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Polycyclic Aromatic Hydrocarbons Affect Functional Differentiation and Maturation of Human Monocyte-Derived Dendritic Cells¹

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Polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene (BP) are environmental carcinogens exhibiting potent immunosuppressive properties. To determine the cellular bases of this immunotoxicity, we have studied the effects of PAHs on differentiation, maturation, and function of monocyte-derived dendritic cells (DC). Exposure to BP during monocyte differentiation into DC upon the action of GM-CSF and IL-4 markedly inhibited the up-regulation of markers found in DC such as CD1a, CD80, and CD40, without altering cell viability. Besides BP, PAHs such as dimethylbenz(a)anthracene and benzanthracene also strongly altered CD1a levels. Moreover, DC generated in the presence of BP displayed decreased endocytic activity. Features of LPS-mediated maturation of DC, such as CD83 up-regulation and IL-12 secretion, were also impaired in response to BP treatment. BP-exposed DC poorly stimulated T cell proliferation in mixed leukocyte reactions compared with their untreated counterparts.

In contrast to BP, the halogenated arylhydrocarbon 2,3,7,8-tetrachlorodibenzo-p-dioxin, which shares some features with PAHs, including interaction with the arylhydrocarbon receptor, failed to phenotypically alter differentiation of monocytes into DC, suggesting that binding to the arylhydrocarbon receptor cannot mimic PAH effects on DC. Overall, these data demonstrate that exposure to PAHs inhibits in vitro functional differentiation and maturation of blood monocyte-derived DC. Such an effect may contribute to the immunotoxicity of these environmental contaminants due to the major role that DC play as potent APC in the development of the immune response. The Journal of Immunology, 2002, 168: 2652–2658.

Polycyclic aromatic hydrocarbons (PAHs)³ are ubiquitous environmental contaminants that are formed through the combustion of fossil fuel and the burning of various substances (1). They are present in significant amounts in automobile exhaust, industrial emissions, tobacco smoke, and charcoal-broiled food. The toxic effects of PAHs have been known for a number of years (2). Indeed, many PAHs, including benzo(a)pyrene (BP), 3-methylcholanthrene (MC), and dimethylbenz(a)anthracene (DMBA), are potent carcinogens (2). This effect is believed to be due to reactive metabolites of PAHs, which are capable of interacting with DNA (3). Such metabolites are generated through the action of cytochromes P450 (CYP), especially CYP1A1. Interestingly, many PAHs can induce CYP1A1 expression in various cell types, thereby increasing their own metabolism. This up-regulation of CYP1A1 is mediated by the arylhydrocarbon receptor (AhR) to which several PAHs bind, thereby triggering translocation of the AhR into the nucleus, association with the AhR nuclear translocator, and, ultimately, interaction with xenobiotic responsive elements found in the 5′-flanking regions of responsive genes, including CYP1A1 (4). Besides PAHs, other agonists of AhR have been described, especially the very potent ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (4–6).

Many PAHs are also potent immunotoxic agents (7–9). PAHs such as BP and DMBA given orally or s.c. to mice suppress humoral and cell-mediated immunity (7, 8, 10). The cellular and biochemical mechanisms by which PAHs produce immunosuppression have not been definitively established. Lymphocytes have been shown to be targets (9–11). DMBA and/or BP have thus been demonstrated to inhibit both murine T and B cell proliferation and to impair T cell cytokine production, tumor-specific cytotoxic T cell induction, and B cell Ab production. In addition, PAHs suppress B cell lymphopoiesis by triggering pre-B lymphocyte apoptosis in an AhR-dependent manner (11–14). Besides lymphocytes, alteration of APCs may contribute to PAH-mediated immunosuppression. Indeed, BP has been reported to impair Ag presentation by mouse macrophages (15). Whether myeloid dendritic cells (DC), which are considered very potent APC (16, 17), may also be affected in response to PAH treatment remains essentially unknown. To address this question, we have investigated in the present study the effects of PAH exposure on monocyte-derived DC differentiation and maturation. Differentiation, performed from human monocytes upon the action of GM-CSF and IL-4, commonly leads to immature DC that express CD1a and have the capacity of efficiently capturing and processing Ags, but have lost CD14 (18). Maturation in response to various compounds, such as LPS, usually results in enhanced secretion of immunomodulatory cytokines, such
as IL-10 and IL-12, and up-regulation of HLA-DR Ags, costimulatory molecules such as CD80 and CD86 (19), and CD83, a marker for functionally mature DC (20); this results in an increased capacity to stimulate T cells (20). Our results demonstrate that exposure to PAHs such as BP during in vitro DC generation from human blood monocytes inhibits functional differentiation and maturation processes through a mechanism that does not seem to be directly dependent on AhR. Such data provide evidence for a new way by which PAHs interfere with the immune system.

