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PCB 126 Induces Monocyte/Macrophage Polarization and Inflammation through AhR and NF-κB pathways

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Abstract

Polychlorinated biphenyls (PCBs) are persistent organic pollutants that contribute to inflammatory diseases such as atherosclerosis, and macrophages play a key role in the overall inflammatory response. Depending on specific environmental stimuli, macrophages can be polarized either to pro-inflammatory (e.g., M1) or anti-inflammatory (e.g., M2) phenotypes. We hypothesize that dioxin-like PCBs can contribute to macrophage polarization associated with inflammation. To test this hypothesis, human monocytes (THP-1) were differentiated to macrophages and subsequently exposed to PCB 126. Exposure to PCB 126, but not to PCB 153 or 118, significantly induced the expression of inflammatory cytokines, including TNFα and IL-1β, suggesting polarization to the pro-inflammatory M1 phenotype. Additionally, monocyte chemoattractant protein-1 (MCP-1) was increased in PCB 126-activated macrophages, suggesting induction of chemokines which regulate immune cell recruitment and infiltration of monocytes/macrophages into vascular tissues. In addition, oxidative stress sensitive markers including nuclear factor (erythroid-derived 2)-like 2 (NFE2L2; Nrf2) and down-stream genes, such as heme oxygenase 1 (HMOX1) and NAD(P)H quinone oxidoreductase 1 (NQO1), were induced following PCB 126 exposure. Since dioxin-like PCBs may elicit inflammatory cascades through multiple mechanisms, we then pretreated macrophages with both aryl hydrocarbon receptor (AhR) and NF-κB antagonists prior to PCB treatment. The NF-κB antagonist BMS-345541 significantly decreased mRNA and protein levels of multiple cytokines by approximately 50% compared to PCB treatment alone, but the AhR antagonist CH-223191 was protective to a lesser degree. Our data demonstrate the involvement of PCB 126 in macrophage polarization and inflammation, indicating another important role of dioxin-like PCBs in the pathology of atherosclerosis.

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Keywords

dioxins; cardiovascular disease; PCB 126; inflammation; atherosclerosis; monocyte macrophage polarization

1. Introduction

Atherosclerosis is the result of chronic inflammation of blood vessels, leading to a progressive narrowing of the arterial wall because of the formation of an atheromatous plaque, which contains a necrotic core, calcified sites, lipids and many cell types (Ross, 1999). Polychlorinated biphenyls (PCBs) are a group of persistent and widespread environmental pollutants, which can contribute to a range of adverse health effects, including atherosclerotic cardiovascular diseases (Choi et al., 2003; Petriello et al., 2016; Wahlang et al., 2017). In fact, we recently observed that exposure to the dioxin-like PCB 126 increased systemic inflammation and accelerated atherosclerosis in a lean LDL receptor-deficient mouse model (Petriello et al., 2018). Numerous studies have also demonstrated that coplanar (or dioxin-like) PCBs can cause vascular endothelial dysfunction as evidenced by an upregulation of inflammatory mediators including the cytokine interleukin-6 (IL-6), vascular adhesion molecule-1 (VCAM-1), and monocyte chemoattractant protein-1 (MCP-1) (Eske et al., 2014; Helyar et al., 2009; Majkova et al., 2009). Furthermore, exposure to coplanar PCBs, such as PCB 77 and PCB 126, caused inflammation in endothelial cells via activation of the NF-κB signaling pathway (Liu et al., 2015).

In addition to endothelial cell activation and dysfunction, accumulating evidence has shown that atherosclerotic lesion formation and growth are effected by both innate and adaptive immune responses (Weber & Noels, 2011; Weber et al., 2008). Although many different types of cells are involved in the initiation and progression of atherosclerosis, macrophages are fundamental contributors, as they migrate from the blood and infiltrate into the intima, release pro-inflammatory cytokines and take up lipids leading to subsequent formation of foam cells. These inflammatory signals in turn result in continuous monocyte recruitment, which is necessary for atherosclerotic plaque maintenance and progression (Weber & Noels, 2011; Weber et al., 2008). Previous studies have shown that all atherosclerosis-related processes (initiation and expansion of lesions, necrosis and rupture, clinical manifestations, resolution and regression of lesions) are influenced by the presence of macrophages (Colin et al., 2014; Tabas & Bornfeldt, 2016).

Serving to protect the organism from infection both in innate and adaptive immune system, macrophages display high plasticity and versatility, in particular, the ability to promote inflammation when needed and switch off inflammatory responses when it is no longer necessary. Such events depend on the fact that macrophages can be activated by the combination of different environmental stimuli. In spite of a spectrum of inflammatory features and functional properties, there are two main groups, classically activated and alternatively activated macrophages, also termed M1 and M2, respectively (Mantovani et al., 2005; Mosser & Edwards, 2008). The macrophage phenotypes and functions can be

assessed by their surface markers and secretome. For example, classical macrophage activation is a response to microbial products (e.g. lipopolysaccharide, LPS) or cytokines (e.g. interferon-γ, IFN-γ; tumor necrosis factor, TNFα). The M1 macrophage is classically characterized by the high secretion of inflammatory cytokines, which is regulated in part via nuclear factor NF-kB signaling (C. P. Liu et al., 2017). Such cytokines include interleukin-1 (IL-1), IL-6, IL-12, IL-23 and TNFα, which play a role in host defense role, and are also considered pro-atherogenic (Martinez & Gordon, 2014). Alternative macrophage polarization (e.g., M2) can be induced by IL-4 and IL-13, or IL-10 cytokines, and are involved in parasite killing, tissue remodeling, and immunoregulation (Martinez & Gordon, 2014).

In atherosclerotic lesions, macrophages are mainly derived from peripheral blood mononuclear cells, which have long been recognized to be heterogeneous. Recent studies show that there is a highly heterogeneous phenotype and environment in atherosclerotic lesions, such as platelet chemokine CXCL4-induced macrophages, M4 (Domschke & Gleissner, 2017; Erbel et al., 2015), oxidized phospholipid-induced macrophages, Mox (Kadl et al., 2010), hemoglobin (Hb)-stimulated macrophages, M(Hb) (Finn et al., 2012) and heme-induced macrophages, Mhem (Boyle et al., 2012). From a functional point of view, M1 and M4 macrophages display a pro-inflammatory profile and are thus pro-atherogenic (Mantovani et al., 2005; Domschke & Gleissner, 2017). Mox macrophages in mice were found to exhibit reduced phagocytic capacity and to express anti-oxidant genes (Kadl et al., 2010). M2, M(Hb), and Mhem showed anti-inflammatory function by preventing foam cell formation (De Paoli et al., 2014; Vinchi et al., 2014). Lesion-associated macrophages interact with a large variety of environmental stimuli, such as cytokines and oxidized phospholipid, which polarize macrophages to a certain phenotype and contribute to atherosclerosis (Chistiakov et al., 2015; Shapouri-Moghaddam et al., 2018).

We previously have shown that PCB 126, a coplanar or dioxin-like PCB and thus an agonist of the aryl hydrocarbon receptor (AhR), can cause vascular endothelial cell inflammation and accelerated atherosclerosis in preclinical models, but a mechanistic investigation into the effects of this pollutant on monocyte/macrophage polarization has been lacking. We hypothesized that PCB 126 can contribute to macrophage inflammation and polarization to a M1-like subtype. To test this hypothesis, THP-1 cells were differentiated to macrophages, and subsequently exposed to different types of PCBs, e.g., coplanar PCB 126, mixed type congener PCB 118, or non-coplanar PCB 153. THP-1 is a human monocytic cell line derived from an acute monocytic leukemia male patient, which is one of the most well established and extensively used cell models to mimic the function and mechanisms of monocytes and macrophages (Chanput et al., 2014; Qin, 2012). Inflammatory cytokines and genes associated with oxidative stress were examined by qPCR and multiplexed protein analysis. As in our previous studies using endothelial cell models, here we observed significant induction of multiple cytokines, chemokines, and inflammatory mediators in human macrophages exposed to PCB 126 but not to PCB 118 or PCB 153.

