

Perinatal oral exposure to low doses of bisphenol A, S or F impairs gut barrier and immune functions in female offspring mice

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Research

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Abstract

Background Bisphenol A (BPA), one of the highest-volume chemicals produced worldwide, has been identified as an endocrine disruptor. Many peer-reviewing studies have reported adverse effects of low dose BPA exposure, particularly during perinatal period (gestation and/or lactation). We previously demonstrated that perinatal oral exposure to BPA (via gavage of mothers during gestation and lactation) has long-term consequences on immune response and intestinal barrier functions. Due to its adverse effects on several developmental and physiological processes, BPA was removed from consumer products and replaced by chemical substitutes such as BPS or BPF, that are structurally similar and not well studied compare to BPA. Here, we aimed to compare perinatal oral exposure to these bisphenols (BPs) at two doses (5 and 50 mg/kg body weight (BW)/day (d)) on gut barrier and immune system in female offspring mice at adulthood (Post Natal Day PND70). **Methods** Pregnant female mice were orally exposed to BPA, BPS or BPF at 5 or 50 µg/kg BW/d from 15th day of gravidity to weaning of pups at PostNatal Day (PND) 21. Gut barrier function and the humoral and cellular immune responses of adult offspring (PND70) were analysed at intestinal and systemic levels. **Results** In female offspring, perinatal oral BP exposure led to adverse effects on intestinal barrier and immune response that were dependant of the BP nature (A, S or F) and dose of exposure. Stronger impacts were observed with BPS at the dose of 5µg/kg BW/d on inflammatory markers in feces associated with an increase of anti-E. coli IgG, revealing a defect of gut barrier. BPA and BPF exposure induced prominent changes at low dose in offspring mice, in term of gut barrier functions and cellular immune responses, provoking an intestinal and systemic Th1/Th17 inflammation. **Conclusion** These findings provide, for the first time, a comparative study of long-time consequences of BPA, S and F perinatal exposure by oral route in offspring mice. This work warns that it is mandatory to consider immune markers and dose in risk assessment associated to new BPA's alternatives. **Keywords:** Bisphenol A, Bisphenol S, Bisphenol F, Immune responses, Perinatal exposure, Intestine, Th1/Th17, immunoglobulin, cytokines

Background

Endocrine disruptors (EDs) are compounds known to impair the functioning of the endocrine system, and their bioaccumulation in humans may cause adverse health effects [1]. Among EDs, bisphenol A (BPA) is widely used as a component of epoxy resins and polycarbonate plastics by industry. BPA is present in plastic food containers, metal cans as epoxy coatings, kitchenware toys, medical devices, and dental composites and sealants [2]. In humans, BPA has been shown to have developmental, reproductive, cardiovascular, immune, and metabolic adverse outcomes [3].

In 2017, BPA was identified as a very high concern substance in the list of the European Chemical Agency (ECHA). Regarding the recent regulations that further restrict the use of BPA in food contact materials [4], food packaging companies are exploring substitutes for the purpose of the gradually BPA elimination from their products [5]. Then, commercialization of BPA-free labeled products is increasing, while BPA analogues are being increasingly used in the manufacturing of consumer products. BPA analogues share the basic bisphenol structure of two benzene rings separated by a short carbon or other chemical chain.

Because Bisphenol S (BPS) is more heat resistant and photo-resistant than BPA, BPS has been chosen by the industry as a replacement for BPA in the production of polycarbonates and epoxy resins for the manufacturing of industrial and consumer products [6]. Thereby, BPS has been detected in personal care product and foodstuffs [7]. Bisphenol F (BPF) is also a BPA analogue with a wide spectrum of industrial uses. BPF is used in epoxy resins and coatings, especially for systems needing increased thickness and durability (i.e., high-solid/ high-build systems). BPF epoxy resins are used for several consumer products such as lacquers, varnishes, liners, adhesives, plastics, water pipes, dental sealants, and food packaging [8].

BPA belongs to the class of EDs since it exerts estrogenic activity, even at concentrations below 1 ng/L. There are a limited number of studies on the BPA analogues' hormonal effects [9]. Some of the BPA substitutes seem to have more estrogenic effects than BPA [8]. *In vitro* studies demonstrated that even though BPS has a similar molecular size and structure than BPA, it has a lower affinity to human nuclear estrogen receptor (ER) α and ER β [10]. This is in agreement with its recently demonstrated lower potent estrogenic activity *via* human ER α and β in comparison with BPA [11]. Additionally, BPS can bind to membrane ERs, and induce non-genomic responses in cultured pituitary cells at very low concentrations (i.e., femtomolar to picomolar) [12]. BPF showed oestrogen (EC₅₀, 4.67 nM) and anti-androgen (IC₅₀, 1.42 nM) activities comparable to those of BPA [13].

Despite the regulatory actions taken in recent years, it appears that one potential hazardous chemical (BPA) is being replaced by others (BPS and BPF) having similar chemical structures and possibly same health outcomes. Human exposure to BPs occurs mainly through diet (food and food contact materials). However, there is few information regarding the occurrence of BP analogues in foodstuffs. Liao and Kannan (2013), performed a study in the United States where they observed the presence of BPA, BPF, and BPS (N = 267) in nine categories of foodstuffs and BPs were found in 75% of the samples tested [14]. The most frequently found BPs were BPA and BPF. The highest total concentrations of BPs (sum of eight different BPs) were found in canned products (27.0 ng/g), followed by fish and seafood (16.5 ng/g), and beverages (15.6 ng/g). Data on BPA analogues occurrence in human samples are scarce. In one hand, Liao et al., (2012) determined the total concentration of BPS in 315 urine samples. They detected BPS in 81% of the samples. The increased frequency of BPS detection in urine samples collected between 2000 and 2014 (N=616) in U.S. adult volunteers reflects the reality of substituting BPA with BPS [15]. On the other hand, free and conjugated BPF were detected in 55% of tested urine samples of anonymous adults in the United States (n=100), with a median concentration of 0.08 mg/L in urine. Lehmler et al., (2018) investigated the association between the presence of BPA, BPF, and BPS in urine samples from adults participating in the National Health and Nutrition Examination Survey (NHANES) 2013-2014 (N = 1808) and children (n = 868). The presence of BPA, BPS, and BPF were respectively observed in 95.7%, 89.4%, and 66.5% of population tested [16].

