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Proinflammatory microglial response is a common mechanism of Aroclor 1254- and Tetrabromobisphenol-A-induced neurotoxicity in immature chronically exposed rats

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Abstract

Polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs) are dominant environmental and food contaminants. Tetrabromobisphenol A (TBBPA) is the most widely used BFR in the world to improve the fire safety of laminates in electrical and electronic equipment. Aroclor 1254, one of the PCBs, is widely distributed in the environment due to its extensive use in industrial applications around the world. Both groups of substances are potent toxicants. There is also increasing evidence that they have neurotoxic effects. In this study we tested the pro-inflammatory effects of Aroclor 1254 and TBBPA based on markers of microglial reactivity and levels of pro-inflammatory factors in the brain of immature rats. Aroclor 1254 or TBBPA were administered to the rats by oral gavage for two weeks at a dose of 10 mg/kg b.w. Both light and electron microscopy studies revealed features indicative of microglia activation in brains of exposed rats. Morphological changes were associated with overexpression of pro-inflammatory enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Analysis of cytokine/chemokine array revealed significant secretion of inflammatory mediators following exposure to both TBBPA and Aroclor 1254, which was stronger in the cerebellum than in the forebrain of exposed immature rats. The results indicate a pro-inflammatory profile of microglia activation as one of the neurotoxic mechanisms of both examined toxicants.

Key words: *proinflammatory cytokines, COX-2, i-NOS, microglial activation, chemokines, neuroinflammation.*

Introduction

Over the last decades, a number of toxic substances has been widely released into the environment, some of which have properties that favour their bioaccumulation in living organisms. The dominant environmental pollutants include polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs). Despite the decline in production and use of these highly toxic compounds, they still pose a health problem due to their high persistence in the environment [27]. Significant amounts of BFRs have spread throughout the world, even to West Africa [6] and the polar regions [29]. Several epidemiological reports have demonstrated the presence of these organic compounds in humans and wildlife [22,35,39] emphasizing their ability to accumulate in many tissues [16,19,38,42]. Both groups of compounds are character-

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ized by a high degree of lipophilicity, which allow them to preferentially accumulate in lipid-rich regions of the body such as adipose tissue and the brain. Therefore, they belong to a class of persistent bio-accumulative toxicants that have been shown to be harmful to the central nervous system (CNS) contributing to the induction of neurological deficits, including changes in cognitive and neurobehavioral functions [7]. However, the mechanisms of neurotoxic effects induced by PCBs and BFRs are still not completely understood.

It has been postulated that inflammatory processes may be involved in the toxicant-induced pathology in peripheral tissues. PCBs have been shown to activate a pro-inflammatory signalling cascade associated with atherosclerosis [4] and hepatic disorders [19], and participate in the development of inflammatory diseases [25]. 2,2',4,4',5,5'-heksachlorobiphenyl (PCB 153) has been shown to up-regulate the inflammatory enzyme cyclooxygenase-2 (COX-2) and the proinflammatory cytokines in human mast cells [20]. Similarly, the BFR member, tetrabromobisphenol A (TBBPA), significantly increased the levels of tumor necrosis factor α (TNF-α), interleukin 6 (IL-6) and interferon γ (IFN-γ), altering the immune response to infection in mice [38] and induced inflammation of the gastric mucosa [41], and lungs of mice [46]. However, no comprehensive studies of such mechanisms in the brain have been conducted so far.

Microglia are resident immune cells in the CNS that exhibit homeostatic activity under physiological conditions. In response to pathological stimuli, they are readily activated and play a key role in the development of the inflammatory response in the brain. Activated microglial cells can induce detrimental neurotoxic effects by releasing proinflammatory factors (cytokines, prostaglandins) and oxidative stress factors (nitric oxide, reactive oxygen species) [44], thus contributing to the onset of inflammation and tissue damage in many brain disorders [11,45]. Under physiological conditions, when microglia are in a resting state, the expression of cytokines and chemokines in the CNS is low, but increases rapidly when exposed to various pathological stimuli [11], including toxic agents such as lead (Pb) [10,32]. Activated microglia also express cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS), which are important factors contributing to the inflammatory response.

Cyclooxygenase-2 is involved in the formation of prostaglandins (PGs), which are critical inflammatory mediators [37] while iNOS plays an important role in promoting nitrosative stress thus leading to neuronal damage [17]. The inflammatory cascade can therefore contribute to destructive processes in the brain.

The present study aimed to investigate the microglial response induced by chemicals representative of two groups of toxicants, PCBs and BFRs. We examined whether chronic exposure to both types of neurotoxicants, Aroclor 1254 (commercial mixture of polychlorinated biphenyl congeners) or TBBPA (a major BFR), could activate microglia and induce a proinflammatory response in the brains of exposed immature rats. The effect of exposure to Aroclor 1254 and TBBPA on the expression of inflammatory enzymes (COX-2, i-NOS) and pro-inflammatory factors, such as cytokines and chemokines, in the rat brain was investigated.

Material and methods

Animals

All procedures using animals were carried out in strict accordance with the EU Directive for the Care and Use of Laboratory Animals (Directive 2010/63/EU) and were approved by the Local Experimental Animal Care and Use Committee in Warsaw.

The research was carried out on two-week-old Wistar rat pups of both sexes (n = 42) purchased from the Animal House of the Mossakowski Medical Research Institute, Polish Academy of Sciences (Warsaw, Poland). Pups at postnatal day 14 (PND 14) were randomly allocated into three groups: 1) control, 2) Aroclor 1254-treated and 3) TBBPA-treated. The compounds (purchased from LGC Standards, Lomianki, Poland) were dissolved in edible rapeseed oil and administered by oral gavage at a dose of 10 mg/kg b.w./day for two weeks. Control animals received only vehicle. Oral administration was chosen to resemble human exposure *via* the food chain.

