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Bisphenol A suppresses Th1-type immune response in human peripheral blood mononuclear cells *in vitro*

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Abstract

Bisphenol A (BPA) is a widely used plasticizer, which came into focus because of its genotoxic and sensitizing potential. Besides its toxic properties, BPA is also well-known for its antioxidant chemical properties.

This *in vitro* study investigated the interference of BPA with interferon- γ (IFN- γ)-induced tryptophan breakdown and neopterin production in human peripheral blood mononuclear cells (PBMC). The pro-inflammatory cytokine IFN- γ induces the conversion of the essential amino acid tryptophan into kynurenine *via* the enzyme indoleamine-2,3-dioxygenase (IDO-1). In parallel, GTP-cyclohydrolase produces neopterin, a marker of immune activation. A model system of phytohaemagglutinin (PHA)-stimulated PBMC was used to assess potential immunomodulatory properties of BPA.

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Competing interests

The authors declare that there are no competing interests.

Authors' contributions

JMG, ER and KB collected experimental data, performed immunoassays, wrote the manuscript and agreed to the final submitted version. ÜF drafted the manuscript and agreed to the final submitted version. HS organized blood donations used in the study, drafted the manuscript and agreed to the final submitted version. JEP performed FACs measurements and agreed to the final submitted version. JMG and DF designed the study, performed statistical analysis, interpreted results, drafted the manuscript and agreed to the final submitted version. All authors read and approved the final manuscript.

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Treatment of cells with BPA [12.5–200 μ M] resulted in a significant and dose-dependent suppression of mitogen-induced tryptophan breakdown and neopterin formation along with a decrease of IFN- γ levels. Similar but less pronounced effects were observed in unstimulated cells.

We postulate that the inhibitory effects of BPA on both T-cell activation and IDO-1 activity that we describe here may be critical for immune surveillance and is likely to influence T helper (Th) type 1/Th2 balance. Such immunosuppressive effects likely contribute to counteract inflammation.

Further studies are required to address the *in vivo* relevance our *in vitro* findings.

Keywords

Bisphenol A; Neopterin; Indoleamine 2,3-dioxygenase; Tryptophan; Human PBMC

1 Introduction

Bisphenol A (BPA) is a widely used chemical, which has been extensively investigated in a number of risk-benefit assessment studies, due to reported endocrine disrupting properties as well as systemic and immunotoxicity [1]. In the search for synthetic estrogens, the first weak estrogen-like activities of BPA were reported in the 1930s [2]. Thus, further investigations on BPA were focused mainly on the analysis of its hormone-like signaling properties. In the 1950's, BPA was rediscovered for the production of plastics. Due to its advantageous physical and chemical properties including optical clarity, shatter-resistance, light weight, durability, high tensile strength and elasticity in addition to low manufacturing costs, BPA was included in several everyday products. However, although BPA is heat-resistant and only moderately water soluble, its application is controversial, especially regarding food packaging, paper or dentistry products, owing to incomplete polymerization, polymer degradation and seepage out from those materials [3–5]. BPA has been detected in dust and air particles and even in water [6,7], but also in human biofluids [8].

Following ingestion, BPA is metabolized by the formation of glucurono- or sulfo-conjugates, which are then excreted. Both BPA-conjugates and biologically active free BPA are detectable in the urine and in the blood, with serum concentration of total BPA reported to reach upwards of 9 ng/ml [9,10]. BPA exposure has been associated with a negative impact on human health. Several *in vitro* studies, animal experiments as well as association studies of environmental exposure in human have been discussed regarding the endocrine disrupting properties of the chemical as primary cause for its biological effects [11]. Additional mechanisms of action have also been identified, such as interference with redox homeostasis, immunotoxicity or mitochondrial dysfunction [12,13]. Exposure to BPA is suggested to promote various clinical conditions and chronic diseases including not only allergy and skin sensitization [14], diabetes [15], obesity or insulin resistance but also cancer [16]. All these pathologies are related to immune system activation. Thus, investigating the potential interferences of BPA with central immunoregulatory pathways we prove helpful in deciphering BPA immunotoxicity.

