model. When they examined lung histology, only adult female offspring consistently demonstrated enhanced whole-lung inflammation when compared with the controls.

Petzold et al. gave 5 µg/ml of BPA in the drinking water of their pregnant BALB/c females (F0) and continued this until 3 weeks after they delivered their pups. Their adult offspring were sensitized with 20 µg OVA by i.p. injection at 6 and 20 weeks of age, and 40 µg of nasal OVA at 8 and 9 weeks old. At 9 weeks of age their AHR, eosinophil number in BALF, OVA-specific IgE and cytokine production from splenocytes were assessed. They did not find any effects of these maternal BPA exposures on the development of asthma in these adult offspring. No histological data are described for the mice with pre- and perinatal exposure to BPA.

O'Brien et al., gave 50 ng, 50 µg or 50 mg/Kg (low, moderate and high) doses of BPA in rodent chow to BALB/c mice, starting from 2 weeks before mating, until the weaning of the offspring [33]. These BPA doses translate into about 8 ng, 8 µg and 8 mg BPA/Kg BW/day, based on the chow consumption data from Bachmanov [38]. They then analyzed the effects of these three different doses of BPA on the serum IgE anti-OVA antibodies, cytokine and chemokine production, differential cell numbers and lung histology, in their male and female offspring. They detected modest increases in serum IgE anti-OVA in low BPA exposure group and 2-fold increase in moderate and high BPA exposed groups. IL-13 was increase in the moderate BPA exposure group and IFN^{γ} production from splenocytes from all the BPA exposure groups was increased. Total leukocyte number in the BALF from female in their high BPA exposure group, and RANTES in lung homogenate in low BPA exposure groups were increased.

Nygaard et al., fed 0, 10, or 100 µg/ml BPA in the maternal drinking water to BALB/c OlaHsd mice (about 0, 2 or 20 mg BPA/KgBW/day). Their drinking water consumption was about 3 ml/day during gestation and 12 ml/day during lactation. They used these data to calculate BPA intakes: 1.4 (during gestation) and 14 (during lactation) mg/Kg BW/day for those who received 10 µg/ml BPA in their drinking water and 14 during (gestation) and 44 (lactation) for those receiving 100 µg/ml BPA. The offspring were sensitized with 10 µg OVA by i.p. injection, without any adjuvant, on PND 4 and 18, and on PND 25, 10 µg OVA i.n. The pups were analyzed for the number of eosinophil in

their BALF, serum IgE, IgG1, IgG2 anti-OVA antibodies, and cytokine concentrations in BALF and cytokine production from splenocytes on PND 30. They detected significant increases of eosinophil numbers in BALF and a trend of high serum IgE anti-OVA in the 100 μg/ml exposure group.

Potential mechanism of effects of early BPA exposure

Fetal/newborn cells are skewed toward a Th2 pattern, partially due to low production of IL12 and the propensity of Th1 cells to undergo apoptosis after antigen exposure [39,40]. "Immune-maturing" infections promote a subsequent shift toward Th1 responses in most children, while an increasing proportion remain prone to develop pathological Th2 responses [41]. Most cases of asthma develop in early childhood [42] and often continue into adulthood, with a very large impact on public health and health care delivery. Thus a better understanding of the roles of genetics and environmental exposures during early life is very important [43].

Studies have shown the effects of BPA on the epigenetic alterations on several body systems in humans and rodent. These are focused mainly on endocrine/reproductive/metabolic system, [44-53] developmental cells, [51,54-57] cancer, neuro/behaviour [58,59] and cardiac [60]. Genome-wide CpG methylation analyses of "saliva DNA" from 60 Egyptian girls aged 10-13 and urinary BPA in spot samples shows general association of higher urinary BPA concentration with less genomic methylation [61]. The pathway analyses shows reduced methylation involvement in immune function with increasing urinary BPA in this study.