After two weeks of exposure, the animals were sacrificed by decapitation, the brains were removed, placed on ice and dissected into two regions: the cerebellum and the forebrain cortex. Each part of the brain was homogenized separately in sucrose medium (0.32 M sucrose, 1 mM EDTA) and centrifuged at 1000 × g for 10 min. The supernatants were used for immunoblotting analysis.

Western blot analysis

Protein concentration was determined according to the method of Lowry *et al*. [24] using bovine albumin as a standard. Samples containing 100 µg of proteins were subjected to SDS-polyacrylamide gel (10%) electrophoresis. Samples were transferred onto nitrocellulose membranes and incubated overnight (4°C) with the primary antibody: polyclonal anti-COX2, (Abcam; 1 : 400) or polyclonal anti-iNOS (Abcam; 1 : 500). Thereafter, the secondary antibody conjugated with HRP (Sigma-Aldrich) was applied. As an internal standard, polyclonal anti-β actin antibody (Sigma-Aldrich; 1 : 500) was used. Bands were visualized with the chemilumi-

nescence ECL kit (Amersham), exposed to Hyperfilm ECL and quantified using densitometry Image Scanner III (GE Healthcare) with Image Quant TL v2005 program.

Analysis of inflammatory cytokines using RayBio® rat cytokine antibody array

The RayBio® kit provides a simple and highly sensitive (pg/ml) method for simultaneous detection of multiple cytokines. The two regions of freshly isolated brain were homogenized to obtain tissue lysates using the cell lysis buffer provided with the kit. The remaining steps of the procedure were performed according to the attached user manual. After extraction, the samples were centrifuged and the supernatant was left for analysis.

The membranes were first blocked with 2 ml of blocking buffer for 30 min, and then covered with lysate samples (100 µg of brain tissue protein). After overnight incubation at 4°C with gentle rotation, samples were decanted and washed three times with 2 ml of wash buffer I and then with 2 ml of wash buffer II. Blocking buffer was added to the tube containing the mixture of biotin-conjugated anti-cytokine antibodies, which was then applied to each membrane. Membranes were incubated overnight at room temperature. Thereafter, 2 ml of 1000-fold diluted horse radish peroxidase-conjugated streptavidin was added to each membrane and incubated at room temperature for 2 h. Detection buffer C and D provided in the kit were mixed, pipetted onto the membranes, and incubated for 2 min. Signals were detected directly from the membrane using a chemiluminescence imaging system. Dots were quantified using densitometry Image Scanner III (GE Healthcare) with Image Quant TL v2005 program. The signals were compared to the table included in the kit to identify released cytokines.

Immunohistochemical procedure

Animals were anesthetized with ketamine hydrochloride (20 mg/kg b.w.) and perfused through the heart with phosphate-buffered saline (PBS; pH 7.4), and subsequently with 250 ml of ice-cold fixative (4% paraformaldehyde in PBS). Brains were then post-fixed in the same fixative for 1.5 h and cryoprotected (10% sucrose in PBS, overnight; 20% sucrose, 2 days; 30% sucrose, 4 days). Tissues were then frozen and cut on 40-micron thin sections collected in PBS with sodium azide (0.1%). Sections were immunostained using primary anti-Iba-1 antibody (Sigma Aldrich, 1 : 400). Secondary antibodies were conjugated with the fluorescent Alexa Fluor (Invitrogen, 1 : 100). The negative control of immunostaining specificity was performed by omitting primary antibodies in the incubation mixture. Images were obtained using BH2-RFCA Olympus fluorescent microscopy and CC12 Soft Imaging System Camera and Cell software.

Transmission electron microscopy

For electron microscopic studies, animals were anaesthetized with ketamine hydrochloride (20 mg/kg b.w.) and perfused through the heart with fixative solution (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 cacodylate buffer pH 7.4). Two hours after the perfusion, brains were removed, small specimens of tissue were taken from the cerebellum and forebrain cortex which were further subjected to the routine TEM procedure. Ultrathin sections were examined with a JEM 1200 EX electron microscope.

Statistical analysis

The results are expressed as mean ±SD from the number of experiments indicated in figure legends. Inter-group comparison was made using a one-way analysis of variance ANOVA followed by Tukey's or Bonferroni's multiple comparison tests. Significance was established at $p < 0.05$. The data analyses were performed using GraphPad Prism Software, version 6.0 (GraphPad Software, La Jolla, San Diego, CA, USA).

Results

Microglia activation in brain regions of animals exposed to Aroclor 1254 and TBBPA

Microglial activation was assessed by immunohistochemical analysis using antibody against Iba-1, a commonly used microglial marker [23]. We observed increased anti-Iba-1 immunostaining in the brains of rats from both experimental groups (Fig. 1). Increased numbers of microglial cells were seen in the forebrain and cerebellum of both Aroclor 1254- and TBBPA-treated animals compared to untreated control animals, although the proliferation of microglial cells was not very intense. However, the cells exhibited the typical morphological features of the activated state, such as shorter and thicker processes and more intense Iba-1 immunoreaction compared to control cells, which showed a ramified morphology with thin and much longer processes. The intensity of microglial activation in both examined brain structures, the cerebellum and the forebrain cortex, was similar (Fig. 1). Characteristic features of cell activation were also observed in TEM analysis.