There is an increasing amount of research linking long-term, low-level exposure to BPA in early life and adverse health effects in infants and fetuses[17]. BPA, BPS and BPF can cross the human placenta and as such represent a risk for fetus [18]. Indeed, BPA and BPS has been found in maternal and cord blood

serum [19]. Furthermore, exposure of lactating women to BPs is of particular concern, as these chemicals pass from mother to infant *via* breast milk, making this matrix a main target for exposure assessment of critical subpopulations. Breast milk is the main source of energy for babies under six months. In this framework, Deceuninck et al. (2015) investigated the presence of a large group of BPA analogues in breast milk samples of a French cohort (N = 30) but BPS was only detected in one sample at a concentration of 0.23 mg/kg, and the rest of the BPA analogues investigated were not detected [20]. However, Niu et al. (2017) found BPA, BPF and BPS, in breast milk samples from Chinese mothers, with BPA being the most abundant BP, followed by BPF [21].

In recent years, BPA regulations have been tightened, particularly to protect against exposure during the fetal and neonatal period. Indeed, emerging evidence from animal studies suggest that EDs exposure during the critical developmental stages of pregnancy and lactation could adversely affect the developing immune system in the offspring, leading to health defects later in life. Exposure to EDCs has been associated with altered immune function, typically by either suppressing immunity, thereby increasing susceptibility to infections, or by enhancing the immune response and participating to the growing incidence of non-communicable diseases (NCDs) like inflammation, allergies, or autoimmune diseases [22]. Studies have shown that the developing immune system is highly sensitive to BPA exposure. In human, prenatal and postnatal environmental BPA exposure is associated with NCDs during childhood and adulthood [23].

In animals, early-life exposure to BPA may produce considerable adverse effects on the immune system. Indeed, we showed in previous studies that perinatal exposure to BPA increased the risk of food intolerance at adulthood, as well as the susceptibility to intestinal infection and/or to exacerbated mucosal inflammation [24]. More recently, we reported that perinatal exposure to BPA induced intestinal and systemic immune imbalances in male offspring mice at adulthood, through a decrease of Th1/Th17 cell frequencies in the small intestine *lamina propria* (*siLP*) concomitant to an increase of splenic Th1/Th17 immune responses [25]. In comparison, the same BPA perinatal exposure led in female offspring mice to a defect in dendritic cell maturation in the *siLP* and spleen associated with a decrease of activated and regulatory T cells in the *siLP*. Interestingly, a sharp increase in interferon- γ and interleukin-17 production in the intestine and a T helper 17 profile in the spleen were observed [26]. Our results highlighted a sex-specific difference in immune response of offspring after BPA oral exposure of mothers. Both these studies concluded also that low doses of BPA can interfere with the maturing immune system and provide information that warrants serious consideration for human safety [27]. Compiling evidences demonstrated that BPA exposure is associated to risk of metabolic disorders (diabetes and obesity) and immune related diseases (allergy, intestinal bowel diseases, food intolerance). However, few information is available concerning BPA's analogues and their potential adverse effect on immune system development.

Restrictions have been imposed on BPA, but substitutes like BPS and BPF, with very low regulations, are now used leading to the question whether those substitutes are safe. Indeed, the considerable use of BPA analogues and their potential health risks require studies to better understand the complex and

widespread effect of human exposure. In this context, the objective of the present study was to compare the effect of oral exposure during perinatal period (gestation and lactation) at two doses of BPA, BPS and BPF (5 and 50 µg/kg of BW/d) on the gut barrier, and intestinal and systemic immune responses of adult female offspring mice.

Methods

Animals and BPA treatment.

All experiments were approved by the Local Animal Care and Use Committee (TOXCOM 0035/EH-2013), in compliance with the European directive 2010/63/UE. To minimize desertion induced by handling during perinatal period, C3H/HeN (Janvier, Roubaix, France) mice were used. The experiment was conducted on more than three litters/treatment (supplementary data Fig. 1). Perinatal experiment was conducted as previously described [25]. Briefly, nulliparous female C3H/HeN mice (Janvier, Roubaix, France) were mated with male for 5 days and then individually isolated. Pregnant and lactating mice were daily treated orally from 15th day of gravidity to weaning of pups (Post Natal Day 21; PND21) with 5 or 50 µg/kg BW/day of BPA, BPS, BPF or the vehicle alone (0.1% ethanol in corn oil) as control group. For more clarity, these groups will be referred as BPA5, BPA50, BPS5, BPS50, BPF5 and BPF50. All animals (mothers and offspring) were kept at a constant temperature (22±1°C) and maintained on a 12:12h light/dark cycle (light on at 7:30 am). Due to sex-related differences in intestinal BPA effects in rats [28], our study was conducted in females offspring. Body weight (BW) was measured at post-natal day (PND) 10 and PND70. At PND70, female mice were euthanized by cervical dislocation, and blood, jejunum and feces were collected. *Lamina propria* from small intestine (*siLP*) and spleen were sampled for primary cell culture.

Humoral response in plasma and feces.

Blood and feces were sampled. Intracardiac blood was collected with heparinized syringe and recovered plasma was kept at -80°C. Fecal proteins were extracted mechanically in complete antiprotease cocktail (Roche Diagnostic, Meylan, France) and frozen at -80°C. Plasma and fecal IgG and IgA concentrations were measured by ELISA.

Plates were coated overnight at +4°C with 5µg/ml sheep anti-mouse IgA (Sigma-Aldrich) or goat anti-mouse IgG (Southern Biotech, France) in PBS. Plates were blocked with PBS-5% fetal calf serum (FCS) (Invitrogen) before incubation with diluted samples or purified IgA or IgG (Southern Biotech). Horseradish-peroxidase (HRP)-conjugated goat anti-mouse IgA (Sigma-Aldrich) or goat anti-mouse IgG (Southern Biotech) were added, HRP was revealed using TMB (Becton Dickinson, France). Reaction was stopped adding H₂SO₄ 2N and plates were analysed using automatic Infinite M200 microplate reader.

Immunoglobulin specificity against commensal *E. coli* lysate

Maxisorp 96-wells plates were coated with 5µg/ml of protein from C3H/HeN isolated *E. coli* lysate, incubated with plasma (10 µg/mL IgG; 20 µg/mL IgA), and revealed as above-mentioned. Results were expressed as arbitrary units (AU) per 10 µg/mL of IgG ou 20 µg/ml of IgA, in comparison with a standardized immune serum.