During a T helper 1 (Th1)-type (cellular) immune response, activated T cells release large amounts of cytokines such as interleukin-2 (IL-2) and interferon- γ (IFN- γ). IFN- γ , the

major Th1-type cytokine, induces anti-microbial and antitumoral host defenses by the induction of a variety of physiological and cellular responses, *e.g.*, it can trigger the release of large amounts of antimicrobial and cytotoxic reactive oxygen species (ROS) in macrophages [17]. ROS can interfere with various redox-sensitive signaling cascades such as activation of the transcription factor nuclear factor κ B (NF- κ B), which leads to the expression of further pro-inflammatory cytokines, thus amplifying the immune response [18]. In addition, IFN- γ activates the enzyme guanosine-triphosphate-(GTP)-cyclohydrolase (GTP-GCH-I, EC 3.5.4.16) in human macrophages, which produces neopterin, a sensitive indicator of immune activation and oxidative stress [19]. In parallel, IFN- γ induces the enzyme indoleamine 2,3-dioxygenase (IDO-1, EC 1.13.11.52) that converts the essential amino acid tryptophan into kynurenine [20]. Tryptophan deprivation limits protein biosynthesis and presents an anti-proliferative strategy to inhibit the growth of both microbes and tumor cells [21,22] and also T cell proliferation, thus mediating immunosuppression [23]. Alongside this, in activated dendritic cells (DC), IDO-1 function is associated with the upregulation of regulatory T-cells responses, resulting in further immunosuppression [24]. Furthermore, tryptophan is the major precursor for the biosynthesis of the neurotransmitter serotonin, which is involved in the pathogenesis of mood disorders and depression [25]. Increased tryptophan breakdown and high neopterin levels have been observed in patients suffering from diseases associated with chronic immune activation such as infections, autoimmune disease, malignant tumors or neurodegenerative disorders [26]. Tryptophan breakdown rates and neopterin production have also turned out as useful markers for the assessment of immunomodulatory effects for a broad range of drugs and compounds *in vitro* [27]. The tryptophan to kynurenine conversion is the rate limiting step in the tryptophan catabolic pathway along the kynurenine axis. Since the 1990s, Kyn/Trp ratio is used as an estimate of IDO-1 activity in a variety of clinical studies [20,26]. Parallel determination of inflammatory molecules such as neopterin or soluble cytokine receptors links increased Kyn/Trp ratio to inflammation-induced IDO-1, as also other enzymes, such as hepatic tryptophan 2,3-dioxygenase (TDO2) may be involved in tryptophan breakdown.

In this study, the immunomodulatory effects of BPA were evaluated *in vitro* using human peripheral blood mononuclear cells (PBMC) freshly isolated from healthy donors [28]. This model allows the investigation of the interaction between T-cells and macrophages. Furthermore, myelomonocytic THP-1 cells and the NF- κ B reporter cell line THP1-Blue [29] were used to address monocyte/macrophage-related responses.

2 Methods

2.1 Chemicals and reagents

Phytohaemagglutinin (PHA), lipopolysaccharide (LPS) and BPA were obtained from Sigma-Aldrich (Vienna, Austria). PHA and LPS was dissolved in phosphate buffered saline (PBS) and stored at -20°C until use. BPA (Sigma Aldrich, Vienna, Austria) was dissolved in 99.9% ethanol at 200 mM and diluted further in RPMI 1640 medium (Biochrom, Berlin, Germany). BPA solutions were always prepared freshly.

2.2 Ethics statement

Whole-blood samples were drawn from healthy volunteer donors at the Central Institute of Blood Transfusion and Immunology, University Clinics Innsbruck. Donors gave written informed consent that their donated blood was used for scientific purposes, when not used for transfusion (Official Bulletin of the Medical University Innsbruck 2008/09 nr.31, §139 decision by the Ethics Committee regarding the research on anonymized sample material, part B).