Microglial cells in the brain of a control, untreated rat exhibited features typical of the resting state, i.e. sparse cytoplasm and few organelles (Fig. 2A). Microglial cells from the brain of rats treated with Aroclor

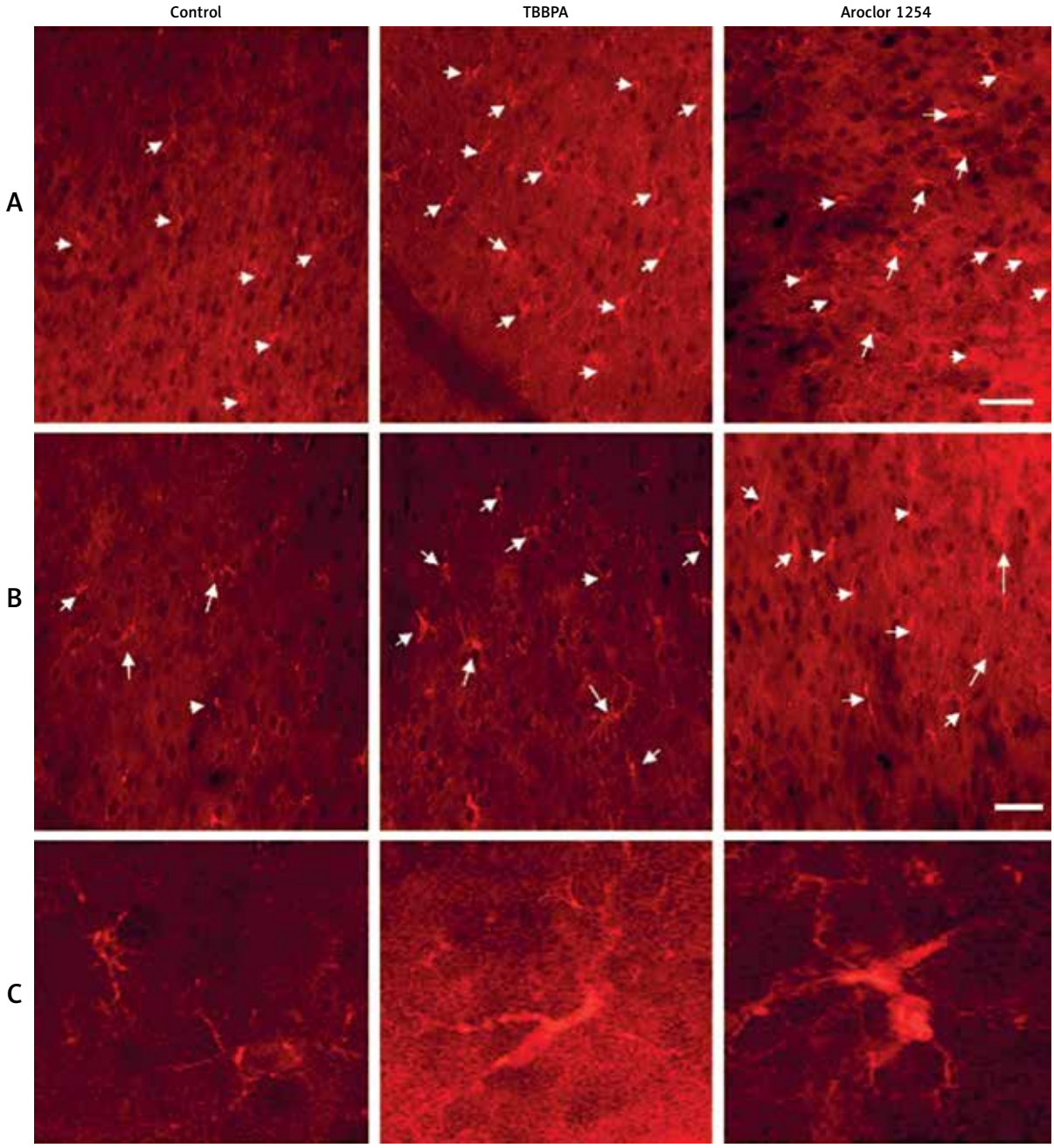


Fig. 1. Representative images of microglia stained with Iba-1 antibody in control, TBBPA- and Aroclor 1254-treated rat brains. Arrows show some of immunolabeled cells present in the forebrain (**A**) or cerebellum (**B**). Increased Iba-1 expression is visible in both exposed groups. Bar = 50 µm. The cells show typical morphological features of the activated state, such as shorter and thicker processes, and more intense Iba-1 immunofluorescence as compared to control cells, which showed ramified morphology with thin and much more longer processes (**C**).

1254 or TBBPA showed morphological characteristic indicative of a reactive state, such as large numbers of mitochondria and enlarged Golgi complexes, and rough endoplasmic reticulum (RER) indicating activation of these organelles (Fig. 2B-D).

Cytokines released in Aroclor 1254- and TBBPA-exposed rat brain

The morphological features of microglia activation observed in exposed rats were accompanied by changes in the relative expression of cytokines detected by