2. Materials and Methods

2.1. Chemicals and reagents

3,3',4,4',5-pentachlorobiphenyl (PCB 126), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) were purchased from AccuStandard Inc. (New Haven, CT, USA). Phorbol 12-myristate 13-acetate (PMA), dimethyl sulfoxide (DMSO), lipopolysaccharide (LPS), BMS-345541 (NF-κB antagonist) were purchased from Sigma (St. Louis, MO, USA). Recombinant human interferon γ (IFN-γ) was purchased from PeproTech (Rocky Hill, NJ, USA). CH-223191(AhR antagonist) was purchased from BioVision (Milpitas, CA, USA)

2.2. Cell culture and differentiation

The human monocytic leukemia cell line THP-1 was used as a model to study monocyte/macrophage functions. THP-1 cells were purchased from the American Type Culture Collection (ATCC® TIB-202™, Manassas, VA, USA), and were cultured in a complete growth medium of RPMI Medium 1640 (ATCC modification) (Gibco™, Grand Island, NY, USA), supplemented with 10% (v/v) ultimate grade fetal bovine serum (VWR Seradigm), 0.05 mM 2-mercaptoethanol (BME; TCI Co., Ltd., Tokyo, Japan), and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Gibco™). The cells were maintained in a humidified atmosphere containing 5 % CO_2 at 37°C. Before PCB treatment, THP-1 cells were washed with serum free media (Macrophage-SFM; Gibco™) and seeded at a density of 1×10^6 cells/well in a 6 – well plate with 20 nM PMA in Macrophage-SFM for 24 h to differentiate.

ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (GenScript, Piscataway, NJ, USA) was used to evaluate the endotoxin level in cell culture reagents.

2.3. PCB treatment

Stock solutions of PCBs were prepared in DMSO, and differentiated THP-1 cells were treated with PCB 126, PCB 118, or PCB 153 at concentrations up to 5000 nM for 4–24 h as described within the figure legends. A cotreatment of 10 ng/mL LPS and 5 ng/mL IFN-γ acted as a positive control. As PCB 118 and PCB 153 elicited no response in initial proinflammatory cytokine induction studies, only PCB 126 was used moving forward with subsequent experimental settings (Supplemental Figure 1). The concentration of DMSO in the media was kept below 0.05%.

2.4. AhR and NF-κB antagonism/inhibition studies

After mature macrophages adhered to the cell culture plate (i.e., PMA treatment for 24 h), cells were pretreated with the AhR antagonist CH-223191 (5 μM) or the NF-kB antagonist BMS-345541 (5 μM) 2 h before PCB 126 (500 nM, 24 h) treatment. Total RNA and cell culture medium were processed and kept in −80 °C for gene expression and cytokine analysis, respectively. mRNA expression of TNFα, IL-1β, CYP1A1 were assessed by real-time PCR, and protein levels for cytokines TNFα, IL-1β, CCL2, IL-8, IL-1RA, CCL3, CCL4 IL-1, IP-10, and IFN-ɣ were measured utilizing Milliplex Map Human Cytokine/Chemokine Magnetic Bead Panel from cell culture medium as instructed.

2.5. Quantitative real-time PCR

Total RNA was extracted from the cells using the TRIzol reagent (Thermo Fisher Scientific Inc., Waltham, MA, USA). RNA purity and quantity were assessed with the Nanodrop 2000/2000c (Thermo Fisher Scientific Inc.) using the Nanodrop 2000 (Version 1.6.198) software. cDNA was synthesized from total RNA using the Quantabio qScrip cDNA SuperMix (QuantaBio Inc., Gaithersburg, MD, USA) and T 100™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). Polymerase chain reaction (PCR) was performed on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad) using the TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific Inc.). Primer sequences mixture from TaqMan Gene Expression Assays (Thermo Fisher Scientific Inc.) were as follows: actin beta (*ACTB*), Hs01060665_g1; interleukin-1 beta (*IL1B*), Hs01555410_m1; interleukin-6 (*IL6*), Hs00174131_m1; IL-8 (*CXCL8*), Hs00174103_m1; tumor necrosis factor alpha (*TNF*), Hs00174128_m1; C-C motif chemokine ligand 2 (*CCL2*), Hs00234140_m1; *CCL3*, Hs00234142_m1; *CCL4,* Hs99999148_m1; cytochrome P450 family 1 subfamily A member 1 (*CYP1A1*), Hs01054796_g1; nuclear factor, erythroid 2 like 2 (*NFE2L2*), Hs00978100_m1; Hs00971716_m1; NAD(P)H quinone dehydrogenase 1 (*NQO1*), Hs01045993_g1; heme oxygenase 1 (*HMOX1*), Hs01110250_m1; glutathione S-transferase theta 1 (*GSTT1*), Hs02512069_s1; glutathione-disulfide reductase (*GSR*), Hs00167317_m1; mannose receptor, C type 1 (*MRC1*), Hs00267207_m1; macrophage scavenger receptor 1 (*MSR1*), Hs00234007_m1.

The levels of mRNA were normalized relative to the amount of *ACTB* mRNA, and expression levels in cells treated with DMSO were set at 1. Gene expression levels were calculated according to the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

2.6. Cytokine/Chemokine assessment

The Milliplex Map Human Cytokine/Chemokine Magnetic Bead Panel (Millipore Corp, Billerica, MA, USA) was utilized to measure cell culture medium cytokines and chemokines Interferon-gamma (IFN-γ), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist (IL-1RA), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-17A (IL-17A), Interferon gamma-induced protein 10 (IP-10), macrophage chemoattractant protein-1 (MCP-1, or CCL2), macrophage inflammatory protein-1 alpha (MIP-1α, or CCL3), macrophage inflammatory protein-1 beta (MIP-1β, or CCL4), and tumor necrosis factor alpha (TNFα). Plates were run and analyzed on the Luminex Xmap MAGPIX system (Luminex Corp, Austin, TX, USA), according to the manufacturer's instructions.

2.7. Statistical Analysis

The results of the cytokines are shown as mean value ± standard error (SEM) as error bars, respectively. Experiments were performed in triplicate. The statistical analysis was performed using GraphPad Prism-6. One-way ANOVA multiple comparison test (as a post-test analysis) was performed with the Tukey test (multiple comparison test comparing every group with every other group). Values are expressed as mean ± SEM. A probability value of $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. PCB 126 elicited inflammation and macrophage polarization

To examine the hypothesis that PCBs can influence macrophage polarization, PCB 126, PCB 118, and PCB 153 were chosen to treat THP-1 derived macrophages. These PCBs represent the three major classes of congeners that humans are exposed to (i.e., coplanar, mixed, and non-coplanar congeners). Initially, both concentration and temporal dose responses were examined. A concentration range of 5, 50, 500 and 5000 nM of the three representative PCBs was used, in addition to vehicle control DMSO and positive control LPS/IFN-γ treatment groups. After 16 h post exposure to PCBs, the expression of two acute inflammatory cytokines TNFα and IL-1 were increased significantly by doses of 50, 500 and 5000 nM of PCB 126 examined, but PCB 118 and PCB 153 had no effect (Figure S2). Then, to examine the effect of exposure duration on cytokine induction, macrophages were treated with two concentrations of PCB 126 (50 and 500 nM) for either 4, 8, 16 or 24 h. Increased IL-6 levels were detected as early as 4 h in PCB 126 (500 nM) treated group, while increased IL-1β levels were only observed at the 24 h time point group. CCL2 mRNA was not a sensitive marker of PCB 126 exposure, but did increase very rapidly in the LPS/ IFN-γ groups. All exposure groups exhibited a time-dependent linear increase in expression of TNFα regardless of concentration, while IL-6 showed a biphasic response to PCB exposure at both concentrations (Figure S3). PCB 126 exposure for 24 h induced expression of inflammatory cytokines in macrophages in both 50 and 500 nM treatment groups, such as TNFα (up to 5 fold), IL-1β (up to 5 fold), CCL3 (up to 3 fold), CCL4 (up to 3 fold), and IL-8 (up to 7 fold) (Figure 1A). Chemokine (C-C motif) ligand 2 (CCL2), referred to as monocyte chemoattractant protein-1 (MCP1), which regulates immune cell recruitment and migration and infiltration of monocytes/macrophages into vascular tissues, also increased in PCB 126 treated macrophages (up to 2 fold) (Figure 1A). All the mentioned inflammatory cytokines increased significantly in PCB 126 and LPS/ IFN-γ co-treatment group. We also investigated two well established markers (MRC1 and MSR1) of the M2 macrophage phenotype, and PCB 126 elicited no significant response (data not shown).

Furthermore, 12 cytokines and chemokines secreted into the cell culture medium, including IFN-γ, IL-1α, IL-1β, IL-1RA, IL-6, IL-8, IL-17A, IP-10, MCP-1(CCL2), MIP-1α (CCL3), MIP-1β (CCL4) and TNFα, were measured by the Milliplex Map Human Cytokine/ Chemokine Magnetic Bead Panel. IL-1β, IL-1RA, IL-8, CCL2, CCL3, CCL4 and TNFα were significantly increased following PCB 126 (500 nM) exposure for 24 h (Figure 1B). IL-6, IL-17A concentrations were below the lower limit of detection (<3.2 pg/mL) (data not shown).