**Materials and Methods**

**Chemicals and reagents**

BP, DMBA, MC, benzo[e]pyrene (Br[e]P), benzanthracene (BA), and FITC-dextran (m.w., 40,000) were provided by Sigma-Aldrich (St. Louis, MO). TCDD was obtained from Cambridge Isotope Laboratories (Cambridge, MA). Chemicals were commonly used as stocked solution in DMSO. The final concentration of the solvent in culture medium did not exceed 0.2% (v/v), and control cultures received the same dose of solvent. [3H]Thymidine (sp. act., 5 Ci/mmol) was purchased from Amersham (Les Ulis, France). Human GM-CSF (sp. act., 1.8 × 10^8 U/mg) was provided by Schering Plough (Lyons, France), whereas IL-4 (sp. act., 2 × 10^8 U/ml) and LPS were obtained from Promocell (Heidelberg, Germany) and Sigma-Aldrich, respectively.

**DC generation and maturation**

DC were prepared from peripheral blood monocytes as previously reported (18). Briefly, mononuclear cells were obtained from blood buffy coats of healthy donors by Ficoll gradient centrifugation. Ten million cells per well were seeded into six-well culture plates in RPMI 1640 medium supplemented with 2 mM glutamine, antibiotics, and 10% FCS. After incubation at 37°C for 2 h, nonadherent cells were removed, and adherent monocyctic cells were further cultured for 7 days with 800 U/ml GM-CSF and 500 U/ml IL-4 for generating DC. Mature DC were further obtained by the addition of LPS (1 µg/ml) for 2 days.

**Cellular viability determination**

Cellular viability was determined by microscopic analysis of cellular exclusion of trypan blue dye and flow cytometric analysis of cellular propidium iodide staining.

**Flow cytometric immunolabeling assays**

Phenotypic analysis of monocyte-derived DC was performed using flow cytometric direct immunofluorescence as previously described (21). Cells were first incubated for 1 h in PBS with 5% human AB serum at 4°C to avoid nonspecific mAb binding. Several mAbs, purchased from Immunotech (Marseille, France), were then used for immunolabeling: PE-conjugated mouse mAbs against CD1a, CD14, and CD40 and FITC-conjugated mAbs directed against HLA-DR, CD86, CD80, and CD83. Isotypic control labeling was performed in parallel. Thereafter, cells were analyzed with a FACSCaliber flow cytometer (BD Biosciences, San Jose, CA) using CellQuest software (BD Biosciences). Results were expressed as the percentage of positive cells or as the mean fluorescence intensity (MFI) calculated according to the formula: mean fluorescence (mAb of interest) – mean fluorescence (control mAb).

**Mixed leukocyte reactions**

The ability of DC to stimulate allogeneic T cells in mixed leukocyte reactions was performed as previously described (22). Briefly, graded numbers of DC cells were cocultured with 1.5 × 10^5 allogenic CD3+ T cells in round-bottom 96-well microtiter plates; the different DC/T cell ratios used were 1/1000, 1/100, 1/50, 1/20, and 1/10. After 5 days of culture, cells were pulsed with 1 µCi [3H]thymidine for 18 h. The incorporation of the radionuclide into DNA was further measured by β-scintillation counting. Results were expressed as radioactivity (cpm) per well.

**Cytokine measurements**

Levels of IL-10 and IL-12 in the supernatants of DC cultures were quantified using ELISA kits obtained from R&D Systems (Abington, U.K.). Analyses were conducted according to the instructions of the manufacturer.

**Endocytosis assay**

DC were incubated with 1 mg/ml FITC-dextran for 30 and 60 min at 4 or 37°C. Cellular uptake of FITC-dextran was then monitored by flow cytometry.