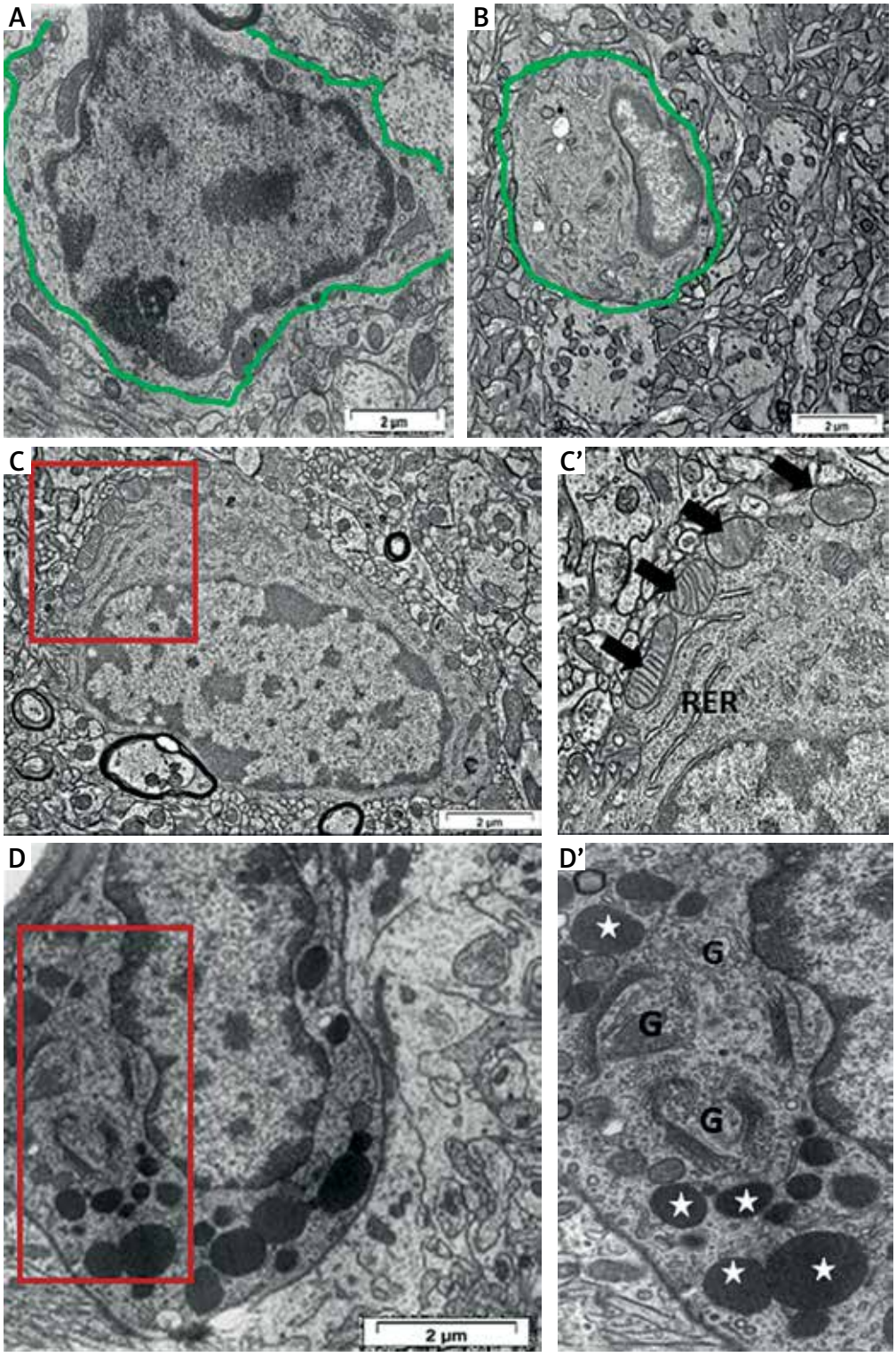
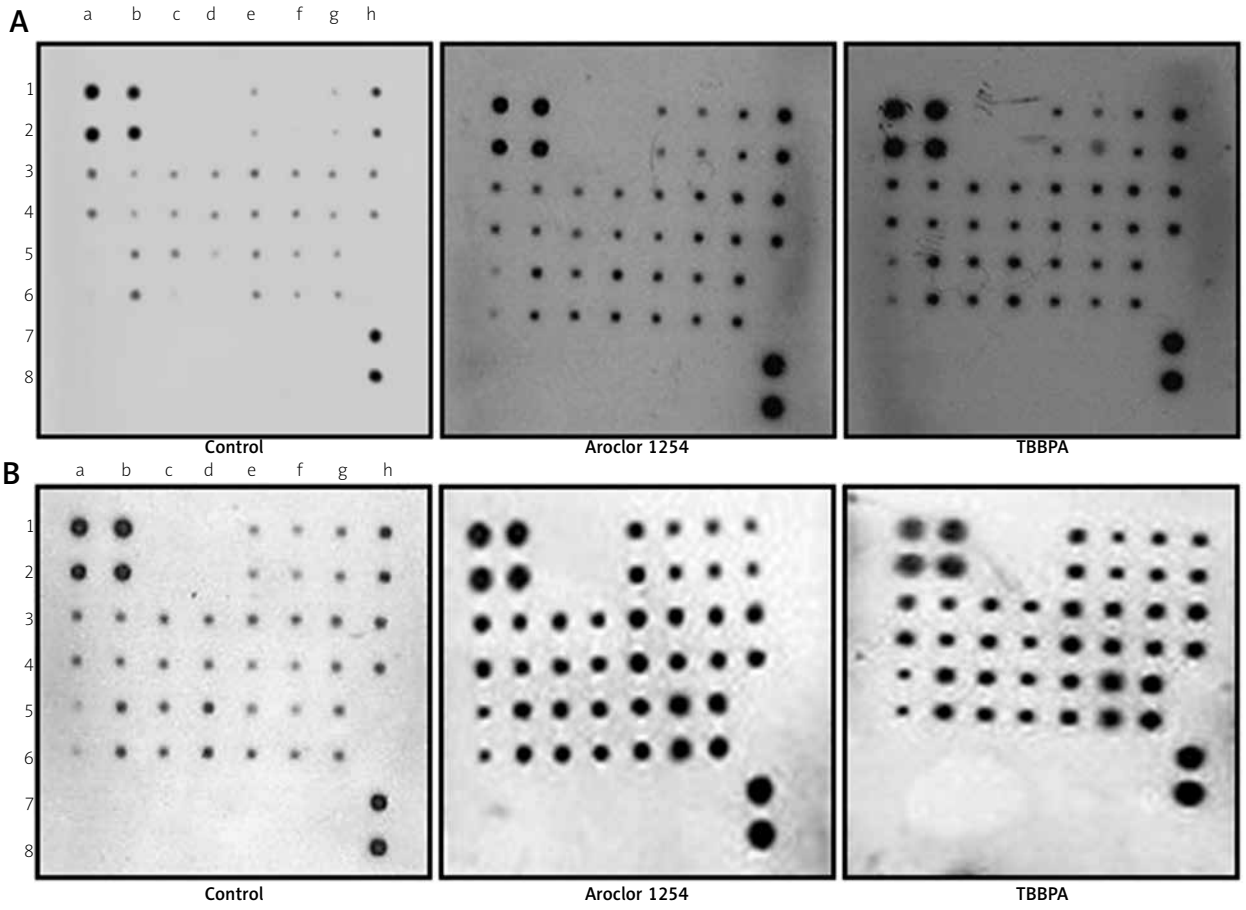