Spleen and small intestine lamina propria (siLP) isolation

Spleens were collected and cells were isolated through 70 µm cell strainer to make a single-cell suspension in PBS-1% KnockOut™ SR (KO SR) (Gibco). Small intestines were washed in cold PBS, cut into 0.5 cm pieces, incubated four times in 30 ml of PBS 3mM EDTA (Sigma-Aldrich) and digested in 20 ml of DMEM added with 20% FCS and 100 U/mL of collagenase (Sigma-Aldrich) for 40 min at 37°C. SiLP cells were purified on a 40%–80% Percoll gradient centrifuged for 15 min at 1800g at room temperature.

Fluorescence-Activated Cell Sorter Analysis

Isolated cells from spleen and siLP were stained as follow: Regulatory T-cells: CD4 (BD), CD25 (BD), Foxp3 (ebioscience); Th17: CD3 (BD), RORyt (BD), IL-17 (BD) and Th1 (CD3 (BD), T-bet (BD) and IFN-γ (BD). The staining protocol was performed as previously described [26]. MACSQuant® Analyzers (Miltenyi Biotec) and VenturiOneÒ (AppliedCytometry) software were respectively used for data collection and analysis.

Cytokines measurement

To culture, cells were seeded on 24-well plates at 1×10^6 cells per well for cytokine assays in Cerrotini culture medium (Dulbecco modified Eagle medium supplemented with 8% Knockout serum replacement, (Gibco, Lifetechnologies, Paisley, UK), 36 mg/l asparagine, 116 mg/l arginine, 10 mg/l folic acid, 1 g/l 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid, 0.05 mmol/l β-mercapto-ethanol, 100 U/ml penicillin, 100 mg/ml streptomycin and 1µg/ml fungizone) in presence or absence of 5µg/ml hamster anti-mouse CD3 and hamster anti-mouse CD28 (BD biosciences) coated wells. After 3 days of stimulation, culture supernatants were collected and frozen at -80°C prior cytokines measurement. Cytokines were measured in supernatant of primary cell culture of spleen or siLP by ELISA: IFN-γ and IL-17 present in primary cells culture supernatant were assayed using commercial enzyme linked immunosorbent assays (ELISA kits; Duoset R&D Systems, Lille, France). Cytokines were measured in feces suspended in RIPA buffer (0.5% deoxycholate, 0.1% SDS and 1% Igepal in TBS) containing complete anti protease cocktail (Roche). Fecal

protein concentrations were measured using BCA uptima kit (Interchim). Lipocalin were assayed using commercial ELISA kits (R&D Systems).

Multivariate data processing.

Mixomics package (6.8.2 version in RStudio software, Boston, MA, 1.0.44 version) was used to build first a principal analysis component based on the compilation of all data present in study. In a second step, a [Partial Least-Squares Discriminant Analysis](#) (PLS-DA) was built to depict immune signature associated with BPs treatment. PLS-DA is a multivariate supervised approach that operates by projecting the samples (X) onto a low-dimensional space of so-called latent variables that maximizes the separation between different groups of samples according to their class labels (Y = mice BPs treatments). Repeated Mfold cross-validations were used to select the optimal number of latent variables for PLS-DA models with minimal error rate.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 6.00 (GraphPad Software, San Diego, California, USA). Results were expressed as means +/- SEM. Multiple group were compared to control group used two-way ANOVA. P-values < 0.05 were considered significant (indicated by asterisks): *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

Results

Body weight of offspring mice

BPA, BPS and BPF after oral exposure did not provoke any change in BW of offspring female at PND10 (Fig. 1a). However a reduction in BW was observed in BPF group at PND70 (Fig. 1b), which is significant for BPF50 group (p<0.05). The average number of offspring/litters for each treatment was dependent on the treatment with a lower number of progenies after BPA50 and BPS5 perinatal exposure (supplementary data Fig. 1a and 1b).

Gut barrier functions of offspring mice

In our previous work, we demonstrated a deleterious impact of mother oral exposure to BPA on intestinal barrier of offspring at PND50 [26]. Then, we wondered if oral exposure of mother with BPA, BPS or BPF has consequences on fecal IgA content in offspring female mice at adulthood. As previously demonstrated [26], we observed a significant reduction of fecal IgA level in BPA50 female offspring mice (p<0.05) (Fig. 2a) associated with a slight but not significant increase of lipocalin level (Fig. 2b).

Interestingly, lipocalin level in feces of BPS group, whatever the dose, was significantly increased ($p < 0.05$) (Fig. 2b). We noticed a significant decrease of plasmatic IgG ($p < 0.01-0.005$) in female offspring mice whatever the dose and BP used compared to vehicle group (Fig. 3a), without any change on total IgA level (Fig. 3b). Only BPS50 offspring mice had a significant increase of specific anti-*E. coli* IgG in plasma compared to vehicle group ($p < 0.05$) (Fig. 3c). No difference in anti-*E. coli* IgA was observed (Fig. 3d).

Intestinal and systemic cellular immune responses

First, we analyzed the frequency of T cells subsets at intestinal level. Interestingly, we observed an increase of Th1 ($CD3^+IFN-\gamma^+T\text{-bet}^+$) subpopulation in *lamina propria* after perinatal oral exposure to BPA5 in female offspring mice, which is significant compared to vehicle group ($p < 0.05$) (Fig. 4a). This effect was associated with a slight but not significant increase of IFN-g secretion in response to anti-CD3/28 *in vitro* restimulation (Fig. 4b). At intestinal level, a significant increase of Th17 ($CD3^+ROR\gamma^+IL-17^+$) frequency in BPA50 and BPF50 groups compared to vehicle group was noticed ($p < 0.05$), associated with an increase of IL-17 secretion in supernatant of siLP cells culture in response to TCR stimulation (anti-CD3/CD28) for BPA50 ($p < 0.05$) (Fig. 4c and 4d). Interestingly, mother exposure to BPA50 *via* oral route during gestation and lactation induced a significant decrease ($p < 0.05$) in regulatory T cell ($CD4^+CD25^+FoxP3^+$) frequency in siLP of offspring (Fig. 4e). All BP treatments after perinatal oral exposure provoked an increase of Th1 frequency in spleen of female offspring mice (Fig. 5a), which was significant for BPA50 and BPF50 ($p < 0.05-0.01$). Interestingly, we noticed a significant rise of IFN-g secretion ($p < 0.001$) in response to anti-CD3/CD28 stimulation for BPA50 group (Fig. 5b). We also analyzed the Th17 frequency at systemic level. We observed a rise of Th17 frequency in BPA50 compared to vehicle group (Fig. 5c). Moreover, a significant increase of IL-17 secretion ($p < 0.05$) was noticed in splenocyte supernatant after anti-CD3/CD28 *in vitro* restimulation for BPA50 group compared to vehicle group (Fig. 5d). The level of IL-17 secretion was also increased for BPF50 offspring mice in comparison to vehicle group. Interestingly, a significant decrease of regulatory T (Treg) cell frequency at systemic level ($p < 0.05$) in BPA50 female offspring mice was observed (Fig. 5e). The other BPs did not have any effect on Treg frequency.