2.3 PBMC isolation and culture

PBMC were isolated by using density centrifugation as described elsewhere [28]. In brief, PBMC consisting mainly of lymphocytes and a small percentage of monocytes were separated from whole-blood in a 30 min centrifugation step at 1500 rpm at 4 °C by using Biocoll separation solution (MedPro, Vienna, Austria). After separation, PBMC were washed twice in phosphate-buffered saline (PBS, Serva, Heidelberg, Germany) solution containing 1 mM ethylenediaminetetraacetate (EDTA) (Merck, Vienna, Austria) and cultivated at a density of 1.0×10^6 cells/ml in RPMI 1640 (Biochrom, Berlin, Germany) supplemented with 10% of heat-inactivated fetal bovine serum (FBS, Invitrogen, Vienna, Austria), L-glutamine (final concentration: 2 mM) and gentamicin (final concentration: 50 µg/ml; both Serva, Heidelberg, Germany). Cells were incubated in a humidified atmosphere (37 °C and 5% CO₂) for 48 h.

2.4 THP-1 and THP1-Blue cell culture

THP1-Blue cells (Invivogen, San Diego, USA) are derived from the myelomonocytic THP-1 cell line and contain a reporter construct expressing secreted embryonic alkaline phosphatase (SEAP), expression of which can be induced *via* NF-κB/activator protein 1 (AP-1) transcription factors. THP-1 cells were maintained in RPMI 1640 medium supplemented with 10% FCS and THP1-Blue culture medium was additionally supplemented with 200 µg/ml zeocin (Invivogen, San Diego, USA).

2.5 Experimental setup

Three independent experiments were performed in duplicate for tryptophan, kynurenine and neopterin measurements or triplicates for NF-κB activity. In the experiments with PBMCs, cells from three different blood donations collected in 3 different weeks were used. Cells were pre-incubated for 30 min with different doses of BPA or vehicle control [(0.11% ethanol (v/v))] or incubated without BPA, and were then left either unstimulated or stimulated with 10 µg/ml of PHA (PBMC) or 1 µg/ml of LPS (THP1-Blue) for 48 h.

2.6 Measurement of cell viability

CellTiter-Blue (CTB) assay (Promega, Mannheim, Germany) was used according to the manufacturer's instructions. After 48 h of incubation, cell viability was measured by determining the metabolic capacity of cells to reduce the indicator dye resazurin to fluorescent resorufin. For this, 10% (v/v) CTB reagent was added to the cultures and after 4 h of incubation fluorescence was measured at 544/590 nm (Fluoroskan Ascent FL plate-

reader, Thermo Labsystems, USA). The half maximal (50% inhibitory concentration (IC_{50}) was calculated using by using the CalcuSyn software (Biosoft, UK) [30].

2.7 Measurement of apoptosis

Apoptosis was analyzed by FITC-Annexin V/TO-PRO3 staining. Briefly, cells were washed twice in ice-cold PBS, resuspended in Annexin binding buffer containing TO-PRO3 (Invitrogen, UK) at a dilution of 1:10,000 and stained with FITC-Annexin V (NBS Biologicals Ltd., Cambridgeshire, UK) according to the manufacturer's instructions. Cell death was analysed by TOPRO-3 exclusion, using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA) acquiring 10,000 events.

2.8 Measurements of tryptophan, kynurenine, neopterin and interferon- γ

Cell supernatants were collected after 48 h by centrifugation and kept frozen at -20°C until analytical measurements. The determination of tryptophan and kynurenine was performed employing high pressure liquid chromatography (HPLC) as described previously [31]. The concentrations of kynurenine, internal standard 3-nitro-L-tyrosine (Sigma-Aldrich, Vienna, Austria) in addition to tryptophan were measured simultaneously performing a reversed-phase HPLC (rp-18) with two detectors connected in series: the first detector (UV-detector SPD-6A, Shimadzu, Vienna, Austria) monitored UV-absorption at a wavelength of 360 nm to determine concentrations of kynurenine and of internal standard nitrotyrosine. The second device was a fluorescence-detector (Pro Star 363, Varian, Palo Alto, CA), by which tryptophan was detected at its maximum excitation wavelength of 286 nm and emission wavelength of 365 nm. To estimate the enzymatic activity of IDO, the ratios of kynurenine to tryptophan (Kyn/Trp) concentrations were calculated and expressed as μmol kynurenine per mmol tryptophan.