Fig. 2. Representative electromicrographs of microglial cells in control (**A**), Aroclor 1254- and TBBPA-exposed (**B-D**) rat brains. Morphological changes characteristic of the reactive state, such as large numbers of mitochondria (black arrows) and enlarged cisternae of the rough endoplasmic reticulum (RER) (**C**, **C'**); enlarged and active Golgi complexes (G), and numerous lysosomes (white asterisks) (**D**, **D'**).



C

	a	b	c	d	e	f	g	h
1	POS	POS	NEG	NEG	CINC-2	CINC-3	CNTF	fractalkine
2	POS	POS	NEG	NEG	CINC-2	CINC-3	CNTF	fractalkine
3	GM-CSF	IFN-γ	IL-1α	IL-1β	IL-4	IL-6	IL-10	LIX
4	GM-CSF	IFN-γ	IL-1α	IL-1β	IL-4	IL-6	IL-10	LIX
5	LEPTIN	MCP-1	MIP-3a	β-NGF	TIMP-1	TNF-α	VEGF	BLANK
6	LEPTIN	MCP-1	MIP-3a	β-NGF	TIMP-1	TNF-α	VEGF	BLANK
7	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	POS
8	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	POS

Fig. 3. Cytokines/chemokines array in the forebrain (**A**) and cerebellum (**B**) of control, Aroclor 1254 and TBBPA-exposed rat brains. The table (**C**) shows the location of antibodies for respective cytokines: cytokine-induced neutrophil chemoattractant (CINC), ciliary neurotrophic factor (CNTF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN), interleukin (IL), lipopolysaccharide-inducible CXC chemokine (LIX), monocyte chemoattractant protein 1 (MCP-1), nerve growth factor (β-NGF), tissue inhibitor of metalloproteinase-1 (TIMP-1), macrophage-inflammatory protein-3α (MIP-3α), metallopeptidase inhibitor 1 (TIMP1) and vascular endothelial growth factor (VEGF).

the RayBio® array. In control animals, the immunodensity of most "dots" was low. However, after exposure to both Aroclor 1254 and TBBPA, the immunodensity increased markedly, indicating protein overexpression of the entire cytokine/chemokine panel in both brain structures examined (Fig. 3). The results of densitometric analysis of immunoreaction intensity revealed statistically significant differences between the control and exposed groups (Fig. 4).

We noted that exposure to Aroclor 1254 or TBBPA induced significant overexpression of key pro-inflammatory mediators such as IL-1β, IL-1α, TNF-α, IL-6, IFN-γ,