Discriminative parameters revealed through multivariate analyses

Based on the compilation of all gut barrier, humoral and cellular immune associated data sets, a non-supervised method (Principal Component Analysis, PCA) was first performed to explore diversity patterns of responses according BP exposure and doses. Sample plot revealed a clear separation of mice according to the BP treatment and the dose used (Fig. 6a). The next step was used to perform a supervised analysis (PLS-DA) on female offspring data set in order to maximize observation of a host-response signature (related to immune response and barrier function) characterizing each nature and dose of BP (Fig. 6b). The model used allowed us to discriminate the Vehicle group (black line) from

treated BPs groups, BPA50 (orange line), and BPF50 (green line) treated mice being the most distant ones. BPA group (blue line) even at low dose of 5 µg/kg BW/d showed a stronger separation from the vehicle group (Fig. 6b). The sample plot revealed a clear separation of BPA50 (orange line), BPA5 (blue line) and BPF50 (green line) groups from the vehicle group (black line). The BPS50 group was less distant from the control group. The loading plot showed that IFN-g level and IL-17 response at systemic and intestinal level and Treg cell frequency were important contributors to the separation between vehicle and BPA50 treated mice. Likewise, body weight and plasmatic IgA level contributed mainly to separate BPF treated mice from vehicle (Fig. 6c).

Discussion

BPS and BPF are the most used BP analogues for BPA. A systematic review that included 32 studies (25 *in vitro* and 7 *in vivo*) revealed that BPF and BPS had similar hormonal effects compared to BPA [8]. The authors concluded that BPS and BPF seemed to have similar mechanisms of action to those of BPA, posing similar health outcomes. Other authors have also reported similarities between BPS, BPF and BPA in terms of their toxicological profiles, including metabolic, carcinogenic, and reproductive effects [29]. In the present study, we chose to work with 5 and 50 µg/kg of BW/d by oral administration of mothers. Indeed, based on the current estimations of infants total exposure to BPA *via* dietary and non-dietary sources, EFSA's latest scientific opinion published in 2015 concluded that children and adolescents are over/above the temporary tolerable daily intake (TDI) of 4 µg/kg BW/d [30]. The widespread contamination had led to high BP exposure in the general population. Exposure to BP can occur through ingestion, inhalation and dermal absorption, however the primary pathway in the human body is considered to be dietary exposure [31]. Then, in the present study, we analyzed effects of two new substitutes, BPS and BPF in comparison to BPA after oral exposure to the mothers. Environmental factor exposure during fetal or neonatal life can interact with genome and maturing immune system, and influence the onset of diseases in adulthood including cancer, infertility, autoimmunity and metabolic disorders. This theory is called "the developmental origins of health and disease" [32]. We previously showed that gestational and lactational exposure to environmental relevant doses of BPA causes adverse effects on immune function in offspring mice [24,25], but no study has investigated the effect of BPS and BPF and its consequences on the immune system of offspring mice. During the neonatal period, the immune system, the intestinal epithelium and the microbiota form one entity, in which all parameters influence each other for their respective development until the equilibrium/homeostasis is reached. To achieve this stage, the immune system is first primed *in utero* by microbial metabolites of the mother, while high intestinal permeability at birth permits lumen-to-mucosa exchanges for further maturation of intestinal immune functions. Intestinal epithelial surface plays a critical role in host protection. Intestinal IgA is involved in the development and maintenance of the homeostasis between microbiota and the host immune system [33]. Interestingly, our results demonstrated that perinatal exposure to BPA50 induced a fall of fecal IgA in offspring adult mice, but no effect was observed after BPS and BPF exposure. These results are in accordance with our previous studies showing a reduced IgA production after perinatal exposure of BPA [26]. However, a significant increase of lipocalin level, an inflammatory marker, in feces

was detected after BPS perinatal exposure at both doses 5 and 50 µg/kg of BW/d, highlighting the fact that the effect of this bisphenol on gut barrier involves a different mechanism compared to BPA. At plasmatic level, we observed an increase of anti-*E. coli* IgG in offspring after mother's exposure to BPA5 and BPS50. We obtained similar results after oral administration of BPA in female offspring mice [26]. Interestingly, low dose of BPS provoked an increase of anti-*E. coli* IgG in offspring mice correlated with high lipocalin level in feces, adding evidence of impaired intestinal barrier in offspring mice exposed to BPA analogues.

In the gut, CD4⁺ T cells contribute to immunity by differentiating into various subsets, notably inflammatory (Th17/Th1) and regulatory T cells (Treg), Th17 cells being the most abundant CD4⁺ T cells in mucosal tissues. They secrete isoforms of IL-17 and/or IL-22, which confer protection against fungi and pathogenic bacteria. Our study reveals the ability of BPA and BPF to provoke a sharp increase in Th1 and Th17 frequency associated with an increase of IL17 and IFN-g level production after *in vitro* anti-CD3/CD28 restimulation of intestinal immune cells of female offspring mice. These results are in accordance with our previous studies demonstrating that perinatal exposure to BPA after oral administration induces a potent Th1/Th17 signature at local level [26]. Interestingly, the present study induced Th1/Th17 cytokines production even at low dose i.e. 5µg/kg of BW/d. At the systemic level, we also reported an increase of IFN-g and IL-17 cytokines production after anti-CD3/CD28 restimulation of splenocytes from BPA and BPF-exposed offspring mice. This effect was associated with an increment of Th1 and Th17 frequency but only with higher BP dose (50 µg/kg BW/d). Luo (2016) recently reported similar observation after gestational and lactational exposure to BPA [34]. Others studies revealed an imbalance in immune responses after exposure of pregnant female rodents to varying relevant human-exposure levels of BPA [35]. Interestingly, in these studies, they observed an increase in a pro-inflammatory Th1 response in the offspring. It is well known that immune tolerance requires the participation of Treg cells [36]. We only observed a decrease in Treg cells isolated from siLP or spleen in offspring mice exposed to BPA50. This result is in accordance with those obtained by Malaisé (2018) in female offspring mice after BPA perinatal exposure by oral route [26].