The concentration of neopterin was determined by enzyme-linked immunosorbent assay (ELISA; BRAHMS GmbH, Thermo-Fisher Scientific, Henningsdorf, Germany) according to the manufacturers' instructions, with a detection limit of 2 nM. Measurements of optical density (OD) were performed at absorption at 405 nm using an Anthos plate reader (Biochrom Ltd., Cambridge, UK). IFN- γ concentrations were determined by ELISA (R&D Diagnostics, Biomedica, Vienna, Austria) according to the manufacturers' instructions, with a limit of detection of 8 pg/ml. Due to limited sample volumes available, IFN- γ measurements were performed in a smaller set of BPA-treated samples only.

2.9 Measurement of NF- κB activity

Expression of SEAP can be induced in THP1-Blue cells upon stimulation of cells with LPS [29]. SEAP activity, as a measure of NF- κB activity, was determined after 48 h in a colorimetric assay by using Quantibblue detection reagent (Invivogen, Vienna, Austria) at 635 nm according to the manufacturer's instructions.

2.10 Statistical evaluation

All data is shown as mean $\pm$ SD. Results of viability, kynurenine, Kyn/Trp, neopterin and NF- κB assessments are shown as percentage of the baseline of the respective unstimulated and stimulated vehicle controls. For tryptophan, the initial concentration of the culture

medium (RPMI = 37.0 μ M) was used as baseline and set as 100%. Since not all data sets displayed a normal distribution, non-parametric and parametric methods were used for statistical analysis. Comparison of more than two groups was performed with the Friedman test. Wilcoxon signed-rank test was applied to compare the individual groups to the baseline values.

3 Results

3.1 Influence of bisphenol A on PBMC viability

PHA-stimulation induced a 1.5-fold increase in viability after 48 h of incubation (Fig. 1A). A dose-dependent reduction in cell viability was observed upon BPA treatment, with the effect more pronounced in PHA-stimulated cells (Fig. 1B). In both unstimulated as well as in PHA-stimulated PBMCs, the cell viability decreased to values less than 50% of control cells at the highest treatment concentration of 200 μ M BPA. Calculated IC₅₀ values were 271 (102–720, 95% CI) μ M for unstimulated and 136 (99.6–187) μ M for PHA-stimulated cells (Table 1).

3.2 Influence of PHA on tryptophan metabolism in PBMC

After 48 h of incubation, mean tryptophan concentrations in culture supernatants of unstimulated PBMC were 23.1 ± 4.2 μ M (=62.5% of initial medium content; Table 2). Mean Kyn/Trp was 79.2 ± 54.8 μ M/mM, and mean kynurenine concentration were 1.8 ± 1.1 μ M (all data mean $\pm$ SD). Upon stimulation with PHA, tryptophan concentrations declined to 7.2 ± 2.2 μ M, which corresponds to 19.5% of the initial medium content. In contrast, kynurenine concentrations increased to 17.8 ± 6.5 μ M. As a result, the average Kyn/Trp ratio following PHA stimulation increased to 2938 ± 1808 , which is an approximate 37-fold increase compared to unstimulated controls. The effect of PHA-stimulation in vehicle treated cells was comparable to PHA-stimulation only (Table 2).

3.3 Influence of bisphenol A on estimated indoleamine 2,3-dioxygenase (IDO-1) activity

In PHA-stimulated cells, exposure to BPA resulted in a dose-dependent increase of tryptophan concentrations in the concentration range of 25–200 μ M BPA (Fig. 2A). In parallel, a significant decrease of kynurenine formation was observed, starting with 50 μ M BPA treatment (Fig. 2B). Thus, in PHA-stimulated cells, BPA exposure led to a significant and dose-dependent suppressive effect on tryptophan breakdown (Kyn/Trp ratio) at concentrations of 25 μ M and above (Fig. 2C). *e.g.*, Kyn/Trp ratio decreased to $47.1 \pm 3.2\%$ upon exposure to 50 μ M BPA, compared to stimulated controls, and IDO-1 activity was nearly completely suppressed with 200 μ M BPA (decrease to $4.0 \pm 1.1\%$). The calculated IC₅₀ concentration for Kyn/Trp was 43.3 μ M (40.8–46.0 μ M) (Table 1).