**RNA isolation and RT-PCR analysis**

Total RNA was isolated from cells using the TRIzol reagent (Life Technologies, Cergy Pontoise, France). RT-PCR analysis of CYP1A1, CD1a, and β-actin expression was then performed as previously reported (23). The primers used for CYP1A1 and CD1a detection were exactly those used by Baron et al. (23) and van den Berg et al. (24), respectively, and have been designed to amplify at least one intron in the genes to exclude contamination of cDNA with genomic DNA. The primers used for the β-actin detection were: sense, 5′−GCCCCAGAGCAAGAGAG−3′ and antisense, 5′−GGCATCTCTTGGCTCG−3′. PCR products were separated on 1.2% agarose gels and stained with ethidium bromide.

**Statistical analysis**

Data were analyzed with the paired Student’s t test. The level of significance was p < 0.05.

**Results**

PAHs interfere with functional differentiation of blood monocytes into DC

Blood monocytes cultured for 7 days in the presence of GM-CSF and IL-4 progressively exhibited a typical phenotype of immature DC, especially up-regulation of CD1a and down-regulation of CD14 (Fig. 1). In the presence of BP, cultured monocytes also lost CD14, but the increase in CD1a was strongly reduced. Such an alteration was already observed in 3-day cultured cells and persisted for cells cultured for 5 and 7 days (Fig. 1), indicating that phenotypic differentiation of monocytes into immature DC was unequivocally impaired in response to BP. Thus, CD1a-related MFI in cells exposed to the PAH for 7 days approximately corresponded to 25% of the value found in control DC (Table I), and the
percentages of CD1a+ cells were 86 ± 12 and 28 ± 14% in control and BP-treated cell cultures, respectively; such a difference between the percentages of CD1a+ cells was statistically significant (*p < 0.05). Exposure to BP also down-regulated the expression of CD80 and CD40. Indeed, CD80 and CD40 MIFs were reduced in BP-treated cells (Table I), and the percentages of CD80- and CD40- cells found in BP-exposed cultures (20 ± 7 and 41 ± 13%, respectively) were lower than the percentages of CD80- and CD40- cells found in control untreated cells (70 ± 17 and 79 ± 13%, respectively). By contrast, CD86 and HLA-DR were not impaired (Table I). BP treatment did not result in alteration of cell viability, as assessed by determination of trypan blue exclusion (Fig. 2A) and by measurement of the percentage of propidium iodide-stained cells (Fig. 2B), indicating that BP-induced alteration of DC phenotypic markers was not a consequence of a general toxicity due to the PAH. The effects of BP on CD1a levels were dose-dependent (Fig. 3). Indeed, 0.1 μM BP only marginally decreased CD1a expression, whereas CD1a down-regulation was greater and statistically significant for doses of 1 and 5 μM. The use of 10 μM was required to obtain a maximal effect on CD1a protein expression; this BP dose, however, failed to obviously down-regulate CD1a mRNA levels as assessed by RT-PCR analysis (data not shown). Besides BP, other PAHs also inhibited CD1a expression in blood monocytes cultured in the presence of GM-CSF and IL-4 (Fig. 4). DMBA and BA used at 10 μM were as efficient as BP; B(e)P was less effective, whereas MC had only a minor, although significant, effect (Fig. 4). We further determined whether the effect of BP on DC differentiation was also functionally significant. For this purpose, blood monocytes were first treated with GM-CSF and IL-4 in the absence or the presence of 10 μM BP for 7 days, and their endocytic activity was then measured by flow cytometry. As shown in Fig. 5, BP-treated cells incubated for 30 or 60 min with FITC-dextran at 37°C displayed reduced endocytosis of the fluorescent substrate compared with their untreated counterparts.

FIGURE 2. Effect of BP treatment on cellular trypan blue exclusion (A) and propidium iodide staining (B). Blood monocytes were cultured with GM-CSF and IL-4 in the absence or the presence of 10 μM BP for 7 days. Cellular viability was then determined by analysis of cellular trypan blue exclusion (A) or by flow cytometric measurement of propidium iodide-stained cells (B). Data shown are expressed as the percentage of trypan blue-negative cells compared with total cells (A) or as flow cytometric graphs indicating the proportion of propidium iodide-positive cells (B); they are the mean ± SD of five independent experiments (A) or are representative of three independent experiments (B).