Non-genomic signaling is a potentially important mechanism of the effects of environmental estrogen exposure, which induces epigenetic modifications [62,63]. This response is initiated by ligand binding to receptors, ERα, ERβ or G protein coupled receptor (GPR)30, and activating signaling cascades that eventually alter kinase activities [62]. These kinases, phospho AKT, protein kinase A (PKA), protein kinase C (PKC) and extracellular signal-regulated kinase (ERK) phosphorylate one of the polycomb proteins, a histone methyltransferase (HMT) termed EZH2, [62,64] enhancer of zeste homolog 2 (EZH2) preferentially methylates histone H3K27, thus generating a binding site for the polycomb repressive complex (PRC)1 [65-68]. EZH2 also recruits DNA methyltransferase (DNMT) [68] and is a member of PRC2. Our group detected ERα on T-cells, mast cells and basophils [69,70]. The role of the PRC2 components in T-cell differentiation is somewhat controversial with observations indicating that they can have either activating [71,72] or repressive activities [73-76]. If these alterations persist, they can produce permanent epigenetic consequences (DNA methylation) within those sequences, therefore causing the development of asthma.

DISCUSSION

We have summarized here the results from eight human epidemiological studies and six experimental mouse models, all of which are related to the effect of early life BPA exposure on the development of allergic asthma. The reason for summarizing these together was our recognition that both experimental approaches have strengths and weaknesses in understanding the effects of BPA, and potentially other environmental estrogens, on the development of asthma in humans. For instance, direct human studies are the most important for understanding the relationship between exposures to chemicals like BPA at various phases of life, and the development and timing and manifestations of asthma. However, human studies have the drawback that their measurements of urinary BPA in the children may only be a biomarker for exposure to other chemicals that co-exist with BPA in certain environments. Further, while urinary measurements and excretion rates of chemicals like BPA are convenient, they only represent the conjugated (water soluble) BPA, which our group and others have shown are not active in cellular assays, most likely because they cannot bind to ERs [17,77].

Carefully controlled and performed animal studies should overcome some of these shortcomings, since the major difference between the experimental and control groups should be the extent of their exposure to purified BPA. While we and others have tried to carefully design the studies to closely imitate the human exposure, by delivering appropriate amounts of BPA through the oral route to achieve burdens of free BPA in blood and tissues of genetically susceptible mice, it is also important to consider the appropriate time for testing for the asthma phenotype. Further, the choice of the strain of mice to be used in these studies should match the genetic susceptibility to allergic disease of humans at the same stage of development. Thus, it is not surprising that results from the more sensitive strain of mice might be more likely to recapitulate findings in human infants that are known to be susceptible to allergic sensitization.

Thus, one of the reasons for reviewing mouse experiments and comparing their findings with human epidemiological studies was to begin to confirm the relevance of the human studies well enough to consider hypothetical mechanisms for these common disease features and plan experiment to test these. For instance, this may be the case when we compare the results of our and Nygaard's mouse studies with those of the human studies of Spanier et al. [21]. It may also be useful to look at the effect of postnatal BPA exposure of humans (e.g. Vaidya) in the context of current mouse studies that assessed the effect of BPA on asthma outcomes in childhood, adolescences and adults life. Clearly more thought and the development of new study designs may improve our understanding of this important process.

Yet another approach that we and others are embarking on is to define the mechanistic steps between pre and postnatal BPA exposures and the development of asthma. We anticipate that this approach may also define biomarkers of BPA's effects, which will be consistent between humans and mice. Preliminary results from these approaches suggest that exposure to BPA, especially during the prenatal period, may cause epigenetic alterations that could last throughout the lifetime of the mice and perhaps humans and even be passed to subsequent generations. Such studies may also help to explain some to the age and gender difference that are so prominent in human asthma, which starts as a male dominant disease in childhood, but are overtaken by women around the time of their menarche. This pattern may explain some of the gender effects in several of the human studies of pre and postnatal BPA exposures [19,23].

CONCLUSION

We have reviewed here the effects of early BPA exposure on allergic asthma development in mouse models and human epidemiological studies. The effects of early BPA exposure were observed as an enhanced development of asthma before adolescent in the animal model. All of the human studies suggest that BPA exposure in early life enhances the likelihood of developing asthma on at least one of the study groups. In contrast, maternally derived BPA exposures in the various mouse systems seem to manifest themselves only during infancy. Comparing the age of wheezing illness in humans, suggest that BPA exposure might be an important component in bronchiolitis, a wheezing illness associated with concurrent viral infections, as well a risk factor the later development of asthma. Further studies on the timing of the onset of asthma as well as the molecular mechanisms of BPA's effect may help in planning approaches for preventing these very common human diseases.