This study compared for the first time, the effect of three bisphenols on immune response and gut barrier functions in adult offspring after oral perinatal exposure. It permits to reveal a specific effect of BPS on IgG response toward commensal microbiota (anti-*E. coli*). Gestational and lactational exposure to BPA and BPF was found to induce more prominent changes in female offspring mice in term of gut barrier functions and cellular immune responses, inducing an intestinal Th1/Th17 inflammation. Our findings suggest that perinatal exposition to relevant environmental doses of BPA and BPF results in changes of Th1 and Th17 development, which may contribute to developmental immunotoxicity. In fact, IL-17-secreting Th17 cells are key players to promote inflammatory diseases in mice [37]. Strong evidence revealed that Th17 cells represent a distinct subset of CD4⁺ T lymphocytes that plays a critical role in chronic inflammation and autoimmunity in mice [38]. Indeed, while the pro-inflammatory properties of IL-17 are key to its host-protective capacity, unrestrained IL-17 signaling is associated with immunopathology, autoimmune diseases and cancer progression [39].

Conclusions

These experimental findings warrant further epidemiological studies to assess the effects of BPA and BPF burden in mothers on the risk of developing childhood and adult immune-mediated diseases in the female offspring mice. An uncontrolled acceleration of the system or failure of the brakes can both lead to persistent inflammation resulting in tissue damage and NCDs later on. In summary, we demonstrated that BPA substitutes BPS and BPF after gestational and lactational exposure, are able to affect intestinal and systemic immune systems of adult offspring mice, at both 5 and 50µg/kg BW/d involving different or similar mechanisms compared to BPA, questioning their safety and the rationale of their use to replace BPA.

Abbreviations

BP: Bisphenol; BW: Body weight; d: day; E: Escherichia; ED: endocrine disruptor; ELISA: Enzyme linked immunosorbent assay; FCS: fetal calf serum; Ig: immunoglobulin; IL: interleukine; PLS-DA: Partial Least-Squares Discriminant Analysis; PCA: Principal Component Analysis; PND: Postnatal day; siLP: small intestine lamina propria; Th: T helper; Treg: regulatory T.

Declarations

Ethics approval

All experimental procedures involving live animals were approved by the Local Animal Care and Use Committee (TOXCOM 0035/EH-2013), in compliance with the European directive 2010/63/UE.

Consent for publication

Not applicable

Availability of data and materials

Please contact the corresponding authors with all requests

Competing interests

Not applicable

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Authors' contributions

YM, SM, CL and CC performed the experiments and collected the data. MO performed ACP analysis. YM, SM and LG analyzed the data. LG wrote the first draft of the manuscript. YM, SM and LG edited the manuscript. YM, MO, SM and LG reviewed the manuscript prior to submission. All authors read and approved the final manuscript.

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Additional File Legend

Supplementary Fig. 1. Consequence of oral exposure to bisphenols on birth rate offspring mice

(a) Average of number of offspring and sex in vehicle, BPA, BPS and BPF groups (male: grey box; female: black box). The number at the top of each column correspond to the number of mothers used for each treatment group. (b) Offspring number for each group used in the study.

Figures

Figure 1.

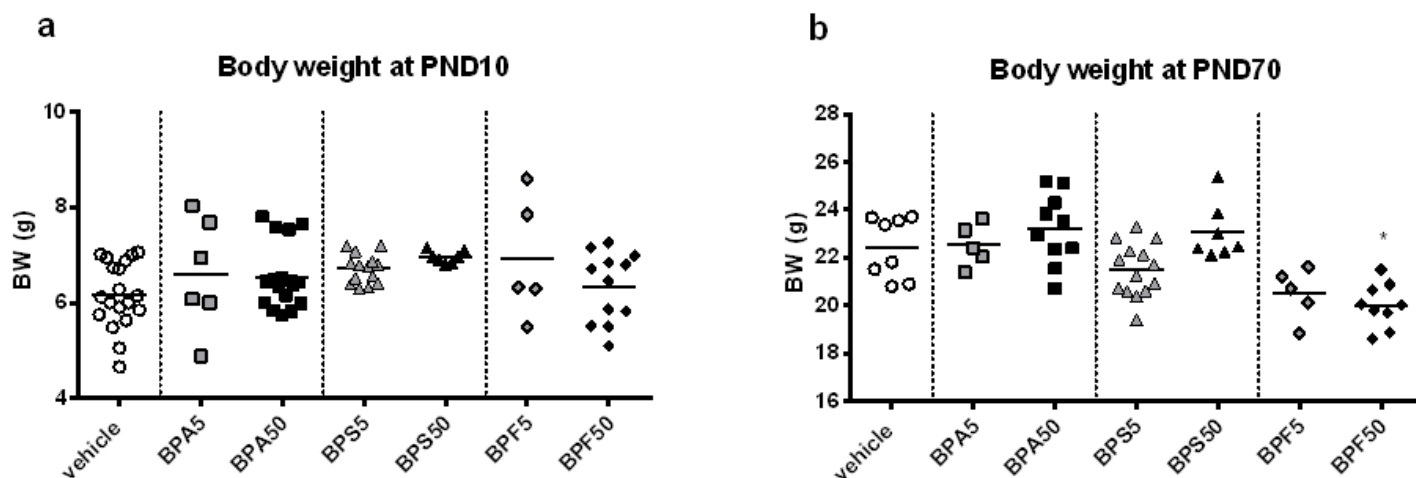


Figure 1

Body weight of young and adult offspring mice (a) Body weight of female offspring mice in different treatment groups measured at PND10 and (b) at PND70. * $P < 0.05$ vs. vehicle group.

Figure 2.