FIGURE 3. Dose-dependent down-regulation of CD1a expression in response to BP. Monocytes were cultured with GM-CSF and IL-4 for 3 days in the absence or the presence of various concentrations of BP. CD1a expression was then analyzed by flow cytometry. Data are expressed as the percentage of CD1a MFI values of cells not exposed to BP and are the mean ± SD of seven independent experiments. *p < 0.05 compared with MFI of untreated cells.

FIGURE 4. Effects of various PAHs on CD1a expression during monocyte-derived DC differentiation. Monocytes were cultured with GM-CSF and IL-4 for 3 days in the absence or the presence of various PAHs, such as BP, MC, DMBA, B(e)P, and BA. All PAHs were used at 10 μM. CD1a expression was then analyzed by flow cytometry. Data are expressed as the percentage of CD1a MFI values found in cells not exposed to BP and the mean ± SD of four independent experiments. *p < 0.05 compared with the MFI of untreated cells.
PAH effects on differentiated monocyte-derived DC

To investigate PAH effects on differentiated DC, blood monocytes were first cultured with GM-CSF and IL-4 for 7 days, and the differentiated immature DC obtained were maintained in the absence or the presence of 10 μM BP for 2 days. Analysis of phenotypic markers such as CD1a, CD14, CD80, CD86, CD40, and HLA-DR was then performed (Fig. 6). BP treatment failed to alter the expression of these markers expressed by differentiated DC. It also did not decrease DC viability (data not shown).

PAH effects on maturation of DC

To evaluate the capacity of PAHs to interfere with the maturation of DC, blood monocytes were first cultured with GM-CSF and IL-4 for 7 days, and the immature DC obtained were then stimulated with LPS in the absence or the presence of 10 μM BP. As shown in Fig. 7, exposure to LPS alone resulted in DC maturation, i.e., changes in some phenotypic markers, such as up-regulation of CD80, CD83, CD86, CD40, and HLA-DR, compared with immature DC; such alterations are in agreement with previous reports (19, 25). In the presence of BP, CD83 expression was significantly decreased, whereas the other markers were not, or were only slightly, affected (Fig. 7).

We next investigated the effects of continuous exposure to BP, i.e., during the differentiation process from blood monocytes and LPS-triggered maturation, on the expression of phenotypic markers of DC maturation. For this, the capacity of DC previously generated from monocytes in the presence of BP to further mature in response to LPS was analyzed. As shown in Fig. 7, these BP-treated cells show no or only marginal up-regulation of DC maturation markers such as CD83, CD80, CD40, CD86, and HLA-DR compared with DC generated in the absence of BP.

The effect of BP on the up-regulation of IL-10 and IL-12 secretion occurring in LPS-stimulated DC was further studied. As shown in Fig. 8, DC previously generated without BP displayed similar LPS-induced production of IL-10 in the absence or the presence of the PAH. By contrast, IL-10 secretion, which was barely detectable from immature DC differentiated in the presence of BP, remained very low after LPS treatment of these cells (Fig. 8). IL-12 secretion was markedly decreased in response to BP both when the PAH was added only during the LPS-triggered maturation process and when it was present during the differentiation and maturation phases (Fig. 8).

We finally investigated the effect of BP on the ability of mature DC to induce proliferation of allogeneic T lymphocytes through MLRs. As indicated in Fig. 9, cells continuously exposed to BP during differentiation and LPS-mediated maturation very poorly stimulated DNA synthesis in T lymphocytes compared with their untreated counterparts. The ability to induce T cell proliferation for DC exposed to BP only during the maturation process, although slightly increased compared with that of cells continuously exposed to PAH, was also markedly decreased compared with that of BP-untreated cells (Fig. 9). In such MLR assays, parental monocytes not exposed to GM-CSF and IL-4 also failed to trigger T cell proliferation (data not shown).