3.2. PCB 126 induced CYP1A1 and NFE2L2 in macrophages

Since it is well established that PCB 126 can activate the AhR and increase reactive oxygen species in other cell types, we next examined mRNA expression for cytochrome P450 1A1 (CYP1A1), nuclear factor (erythroid-derived 2)-like 2 (NFE2L2) and downstream genes, such as heme oxygenase 1 (HMOX1), NAD(P)H quinone oxidoreductase 1 (NQO1), glutathione S-transferase (GST) and glutathione-disulfide reductase (GSR) by real-time PCR after PCB 126 exposure for 24 h. CYP1A1 increased significantly in the 500 nM PCB 126

treated group and PCB 126+ LPS/ IFN-γ co-treatment group compared to all other groups (Figure 2). The expression of most antioxidant genes, such as Nrf2 (alias for NFE2L2), HMOX1, NQO1 expression were upregulatedd in PCB 126 treated groups and PCB 126+ LPS/ IFN-γ co-treatment group (Figure 2), indicating induction of xenobiotic-linked defensive mechanisms.

3.3. PCB 126 exacerbated inflammation in LPS exposed monocytes

Since undifferentiated monocytes may come into contact with both PCBs and other inflammatory stressors such as LPS in the blood simultaneously (before they migrate to the intima or tissues, and differentiate to macrophages), we next examined the 24 h treatment effect of PCB 126, LPS/IFN-γ, or PCB 126 + LPS/IFN-γ on THP-1 cell activation. Unlike with the macrophage studies described above, PCB 126 alone had minimal effect on TNFα or IL-1β mRNA expression in undifferentiated monocytes. Interestingly, and as expected, the positive control group of LPS/IFN-γ displayed increased expression of these pro-inflammatory cytokines, but this was synergistically increased when PCB 126 was concomitantly added (Figure 3A). CYP1A1 was induced by PCB 126 in monocytes, but there was no synergistic increase in the presence of LPS/IFN-γ (Figure 3B), confirming our previous findings that coplanar PCBs, like PCB 126, upregulate CYP1A1 expression (Hennig et al., 2002).

3.4. PCB 126-induced oxidative stress and inflammation involved both AhR and NF-κB signaling pathways

To examine signaling mechanisms that may be responsible for the observed PCB 126-induced inflammation of macrophages, both the AhR antagonist (CH-223191, 5 M) and NF B antagonist (BMS-345541, 5 M) were utilized. mRNA expression for TNFα, IL-1β, CYP1A1 were assessed by real-time PCR (Figure 4A), and protein levels of cytokines TNFα, IL-1β, IL-8, CCL2, CCL3, CCL4, IL-1RA, IL-1, IP-10, and IFN-γ were measured utilizing Milliplex Map Human Cytokine/Chemokine Magnetic Bead Panel from cell culture medium (Figure 4B). The mRNA expression of TNFα and IL-1β were reduced by 2–3 fold in both AhR and NF-κB antagonist groups co-treated with PCB 126 (Figure 4A). At the protein level, concentrations of 6 inflammatory cytokines (TNFα, IL-1β, IL-8, IL-1RA, CCL3, CCL4) were decreased in the presence of the NF-κB antagonist while only 2 cytokines (TNFα, IL-8) were reduced in the presence of the AhR antagonist (Figure 4B).

4. Discussion

It is well documented that macrophage accumulation within the arterial wall is a hallmark of atherosclerosis (Geovanini & Libby, 2018; Z. Liu et al., 2017; Moore & Tabas, 2011; Zeller & Srivastava, 2014). A chronic inflammatory response in the vascular wall, linked to uptake of low-density lipoprotein by macrophages and their subsequent transformation into foam cells, are characteristic events in the pathology of atherosclerosis. Monocyte-derived macrophages are a homogeneous cell population and present a spectrum of phenotypes according to different micro-environmental stimuli. Accumulating evidence suggests that macrophages can show high plasticity in atherosclerotic plaques, and more macrophage subtypes have been profiled, such as M4 (Domschke & Gleissner, 2017; Erbel et al., 2015),

Mox (Kadl et al., 2010), M(Hb) (Finn et al., 2012) and Mhem (Boyle et al., 2012). Numerous stimuli, such as inflammatory cytokines or oxidized phospholipids, can polarize macrophages to a certain phenotype and contribute to atherosclerosis (Chistiakov et al., 2015; Shapouri-Moghaddam et al., 2018). The current study focuses on the pro-inflammatory macrophage phenotype M1, a major mediator of inflammation during atherogenesis (Martinez & Gordon, 2014).

There is evidence that NF-kB signaling pathways are involved in monocyte activation and macrophage polarization (Brasier, 2010; C. P. Liu et al., 2017; Majdalawieh & Ro, 2010). In fact, NF-kB comprises a family of transcription factors that are oxidative stress sensitive and involved in the pathology of various inflammatory diseases, such as atherosclerosis (Pamukcu et al., 2011; Robbesyn et al., 2004; Yamamoto & Gaynor, 2001). Thus, inhibition of the NF-kB signaling pathways may reduce inflammation, attenuate atherosclerosis and prevent its complications (Pamukcu et al., 2011). There is also evidence that AhR plays a role in the pathogenesis of cardiovascular diseases (Yi et al., 2018). This may be due to its ability to modulate biological responses or other signaling pathways within different cells types, e.g., macrophages, in the cardiovascular system (Hewitson et al., 2004; Ogando et al., 2016; Wang et al., 2017). Interestingly, at least in mice, it appears that loss of AhR signaling can also lead to dysregulated inflammatory signaling. For example, AhR whole body knockouts display increased expression of TNF-α, IL-6 and IL-1β in an experimental model of autoimmune Uveitis, and peritoneal macrophages isolated from AhR null mice also displayed increased levels of these cytokines (Climaco-Arvizu et al., 2016; Huang et al., 2018). Importantly, these studies utilized mice that were deficient in AhR from birth and thus may have exhibited overall developmental differences of the immune system (Li et al., 2017). Also, as has been shown using cell-based assays, murine and human AhR can differ in ligand binding capabilities (Bock, 2018). Future work utilizing conditional or inducible knockouts will be critical to investigate AhR's role in inflammation in adult mice. Interestingly, interactions between AhR and NF-kB pathways could be critical in regulating inflammatory responses and associated diseases (Vogel & Matsumura, 2009).

PCB 126 and other dioxin-like pollutants may also elicit toxic effects through additional signaling pathways. One possible explanation is the growing body of evidence of AhR crosstalk with multiple other inflammatory regulators, including NF-kB and Nrf2. For example, AhR appears to interact with two critical subunits of NF-kB, RelA and RelB, to modulate inflammation through the expression of many important cytokines (Vogel & Matsumura, 2009; Vondracek et al., 2011). In addition, there appears to be bidirectional interactions between AhR and the master regulator of antioxidant response, Nrf2. Interestingly, there appears to be AhR binding sequences within the Nrf2 promoter, and optimum upregulation of certain downstream genes, such as NQO1, requires both Nrf2 and AhR. (Kahan et al., 1988). Crosstalk between AhR and Nrf2 may explain in part the results presented in Figure 2, which indicate simultaneous upregulation of CYP1A1, NQO1 and HMOX1, following exposure to PCB 126.

There is accumulating evidence suggesting that exposure to certain environmental contaminants, such as polyhalogenated aromatic hydrocarbons, e.g., TCDD or PCBs (coplanar PCBs in particular), can be involved in the development of atherosclerosis.

Previous studies have shown that exposure to PCBs can be pro-atherogenic by inducing pro-inflammatory reactions, causing endothelial cell dysfunction (Choi et al., 2003; Eske et al., 2014; Liu et al., 2015; Petriello et al., 2016). In the present study, we provide evidence that coplanar PCBs (PCB 126) can induce or exacerbate macrophage/monocyte inflammation, and thus can promote atherogenisis. Our findings add to this growing mechanistic understanding of how dioxin-like pollutants may contribute to the pathologies of cardiovascular disease, such as atherosclerosis (Mohsenzadeh et al., 2018; Petriello et al., 2018). Historically, the toxicity equivalents of PCB 126 and related dioxin-like pollutants have been determined using cell-based assays of rat or mouse origin, and it is critical for proper risk assessment paradigms to utilize human cell-based approaches since PCB 126 may be a less efficient AhR ligand as once thought (Larsson et al., 2015).

