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Early life exposure to bisphenol A investigated in mouse models of airway allergy, food allergy and oral tolerance

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ABSTRACT

The impact of early life exposure to bisphenol A (BPA) through drinking water was investigated in mouse models of respiratory allergy, food allergy and oral tolerance. Balb/c mice were exposed to BPA (0, 10 or 100 μg/ml), and the offspring were intranasally exposed to the allergen ovalbumin (OVA). C3H/HeJ offspring were sensitized with the food allergen lupin by intragastric gavage, after exposure to BPA (0, 1, 10 or 100 μg/ml). In separate offspring, oral tolerance was induced by gavage of 5 mg lupin one week before entering the protocol for the food allergy induction. In the airway allergy model, BPA (100 μg/ml) caused increased eosinophil numbers in bronchoalveolar lavage fluid (BALF) and a trend of increased OVA-specific IgE levels. In the food allergy and tolerance models, BPA did not alter the clinical anaphylaxis or antibody responses, but induced alterations in splenocyte cytokines and decreased mouse mast cell protease (MMCP)-1 serum levels. In conclusion, early life exposure to BPA through drinking water modestly augmented allergic responses in a mouse model of airway allergy only at high doses, and not in mouse models for food allergy and tolerance. Thus, our data do not support that BPA promotes allergy development at exposure levels relevant for humans.

1. Introduction

Bisphenol A (BPA), an endocrine disruptor with estrogenic activity, is a commonly used component in polycarbonate plastic and epoxy resins. It has been detected at low levels in food, beverages, cigarette filters, as well as in soap and detergents (Dodson et al., 2012; Sakhi et al., 2014). Exposure of humans is ubiquitous, and BPA has been detected in more than 95% of all urine samples analysed in some epidemiological studies (Vandenberg et al., 2010). The major source for human BPA exposure is via food and beverages, while exposure through dust ingestion, dental surgery and dermal absorption from thermal paper has been estimated to remain below 5% (Geens et al., 2012).

Airway and food allergies have prevalences of about 5–25% and 5–10%, respectively, and there has been an increase in the prevalence of allergies worldwide during the last centennial (Pawankar et al., 2011; Sicherer and Sampson, 2014). The increased incidence of allergic disease has been linked to changes in human lifestyle and the environment, such as various components in our diet and outdoor and indoor air, and is also coinciding with increased exposures to endocrine disruptors (ED). Allergic individuals mount Th2–dominated responses and specific IgE antibodies against proteins due to a failure of the normal tolerance responses seen in healthy individuals. Compounds that contribute to irritation, inflammation, altered microflora, altered allergen processing and presentation, or directly alter immune cell function or differentiation via other mechanisms, may contribute to break of tolerance, and thereby promote development of allergic responses towards certain proteins (i.e. act as adjuvants). Since the immune system

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develops throughout early life, both *in utero* and early life exposure to compounds affecting the immune system can give long-lasting adverse consequences (Dietert and Zelikoff, 2008).

Possible negative effects of BPA exposure on human health are widely debated, partly because of the very low levels of BPA detected in different food items, diverging results from epidemiological studies and occasional non-monotone dose–response curves in experimental studies (Casey and Neidell, 2013; EFSA, 2015; FitzGerald and Wilks, 2014). Also with regard to immune-related endpoints, epidemiological and experimental studies are inconsistent, but several authors have reported associations between urinary BPA concentrations in mother or child and the incidence of asthma and allergy (Donohue et al., 2013; Gascon et al., 2014; Kim et al., 2014; Spanier et al., 2012; Vaidya and Kulkarni, 2012). In experimental rodent studies, BPA has been reported to enhance allergic airway inflammation in mouse allergy models (Bauer et al., 2012; Midoro-Horiuti et al., 2010; Nakajima et al., 2012; O'Brien et al., 2014a; Petzold et al., 2014), impair non-allergic food tolerance induction (IgG) or certain mechanisms underlying oral food allergy tolerance (Menard et al., 2014b; Ohshima et al., 2007; Yamashita et al., 2003) and accelerate autoimmune diabetes development in non-obese diabetic (NOD) mice (Bodin et al., 2014, 2013). However, an impact of BPA on the development of food allergy, or tolerance tested in a food allergy model, have to our knowledge not been investigated. The risk of human adverse effects of BPA exposure has recently been critically reviewed by the European Food Safety Authority (EFSA), concluding with a lowering of the recommended tolerable daily intake level of 50 μg/kg body weight per day to 5 μg/kg body weight per day (EFSA, 2015). Recent studies report immune effects at BPA levels at or below this exposure level, supporting the need for further insight into adverse immune effects of BPA (Menard et al., 2014a, 2014b).