In unstimulated PBMCs, the Kyn/Trp ratio was also affected but to a lesser extent (Fig. 2C). Tryptophan levels in unstimulated cells were not significantly affected upon BPA treatment, however kynurenine levels decreased to about 60% compared to control cells (Fig. 2B). Significant effects on kynurenine formation were observed at a concentration of 50 μ M BPA. Kyn/Trp ratios decreased in a dose-dependent manner from 50 μ M BPA onwards, with maximum inhibition observed at 200 μ M BPA to $59.1 \pm 16.2\%$ of control values (Fig. 2C).

3.4 Influence of bisphenol A on neopterin production in PBMC

Mean concentrations of neopterin were 2.9 ± 0.4 nM in the supernatants of unstimulated PBMC and increased to 10.0 ± 3.7 nM upon stimulation with PHA (Table 2).

Neopterin formation of PBMC was influenced by BPA in both unstimulated and PHA-stimulated cells. In unstimulated cells, the highest dose of 200 μ M BPA significantly decreased neopterin concentrations to about 75% of control values. A similar but more marked effect was seen in the PHA-stimulated cells, with neopterin concentrations decreasing in a dose-dependent manner to lower levels, down to $37.3 \pm 7.6\%$ at 200 μ M BPA, compared to stimulated controls (Fig. 2D). The decrease in neopterin was significant with concentrations of 12.5 μ M in both stimulated and unstimulated cells.

3.5 Influence of bisphenol A on interferon- γ in PBMC

PHA-induced formation of IFN- γ was dose-dependently suppressed upon addition of BPA reaching the level of significance at concentrations of 12.5 μ M BPA (Fig. 3). In unstimulated control cells, IFN- γ levels were below the limit of detection.

3.6 Influence of bisphenol A on THP1-Blue cells

LPS-stimulation of THP1-Blue cells did not affect cell viability after 48 h, although solvent treatment decreased the viability of unstimulated cells to about 7% (Supplementary material, Fig. S1). BPA treatment reduced cell viability as measured by resazurin to resorufin reduction in both stimulated and unstimulated cells with IC_{50} values of 87.0 μ M (54.6–139) μ M and 79.6 μ M (50.6–125) μ M, respectively (Supplementary material, Table S1). Analysis of the effect of BPA treatment on apoptosis and necrosis, as estimated by flow cytometry using FITC-Annexin binding and TO-PRO3 staining, showed that at concentrations of 12.5 and 50 μ M, viability was not affected in stimulated cells and only marginally affected in unstimulated cells at 50 μ M BPA (Supplementary material, Fig. S2), indicating that resazurin reduction is a very sensitive method that detects decreases in cell metabolic activity before apoptotic events occur.

Kynurenine levels were elevated approximately 2-fold in stimulated cells whereas tryptophan concentrations were not significantly influenced upon LPS-stimulation after 48 h of treatment. The Kyn/Trp ratio increased from 60.5 ± 19.5 to 120 ± 23.3 μ M/mM (Supplementary material, Table S2). The effect of stimulation was similar in vehicle-treated cells.

BPA treatment significantly affected tryptophan levels at the highest concentrations of 100 and 200 μ M in both unstimulated and LPS-stimulated cells (Supplementary material, Fig. S3A). A dose-dependent reduction of kynurenine and Kyn/Trp was observed in LPS-stimulated cells only, at treatment concentrations from 50 to 200 μ M (Fig. S3B and S3C). The calculated IC_{50} concentration for Kyn/Trp ratios in LPS-stimulated THP1-Blue cells was 97.0 μ M (71.0–130) μ M (Supplementary material, Table S1).

NF- κ B/AP-1 activation, as indicated by SEAP activity, was significantly upregulated in THP1-Blue cells upon LPS stimulation of about 4-fold (Supplementary material, Fig. S4A). BPA treatment did not affect basal NF- κ B in unstimulated cells but exerted a biphasic effect

on LPS-stimulated cells. Treatment with 12.5 μM BPA lead to a 1.3-fold increase of NF- κB /AP-1 activity compared to LPS-treated control. Higher concentrations of 100 and 200 μM BPA lead to a decrease in NF- κB /AP-1 activity (Supplementary material, Fig. S4B). Calculated IC_{50} concentrations was 139 μM (134–144 μM).