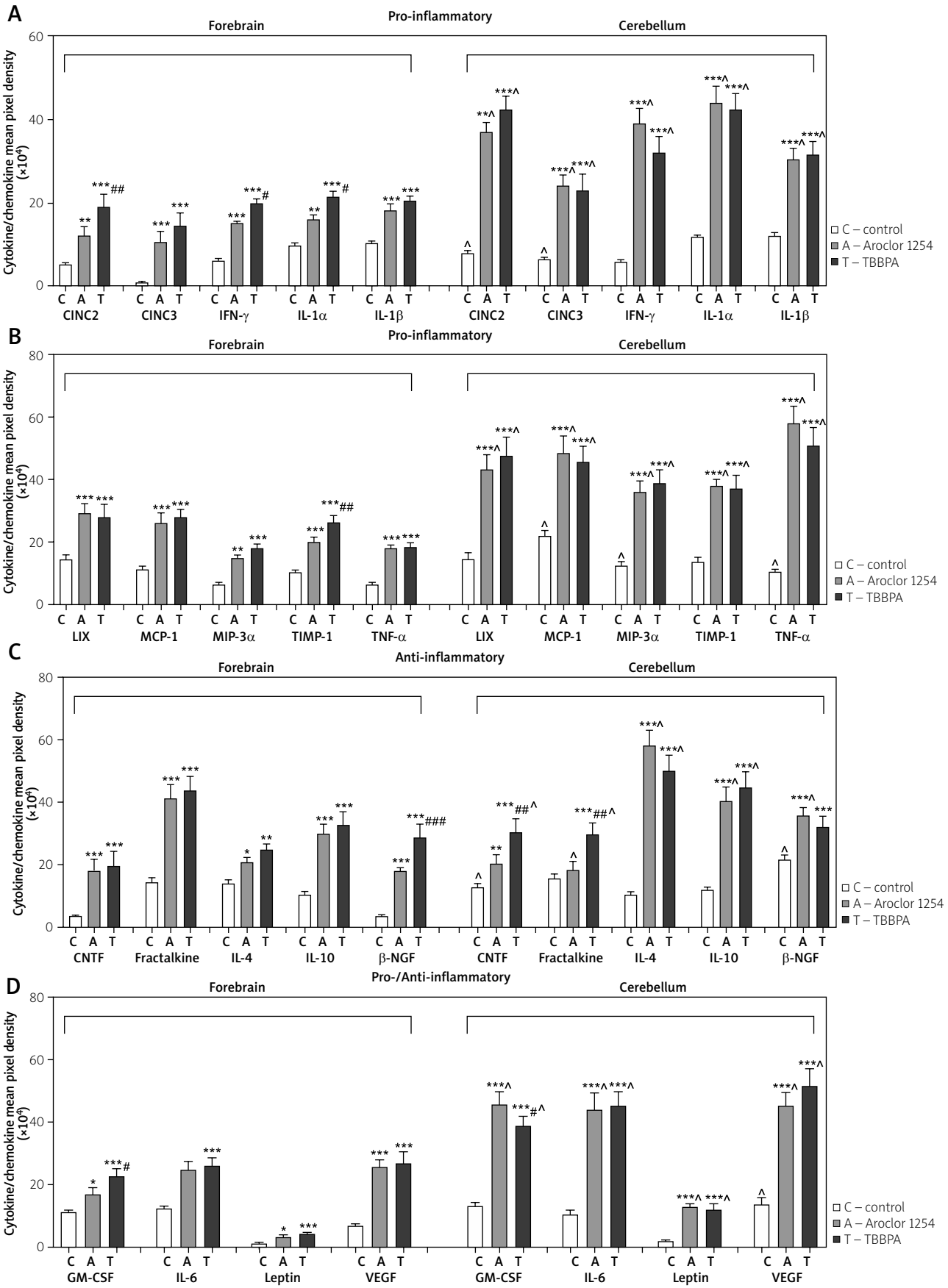


Fig. 4. The graph shows the results of densitometric measurements of dots detected on Ray Bio© membranes, which represent the respective cytokines/chemokines secreted in the control group and toxicant-exposed groups in both brain regions examined. Measurements were performed on 4 membranes each using individual brain samples, forebrain or cerebellum, respectively. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. Aroclor 1254 group; $^{\wedge}p < 0.05$ vs. forebrain (one-way ANOVA with post hoc Bonferroni's multiple comparison test).

Table I. The changes in the cytokines/chemokines profile in the forebrain and cerebellum of rats treated with Aroclor 1254 and TBBPA vs. control rats

Fold of the control immunodensity					
Cytokine/ chemokine	Pro-/anti-inflammatory	Forebrain		Cerebellum	
		A 1254	TBBP-A	A 1254	TBBPA
CINC-2	Pro	2.3	3.6	4.7	5.4
CINC-3	Pro	9.9	13.5	3.7	3.5
CNTF	Anti	4.5	4.9	1.6	2.3
Fractalkine	Anti	2.8	3.0	1.2	1.9
GM-CSF	Pro/anti	1.5	2.0	3.5	2.9
IFN-γ	Pro	2.4	3.2	6.8	5.6
IL-1α	Pro	1.6	2.2	3.8	3.6
IL-1β	Pro	1.7	2.0	2.5	2.6
IL-4	Anti	1.5	1.7	5.5	4.8
IL-6	Pro/anti	2.0	2.1	4.2	4.2
IL-10	Anti	2.8	3.1	3.3	3.7
LIX	Pro	2.0	1.9	3.0	3.3
Leptin	Pro/anti	2.3	3.0	6.3	6.0
MCP-1	Pro	2.3	2.5	2.2	2.0
MIP-3α	Pro	2.3	2.8	2.8	3.0
β-NGF	Anti	5.0	7.9	1.7	1.5
TIMP-1	Pro	1.9	2.5	2.7	2.7
TNF-α	Pro	2.7	2.8	5.4	4.8
VEGF	Pro/anti	3.7	3.8	3.3	3.8

The table presents the results of densitometric analysis of pro- and anti-inflammatory cytokines/chemokines as a fold of the control value. The mean immunodensity of each cytokine/chemokine secreted in animals under exposure to the toxins (presented in Fig. 4) is referred to the mean immunodensity of the respective control, which is taken as 1.

as well as chemokines involved in leukocyte recruitment during inflammation, i.e. cytokine-induced neutrophil chemoattractants CINC-2 and CINC-3 and monocyte chemoattractant protein-1 (MCP-1) (Fig. 4). In parallel, we also observed the induction of anti-inflammatory mediators such as IL-4, IL-10, β-NGF, ciliary neurotrophic factor (CNTF) or fractalkine in both exposed groups.

An interesting observation is that the intensity of the response was similar regardless of the type of the toxicant, but differed significantly between the structures examined. In the cerebellum, the relative protein levels of several secreted cytokines were significantly higher than in the forebrain. In this structure, the levels of proinflammatory cytokines such as IL-1α, IL-6, TNF-α and INF-γ increased 4-6 times compared to control levels, while in the forebrain they exceeded control levels only 2-3 times (Table I). Among other pro-inflammatory factors, strong overexpression of CINC-2, LIX, leptin or MIP-3α was found. In turn, the induction of some anti-inflammatory mediators, such as fractalkine, CNTF or β-NGF was lower in the cerebellum than in the forebrain. This indicates a more intense inflammatory response along with a disturbed balance between pro- and anti-inflammatory factors, suggesting that the cerebellum is much more vulnerable to the tested toxic substances during developmental exposure than the forebrain.