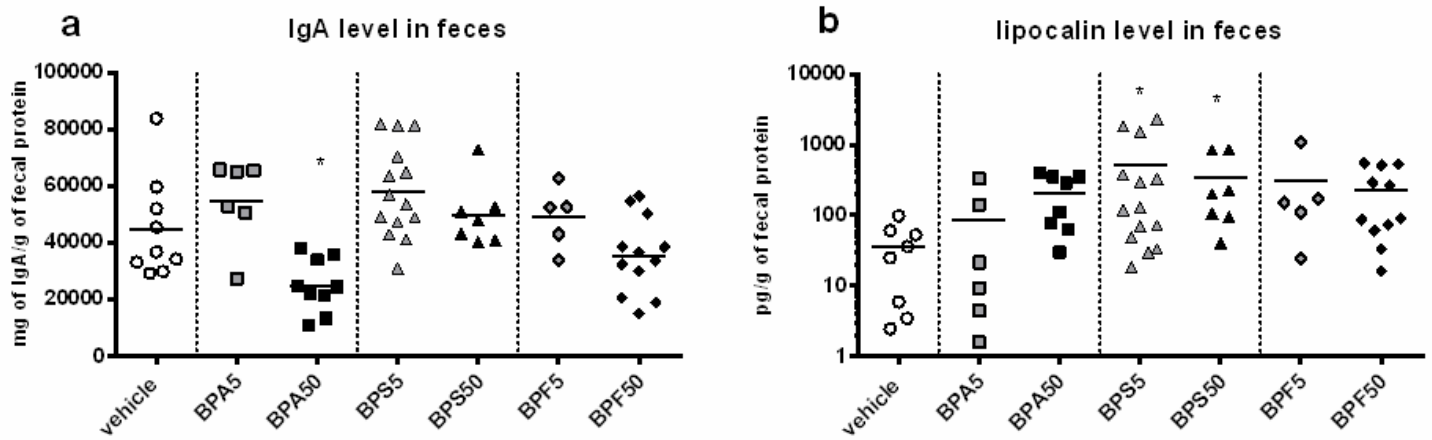


Figure 2

Perinatal exposure to bisphenols disrupts gut barrier in adult offspring mice (a) Total IgA concentration measured by ELISA in fecal samples of female offspring mice at PND70. (b) Lipocalin level determined in fecal supernatant of female offspring mice at PND70. * P<0.05 vs. vehicle group.

Figure 3.

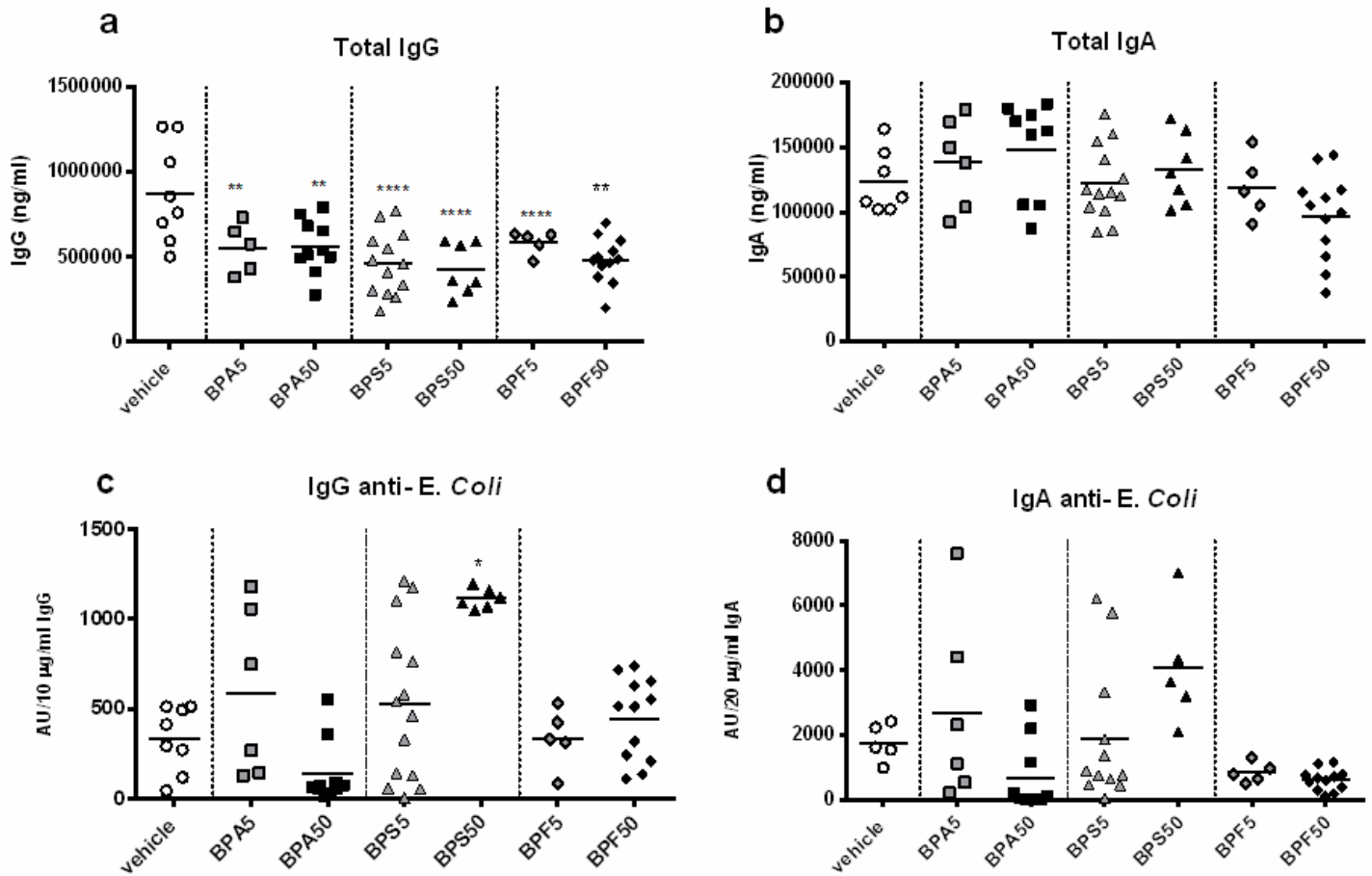


Figure 3

Perinatal exposure to bisphenols disrupts intestinal anti-microbial functions in adult offspring dependent of BP's molecule Plasma IgG (a) and IgA (b) concentrations measured by ELISA in fecal samples of female offspring mice at PND70. IgG (c) and IgA (d) specificity against *E. coli* lysate assessed by ELISA after normalizing to Ig concentrations. The lines represent the median (the 50th percentile). * $P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$ vs. vehicle group.