4 Discussion

This study focused upon the evaluation of the effects of BPA on biochemical pathways related to Th1-type immunity, using mitogen-stimulated human PBMCs as a model cell system. Effects were also analysed in LPS-stimulated myelomonocytic THP1-Blue cells.

Treatment of PBMC with BPA suppressed tryptophan breakdown and increased kynurenine formation in a dose-dependent manner in PHA-stimulated cells, leading to a decrease of Kyn/Trp in the concentration range of 25–200 μM BPA. Kynurenine concentrations and Kyn/Trp ratios were also affected in unstimulated cells, however to only a minor extent and with no obvious dose dependency. Suppressive effects on Kyn/Trp by BPA were similar in LPS-stimulated THP1-Blue cells (50 μM BPA), while Kyn/Trp ratios were not significantly affected in unstimulated cells.

A dose-dependent decrease was found also for neopterin and IFN- γ concentrations in PHA-stimulated PBMC indicating an efficient suppression of biochemical pathways related to Th1-type immunity. Of note, neopterin concentrations were also dose-dependently decreased in unstimulated cells, although less marked. Activated macrophages are reported as the main source of neopterin production [32]. This suggests a direct effect of BPA on the monocyte/macrophage population within the PBMCs, where net effects depend on the activation level of the cells. Thereby, production of IFN- γ is one central but not the only affected pathway. Also, the amounts of ROS production could be influenced. This hypothesis is supported by the BPA-mediated inhibition of estimated IDO-1 activity in the stimulated THP1-Blue cell model system. In this cell system, stimulation is mediated by LPS signaling, and no IFN- γ releasing T cells are present.

In addition, low levels of cell activation might occur also during the PBMCs purification procedure. Of note, treatment concentrations in the lower micromolar range of BPA were observed to reduce mitogen induced IFN- γ secretion in murine splenic mononuclear cells *in vitro* [33] and to reduce NO production and increase apoptotic cell death in murine RAW 264.4 macrophages [34]. Importantly, no significant effect was observed on IL-2 production in activated CD4⁺ Jurkat T cells upon treatment with BPA concentrations from 10 nM to 10 μM [35]. Future studies are necessary to address the effects of BPA on isolated human monocytes/macrophages in more detail. An interesting observation is the biphasic effect of BPA on NF- κB /AP-1 activity in LPS-stimulated THP1-Blue cells, which was superinduced at 12.5 μM BPA while being suppressed at higher concentrations. In our study, BPA treatment affected cell viability in both unstimulated and stimulated PBMCs and THP1-Blue cells in a dose-dependent manner. However, in stimulated cells Kyn/Trp was affected to a greater extent than cell viability.

The measurement of cell viability due to decreased reduction of resazurin to resorufin in living cells is a very sensitive method to investigate substance growth inhibitory and toxicity effects. Loss of metabolic activity is also not necessarily related to cell death [36]. To investigate the effect of bisphenol treatment on induction of apoptosis, a proof of principle experiment was performed using FITC-Annexin V/TO-PRO3 staining (Supplementary material, Fig. S2). Data show that resazurin reduction is affected already at lower concentrations where apoptosis is not yet seen to occur. In line with the observation that metabolic parameters are affected at early stages, we suggest that the modulation of tryptophan breakdown and IDO-1 activity by compounds is a phenomenon that precedes/initiates immunotoxicity. Similar observations have been made with other compounds [37]. Interestingly, it has already been suggested that IDO-1 suppression could serve as a sensitive indicator of cell death [24]. Additionally, as both formation of neopterin, a macrophage product, and IFN- γ secretion were affected in PBMCs, most likely both monocytes/macrophages and T cells are affected by BPA toxicity (Fig. 4).