The finding that PCB 126 causes an inflammatory response in human macrophages through AhR and NF-κB transcriptional factors has not been previously reported. Our study confirmed the hypothesis that dioxin-like PCBs (e.g., PCB 126) can contribute to macrophage inflammation, and thus polarize monocytes to a M1-like phenotype, in contrast to PCB 118 and PCB 153. PCB 126-induced inflammation was both concentration and time dependent. Our data also suggest that PCB 126 is a ligand for the AhR within macrophages as evidenced by induction of CYP1A1 expression. PCB 126 also appears to modulate redox signaling, as the antioxidant regulator Nrf2 as well as its target genes such as HMOX1 and NQO1 were upregulated accordingly. Besides, earlier studies demonstrated that expression of Nrf2 can be directly modulated via ligand-activated AhR, and vice versa (Miao et al., 2005; Shin et al., 2007). AhR-Nrf2 crosstalk also plays a key role in drug metabolism (Haarmann-Stemmann et al., 2012). All these findings suggest that PCB 126 causes oxidant stress and inflammation in macrophages. In the NF-κB and AhR blocking experiments, most of the tested inflammatory cytokines were reduced due to NF-κB inhibition, and two notable acute phase proteins, TNFα and IL-8, were downregulated as a result of AhR blocking.

In concordance with our findings, a recent study showed that PCB 126 activates a vigorous pro-inflammatory state in fat cell precursors (preadipocytes) depending on AhR activation, although induction of the response was delayed compared to upregulation of CYP1A1, and an inhibitor (Bay11–7082, 4 M) of NF-κB partially protected preadipocytes from the effects of PCB 126 (Gourronc et al., 2018). Our previous studies demonstrated that coplanar PCB-induced endothelial cell inflammation is regulated in part through epigenetic modification of the NF-κB subunit p65 (Liu et al., 2015). Nutritional protection against PCB126 induced inflammation can also occur through AhR inhibition (Han et al., 2012) and epigenetic modifications of NF-κB target genes in human endothelial cells (Liu et al., 2016). These previous studies all suggest that both AhR and NF-κB signaling pathways are important in regulating PCB 126-mediated inflammation. Our present study provides evidence that macrophages can significantly contribute to a PCB-mediated inflammatory response through AhR and NF-κB signaling pathways.

In conclusion, we demonstrated that PCB 126 can contribute to macrophage polarization and inflammation, indicating another possible role of dioxin-like PCBs in the pathology of atherosclerosis. We also provide evidence that the observed PCB 126-induced inflammation is regulated in part through both AhR and NF-κB signaling pathways, i.e., critical pathways

involved in the overall pathology of inflammatory diseases, such as atherosclerosis. Our findings have translational implications, suggesting that environmental insults can contribute to chronic diseases by modulating inflammatory cascades within immune cells creating an advantageous environment for cardiovascular disease progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Abbreviations:

PCBs	polychlorinated biphenyls
AhR	aryl hydrocarbon receptor
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
CYP1A1	cytochrome P450 family 1 subfamily A member 1
NFE2L2	nuclear factor, erythroid 2 like 2
NQO1	NAD(P)H quinone dehydrogenase 1
HMOX1	heme oxygenase 1
GST	glutathione S-transferase
GSR	glutathione-disulfide reductase
IFN-γ	interferon-γ
LPS	lipopolysaccharide
PMA	Phorbol 12-myristate 13-acetate
BME	2-mercaptoethanol
TNFα	tumor necrosis factor alpha
IL-1α	interleukin-1 alpha
IL-1β	interleukin-1 beta
IL-1RA	interleukin-1 receptor antagonist
IL-6	interleukin-6
IL-8	interleukin-8

IL-17A	interleukin-17A
IP-10	Interferon gamma-induced protein 10
MCP-1	(CCL2) macrophage chemoattractant protein-1
MRC1	mannose receptor, C type 1
MSR1	macrophage scavenger receptor 1
MIP-1α	(CCL3) macrophage inflammatory protein-1 alpha
MIP-1β	(CCL4) macrophage inflammatory protein-1 beta