Figure 4

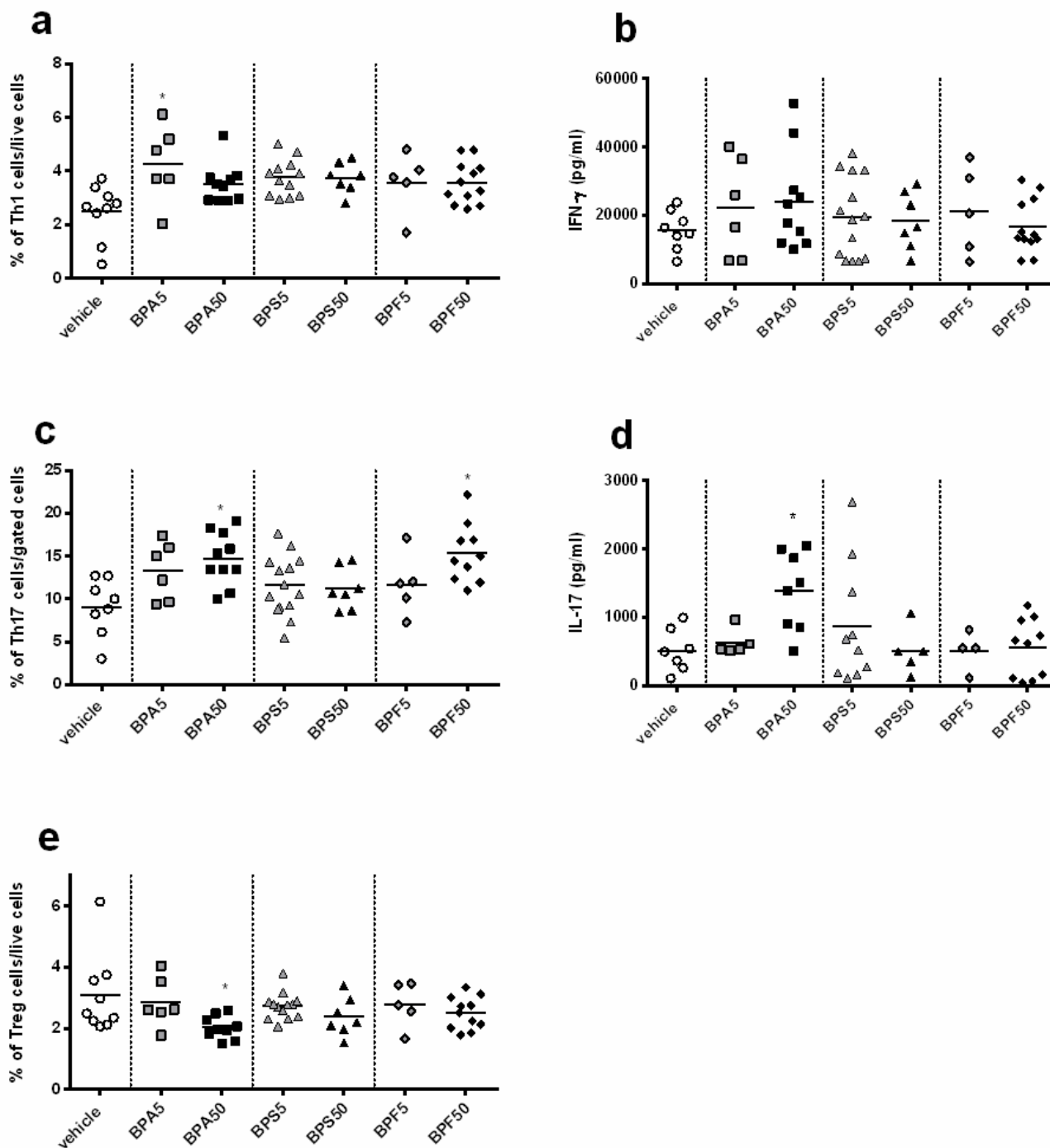


Figure 4

Perinatal exposure to BPA provokes intestinal Th1/Th17 immune response in adult offspring mice. Flow cytometry analysis of Th1 CD3+IFN- γ +Tbet+ (a) or Th17 CD3+RORgt+IL-17+ (c) lymphocytes from siLP. IFN- γ (b) or IL-17 (d) level assessed by ELISA after anti-CD3/CD28 in vitro restimulation of isolated lymphocytes from siLP offspring mice at PND70. (e) Proportion of CD4+CD25+FoxP3+ Treg cells in siLP of offspring mice at PND70. * P<0.05 vs. vehicle group.