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REFERENCES

1. Kahr N, Naeser V, Stensballe LG, Kyvik KO, Skytthe A, et al. Gene-environment interaction in atopic diseases: a population-based twin study of early-life exposures. Clin Respir J. 2015; 9: 79-86. **Ref.:** https://goo.gl/LnKi1t

2. Bonds RS, Midoro-Horiuti T. Estrogen effects in allergy and asthma. Curr Opin Allergy Clin Immunol. 2013; 13: 92-99. **Ref.:** https://goo.gl/1sEYTe

3. Ng SC, Gilman-Sachs A, Thaker P, Beaman KD, Beer AE, et al. Expression of intracellular Th1 and Th2 cytokines in women with recurrent spontaneous abortion, implantation failures after IVF/ET or normal pregnancy. Am J Reprod Immunol. 2002; 48: 77-86. **Ref.:** https://goo.gl/edikvY

4. Piccinni MP, Maggi E, Romagnani S. Role of hormone-controlled T-cell cytokines in the maintenance of pregnancy. Biochem Soc Trans. 2000; 28: 212-215. **Ref.:** https://goo.gl/3JnN5m

5. Iwata M, Eshima Y, Kagechika H, Miyaura H. The endocrine disruptor's nonylphenol and octylphenol exert direct effects on T cells to suppress Th1 development and enhance Th2 development. Immunol Lett. 2004; 94: 135-139. **Ref.:** https://goo.gl/gEpexT

6. Yan H, Takamoto M, Sugane K. Exposure to Bisphenol A prenatally or in adulthood promotes T (H) 2 cytokine production associated with reduction of CD4CD25 regulatory T cells. Environ Health Perspect. 2008; 116: 514-519. **Ref.:** https://goo.gl/AQ3REY

7. Cai Y, Zhou J, Webb DC. Estrogen stimulates Th2 cytokine production and regulates the compartmentalisation of eosinophils during allergen challenge in a mouse model of asthma. Int Arch Allergy Immunol. 2012; 158: 252-260. **Ref.:** https://goo.gl/q8F5EQ

8. Zincke T. Mittheilungen aus dem chemischen Laboratorium der Universitat Marburg. Justus Leibigs Annals Chemie. 1905; 343: 75-99.

9. Dodds Ec, Huang Rl, Lawson W, Robinson R. Synthetic oestrogenic compounds related to stilbene and diphenylethane. III. Proc R Soc Lond B Biol Sci. 1953; 140: 470-497. **Ref.:** https://goo.gl/bwPkuJ

10. Akinbami LJ, Moorman JE, Liu X. Asthma prevalence, health care use, and mortality: United States, 2005-2009. Natl Health Stat Report. 2011; 1-14. **Ref.:** https://goo.gl/Vb5s66

11. Tilson HA. Collection Bisohenol A (BPA). Env Health Perspectives. 2012; 1-37.

12. Hines EP, Mendola P, von Ehrenstein OS, Ye X, Calafat AM, et al. Concentrations of environmental phenols and parabens in milk, urine and serum of lactating North Carolina women. Reprod Toxicol. 2015; 54: 120-128. **Ref.:** https://goo.gl/ahcDqZ

13. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). Reprod Toxicol. 2007; 24: 139-177. **Ref.:** https://goo.gl/oZQTtA

14. Lv Y, Lu S, Dai Y, Rui C, Wang Y, et al. Higher dermal exposure of cashiers to BPA and its association with DNA oxidative damage. Environ Int. 2017; 98: 69-74. **Ref.:** https://goo.gl/FfKhXY

15. Hormann AM, Vom Saal FS, Nagel SC, Stahlhut RW, Moyer CL, et al. Holding thermal receipt paper and eating food after using hand sanitizer results in high serum bioactive and urine total levels of bisphenol A (BPA). PLoS One. 2014; 9: e110509. **Ref.:** https://goo.gl/DCiDgm

16. Nakajima Y, Goldblum RM, Midoro-Horiuti T. Fetal exposure to bisphenol A as a risk factor for the development of childhood asthma: an animal model study. Environ Health. 2012; 11: 1-7. **Ref.:** https://goo.gl/Th2j3o

17. Vinas R, Goldblum RM, Watson CS. Rapid estrogenic signaling activities of the modified (chlorinated, sulfonated, and glucuronidated) endocrine disruptor bisphenol A. Endocrine Disruptors. 2013; 1: e25411. **Ref.:** https://goo.gl/BWoQJ5

18. Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgartten FJ, et al. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ Health Perspect. 2010; 118: 1055-1070. **Ref.:** https://goo.gl/UDJAQk

19. Vaidya SV, Kulkarni H. Association of Urinary Bisphenol A Concentration with Allergic Asthma: Results from the National Health and Nutrition Examination Survey 2005-2006. J Asthma. 2012; 49: 800-806. **Ref.:** https://goo.gl/4D6xWP

20. Spanier AJ, Kahn RS, Kunselman AR, Hornung R, Xu Y, et al. Prenatal exposure to bisphenol a and child wheeze from birth to 3 years of age. Environ Health Perspect. 2012; 120: 916-920. **Ref.:** https://goo.gl/fCj5PZ

21. Spanier AJ, Kahn RS, Kunselman AR, Schaefer EW, Hornung R, et al. Bisphenol A Exposure and the Development of Wheeze and Lung Function in Children Through Age 5 Years. JAMA Pediatr. 2014; 168: 1131-1137. **Ref.:** https://goo.gl/HkVeRi

22. Donohue KM, Miller RL, Perzanowski MS, Just AC, Hoepner LA, et al. Prenatal and postnatal bisphenol A exposure and asthma development among inner-city children. J Allergy Clin Immunol. 2013; 131: 726-742. **Ref.:** https://goo.gl/rcN1Pc

23. Kim K, Kim J, Kwon H, Hong S, Kim B, et al. Bisphenol A Exposure and Asthma Development in School-Age Children: A Longitudinal Study. PLOS One. 2014b; 9: e111383. **Ref.:** https://goo.gl/ab8Tus

24. Whyatt RM, Rundle AG, Perzanowski MS, Just AC, Donohue KM, et al. Prenatal phthalate and early childhood bisphenol A exposures increase asthma risk in inner-city children. J Allergy Clin Immunol. 2014; 134: 1195-1197. **Ref.:** https://goo.gl/YJtU2y

25. Gascon M, Casas M, Morales E, Valvi D, Ballesteros-Gomez A, et al. Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy. J Allergy Clin Immunol. 2015; 135: 370-378. **Ref.:** https://goo.gl/NYK335

26. Wang IJ, Chen CY, Bornehag CG. Bisphenol A exposure may increase the risk of development of atopic disorders in children. Int J Hyg Environ Health. 2016; 219: 311-316. **Ref.:** https://goo.gl/vm1CEs

27. Kim JH, Sartor MA, Rozek LS, Faulk C, Anderson OS, et al. Perinatal bisphenol A exposure promotes dose-dependent alterations of the mouse methylome. BMC Genomics. 2014a; 15: 30. **Ref.:** https://goo.gl/G4fCRz

28. Larsson K, Ljung BK, Palm B, Wennberg M, Kaj L, et al. Exposure determinants of phthalates, parabens, bisphenol A and triclosan in Swedish mothers and their children. Environ Int. 2014; 73: 323-333. **Ref.:** https://goo.gl/Tm5smM

29. Covaci A, Hond ED, Geens T, Govarts E, Koppen G, et al. Urinary BPA measurements in children and mothers from six European member states: Overall results and determinants of exposure. Environ Res. 2015; 141: 77-85. **Ref.:** https://goo.gl/999Gex

30. Midoro-Horiuti T, Tiwari R, Watson CS, Goldblum RM. Maternal exposure to bisphenol A enhances susceptibility to experimental allergic asthma in their pups. Env Health Perspectives. 2010; 118: 273-277.

31. Bauer SM, Roy A, Emo J, Chapman TJ, Georas SN, et al. The effects of maternal exposure to bisphenol A on allergic lung inflammation into adulthood. Toxicol Sci. 2012; 130: 82-93. **Ref.:** https://goo.gl/rHdZxY

32. Petzold S, Averbeck M, Simon JC, Lehmann I, Polte T. Lifetime-dependent effects of bisphenol A on asthma development in an experimental mouse model. PLoS One. 2014; 9: e100468. **Ref.:** https://goo.gl/7h8PLJ