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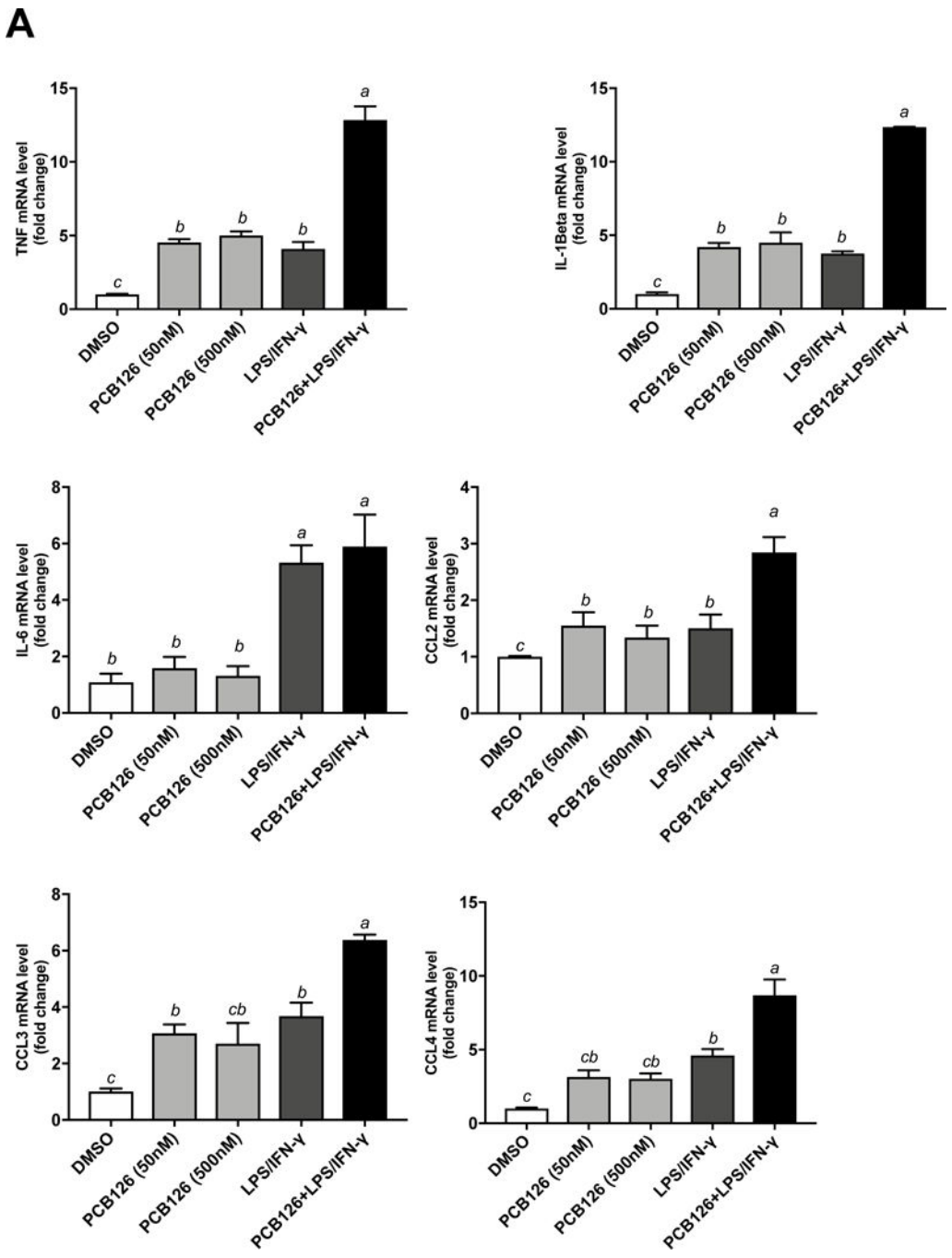
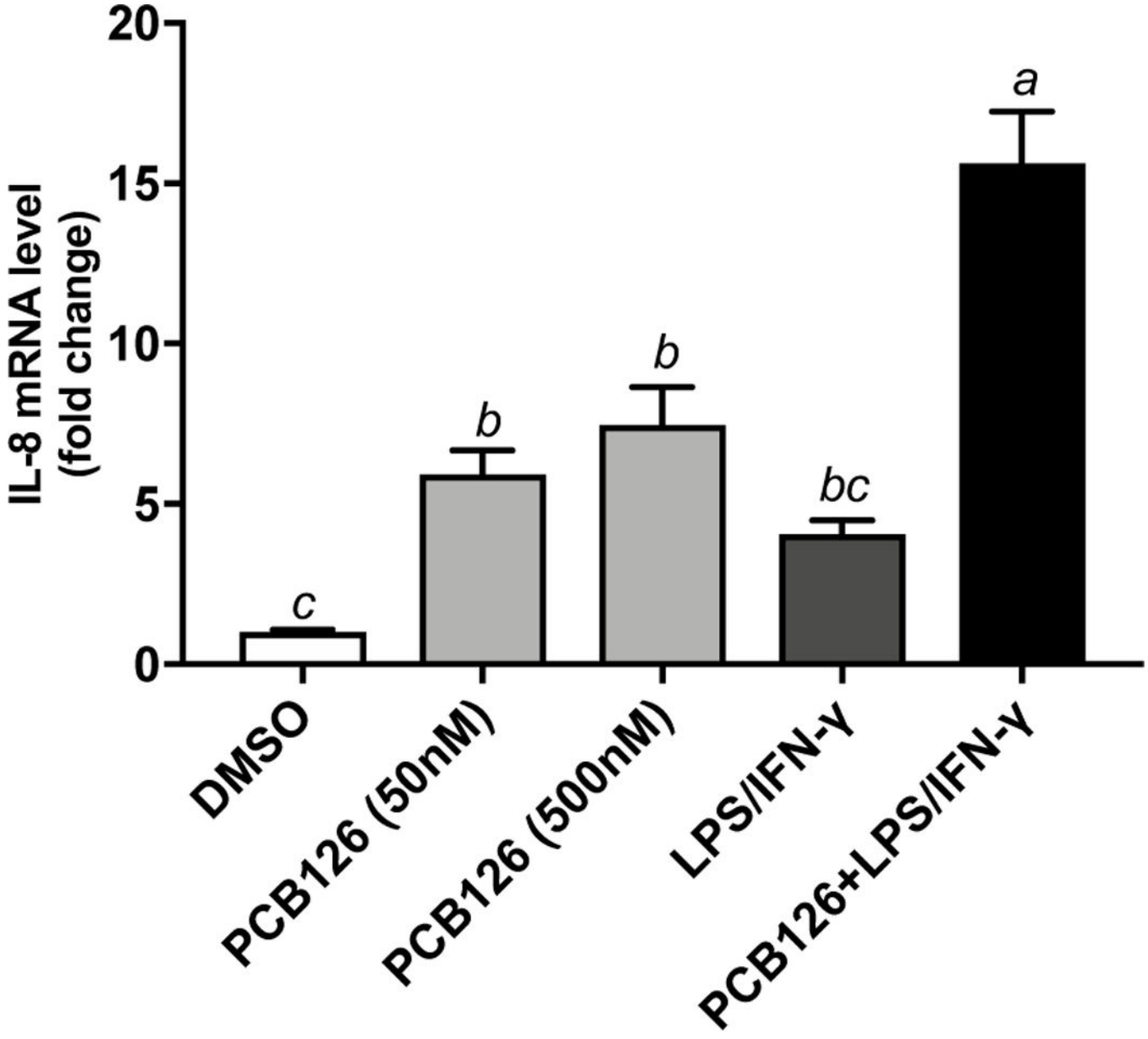
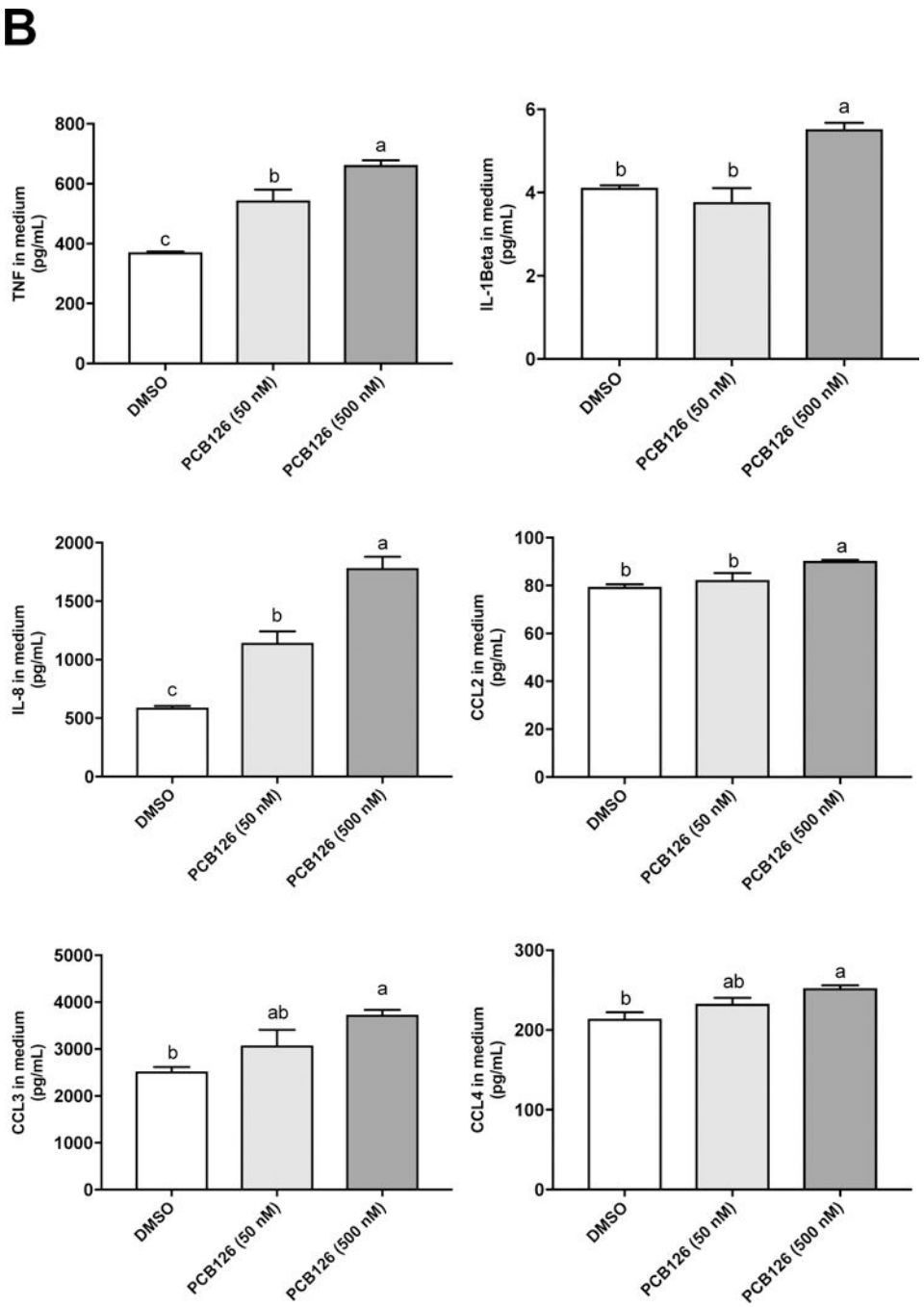
Highlights

- Macrophages can be polarized either to pro-inflammatory or anti-inflammatory phenotypes.
- Dioxin-like PCBs can contribute to macrophage polarization associated with inflammation.
- PCB 126-induced inflammation is regulated in part through both AhR and NF-κB signaling pathways.