Figure 5

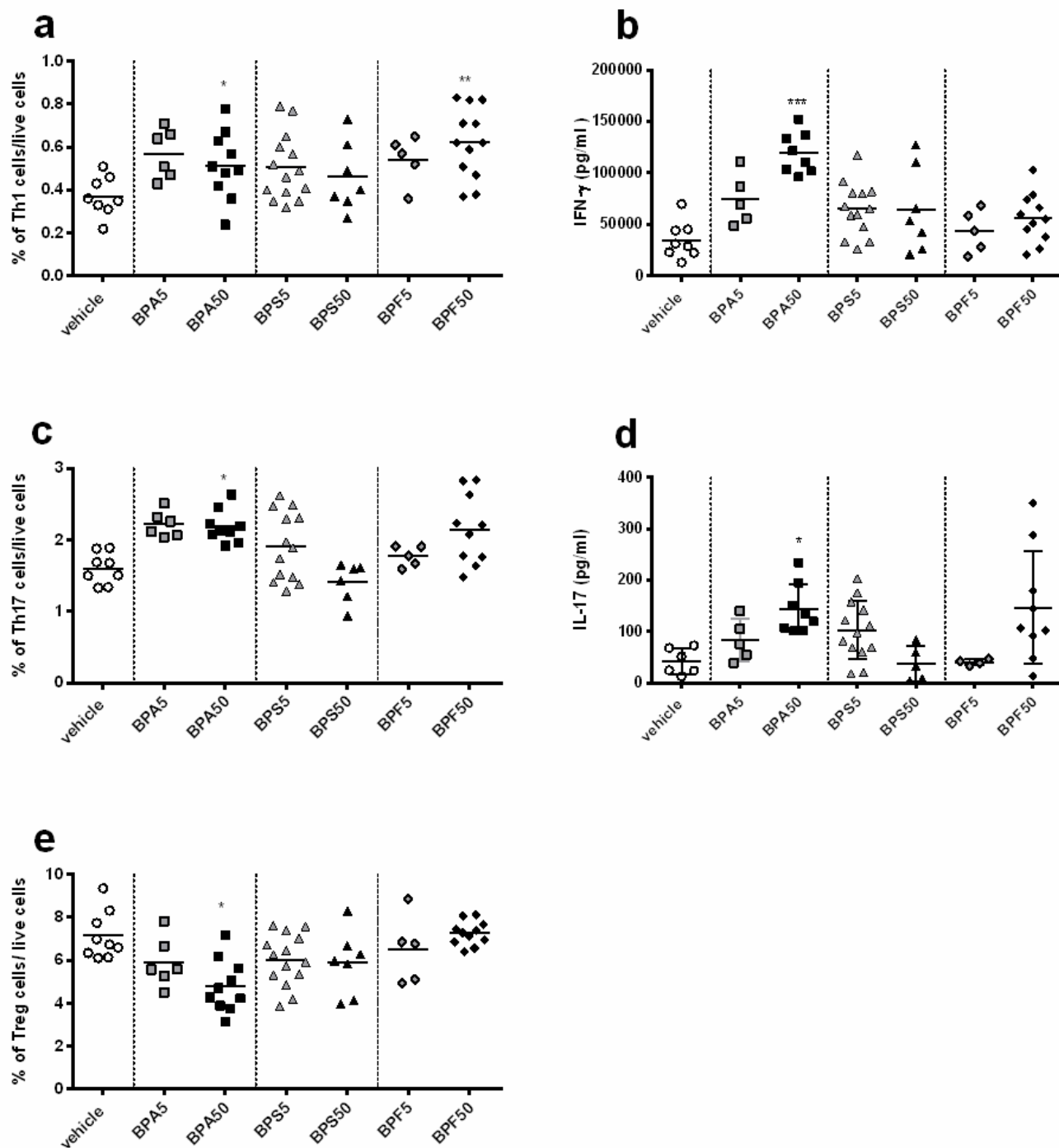


Figure 5

Perinatal exposure to BPA or BPF provokes systemic Th1/Th17 immune response in adult offspring mice. Flow cytometry analysis of Th1 CD3+IFN- γ +T-bet+ (a) or Th17 CD3+RORgt+IL-17+ (c) lymphocytes from spleen. IFN- γ (b) or IL-17 (d) level assessed by ELISA after anti-CD3/CD28 in vitro restimulation of isolated lymphocytes from splenic offspring mice at PND70. (e) Proportion of CD4+CD25+FoxP3+ Treg cells in spleen of offspring mice at PND70. * P<0.05; ** P<0.01; *** P<0.001 vs. vehicle group.

Figure 6.

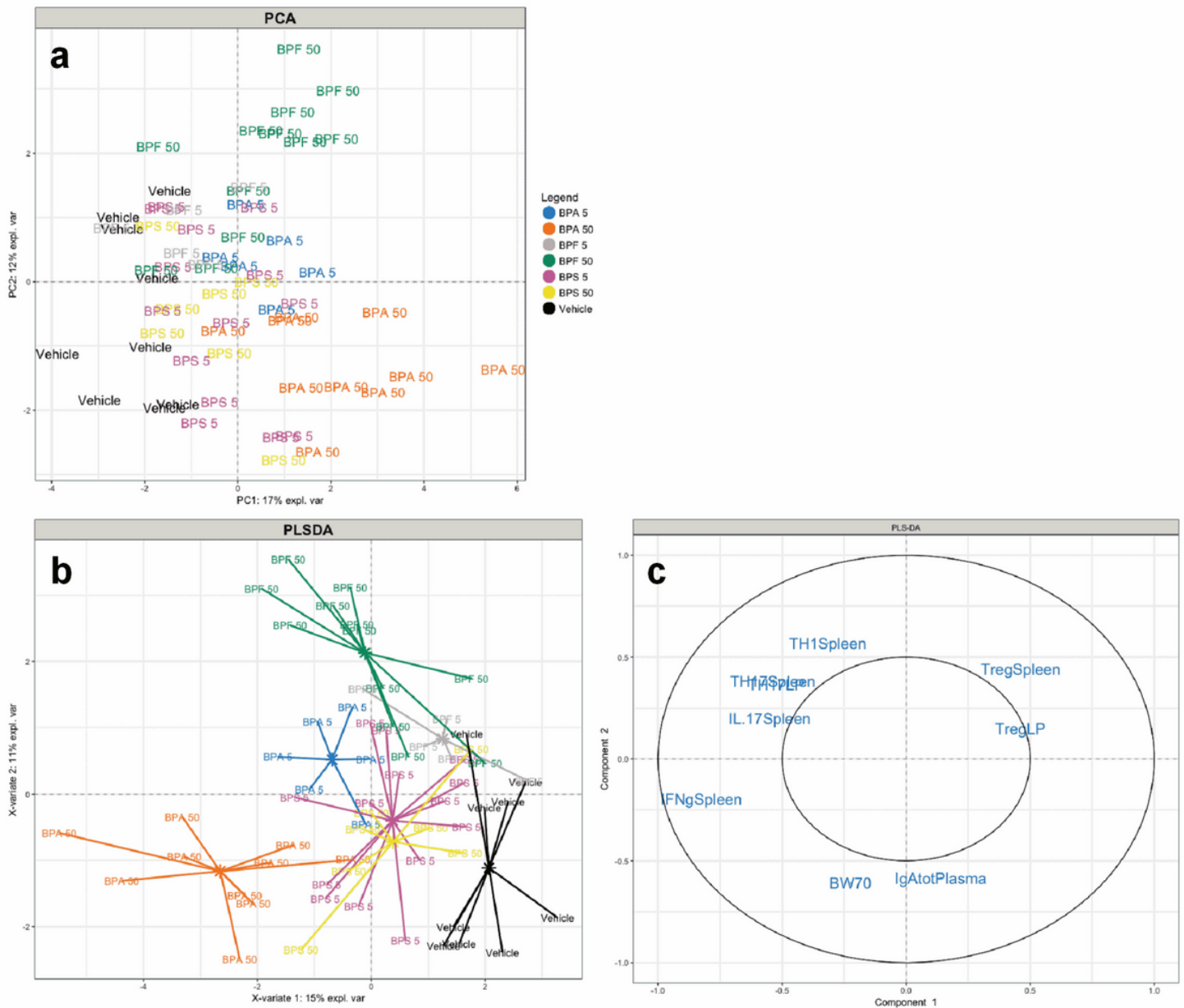


Figure 6

Multivariate analysis representing immune profiles in function of BP perinatal exposure in offspring mice (a) Sample score plot and associated loading plot on the first two PCA components resulting from all data set in offspring mice. Each color indicated groups with 0.85% confidence level ellipse plots. (b) PLS-DA sample score plot and associated loading plot (c) on the first two components derived from data set from all treated groups in offspring mice. Only loadings with correlation threshold >0.5 were represented on the loading plots. % expl var: percentages for each first two components explained by the model.

Supplementary Files

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