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

Johanna M. Gostner[a,d], Emanuel Raggl[b], Kathrin Becker[b], Florian Überall[a], Harald Schennach[c], James E. Pease[d], Dietmar Fuchs[b,*]

[a] Division of Medical Biochemistry, Biocenter, Medical University of Innsbruck, Innrain 80, 6020 Innsbruck, Austria
[b] Division of Biological Chemistry, Biocenter, Medical University of Innsbruck, Innrain 80, 6020 Innsbruck, Austria
[c] Central Institute of Blood Transfusion and Immunology, University Hospital, Anichstrasse 35, 6020 Innsbruck, Austria
[d] Receptor Biology Group, Inflammation, Repair and Development Section, National Heart and Lung Institute, Imperial College London, South Kensington Campus, London SW7 2AZ, UK

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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.

Treatment of cells with BPA [12.5–200 μM] 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.



## 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-conjungates, 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

*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.

* Correspondence author at: Division of Biological Chemistry, Biocenter, Medical University of Innsbruck, Rm 4.03-313, Innrain 80, 6020 Innsbruck, Austria. Fax: +43 512 9003 73110.
*E-mail address:* dietmar.fuchs@i-med.ac.at (D. Fuchs).

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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 cytocidal 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 κB (NF-κB), 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-doxygenase (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-κB 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 $1.0 \times 10^6$ cells/ml in RPMI 1640 (Biochrom, Berlin, Germany) supplemented with 10% of heat-inactived 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% $CO_2$) 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 manufacturerís instructions. After 48 h