33. O'Brien E, Bergin IL, Dolinoy DC, Zaslona Z, Little RJ, et al. Perinatal bisphenol A exposure beginning before gestation enhances allergen sensitization, but not pulmonary inflammation, in adult mice. J Dev Orig Health Dis. 2014; 5: 121-131. **Ref.:** https://goo.gl/Qcr4zF

34. Nygaard UC, Vinje NE, Samuelsen M, Andreassen M, Groeng EC, et al. Early life exposure to bisphenol A investigated in mouse models of airway allergy, food allergy and oral tolerance. Food Chem Toxicol. 2015; 83: 17-25. **Ref.:** https://goo.gl/E65agS

35. Leme AS, Hubeau C, Xiang Y, Goldman A, Hamada K, et al. Role of breast milk in a mouse model of maternal transmission of asthma susceptibility. J Immunol. 2006; 176: 762-769. **Ref.:** https://goo.gl/4FnZaH

36. Midoro-Horiuti T. Environmental estrogens and allergy. Japanese Journal of Pediatric Intaractable Asthma and Allergic Diseases. 2014; 12: 311-314.

37. Bronson FH, Dagg CP, Snell GD. Reproduction. In: Green,E.L. (Ed.), Biology of the Laboratory Mouse. Dover Publications, INC. New York. 2007.

38. Bachmanov AA, Reed DR, Beauchamp GK, Tordoff MG. Food intake, water intake, and drinking spout side preference of 28 mouse strains. Behav Genet. 2002; 32: 435-443. **Ref.:** https://goo.gl/SQPtVE

39. Allam JP, Zivanovic O, Berg C, Gembruch U, Bieber T, et al. In search for predictive factors for atopy in human cord blood. Allergy. 2005; 60: 743-750. **Ref.:** https://goo.gl/Jcsbez

40. Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. J Immunol. 1998; 160: 4730-4737. **Ref.:** https://goo.gl/dV5Mpu

41. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, et al. Development of allergen-specific T-cell memory in atopic and normal children. Lancet. 1999; 353: 196-200. **Ref.:** https://goo.gl/Q6s9MX

42. Gern JE, Lemanske RF Jr, Busse WW. Early life origins of asthma. J Clin Invest. 1999; 104: 837-843. **Ref.:** https://goo.gl/gvTu2v

43. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. Allergy. 2000; 55: 688-697. **Ref.:** https://goo.gl/unJVx8

44. Strakovsky RS, Wang H, Engeseth NJ, Flaws JA, Helferich WG, et al. Developmental bisphenol A (BPA) exposure leads to sex-specific modification of hepatic gene expression and epigenome at birth that may exacerbate high-fat diet-induced hepatic steatosis. Toxicol Appl Pharmacol. 2015; 284: 101-112. **Ref.:** https://goo.gl/wiZny4

45. Maccani MA, Marsit CJ. Exposure and fetal growth-associated miRNA alterations in the human placenta. Clin Epigenetics. 2011; 2: 401-404. **Ref.:** https://goo.gl/LiR6SY

46. Dhimolea E, Wadia PR, Murray TJ, Settles ML, Treitman JD, et al. Prenatal exposure to BPA alters the epigenome of the rat mammary gland and increases the propensity to neoplastic development. PLoS One. 2014; 9: e99800. **Ref.:** https://goo.gl/wM6NP9

47. Li G, Chang H, Xia W, Mao Z, Li Y, et al. F0 maternal BPA exposure induced glucose intolerance of F2 generation through DNA methylation change in Gck. Toxicol Lett. 2014; 228: 192-199. **Ref.:** https://goo.gl/Fj28CP

48. Bastos SL, Kamstra JH, Cenijn PH, van Rijt LS, Hamers T, et al. Effects of endocrine disrupting chemicals on *In Vitro* global DNA methylation and adipocyte differentiation. Toxicol *In Vitro*. 2013; 27: 1634-1643. **Ref.:** https://goo.gl/5H6LWe

49. Warita K, Mitsuhashi T, Ohta K, Suzuki S, Hoshi N, et al. Gene expression of epigenetic regulatory factors related to primary silencing mechanism is less susceptible to lower doses of bisphenol A in embryonic hypothalamic cells. J Toxicol Sci. 2013; 38: 285-289. **Ref.:** https://goo.gl/sCo2Hj

50. Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK. Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. PLoS One. 2013; 8: e55387. **Ref.:** https://goo.gl/DSbpEx