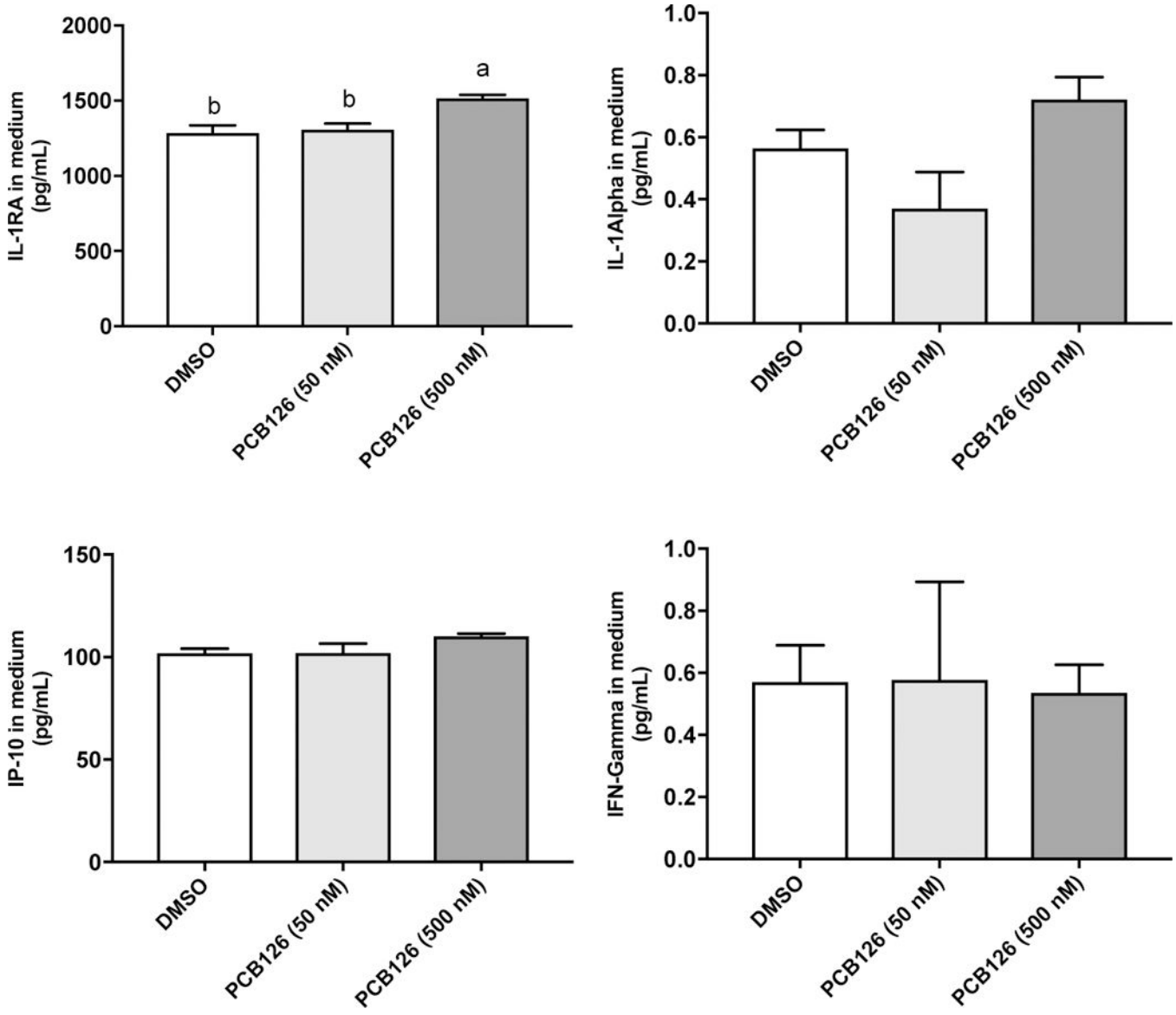


Figure 1. PCB 126 elicited inflammation in macrophages.

(A) THP-1 derived macrophage mRNA expression for TNF, IL-1β, IL-6, IL-8, CCL2 (MCP1), CCL3 (MIP-1α), CCL4 (MIP-1β) were assessed after 24 h treatment of PCB 126, PCB126 + LPS (10 ng/mL)/IFN-γ (5 ng/mL) and DMSO. (B) Protein levels for cytokines TNF, IL-1β, IL-8, CCL2, CCL3, CCL4, IL-1RA, IL-1, IP-10, and IFN-γ were measured utilizing Milliplex Map Human Cytokine/Chemokine Magnetic Bead Panel from cell culture medium after 24 h exposure of PCB 126. IL-6, IL-17A concentrations were below the lower limit of detection (<3.2 pg/mL) (data not shown). Values are mean ± SEM (n=3). Different letters indicate significant difference ($P \leq 0.05$) between treatment groups. Tukey's multiple comparisons test was used.

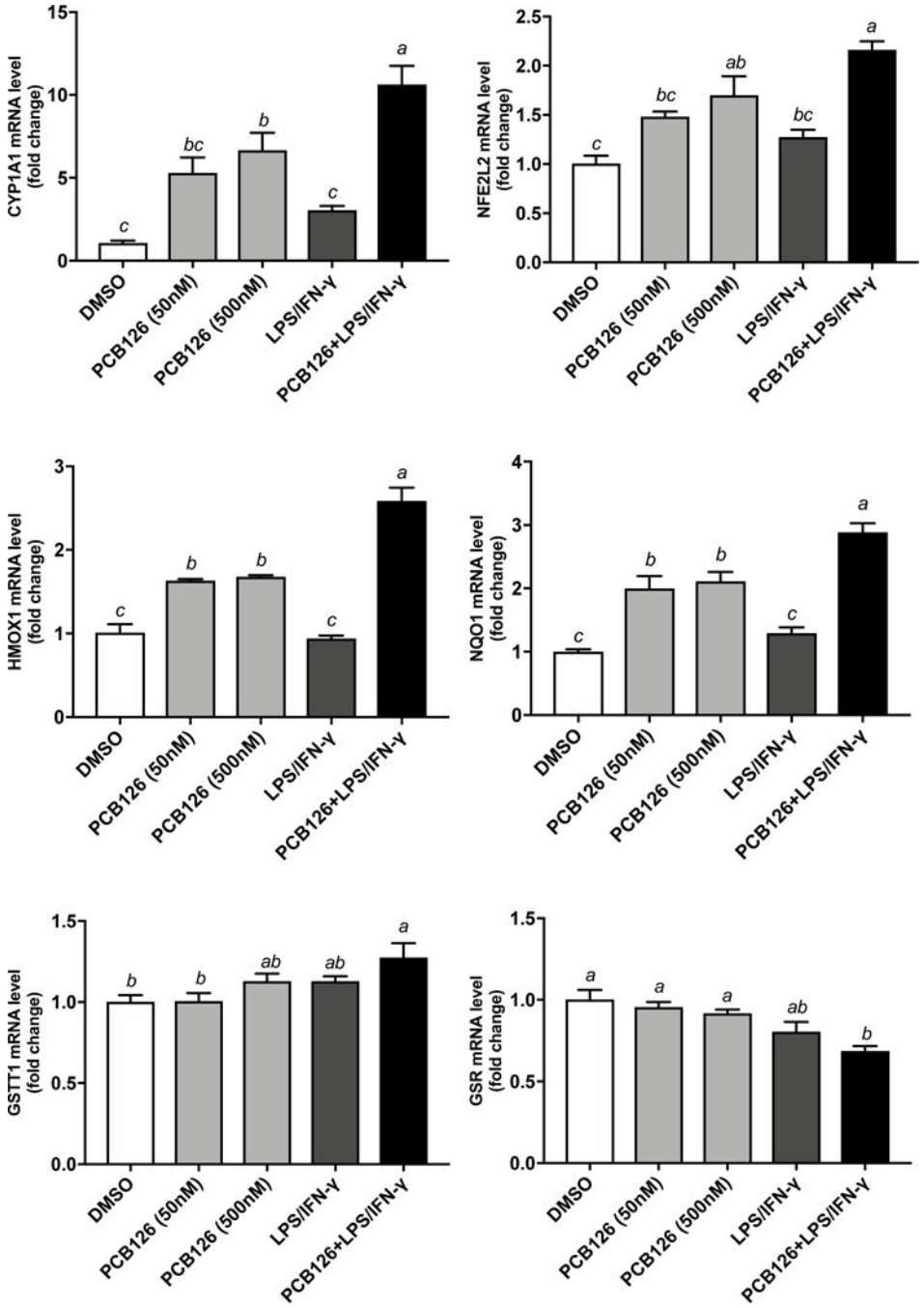


Figure 2. PCB 126 induced CYP1A1 and NFE2L2 in macrophages.

Cytochrome P450 1A1 (CYP1A1) increased significantly in PCB126 (500 nM), and PCB126 + LPS (10 ng/mL)/IFN-γ (5 ng/mL) treated groups compared to all other groups. Nuclear factor (erythroid-derived 2)-like 2 (NFE2L2) was induced with 500 nM PCB126, and PCB126 + LPS/IFN-γ compared to control, while down-stream genes, such as heme oxygenase 1 (HMOX1), NAD(P)H quinone oxidoreductase 1 (NQO1) were significantly increased following 500 nM PCB126 or PCB126 + LPS /IFN-γ exposure compared to all other groups, indicating induction of xenobiotic-linked defensive mechanisms. Values are

mean ± SEM (n=3). Different letters indicate significant difference ($P \leq 0.05$) between treatment groups. Tukey's multiple comparisons test was used.