Kynurenine itself and its metabolites 3-hydroxykynurenine, kynurenic acid and quinolinic acid play an important role in the immune system regulation [38]. Kynurenine is an endogenous ligand of the arylhydrocarbon (AhR) receptor thereby interfering with immunoregulatory cascades in T cells, macrophages and dendritic cells [39], but also mast cells [40]. Interestingly, BPA was also shown to negatively interfere with AhR activity [41]. In summary, our data indicate the suppression of IFN- γ dependent Th1-type immune responses by BPA. Such data confirms and extends results reported earlier on effects of BPA upon cytokine production rates in immuno competent murine cells [33,34].

IDO-1 mediated tryptophan metabolism is a key pathway in immunoregulation, being associated with anti-inflammatory or allergy promoting effects as well as the induction of immune deficiency and tolerance depending on the cellular and systemic context [23,24,26]. Thus, suppression of Th1-type immune response by BPA might promote immunodeficiency, increase the risk of infections and promote the carcinogenic potential of BPA [42–44]. Although no Th2-type cytokines have been measured in this study, it can be hypothesized that a suppression of Th1-type immunity by BPA could be linked to an increase in the risk for developing allergy and asthma, due to the cross-regulation of the Th1- and Th2-type immune compartments. BPA can act as an antioxidant as suggested from its structure, and the suppression of Th1-type responses may originate from this property. Earlier *in vitro* studies have already shown that also other antioxidant compounds, *e.g.*, those contained in food, exert immune suppressive properties on PBMC *via* IDO-1 suppression [45] or NF- κ B inhibition [46]. Recent studies on pre- and post natal exposure to BPA strongly suggest an association between exposure and increased risk of asthma and respiratory tract infections throughout childhood [47–49]. Another study reports an BPA exposure associated increase in Th2-cytokine production and a decrease in regulatory T cell number [50].

However, since antioxidant compounds neutralize overwhelming ROS production, our data may also indicate potential anti-inflammatory properties of BPA in terms of suppressing Th1-type pathways. In this regard, the duration of exposure may play a major role as long term exposure and thus chronic suppression may particularly favor above mentioned adverse effects.

One limitation of our study certainly is that the concentrations effective in our *in vitro* experiments appear to be rather high compared to the concentration range observed in humans, and it is still questionable to what extent one can extrapolate results from *in vitro* experiments using such high dosage to possible effects in humans. Perhaps in the gastrointestinal tract one can assume the presence of these compounds in concentrations, which may make antioxidant reactions likely. Another limitation is that the duration of exposure in our experiments was rather short. Thus, we may conclude that there is a possibility that the antioxidant capacity of ingested BPA may shift the redox equilibrium in the gut, which could then have consequences for the whole organism.

5 Conclusion

The results of the present study show that BPA affects cells of the human immune system by suppressing the Th1-type immune response as demonstrated by the decrease of IFN- γ and neopterin formation and the suppression of tryptophan catabolism by IDO-1 in stimulated immune cells. Both monocyte/macrophages and T cells are targeted by BPA immunotoxicity and analysed pathways were modulated already at sublethal concentrations. This data are derived from *in vitro* investigations using cellular model systems, it would be of interest to analyze the immunoregulatory effect of BPA exposure in more detail by comparing tryptophan and neopterin concentration in epidemiological studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BPA	bisphenol A
CTB	Cell-Titer Blue
EDTA	ethylenediaminetetraacetate
ELISA	enzyme-linked immunosorbent assay
FBS	fetal bovine serum
IDO-1	indoleamine 2,3-dioxygenase 1
GTP-GCH-I	GTP-cyclohydrolase
IFN-γ	interferon- γ
Kyn/Trp	kynurenine to tryptophan ratio
LPS	lipopolysaccharide

NF-κB	nuclear factor kappa B
OD	optical density
PBMC	peripheral blood mononuclear cells
PHA	phytohemagglutinin
PBS	phosphate buffered saline
ROS	reactive-oxygen species
SEAP	secreted embryonic alkaline phosphatase

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Fig. 1.