51. Doshi T, Mehta SS, Dighe V, Balasinor N, Vanage G. Hypermethylation of estrogen receptor promoter region in adult testis of rats exposed neonatally to bisphenol A. Toxicology. 2011; 289: 74-82. **Ref.:** https://goo.gl/8AFvsp

52. Bromer JG, Zhou Y, Taylor MB, Doherty L, Taylor HS. Bisphenol-A exposure in utero leads to epigenetic alterations in the developmental programming of uterine estrogen response. FASEB J. 2010; 24: 2273-2280. **Ref.:** https://goo.gl/kiUQgb

53. Doherty LF, Bromer JG, Zhou Y, Aldad TS, Taylor HS. In utero exposure to diethylstilbestrol (DES) or bisphenol-A (BPA) increases EZH2 expression in the mammary gland: an epigenetic mechanism linking endocrine disruptors to breast cancer. Horm Cancer. 2010; 1: 146-155. **Ref.:** https://goo.gl/ak8jsj

54. Trapphoff T, Heiligentag M, El Hajj N, Haaf T, Eichenlaub-Ritter U. Chronic exposure to a low concentration of bisphenol A during follicle culture affects the epigenetic status of germinal vesicles and metaphase II oocytes. Fertil Steril. 2013; 100: 1758-1767. **Ref.:** https://goo.gl/4HikbX

55. Doshi T, D'souza C, Dighe V, Vanage G. Effect of neonatal exposure on male rats to bisphenol A on the expression of DNA methylation machinery in the postimplantation embryo. J Biochem Mol Toxicol. 2012; 26: 337-343. **Ref.:** https://goo.gl/wWeH99

56. Avissar-Whiting M, Veiga KR, Uhl KM, Maccani MA, Gagne LA, et al. Bisphenol A exposure leads to specific microRNA alterations in placental cells. Reprod Toxicol. 2010; 29: 401-406. **Ref.:** https://goo.gl/AyHLzH

57. Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci USA. 2007; 104: 13056-13061. **Ref.:** https://goo.gl/Sejh9t

58. Yeo M, Berglund K, Hanna M, Guo JU, Kittur J, et al. Bisphenol A delays the perinatal chloride shift in cortical neurons by epigenetic effects on the Kcc2 promoter. Proc Natl Acad Sci USA. 2013; 110: 4315-4320. **Ref.:** https://goo.gl/TmygRn

59. Yaoi T, Itoh K, Nakamura K, Ogi H, Fujiwara Y, et al. Genome-wide analysis of epigenomic alterations in fetal mouse forebrain after exposure to low doses of bisphenol A. Biochem Biophys Res Commun. 2008; 376: 563-567. **Ref.:** https://goo.gl/3BLehT

60. Patella V, Giuliano A, Bouvet JP, Marone G. Endogenous superallergen protein Fv induces IL-4 secretion from human Fc epsilon RI+ cells through interaction with the VH3 region of IgE. J Immunol. 1998; 161: 5647-5655. **Ref.:** https://goo.gl/DxR4TP

61. Kim JH, Rozek LS, Soliman AS, Sartor MA, Hablas A, et al. Bisphenol A-associated epigenomic changes in prepubescent girls: a cross-sectional study in Gharbiah, Egypt. Environ Health. 2013; 12: 12-33. **Ref.:** https://goo.gl/UBMptm

62. Wong RL, Walker CL. Molecular pathways: environmental estrogens activate nongenomic signaling to developmentally reprogram the epigenome. Clin Cancer Res. 2013; 19: 3732-3737. **Ref.:** https://goo.gl/R7FR59

63. Jeng YJ, Watson CS. Combinations of physiologic estrogens with xenoestrogens alter ERK phosphorylation profiles in rat pituitary cells. Environ Health Perspect. 2011; 119: 104-112. **Ref.:** https://goo.gl/boz1T3

64. Chang LC, Lin HY, Tsai MT, Chou RH, Lee FY, et al. YC-1 inhibits proliferation of breast cancer cells by down regulating EZH2 expression via activation of c-Cbl and ERK. Br J Pharmacol. 2014; 171: 4010-4025. **Ref.:** https://goo.gl/gVLMmS

65. Muller J, Hart CM, Francis NJ, Vargas ML, Sengupta A, et al. Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. Cell. 2002; 111: 197-208. **Ref.:** https://goo.gl/iT9CyF