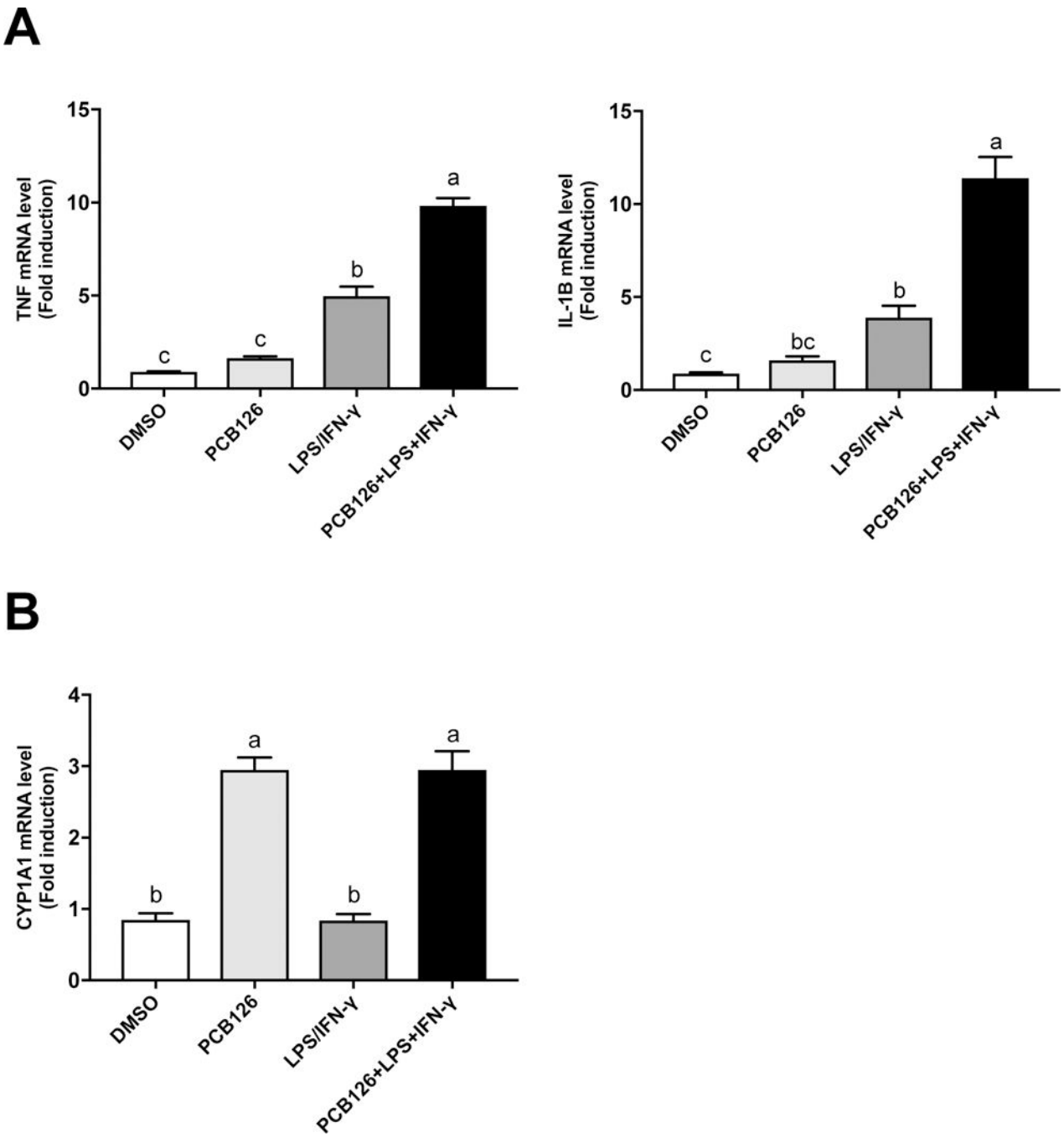
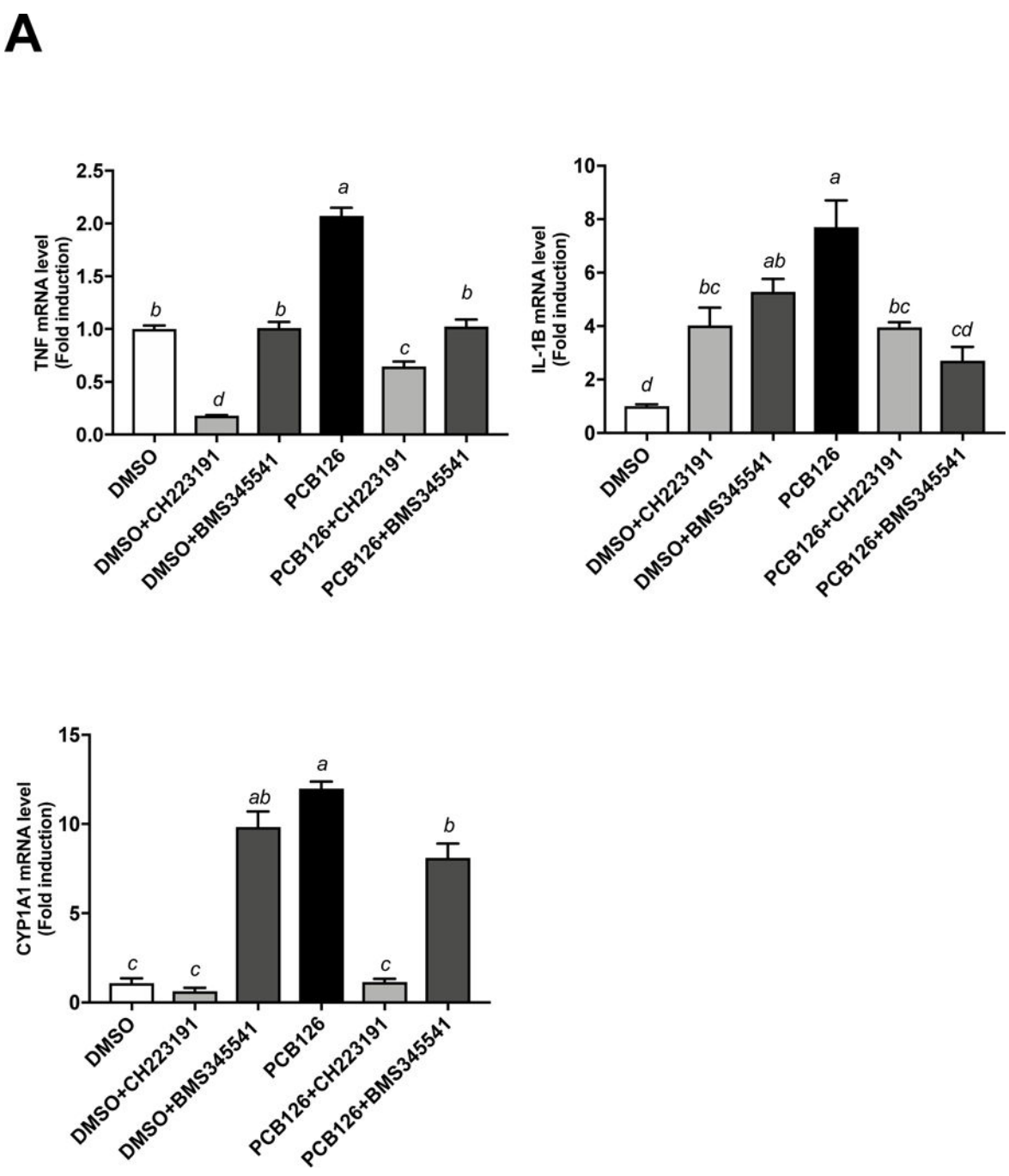
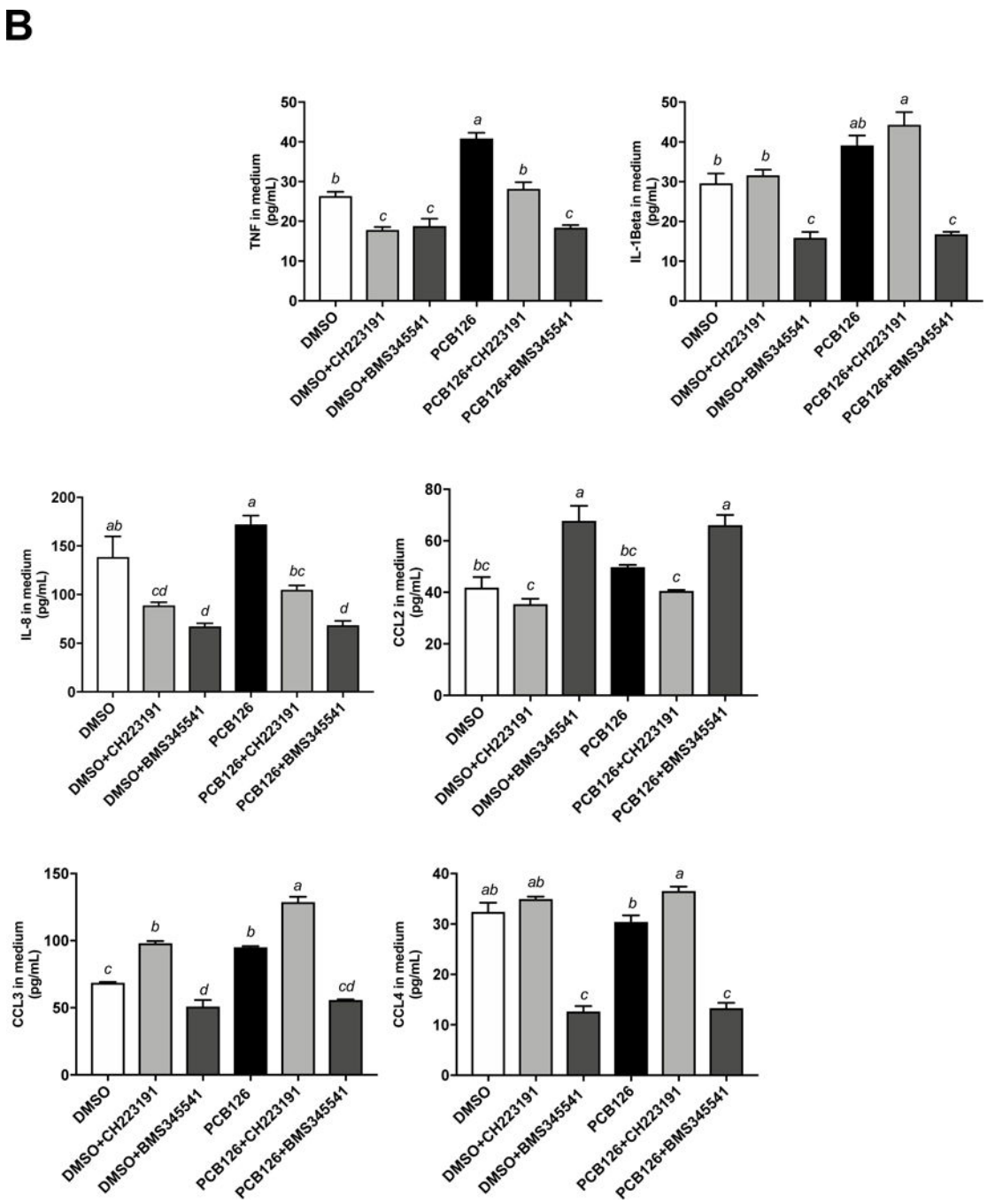