PAH inhibition of DC differentiation is not directly related to AhR

To determine whether AhR was functionally present in monocyte-derived DC cultures, blood monocytes were cultured with GM-CSF and IL-4 in the presence or the absence of 10 μM BP or 20 nM TCDD for 3 days. The expression of CYP1A1 was then analyzed by RT-PCR. As indicated in Fig. 10, CYP1A1 mRNA levels
were hugely induced in TCDD- and BP-treated cells compared with their untreated counterparts, whereas $H_9252$-actin mRNA amounts were not affected by the xenobiotics. This indicates that AhR is most likely fully active in monocyte-derived DC cultures, because this receptor is well known to play a key role in PAH and TCDD regulation of CYP1A1 (4, 5, 26). We further determined the effects of the very potent AhR agonist TCDD on DC differentiation from blood monocytes (Fig. 11). In contrast to BP, TCDD was not found to alter CD1a up-regulation occurring during DC differentiation; indeed, blood monocytes cultured with GM-CSF and IL-4 in the presence or the absence of TCDD for 7 days exhibited similar levels of CD1a (Fig. 11). TCDD treatment also did not obviously impair the expression of CD14 down-regulation. Similarly, TCDD did not obviously impair the expression of CD80 and CD40, in contrast to BP (Fig. 11).

Discussion

Many PAHs are well recognized as potent immunosuppressive agents (7, 10, 27). Although lymphocytes have been shown to be targets (9, 28, 29), the mechanisms by which PAHs exert immunotoxicity remain incompletely understood. In the present study, we provided phenotypical and functional evidence that PAHs are able to inhibit the in vitro differentiation and maturation of human monocyte-derived DC, thereby possibly identifying a new way by which PAHs interfere with the immune system. Indeed, we have

FIGURE 7. Effect of BP on LPS-triggered terminal maturation of immature DC. Immature DC previously generated from blood monocytes in the absence or the presence of 10 μM BP were further incubated for 48 h with LPS in the absence or the presence of 10 μM BP. Phenotypic analysis was then performed as described in Materials and Methods. Results for each marker are expressed as the MFI induction factor, i.e., the ratio of MFI after LPS treatment/MFI of immature DC; they are the mean ± SD of four independent experiments. DC + LPS, control cells not exposed to BP; DC + LPS/BP, cells exposed to BP only during maturation; DC/BP + LPS/BP, cells exposed to BP during differentiation and maturation. *, $p < 0.05$ compared with MFI induction factor for control cells not exposed to BP.

FIGURE 8. Effect of BP on IL-10 (A) and IL-12 (B) secretion from LPS-stimulated DC. Immature DC previously generated from blood monocytes in the absence or the presence of BP were further incubated for 48 h with LPS in the absence or the presence of 10 μM BP. Thereafter, IL-10 (A) and IL-12 (B) secretions in culture medium were analyzed by ELISA. Data are the mean ± SD of three independent experiments. DC, control DC exposed to neither LPS nor BP; DC + LPS, DC exposed to LPS; DC + LPS/BP, DC exposed to LPS and BP; DC/BP, DC differentiated in the presence of BP; DC/BP + LPS/BP, DC exposed to BP during differentiation and LPS-mediated maturation. *, $p < 0.05$ compared with DC + LPS.

FIGURE 9. Effect of BP on the stimulatory activity of monocyte-derived DC in MLR. DC previously generated from blood monocytes in the absence or the presence of 10 μM BP were further incubated for 48 h with LPS in the absence or the presence of 10 μM BP. MLR were then conducted as described in Materials and Methods. Data from a representative experiment performed in triplicate and expressed as cpm per well are shown; similar results were obtained in three other experiments. DC + LPS, DC exposed to LPS; DC + LPS/BP, DC exposed to LPS and BP; DC/BP + LPS/BP, DC exposed to BP during differentiation and LPS-mediated maturation.