Overexpression of pro-inflammatory enzymes in the brain of rats exposed to Aroclor 1254 and TBBPA

In the next step of the study, we assessed the expression of COX-2 and iNOS, the enzymes involved in the mechanisms of inflammatory response. Western blot analysis revealed a similar trend of changes in the relative content of both enzymes in the examined brain regions. Exposure to Aroclor 1254 led to significant overexpression of COX-2 protein by about 30% compared to the control group in both the cortex and cerebellum. Similarly, exposure to TBBPA increased COX-2 expression by about 40-20% relative to controls in the cortex and cerebellum, respectively (Fig. 5A). Also iNOS protein was overexpressed in exposed rats. Both substances significantly increased the expression of

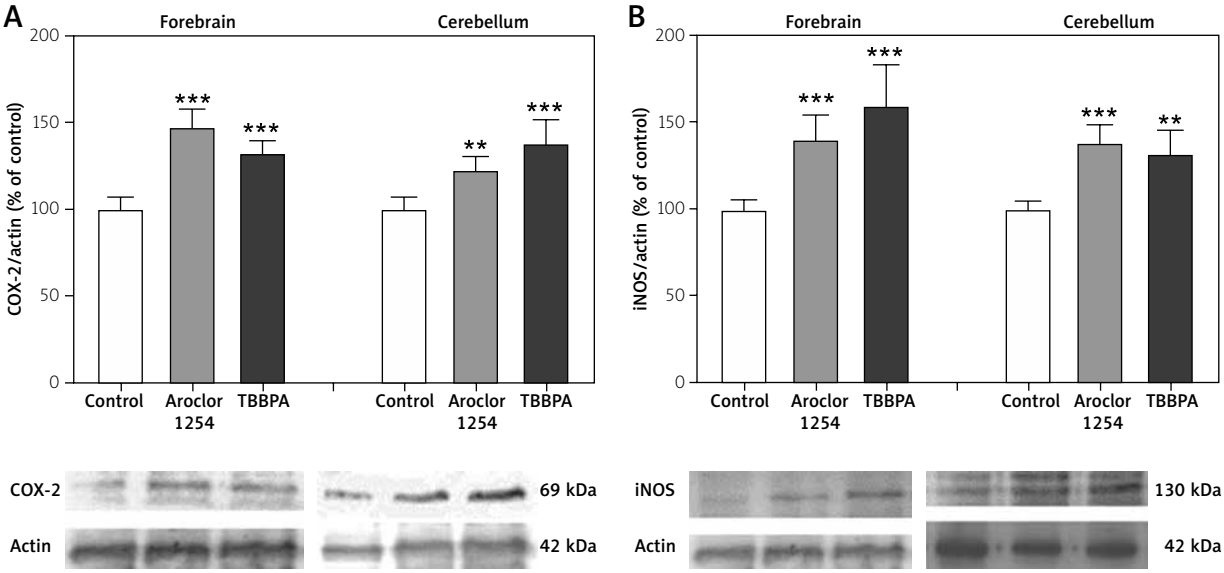


Fig. 5. Expression of COX-2 (**A**) and iNOS (**B**) proteins in the forebrain and cerebellum of the young rat brain after prolonged exposure to Aroclor 1254 or TBBPA. Representative immunoblots and graphs presenting the results of densitometric measurements of immunoblots performed on individual brain samples ($n = 4$), normalized against β-actin and expressed as a percentage of respective controls. *$p < 0.05$ (one-way ANOVA with post hoc Tukey test).

iNOS protein in the cerebellum and forebrain by about 20-25% (Aroclor 1254) or 20-30% (TBBPA) (Fig. 5B).

Discussion

Organohalogenated pollutants such as PCBs and BFRs are widespread in nature and display a complex spectrum of toxicological properties, including neurotoxicity. In the brain, which is susceptible to the accumulation of organic toxicants due to the high content of fatty acids, they contribute to a number of adverse effects. Exposure to Aroclor 1254 was associated with substantial neuronal loss and inhibition of NMDA receptor-mediated glutamatergic signalling in the rat hippocampus [15]. It has also been found to markedly reduce the expression of genes involved in dopamine, serotonin, and opioid systems [1], as well as downregulate the main glial glutamate transporter GLT-1, which may potentially lead to disturbances in glutamate clearance followed by excitotoxicity [33]. In turn, exposure to TBBPA impaired hippocampal neurogenesis and cognitive function in mice [18].

Although the induction of an inflammatory response by PCBs and BFRs in peripheral tissues has been confirmed [4,20,41,46,47], the neuroinflammatory effects have not been thoroughly investigated. Moreover, the mechanisms of developmental neurotoxicity of these substances are poorly defined.

Therefore, in the present study we investigate the potential of a commercial mixture of PCBs (Aroclor 1254) and the most commonly used BFRs (TBBPA) to induce proinflammatory microglial activation. Since immature organisms are much more susceptible to neurotoxicants than adults when exposed prenatally or early postnatally, an *in vivo* model of developmental exposure was used in the study.

Microglia are typically in a resting state characterized by a ramified morphology. In response to certain pathological conditions, brain microglia become activated and undergo morphological transformation from their ramified state into an amoeboid morphology [44]. The results of microscopic studies indicate that exposure to both examined toxins leads to the activation of the microglial pool of cells confirmed by the overexpression of the microglial marker Iba-1 and cell morphology (Figs. 1, 2).