66. Kuzmichev A, Nishioka K, Erdjument-Bromage H, Tempst P, Reinberg D. Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. Genes Dev. 2002; 16: 2893-2905. **Ref.:** https://goo.gl/jS6fmB

67. Czermin B, Melfi R, McCabe D, Seitz V, Imhof A, et al. Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. Cell. 2002; 111: 185-196. **Ref.:** https://goo.gl/mLmecg

68. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, et al. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science. 2002; 298: 1039-1043. **Ref.:** https://goo.gl/BuhaKb

69. Lambert KC, Curran EM, Judy BM, Milligan GN, Lubahn DB, et al. Estrogen Receptor {alpha} (ER{alpha}) Deficiency in Macrophages Results in Increased Stimulation of CD4+ T Cells while 17{beta}-Estradiol Acts through ER{alpha} to Increase IL-4 and GATA-3 Expression in CD4+ T Cells Independent of Antigen Presentation. J Immunol. 2005; 175: 5716-5723. **Ref.:** https://goo.gl/nkpqej

70. Zaitsu M, Narita S, Lambert KC, Grady JJ, Estes DM, et al. Estradiol activates mast cells via a non-genomic estrogen receptor-alpha and calcium influx. Mol Immunol. 2007; 44: 1987-1995. **Ref.:** https://goo.gl/s2idwm

71. He S, Xie F, Liu Y, Tong Q, Mochizuki K, et al. The histone methyltransferase Ezh2 is a crucial epigenetic regulator of allogeneic T-cell responses mediating graft-versus-host disease. Blood. 2013; 122: 4119-4128. **Ref.:** https://goo.gl/4yG6zo

72. Jacob E, Hod-Dvorai R, Schif-Zuck S, Avni O. Unconventional association of the polycomb group proteins with cytokine genes in differentiated T helper cells. J Biol Chem. 2008; 283: 13471-13481. **Ref.:** https://goo.gl/QDd14d

73. Tumes DJ, Onodera A, Suzuki A, Shinoda K, Endo Y, et al. The polycomb protein Ezh2 regulates differentiation and plasticity of CD4(+) T helper type 1 and type 2 cells. Immunity. 2013; 39: 819-832. **Ref.:** https://goo.gl/EpeHw1

74. Koyanagi M, Baguet A, Martens J, Margueron R, Jenuwein T, et al. EZH2 and histone 3 trimethyl lysine 27 associated with Il4 and Il13 gene silencing in Th1 cells. J Biol Chem. 2005; 280: 31470-31477. **Ref.:** https://goo.gl/HjZGog

75. Bredfeldt TG, Greathouse KL, Safe SH, Hung MC, Bedford MT, et al. Xenoestrogen-induced regulation of EZH2 and histone methylation via estrogen receptor signaling to PI3K/AKT. Mol Endocrinol. 2010; 24: 993-1006. **Ref.:** https://goo.gl/2KbVSw

76. Greathouse KL, Bredfeldt T, Everitt JI, Lin K, Berry T, et al. Environmental estrogens differentially engage the histone methyltransferase EZH2 to increase risk of uterine tumorigenesis. Mol Cancer Res. 2012; 10: 546-557. **Ref.:** https://goo.gl/ztTtxa

77. Shimizu M, Ohta K, Matsumoto Y, Fukuoka M, Ohno Y, et al. Sulfation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF7 cells. Toxicol *In Vitro*. 2002; 16: 549-556. **Ref.:** https://goo.gl/tPxh8V

78. Fedulov AV, Kobzik L. Allergy risk is mediated by dendritic cells with congenital epigenetic changes. Am J Respir Cell Mol Biol. 2011; 44: 285-292. **Ref.:** https://goo.gl/kUEGHz

79. Hollingsworth JW, Maruoka S, Boon K, Garantziotis S, Li Z, et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. J Clin Invest. 2008; 118: 3462-3469. **Ref.:** https://goo.gl/438pQB

80. Liu J, Ballaney M, Al-alem U, Quan C, Jin X, et al. Combined inhaled diesel exhaust particles and allergen exposure alter methylation of T helper genes and IgE production in vivo. Toxicol Sci. 2008; 102: 76-81. **Ref.:** https://goo.gl/pCAKzp