(A) Influence of phytohaemagglutinin (PHA, black bars) upon cell viability compared to unstimulated (white bars) control and vehicle [0.11% (v/v) EtOH] treated cells. $*p < 0.05$ compared to unstimulated control (set as 100%). (B) Influence of bisphenol A (BPA) on cell viability in unstimulated and PHA-stimulated cells. Unstimulated control and PHA-stimulated control cells are set to 100%, respectively; $*p < 0.05$ compared to control cells without added BPA. Cell viability is shown as mean values % of baseline $\pm$ SD of three independent experiments run in duplicates.

Fig. 2.

Influence of bisphenol A (BPA) on tryptophan (A), kynurenine (B) and neopterin (D) concentrations as well as on indoleamine 2,3-dioxygenase (IDO-1) activity, indicated by the kynurenine to tryptophan ratio (Kyn/Trp) (C), in unstimulated in peripheral blood mononuclear cells (PBMC; white bars) as well as in PBMC stimulated with phytohaemagglutinin (PHA; black bars) for 48 h. Values shown are mean $\pm$ SD of three independent experiments run in duplicates, expressed as % of unstimulated or PHA-stimulated control, respectively, for kynurenine, Kyn/Trp and neopterin, and expressed as % of initial medium content for tryptophan. (* $p < 0.05$ compared to cells without added BPA).

Fig. 3.

Influence of bisphenol A (BPA) on production of interferon- γ in peripheral blood mononuclear cells (PBMC) stimulated with phytohaemagglutinin (PHA) for 48 h. Bars show mean $\pm$ SD in % of PHA controls of three independent experiments run in duplicates. * $p < 0.05$ compared to PHA-stimulated cells without added BPA.

Fig. 4.

Potential interactions of bisphenol (BPA) with PBMCs. BPA treatment affected interferon- γ (IFN- γ) secretion as well as phytohemagglutinin (PHA)-induced neopterin formation and tryptophan breakdown. Mitogen treatment induces GTP-CH-I activity and IDO-1 expression and activity. The threshold for stimulation as well as enzyme expression and function may be affected by BPA treatment. As both IFN- γ and neopterin levels are affected, BPA immunotoxicity targets both T cells and macrophages.

Table 1

Comparison of the half-maximal (50%) inhibitory concentrations (IC₅₀) (median effect dose, 95% confidence interval) of bisphenol A treatment upon cell viability, IDO activity as determined by the kynurenine to tryptophan ratio (Kyn/Trp), as well as neopterin and interferon- γ (IFN- γ) formation in unstimulated and phytohaemagglutinin (PHA)-stimulated PBMCs.

	Unstimulated	PHA [10 μ g/mL]
Viability	271 μ M (102–720)	136 μ M (99.6–187)
Kyn/Trp	NA (>200 μ M)	43.3 μ M (40.8–46.0)
Neopterin	NA (>200 μ M)	138 μ M (72.6–261)
IFN- γ	NA	41.9 μ M (23.0–76.3)

Table 2

Concentrations of tryptophan, kynurenine, kynurenine to tryptophan ratio (Kyn/Trp) and neopterin in the supernatant of unstimulated PBMCs and in cells stimulated with 10 µg/ml phytohaemagglutinin (PHA) for 48 h. Results shown are the mean values $\pm$ SD of three independent experiments run in duplicates (* $p < 0.05$, compared to unstimulated cells, respectively).

	Tryptophan (µmol/L)	Kynurenine (µmol/L)	Kyn/Trp (µmol/mmol)	Neopterin (nmol/L)
Unstimulated	23.1 $\pm$ 4.2	1.8 $\pm$ 1.1	79.2 $\pm$ 54.8	2.9 $\pm$ 0.4
PHA [10 µg/mL]	7.2 $\pm$ 2.2*	17.8 $\pm$ 6.5*	2938 $\pm$ 1808*	10.0 $\pm$ 3.7*
Unstimulated + EtOH [0.11%]	24.6 $\pm$ 1.8	1.3 $\pm$ 0.7	55.6 $\pm$ 32.3	2.8 $\pm$ 0.4
PHA [10 µg/mL] + EtOH [0.11%]	7.1 $\pm$ 1.9*	17.6 $\pm$ 6.6*	2816 $\pm$ 1663*	10.4 $\pm$ 4.1*