Activated microglial cells can secrete a number of mediators, including proinflammatory factors (TNF-α, IL-1β, IL-6, prostaglandins) and oxidative stress factors (nitric oxide, hydrogen peroxide) [13]. Here we show that microglial activation induced by Aroclor 1254 and TBBPA results in the release of a number of cytokines and chemokines, including those of proinflammatory potential, such as TNF-α and IL-1β, which play a significant role in initiating and regulating cytokine cascade during the inflammatory response [48], as well as IFN-γ, IL-6, LIX, leptin, MCP-1, MIP-3α, CINC-2, CINC-3 (Figs. 3, 4,

Table I). Some of these proteins regulate inflammatory cell trafficking during inflammation [26,40]. Previous studies have suggested that CINCs are involved in acute inflammation by playing an important role in neutrophil recruitment [34]. In turn, MIP-3α is overexpressed in response to inflammatory mediators such as IL-1β and TNF-α [31].

Analysing the profile of cytokines/chemokines induced by exposure to Aroclor 1254 and TBBPA, we also noted an increase in IFN-γ. Interferon γ-induced effects include activation of i-NOS and stimulation of the production of other cytokines. Other stimuli, including IL-1, lipopolysaccharide (LPS), and TNF-α have also been shown to induce i-NOS in both astrocytes and microglia [12]. Another factor is leptin, which we found to be overexpressed concomitantly with iNOS, particularly in the cerebellum. The pro-inflammatory properties of leptin are expressed through upregulation of multiple inflammatory cytokines, including TNF-α, IL-6 and IL-12, and promotion of NO, iNOS, COX-2 and prostaglandin E production [21].

Prostaglandins (PGs) are largely produced during brain inflammation by activated microglia and reactive astrocytes from arachidonic acid (AA) by two isoforms of the cyclooxygenase enzyme, COX-1 and COX-2. Through the production of PGs, cyclooxygenases perform various critical functions in the regulation of inflammatory pathways [12]. In particular, COX-2 expressed in inflammatory cells has attracted much attention as the isoform highly involved in the acute and chronic inflammatory stages [37,43].

Our studies revealed that exposure of immature rats to both Aroclor 1254 and to TBBPA increases expression of COX-2 by about 30% (Fig. 5). The results are consistent with previous reports showing elevated levels of COX-2 in mouse liver [30] and in human leukemic mast cell line [20] exposed to PCBs.

Once activated, microglia release a number of cytokines that act in a complex manner *via* a vicious circle mechanism. Our results demonstrate that exposure of immature rats to both Aroclor 1254 and TBBPA significantly increases the levels of iNOS and COX-2 proteins, which may be the result of the orchestrated activity of various cytokines/chemokines acting to potentiate neuroinflammation and subsequent neuronal damage.

In parallel with the overexpression of inflammation-mediating factors, we also observed the release of cytokines/chemokines of protective anti-inflammatory nature, including fractalkine (CX3CL1 chemokine). Since fractalkine is highly expressed in neurons in the healthy brain, it is postulated to play a protective role [2], acting as an anti-inflammatory factor by inhibiting microglia activation and maintaining cells in a state of low responsiveness [49]. It has been reported to suppress the production of NO and pro-inflammatory cytokines such as TNF-α by activated microglia [28]. We found that fractalkine is highly overexpressed (about 3-fold) in the forebrain of rats exposed to Aroclor 1254 and TBBPA, while it is only slightly induced in the cerebellum. This suggests that this protective mechanism is not effective in the cerebellum.

Similarly, β-NGF did not increase significantly in the cerebellum compared with the forebrain. NGF has been reported to attenuate pro-inflammatory response in microglia by downregulating the pro-inflammatory cytokines and NO and thereby contributing to the regulation of microglia-mediated neuroinflammation [9].

Our comprehensive results indicating overexpression in a panel of cytokines/chemokines are in accordance with previous reports selectively showing the presence of individual inflammatory mediators in animals exposed to various compounds belonging to PCBs and BFRs groups. Hayley and coworkers [14] described that exposure to PCB significantly elevated hypothalamic IL-6, TNF-α and anti-inflammatory cytokine, IL-10. It has also been shown that PCBs markedly upregulate the expression of MCP-1 [3] and VEGF [5,8] in human vascular endothelial cells. Similarly, increased levels of TNF-α, IL-6, INF-γ were reported in BFR-exposed mice [38]. Exposure to TBBPA significantly increased the levels of the pro-inflammatory factors TNF-α, IL-1β, and IL-6 and decreased the level of the anti-inflammatory factor IL-10 in the gastric mucosa [41].

The final effect of the inflammatory response is the result of the balance between pro- and anti-inflammatory mediators. Pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α are involved in early response and amplify the inflammatory reaction, while anti-inflammatory cytokines, such as IL-4, IL-10, and IL-13 limit it. Our results indicate a significant imbalance between opposing factors during neuroinflammation induced by both A1254 and TBBPA, particularly in the cerebellum, where a severe pro-inflammatory reaction is combined with an insufficient anti-inflammatory response. Such an imbalance is usually associated with the so-called cytokine storm or cytokine release syndrome (CRS) characterized by loss of immune homeostasis. The key factors identified in cytokine storm pathology are TNF-α, interferons, IL-1β, MCP-1 (CCL2), and most importantly IL-6 [36].

In conclusion, the present study provides evidence that prolonged exposure to Aroclor 1254 and TBBPA causes microglial activation in the brain of immature rats associated with the release of a number of cytokines/chemokines with both pro- and anti-inflammatory nature.

In parallel, both toxins lead to the overexpression of pro-inflammatory enzymes, iNOS and COX2. The re-

sults clearly indicate that the inflammatory response of activated microglia is a common reaction shared by the examined representatives of the two groups of environmental toxicants, PCBs and BFRs. However, the observed neurotoxic effect is region-dependent and is more strongly expressed in the cerebellum than in the forebrain. Our findings strongly suggest that both Aroclor 1254 and TBBPA are neurotoxicants involved in neuroinflammatory events in the developing brain, which may likely contribute to brain injury.

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Disclosure

The authors report no conflict of interest.

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