In this study, we further investigated the hypothesis that pre- and postnatal BPA exposure promotes allergy development. The impact of early life exposure to different doses of BPA in the drinking water was investigated in three different mouse models related to allergy development; i) an airway allergy model with an intraperitoneal (i.p.) OVA sensitization protocol without adjuvant followed by intranasal OVA challenge, ii) a lupin food allergy model using an intragastric (i.g.) sensitization regime followed by allergen challenge i.p. or i.g., iii) a model inducing oral tolerance to the induction of lupin food allergy.

2. Material and methods

2.1. Study design

2.1.1. Airway allergy model

In a previous study of interactions between allergen dose, sex and age in an airway model, Hansen et al., 2011a Hansen et al., 2011b found that the optimal OVA allergen dose for mice being immunized for the first time at 1-week of age was 10 μg. In the present study, we therefore used this dose, but without the use of adjuvant to induce a suboptimal sensitization allowing detection of a potential increased sensitization by the BPA exposure. BALB/c dams were exposed to BPA (0, 10 and 100 μg/ml) in drinking water from time of mating and until the end of the lactation period. Female and male Balb/c offspring (n = 14–15) were weighed at 5 weeks of age, and went through the suboptimal sensitisation protocol administering 10 μg OVA intraperitoneally (i.p.) without adjuvant at postnatal days (PND) 4 and 18. At PND 25 they were anaesthetized with isoflurane and challenged with 10 μg OVA intranasally (i.n.). Five days later (PND30), the mice were anaesthetized with ZRF before the chest was opened and blood sample drawn by heart puncture. The spleen and bronchoalveolar lavage fluid (BALF) was collected, and kept on ice until cell isolation or staining as previously described (Samuelsen et al., 2009).

2.1.2. Food allergy model

C3H/HeJ dams and offspring were exposed to BPA (0, 1, 10 and 100 μg/ml) in the drinking water from the time of mating and throughout the experiment. Water consumption and animal weights were determined during gestation, lactation and for offspring at age 4 weeks. Food allergy with clinical anaphylaxis was induced in female C3H/HeJ offspring (n = 10–13) according to a previously established protocol (Vinje et al., 2009), starting immunisations at 4 weeks of age. All mice received intragastric gavage of 5.7 mg lupin extract (LE), with 10 μg cholera toxin (CT) as adjuvant to break the oral tolerance, on days 0, 1, 2, 7, 21 and 28. All mice were bled at day 34, and at day 35 the mice were challenged with either a single dose of 5 mg LE i.p. or 25 mg LE divided on two i.g. administrations given 30 min apart. Anaphylaxis was assessed during a 30 min observation period after challenge (see below), thereafter the mice were anesthetized with isofluorane gas, blood was drawn by heart puncture and spleen was collected and kept on ice until further preparation.

2.1.3. Oral tolerance model

From each dam described for the food allergy model, exposed to BPA (0, 1, 10 and 100 μg/ml) in the drinking water from the time of mating and throughout the experiment, individual offspring entered the oral food allergy tolerance model (n = 10–13). Oral tolerance to lupin was induced by i.g. gavage of 5 mg LE in 3 weeks old offspring, before they entered the same sensitization, i.p. challenge and endpoint assessment protocol as in the food allergy model.

2.2. Animals

For the airway allergy model, female (6 weeks old) and male (7 weeks old) Balb/cOlaHsd mice were obtained from Harlan (Horst, the Netherlands). For the food allergy and tolerance model, female (6 weeks old) and male (8 weeks old) C3H/HeJ mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). The animals were housed on NESTPAK bedding (Datesand Ltd, Manchester, England) in disposable, BPA-free IVC cages in IVC racks (Innovive Inc, San Diego, CA, USA) and exposed to a 12 h/12 h light/dark cycle at room temperature (20 ± 2 °C) with 40–60% relative humidity. The mice were given ad libitum HT2019 (Harlan) pelleted feed with minimal content of phytoestrogens and drinking water in BPA-free PET bottles (Innovive Inc). In all models, water consumption was determined at various instances during the experiments, by weighing water bottles for all cages with regular intervals, and calculating the average water consumption per mouse per day. The experiments were performed in conformity with the laws and regulations for experiments with live animals in Norway, and were approved by the Norwegian Animal Research Authority under the Ministry of Agriculture.

2.3. Allergens, adjuvant and anaesthetics

Ovalbumin (OVA) (Gal d1; chicken egg albumin, grade VII; Sigma–Aldrich, St. Louis, MO, USA) was used as allergen in the airway allergy model, after removal of endotoxin by Detoxi-Gel Endotoxin Removing Gel (Pierce, Rockford, IL). The lupin extract (LE) used as allergen in the food allergy and tolerance models was provided by the National Veterinary Institute of Norway and was made by extracting homogenised lupin (*Lupinus angustifolius*) in Tris/glycine buffer, pH 8.7, overnight followed by centrifugation. The total protein concentration of the extract was measured by

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