FIGURE 10. CYP1A1 mRNA up-regulation in BP- and TCDD-treated monocyte-derived DC cultures. Monocytes were cultured with GM-CSF and IL-4 in the presence or the absence of 10 μM BP or 20 nM TCDD for 3 days. The expressions of CYP1A1 and $β$-actin were then analyzed by RT-PCR as described in Materials and Methods.
found that exposure to PAHs such as BP, BA, and DMBA impaired phenotypic markers of monocyte-derived DC. CD1a protein induction occurring during the DC differentiation process was notably strongly altered; such a regulation may involve translational or posttranslational mechanisms since we have detected similar levels of CD1a mRNAs in both untreated and BP-treated cells. Moreover, endocytosis capacity, which represents a key feature of immature DC (16, 17), was strongly decreased in response to BP. Cells differentiated in the presence of BP also displayed altered maturation in response to LPS, i.e., reduced or abolished up-regulation of phenotypic markers such as CD83, CD80, CD86, CD40, and HLA-DR, and decreased secretion of IL-10 and IL-12, compared with their untreated counterparts. Addition of BP only during the maturation process was found to result in more limited alterations, i.e., down-regulation of CD83 expression and reduced IL-12 secretion. However, such changes were functionally significant, because the addition of BP only during maturation was sufficient for markedly decreasing the ability of DC to stimulate the proliferation of allogeneic T cells in MLR assays; cells continuously exposed to BP, i.e., during differentiation and maturation, also very poorly induced T cell proliferation. This suggests that both DC differentiation and maturation are markedly affected by PAHs. However, we have failed to detect any phenotypic changes in differentiated DC exposed to BP; in particular, CD1a expression, which was strongly decreased when BP was used during the differentiation process from blood monocytes, was not altered when PAH was added after the differentiation occurred. This indicates that if PAHs can prevent DC differentiation and maturation from blood monocytes, they probably cannot act, or can act only marginally, on immunophenotypical features of established DC.

The mechanisms by which PAHs inhibit functional differentiation of monocyte-derived DC remain to be identified. The involvement of an unspecific and major toxicity due to PAHs can be ruled out, because we did not find any loss in cell viability in response to BP treatment. The fact that BP did not interfere with phenotypical features of established DC not previously exposed to PAHs during the differentiation process also argues against a major cytotoxic effect of PAHs. A role for IL-10, which is known to inhibit DC differentiation from monocytes (30, 31), can also be discarded, because we have failed to detect an up-regulation of IL-10 secretion in response to BP. The AhR, which is known to mediate many effects of PAHs, including immunotoxic effects (32), could be implicated. Indeed, it is present and functional in monocyte-derived DC, as indicated by RT-PCR detection of CYP1A1 up-regulation in BP- and TCDD-treated cells. However, several points do not support a direct involvement of AhR in PAH-related inhibition of DC differentiation: 1) the very potent AhR ligand TCDD failed to alter monocyte-derived DC differentiation; 2) the dose-effect relationship for BP-related inhibition of CD1a expression did not strictly parallel that reported for BP-mediated induction of AhR-regulated genes such as CYP1A1, i.e., low doses of BP, such as 0.1 μM, failed to significantly decrease CD1a levels, whereas they have been reported to be sufficient to induce CYP1A1 expression (26); 3) 3-MC, which displays a better affinity for AhR than BP (33), only weakly affected DC differentiation compared with BP; and 4) B(e)P, a PAH that is not thought to interact with AhR (34), displayed some inhibitory effects toward DC differentiation. Interestingly, the inhibitory effects of PAHs, such as BP on DC differentiation, are close to those reported for the nonsteroidal antiestrogens tamoxifen and toremifene (35). Indeed, these compounds have been shown, like BP, to markedly decrease CD1a expression without altering CD14 down-regulation in blood monocyte-derived DC; they also reduced CD80 and CD40 expression. Such effects have been shown to be independent of their antiestrogenic properties; they have been hypothesized to be linked to inhibition of protein kinase C activity (35). PAHs have also been shown to block protein kinase C activity (36). Whether such an effect can be the basis of PAH-related inhibition of DC differentiation deserves further study.

Due to the central role played by DC in the development of the immune response (16), PAH-altered differentiation of blood monocyte DC could significantly contribute to the known immunosuppressive properties of these environmental contaminants. Interestingly, BP, which strongly inhibits DC differentiation, has also been shown to affect Ag presentation through alteration in macrophage function in BP-treated mice (15). These data suggest a general inhibitory action of PAHs on APCs. It is also noteworthy that cutaneously applied DMBA abrogates afferent lymph DC function in the sheep (37–40), indicating that DC are probably in vivo targets of PAHs. In addition, it may be underlined that impairment of DC differentiation and function in response to PAHs could favor tumor development due to decreased immunological surveillance. This last point is important to consider, because PAHs are potent chemical carcinogens (1, 2), and therefore their immunosuppressive properties could indirectly contribute to their carcinogenic action, primarily due to their genotoxicity (3).

In summary, our data indicate that PAHs inhibit functional differentiation and maturation of blood monocyte-derived DC. Such an effect does not seem to directly involve the AhR. It could contribute to the known immunosuppressive properties of PAHs.

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