Figure 3. PCB 126 exacerbated inflammation in LPS exposed monocytes and induced CYP1A1.

THP-1 cells were treated with PCB126, LPS/IFN-γ, and PCB126 + LPS/IFN-γ respectively for 24 h, and the results showed that the inflammatory cytokines, such as TNF and IL-1β, increased in LPS/IFN-γ and PCB126 + LPS/IFN-γ treated groups. However, CYP1A1 increased significantly in PCB126 and PCB126 + LPS/IFN-γ treated groups. Values are mean ± SEM (n=3). Different letters indicate significant difference ($P \leq 0.05$) between treatment groups. Tukey's multiple comparisons test was used.





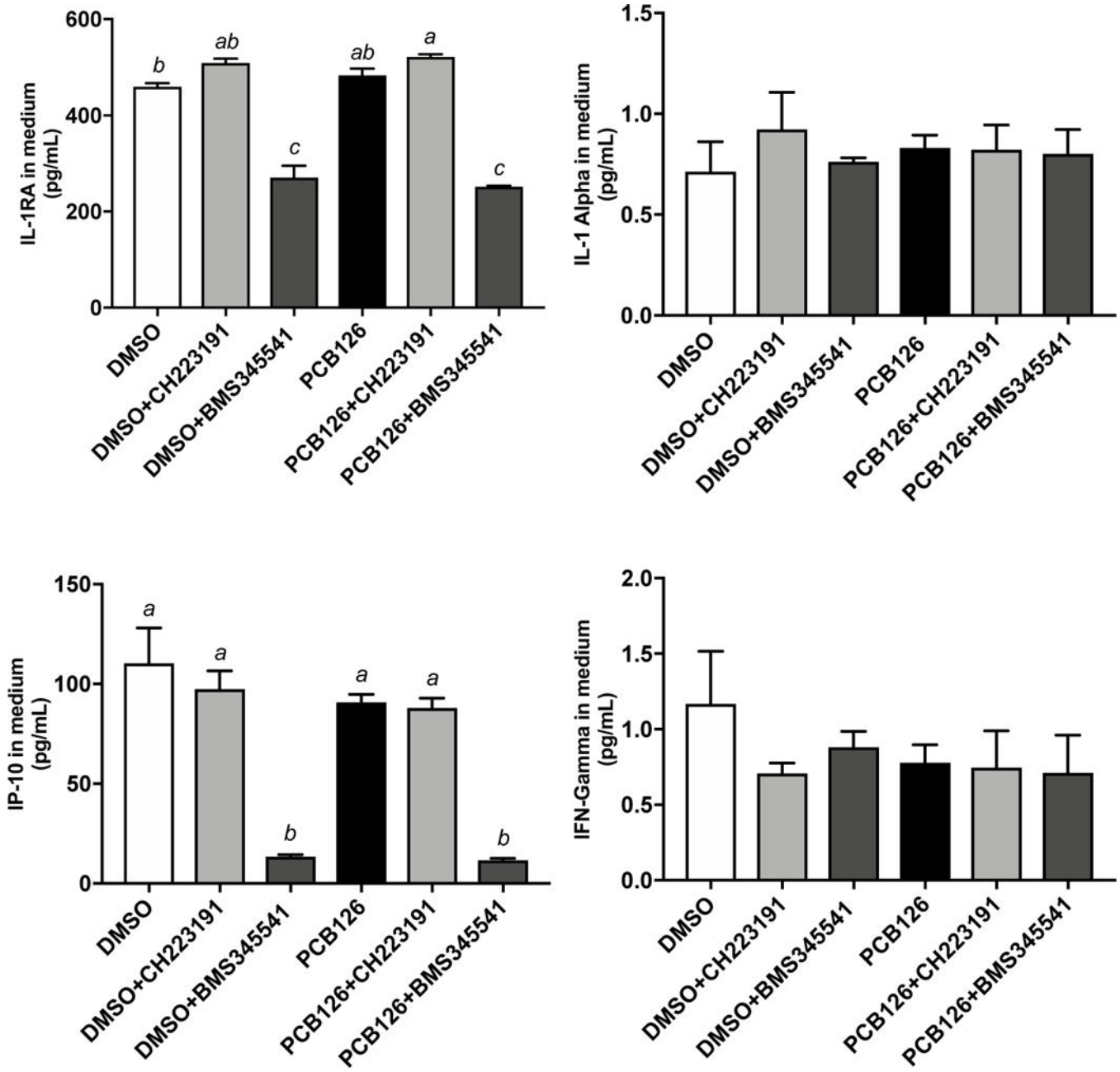


Figure 4. PCB 126 induced inflammation and oxidative stress involved both AhR and NF B signaling pathway in macrophages.

THP-1 derived macrophages were pretreated with the AhR antagonist CH-223191 (5 M) or the NF B antagonist BMS-345541 (5 M) for 2 h before PCB 126 (500 nM, 24 h) exposure. (A) mRNA expression for TNF, IL-1β, CYP1A1 were assessed. (B) Protein levels for cytokines TNF, IL-1β, IL-8, CCL2, CCL3, CCL4, IL-1RA, IL-1, IP-10, and IFN-ɣ were measured utilizing Milliplex Map Human Cytokine/Chemokine Magnetic Bead Panel from cell culture medium. IL-6, IL-17A concentrations were below the lower limit of detection

(<3.2 pg/mL) (data not shown). Values are mean ± SEM (n=3). * $P \leq 0.05$. Tukey's multiple comparisons test was used.